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Behavioral and Molecular Effects of Magnesium and Vitamin B6 Complex in a Mouse Model of Post-Traumatic Stress Disorder

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Abstract

Exposure to severe or uncontrollable traumatic stress factors often results in long-lasting alterations in emotional regulation and memory, leading to development of symptoms of post-traumatic stress disorder (PTSD). The present study evaluated the behavioral and neurobiochemical effects of a magnesium-vitamin B6 complex in a mouse PTSD model of PTSD induced by inescapable electric foot-shock. Male C57BL/6J mice were randomly divided into three groups: Control, Stress (exposure to electric foot shock), and Stress+Mg-B6 (electric foot shock with poststress administration of Mg-B6 complex with increasing dose from 100 to 200 mg/kg body mass). To assess the development of PTST-like symptoms, a battery of behavioral tests was used, including the aversive context test, open field test, light-dark box, elevated plus maze, splash test, and tail suspension test. Mice exposed to electric shocks demonstrated markedly longer freezing times compared with controls, indicating enhanced fear memory. Treatment with Stress+Mg-B6 resulted in reduced locomotor activity and shorter time spent in open areas in both the open field and elevated plus maze, suggesting transient behavioral inhibition. The Mg-B6 complex also reduced grooming behavior in splash test, which may indicate a lower motivational drive or suppression of stress-induced arousal rather than depressive-like symptoms. Electrical shock caused a decrease in total leukocyte levels but an increase in plasma myeloperoxidase activity. These changes were not modulated by the Mg-B6 complex. No significant changes in paraoxonase activity, IL-1β levels in blood, or cortex lipid peroxide levels were found in any of the experimental groups. Overall, the study suggests that high doses of Mg-B6 can modulate stress-related neurobehavioral responses, which appear to transiently suppress exploratory and motivational behaviors, possibly due to the inhibitory action of magnesium on neuronal excitability.

Keywords: post-traumatic stress disorder, electric footshock, mice, magnesium, vitamin B6, oxidative stress, inflammation

1. INTRODUCTION

Post-traumatic stress disorder (PTSD) occurs as a delayed response to a traumatic experience associated with threatened danger to life (Smid et al. 2022). PTSD is characterized by a complex set of symptoms, including heightened fear, intrusive thoughts, sleep disturbances, and significant anxiety (Bremner 2006). Although the pathophysiology of PTSD has been studied for decades, the available pharmacological treatments remain limited in efficacy and are frequently associated with adverse effects. This highlights the need for experimental studies aimed at identifying safer and more effective therapeutic strategies.

Accumulating evidence indicates that oxidative stress and neuroinflammation play an essential role in the development and maintenance of PTSD-like symptoms (Lushchak et al. 2023a, b; Dmytriv et al. 2023). Traumatic stress disrupts the balance between pro-oxidant and antioxidant systems, resilting in oxidative damage to neurons (Karanikas 2021). Concomitant activation of microglia, which releases inflammatory mediators, further contributes to neuronal dysfunction and behavioral abnormalities. However, the interaction between oxidative damage, inflammatory response, and behavior manifestation in PTSD-like states is still not fully understood.

Animal models that are based on exposure to acute inescapable traumatic stress, such as electric foot-shock, reproduce several key behavioral and neurobiological features of PTSD in humans, including conditioned fear responses, freezing behavior, and long-term alterations in emotional regulation (Verbitsky et al. 2020). These models provide a useful tool for studying mechanisms of trauma-related psychopathology and for testing potential protective compounds under controlled laboratory conditions.

Magnesium is a vital micronutrient that participates in numerous enzymatic reactions and regulates neuronal excitability through its effects on glutamatergic transmission. Magnesium blocks the calcium channel in the N-methyl-d-aspartate (NMDA) receptor and, in turn, prevents glutamatergic excitatory signaling (Kirkland et al. 2018). Deficiency of magnesium has been linked to increased glutamate excitotoxicity, followed by vulnerability to stress and anxiety-related behaviors (Pickering et al. 2020). Dietary supplementation with magnesium, especially in combination with vitamin B6, has been shown to enhance neurochemical stability, mitigate oxidative stress, and support synaptic plasticity (Noah et al. 2021; Patel et al. 2024). Vitamin B6 is a cofactor of gamma-glutamate decarboxylase, which produces inhibitor gamma-butyric acid (GABA). Despite the potential anxiolytic properties, the influence of dietary magnesium-B6 supplementation on PTSD-like behavioral and biochemical changes has not been adequately investigated.

The present study aimed to examine the protective effects of a magnesium-vitamin B6 complex in a mouse PTSD model induced by electric foot-shock exposure. Behavioral tests reflecting anxiety- and depression-related responses were combined with biochemical assays of oxidative and inflammatory markers, including blood paraoxonase (PON), myeloperoxidase (MPO), IL-6, and IL-1β. This experimental approach allows an integrated evaluation of both neurobehavioral and peripheral mechanisms potentially involved in the modulation of trauma-related disorders.

2. MATERIALS AND METHODS

2.1. Animals and housing

Adult male C57BL/6J mice were used in this study. Mice were obtained from Bogomolets Institute of Physiology (Kyiv, Ukraine) and then bred at animal house under controlled conditions (temperature: 22 ± 2 °C; humidity: 50-60%; 12:12 h light/dark cycle, 6 a.m./6 p.m.) with food and water available *ad libitum*. The animals were fed a standard rodent chow "KombyKorm", commercial food (PF Vita, Ukraine).

2.2. Experimental design

Animals at the age of 4 months were randomly assigned to three experimental groups (n = 8 per group):

1) Control group (without exposure to stress),
2) Stress group , in which mice were exposed to electric foot shock.
3) Stress+Mg-B6 group, which was exposed to to electric food shock with administration peroral Mg-B6 complex.

On Day 0 and Day 1, mice of the Stress and Stress+Mg-B6 groups were placed individually into a chamber with two walls and a lid made of transparent plexiglass, and two walls made of white plexiglass with a metal mesh floor, connected to a stimulus generator. Each mouse was subjected to 15 electric stimuli (intensity 0.8 mA; duration 10 s; interval between sessions 10 s) for 7 minutes in total. Immediately after each shock session, animals were returned to their home cages. Mice in the Control group were placed in the same chamber for 7 minutes on Days 0 and 1, but did not receive shocks.

Starting on Day 7, mice of the Stress+Mg-B6 group were exposed to daily oral gavage by the testing compound Mg-B6 for seven consecutive days (Days 7-13). The compound was dissolved in a 5% sucrose solution and administered at a dose of 100 mg/kg of body weight (50 μL). Mice of the Control and Stress groups received equivalent gavage volumes of 5% sucrose solution (vehicle) without the active compound. After open field test and splash-test, the dose of Mg-B6 (200 mg/kg) was elevated by two-fold and treatment was continued for five days. Mice were weighed before treatment to ensure accurate dose calculations.

To minimize handling-related stress, all mice were handled gently by the experimenters for 5 minutes each day during the 7-10 days immediately before the start of experiments. This pre-exposure to handling allowed animals to acclimate to human contact before any behavioral testing or stress procedures. All experimental protocols were approved by the Animal Experiments Committee of Vasyl Stefanyk Precarpathian National University, Ukraine, and were conducted in accordance with Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes.

2.3. Contextual Fear Conditioning Test

Contextual fear memory was assessed on Day 3 (Fig. 1A). Electric foot shock was combined with contextual trauma reminder through re-exposure to shock chambers, so-called the aversive context procedure. Freezing behavior, defined as the complete absence of movement except for breezing, was recorded as an index of contextual memory recall (Verbitsky et al. 2020). No additional stimuli were applied during the aversive context session.

2.4. Behavioral Testing

A battery of behavioral tests was conducted to evaluate locomotor activity, anxiety-like behavior, and depression-like behavior (Fig. 1A). Mice were allowed to acclimate to the testing room for 30 minutes before each session. Different researchers conducted the stress induction and the behavioral testing to ensure that the researcher conducting behavioral assessments was blinded to the treatment groups.

Open field test (Day 14). Locomotor activity and anxiety-like behavior using an open-field arena were measured as described previously (Balatskyi et al. 2025a, b). Each mouse was placed in the center of a square plexiglas box (40 cm × 40 cm) and allowed to explore for 10 minutes. Automated tracking ToxTrac software (version 2.98) developed by Magnus Andersson's team (Rodriguez et al. 2018) was used to analyze locomotor and anxiety activity, which processed a pre-recorded 10-minute video (Seibenhener and Wooten 2015). Defecation rate was also recorded.

Splash-test (Day 15). Depression-like behavior was assessed using the sucrose splash-test. A 10% sucrose solution was gently sprayed on the dorsal fur of each mouse, and grooming was

recorded for 5 minutes. Parameters scored included latency to the first grooming, duration of grooming, and grooming frequency (Bouguiyoud et al. 2022). Reduced grooming (longer latency or shorter duration) is interpreted as a depression-like apathy.

Elevated Plus Maze (Day 21). Anxiety-like behavior was further assessed using an elevated plus maze (EPM) (Bouguiyoud et al. 2022). The maze had two open arms and two closed arms (each 5 cm wide × 30 cm long; closed arm walls 15 cm high), elevated above the floor (40 cm). Each mouse was placed in the central area facing an open arm and allowed to explore for 5 minutes (after 1 min of adaptation). The time spent in open versus closed arms and the number of entries into each were recorded by a researcher.

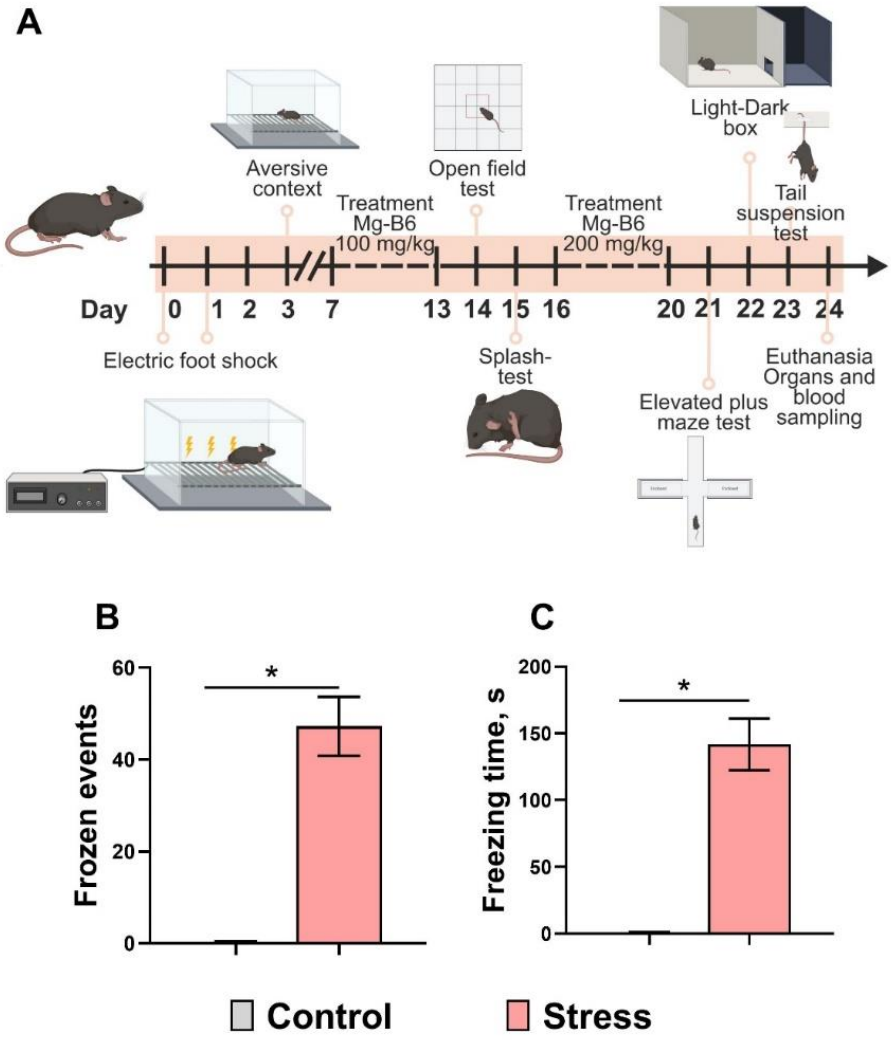

Fig. 1. Experimental design and freezings in the contextual fear conditioning test (aversive context test). (A) Four-month-old male C57BL/6J mice were randomly distributed into three groups: Control (n = 10 mice), Stress (n = 11) and Stress+Mg-B6 (n = 12 mice). Mice from the Stress and Stress+Mg-B6 groups were subjected to the electric foot shock procedure as described in the Materials and Methods. The aversive context test was performed on the third day after stress. Treatment with Mg-B6 (100 mg/kg) was started on day 7 and lasted for 7 consecutive days. The open field test was conducted on day 14 of the experiment. The elevated plus maze test (EPM) was performed on day 15 of the experiment. The second round of treatment with Mg-B6 (200 mg/kg) starts on day 16 and lasts for 5 days. The EPM was performed on day 21. Light-dark box and tail suspension tests were conducted on days 22 and 23, respectively. (B) Number of frozen events in the aversive context test, and (C) total freezing time in the aversive context. Data are presented as

mean ± SEM, n = 10-12. Significant differences (*$p < 0.05$) between the control and experimental groups were determined using Kruskal-Wallis test.

Light-Dark Box (Day 22). Anxiety-like behavior was also assessed in the light-dark box. The apparatus consisted of two compartments: one brightly lit and one darkened, connected by a small opening. Each mouse was placed in the light part and allowed to move freely between compartments for 7 minutes. Time spent in the light area and the number of transitions were recorded.

Tail Suspension Test (Day 23). Depression-like symptoms were measured by the tail suspension test. Each mouse was suspended by the tail (using adhesive tape ~1 cm from the tip) at a height of 50 cm above the floor within a custom suspension box. The duration of immobility was recorded during a 6-minute test as an index of behavioral despair (Bouguiyoud et al. 2022). Mice were defined as immobile when they hung passively and motionless.

2.5. Blood and Tissue Sampling

On Day 24, all mice were euthanized using light carbon dioxide anesthesia in a chamber, followed by manual cervical dislocation (Bayliak et al. 2022). Blood was collected by puncturing the right retro-orbital sinus and divided into two portions. The first portion was placed in heparin-coated tubes and centrifuged to separate plasma and cells. The plasma was stored on ice (0-4 °C) until biochemical analysis. The second portion was used immediately for leukocyte count. After euthanasia and blood sampling, mice were decapitated. The cerebral cortex was removed, washed in 0.9% NaCl, frozen in liquid nitrogen, and stored at -80°C for subsequent biochemical analyses. The total number of leukocytes was determined manually using Goryaev's chamber under a light microscope (Evolution LUM LS-8530) (Sorochynska et al. 2019).

2.6. Measurement of Plasma Paraoxonase, Myeloperoxidase, and Cytokine Levels

Plasma paraoxonase (PON) activity was determined by monitoring the rate of p-nitrophenol generation from p-nitrophenyl acetate at 405 nm, indicating the hydrolytic capacity of the enzyme (Vatashchuk et al. 2022). Myeloperoxidase (MPO) activity was assessed based on its ability to catalyze hydrogen peroxide-dependent oxidation of 3,3′,5,5′-tetramethylbenzidine, and the resulting chromogenic reaction was quantified spectrophotometrically (Yadav et al. 2014).

In addition, plasma concentrations of interleukin-1 beta (IL-1β) were quantified as described previously (Hurza et al. 2025). Briefly, blood plasma was diluted 1:5 in PBS, and 100 µL of each sample was added per well. Plates were incubated at 4°C for 2 h at room temperature to allow protein binding, then washed three times with PBS. Non-specific binding was blocked with 200 µL of 4% BSA in PBS for 2 h at room temperature, followed by washing. Primary anti-IL-1β antibody (Abcam, #ab9722) was applied (diluted 1:100 in 4% BSA) for 2 h at room temperature. After washing, HRP-conjugated secondary antibody (Cell Signaling Technology, #7074S; 1:2500 in 4% BSA) was added for 2 h at room temperature. HRP substrate (TMB) was then applied, incubated at 37°C for 22 min, and the reaction was stopped with 2 M H_2SO_4. Absorbance was measured at 450 nm, and IL-1β concentrations were calculated from standard curves.

Mouse plasma IL-6 levels were quantified using the Quantikine® Mouse IL-6 ELISA kit (R&D Systems, M6000B) according to the manufacturer's instructions. All reagents and samples were equilibrated to room temperature. Plasma samples were assayed in duplicate. Standards were prepared by serial dilution. Fifty microliters of Assay Diluent and 50 µL of either standard or sample were added to each well and incubated for 2 hours. After washing, IL-6 conjugate was added for a further 2-hour incubation. Following a second wash, substrate was added and the reaction was stopped after 20 minutes. Absorbance was read at 450 nm, and IL-6 concentrations were calculated from the standard curve.

2.7. Determination of Lipid Hydroperoxides

Lipid hydroperoxide (LOOH) concentrations in the cerebral cortex of experimental mice were evaluated spectrophotometrically. Frozen samples were homogenized in ice-cold 96% ethanol (1:10, w/v) and centrifuged to obtain clear supernatants. The content of lipid peroxides was then determined using the ferrous oxidation-xylenol orange (FOX) assay, which detects hydroperoxide-dependent oxidation of ferrous ions (Lushchak et al. 2005).

2.8. Statistical analysis

Statistical analysis and visualization were performed using GraphPad Prism (version 8.0.0 for Windows, GraphPad Software, Boston, Massachusetts USA, www.graphpad.com). Data of behavioral tests were analyzed by Kruskal-Wallis test with $p < 0.05$ as statistically different. Data of biochemical parameters were analysed by Tukey test with $p < 0.05$ as statistically different. The chosen sample size was based on power analysis calculations (https://wnarifin.github.io/ssc/ssanimal.html).

3. RESULTS

3.1. Behavioral effects of Mg-B6

Exposure to inescapable foot-shock resulted in a pronounced enhancement of conditioned freezing behavior. Mice subjected to electrical shock exhibited approximately 47-fold more freezing episodes (Fig. 1B) and spent significantly longer total time in the frozen state compared with the control group that was not exposed to stress (Fig. 1C). No significant freezing events were observed in the control animals placed in the same chamber without electrical stimuli (Fig. 1B, C).

In the open field test, mice that were treated with Mg-B6 (100 mg/kg) for 7 days spent 3.2-fold less time in the central zone of the arena compared with the Control group and 5.3-fold less compared with the mice of Stress group (Fig. 2A). Mice exposed to Mg-B6 treatment also displayed a 27% reduction in total distance traveled in open field arena relative to controls (Fig. 2B), indicating decreased locomotor and exploratory activity. It can be an indicative of sedative effects ofMg-B6 compound. Both the Stress and Stress+Mg-B6 groups exhibited 7.9-fold more freezing episodes compared with the control group (Fig. 2D).

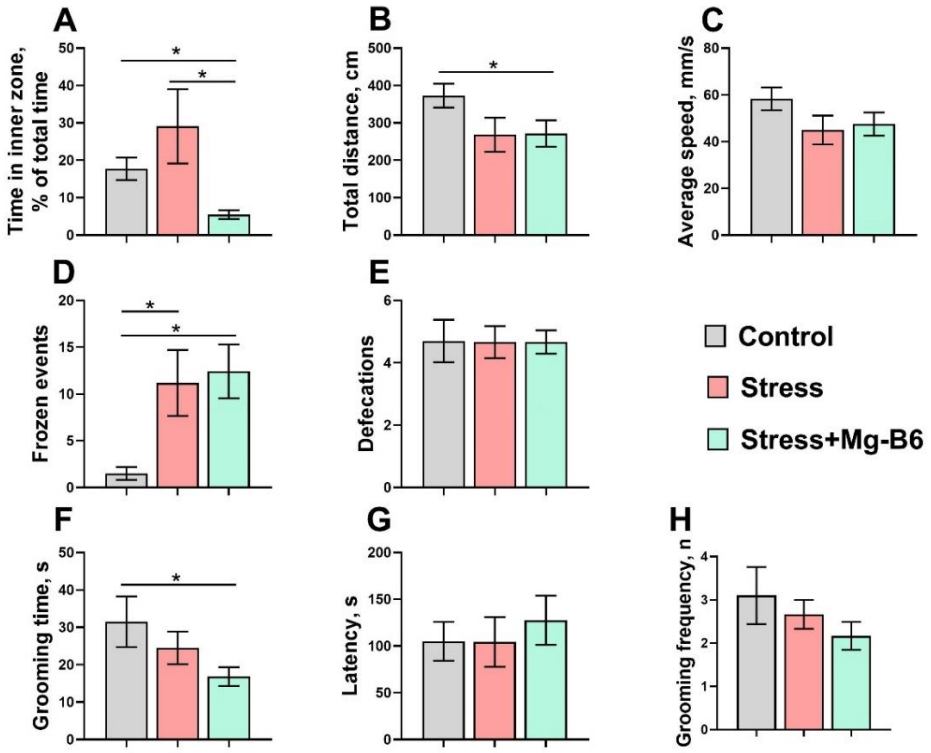

Fig. 2. Open field test (A-E) and splash-test (F-H) in mice exposed to electic footshock and treated with with Mg-B6 in the first round. (A) Time spent in the central squares of the open field, (B) total distance traveled, (C) average speed of mice, (D) frozen events in the open field, and (E) number of fecal boli per mouse in the open field. (F) Grooming time (G), latency to first grooming, and (H) grooming frequency in the splash-test. Data are presented as mean ± SEM, n = 10-12. Significant differences (*$p < 0.05$) between the control and experimental groups were determined using the Kruskal-Wallis test. Another information as in Fig. 1.

In the splash test, mice treated with Mg-B6 demonstrated 46% less total grooming time compared with control animals (Fig. 2F). Although there was also a tendency toward a reduced number of grooming events, this effect did not reach statistical significance (Fig. 2H).

In the EPM test, mice exposed to electric stress and subsequently treated with magnesium spent 3.7-fold less time in the open arms compared with the control group (Fig. 3A). They also made 2.7-fold fewer entries into the open arms (Fig. 3B). Additionally, the total number of arm transitions was 2-fold lower in the Mg-B6-treated mice compared with controls (Fig. 3C), reflecting a general reduction in exploratory and locomotor activity.

In the light-dark box test, mice of both the Stress and Stress+Mg-B6 groups exhibited 2.1-fold lower numbers of entries into the light part of the apparatus compared with the Control group (Fig. 3E). Similarly, mice of the Stress and Stress+Mg-B6 groups spent significantly less time in the light zone (Fig. 3F), indicating increased anxiety-like behavior and reduced exploratory behavior.

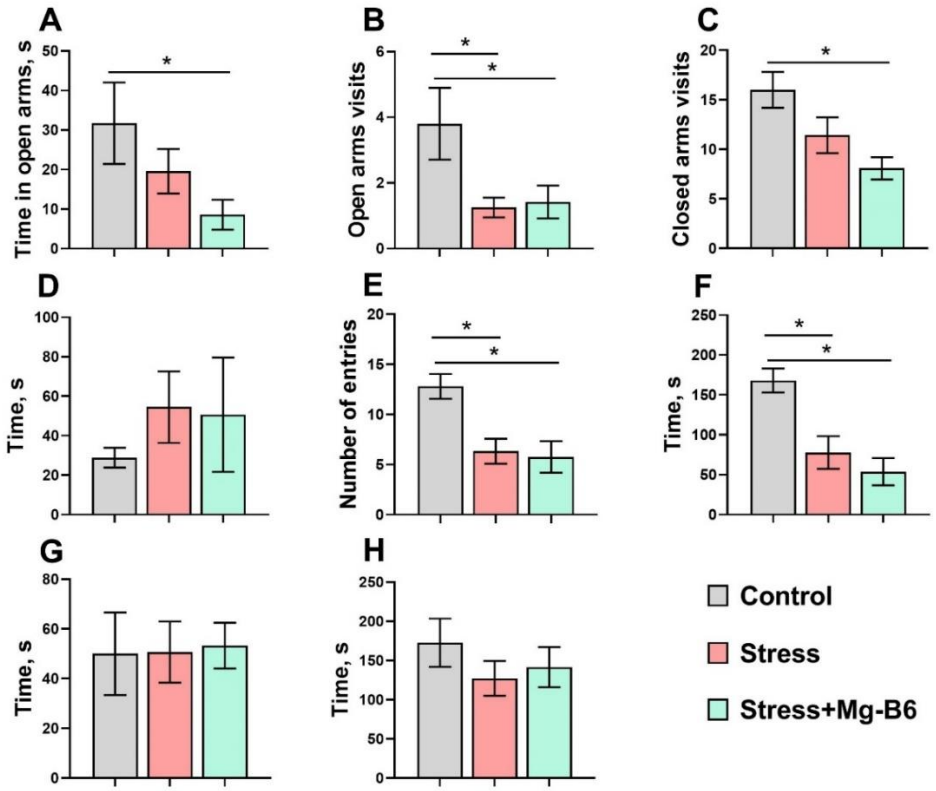

Fig. 3. Elevated plus maze (A-C), light-dark box test (D-F) and tail suspension test (G-H) after the second round of treatment with Mg-B6. (A) Time spent in the open arms of the elevated plus maze, (B) open and (C) closed arms visits. (D) latency to the entry into the light part of the light-dark box, (E) number of entries, and (F) time spent in the light part. (G) Latency before firs immobility episode and (H) immobility time in tail suspension test. Data are presented as mean ± SEM, n = 10-12. Significant differences (*$p < 0.05$) between the control and experimental groups were determined using the Kruskal-Wallis test. Another information as in Fig. 1.

In the tail suspension test, no significant differences were observed among the groups (Fig. 3G, H). Both the latency before first immobility episode and duration of immobility remained comparable across all experimental conditions (Fig. 3G, H).

3.2. Hematological and Biochemical Parameters

To assess systemic inflammatory and oxidative responses following stress exposure and Mg-B6 treatment, a series of hematological and biochemical analyses were performed on blood plasma and cerebral cortex samples from experimental animals. Mice exposed to stress exhibited a marked reduction in blood leukocyte count, which was approximately 16% lower than in the control mice (Fig. 4A), indicating potential stress-induced immunosuppression.

Myeloperoxidase (MPO) activity was by 4.5-fold higher in mice of both the Stress and Stress+Mg-B6 groups compared with the Mg-B6-Control group (non-stressed animals receiving magnesium) (Fig. 4B). In contrast, paraoxonase (PON) activity did not differ significantly among groups (Fig. 4C), suggesting relative stability of plasma antioxidative defenses. Lipid peroxide (LOOH) levels in the cerebral cortex were not affected by stress exposure. Furthermore, Mg-B6 treatment did not affect LOOH levels, demonstrating that neither stress nor Mg-B6 modulated cortical lipid oxidation under the conditions of this experiment (Fig. 4D). Similarly, interleukin-1β (IL-1β) and interleukin-6 (IL-6) concentrations remained unchanged (Fig. 4E, F), indicating that the applied stress paradigm and magnesium supplementation did not substantially alter systemic cytokine-mediated inflammation.

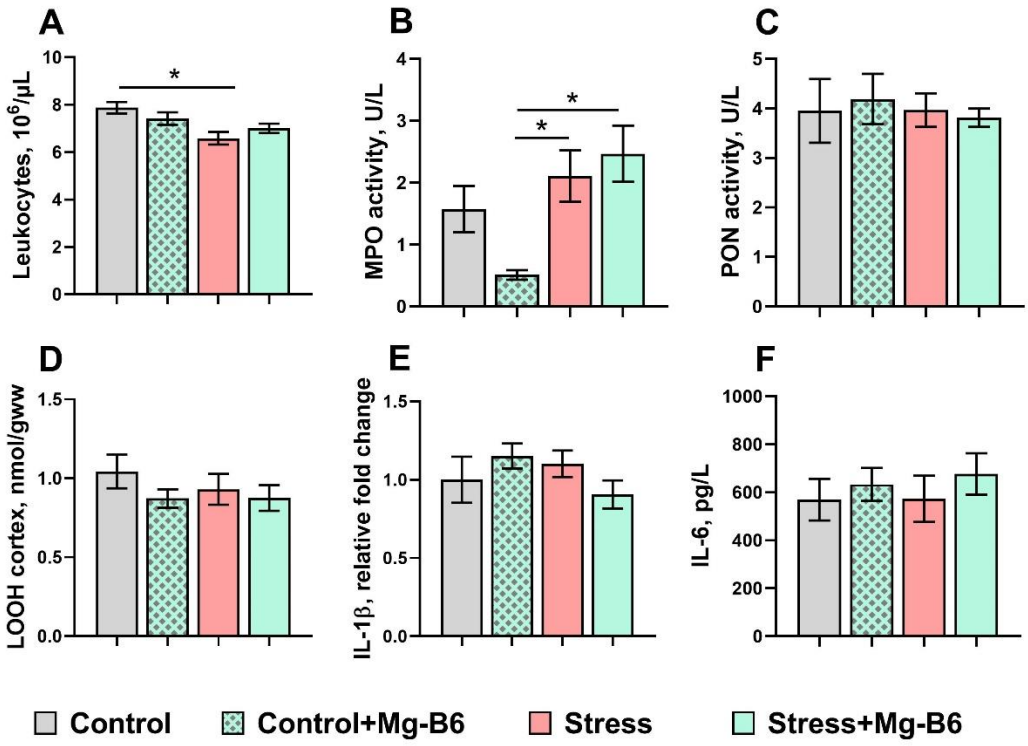

Fig. 4. Peripheral inflammatory and oxidative markers following the second round of Mg-B6 treatment after electric foodshock. (A) Total leukocyte count; (B) plasma myeloperoxidase (MPO) activity; (C) paraoxonase (PON) activity; (D) interleukin-1β (IL-1β) concentration; (E) lipid peroxide (LPO) levels in the cerebral cortex. Data are expressed as mean ± SEM (n = 5-12 per

group). Statistical differences between groups were evaluated using Tukey's post hoc test after one-way ANOVA; *$p < 0.05$ was considered statistically significant.

4. DISCUSSION

The damaging effects of traumatic stress are realized via a combination of nervous, endocrine, and immune responses, making PTSD a multisystemic disease with a significant component of immunoregulation and oxidative stress (Dmytriv et al. 2023). In this regard, studying the effects of potential neuromodulators such as the combination of magnesium and vitamin B6 (Mg-B6) has not only behavioral but also biochemical rationale. The combination of effects on NMDA sensitivity, neurotransmitter synthesis, and systemic antioxidant status makes Mg-B6 a promising candidate for modulating stress responses (Pickering et al. 2020).

The present findings confirm that inescapable foot-shock produces a robust conditioned fear response in mice. Indeed, animals exposed to electric foot shock showed markedly longer freezing during contextual re-exposure than controls, consistent with the formation of a persistent fear memory. Freezing as an index of conditioned fear is well established and was scored here by the standard criterion of immobility (Curzon et al. 2009). Treatment with the Mg-B6 complex after stress in our study results in a mixed behavioral profile. On one hand, Mg-B6-treated mice did not show anxiolytic effect, indicating that short-term Mg-B6 administration may be insufficient to modify stress-induced anxiety-related behavioral outcomes. On the other hand, treated animals displayed reduced exploratory activity in the open-field and spent less time on the open arms of the elevated plus maze, together with decreased grooming in the splash test. These changes point to a transient suppression of behavioral drive and exploration rather than a simple anxiolytic effect. This bidirectional pattern can be associated with known actions of magnesium on neuronal excitability. Magnesium ions modulate glutamatergic transmission by blocking NMDA receptor channels and also facilitate GABAergic function indirectly (Huang et al. 2025). NMDA blockade by extracellular Mg^{2+} lowers postsynaptic Ca^{2+} influx and synaptic potentiation, enhancing inhibitory neurotransmission. Pyridoxal phosphate (vitamin B6) is an essential cofactor for glutamate decarboxylase and thus facilitates GABA synthesis. Mg-B6 complex shifts the excitation-inhibition balance toward greater inhibitory tone, which results in suppression of decreased exploratory locomotion and motivation engagement observed in our study.

Preclinical studies showed that treatment with magnesium alleviates anxiety-like phenotypes in rodents (Coffman et al. 2024). A battery of anxiety tests, including the open field test, the light/dark test, the stress-induced hypothermia test, and the hyponeophagia test, used in the study by Sartori and colleagues (2011), showed enhanced anxiety-like behavior in mice with magnesium deficiency. Increased immobility time in the forced swim test was caused by a low magnesium diet, indicating enhanced depression-like behavior (Singewald et al. 2004). Manipulations that raise magnesium level in the organism can reduce excitability and, at higher exposure, suppress spontaneous activity (decreased locomotion or reduced explorative rate) depending on dose and formulation (Sartori et al. 2011). It was previously shown that magnesium regulates the activity of the hypothalamic-pituitary adrenal (HPA) axis (Murck and Steiger 1998), that the main neurobiological mechanism of PTSD and stress response. Magnesium reduces HPA axis activity by suppression of adrenocorticotropic hormone (ACTH) secretion (Murck and Steiger 1998).

Clinical and translational studies further support a beneficial role for combined magnesium and pyridoxine in stress-related outcomes. Randomized and observational data indicate more pronounced stress reduction when vitamin B6 is added to magnesium (Pouteau et al. 2018). The behavioral outcomes will depend critically on dose, timing, and the physiological state of the organism.

Traumatic stress is known to engage peripheral inflammatory pathways and alter oxidative-detoxifying systems. The decrease in absolute white blood cell count in the stress group is consistent with the known effects of chronic or intense stress that involves restructuring of immune

populations and redistribution of cells between tissues and circulation, mediated by glucocorticoid and sympathetic signaling. This phenomenon is described in studies where chronic stress suppresses the proliferation of immune cells by increasing corticosteroid levels (Dhabhar et al. 2012). The fact that Mg-B6 did not restore white blood cell counts in our experiment suggests two possible interpretations: (1) the hematological effects of stress in our model are persistent and short-term oral therapy is unable to correct them; (2) the neuromodulatory mechanisms of action of Mg-B6 are less influential on the systemic cell population at the time intervals we investigated. This is consistent with studies demonstrating a time-dependent response of the immune system to stress (Barrett et al. 2021).

An important observation is the significant increase in MPO activity in the Stress and Stress+Mg-B6 groups compared to Mg-Control. MPO is a marker of neutrophil activation and tissue inflammation. An increase in MPO activity under stress has been demonstrated in several models as an indication of enhanced oxidation and immune activation (Chen et al. 2020). Notably, the elevation in MPO activity in the Mg-B6 group suggests that short-term supplementation does not suppress early myeloid activation. At the same time, systemic IL-1β levels and plasma paraoxonase (PON) activity remained unchanged, demonstrating stress-induced shift toward rapid neutrophil oxidative activation, while cytokine signaling and systemic antioxidant compensation remain unaffected. Previous clinical and preclinical studies showed that Mg-B6 can alleviate subjective stress and behavioral manifestations of anxiety in humans and animals (Pouteau et al. 2018; Noah et al. 2021). However, most clinical studies have evaluated subjective parameters or long-term supplementation, whereas our results emphasize that short-term oral administration may not affect early systemic immune activation (MPO) or hematological changes. By contrast to studies that demonstrated a decrease in brain oxidation markers under the influence of magnesium in models of acute damage (e.g., ischemia or trauma), in our model, cortical LOOH remained unchanged, emphasizing the effect of the type of injury, magnesium regimen and dose, and treatment duration.

Taken together, the behavioral suppression observed here after Mg-B6 treatment indicates a dose-sensitive inhibitory action on neural circuits. At moderate doses, magnesium appears to be anxiolytic and protective, but higher acute exposures may transiently reduce motor and motivational output. This may help to explain apparent contradictions in the previous studies, where some preclinical studies report anxiolytic and antidepressant-like effects of magnesium (Poleszak et al. 2004) while others note reduced activity or sedative effects at different doses or delivery methods (Julio et al. 2017).

Limitations of the current study may restrict any strong mechanistic conclusions. The experiment used a single dosing regimen and one post-stress treatment window. Dose-response dependence and longer follow-up are needed to distinguish short-term motor suppression from longer-term anxiolytic or resilience-promoting effects. Finally, although peripheral markers such as MPO and PON can indicate systemic oxidative and inflammatory status, direct measures within discrete brain nuclei (amygdala, hippocampus) and neurochemical assays (NMDA/GABA receptor function, synaptic proteins, neurotransmitter levels) would strengthen mechanistic inferences.

5. CONCLUSIONS

In conclusion, our data indicate that a Mg-B6 complex modulates behavioral and biochemical responses after traumatic stress in a complex manner. The compound may reduce exploratory and motivational behaviors. Future studies should map dose-response curves, extend observation windows, include both sexes, and combine behavioral profiling with targeted neurochemical and regional brain analyses to clarify whether the observed suppression represents an adaptive dampening of hyperarousal or an undesirable sedative side effect.

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induce PTSD-like symptoms in mice. We thank V. Balatskyi for providing expert consultation on the experimental design and for valuable advice regarding the handling and care of mice. The authors also acknowledge H. Cherevata for animal care, and R. Shuliar, M. Mishchur, and T. Prysiazhniuk for constructing the chamber for the design and construction of the electric foot shock apparatus used for stress induction. Special thanks are extended to our student Oksana Vasylyshyn for her skilled technical assistance in tissue collection and hematological analyses.

Author contributions. M.B. and O.S. developed the idea for the research and edited the manuscript. V.B., V.D., V.H. and M.V. performed all the experiments and collected the data. O.S. and M.L. analyzed and interpreted the data. O.S. was the major contributor to the writing of the manuscript. All authors read and approved the final manuscript.

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Data availability. The part of the original data reported in this study are available in the DataSet repository at the following link: https://dataset.pnu.edu.ua/records/2380f-tcp48. Other relevant datasets can be obtained from the corresponding author upon reasonable request.

Declarations

Conflict of interest. The authors have no competing interests to declare relevant to this article's content.

Research involving human participants and/or animals. All mouse protocols were approved by the Animal Experimental Committee of Vasyl Stefanyk Carpathian National University and were conducted in accordance with the Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. The current study complies with the ARRIVE Guidelines for reporting in vivo experiments (https://arriveguidelines.org/arrive-guidelines).

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Стрільбицька ОМ, Гурза ВВ, Ватащук МВ, Деркачов ВП, Березовський ВВ, Лилик МП, Байляк ММ (2025) Поведінкові та молекулярні ефекти комплексу магнію та вітаміну B_6 у моделі посттравматичного стресового розладу у мишей. *Журнал Прикарпатського національного університету імені Василя Стефаника. Біологія* 12: 60-73.

Вплив тяжких або неконтрольованих травматичних стресових факторів часто призводить до тривалих змін емоційної регуляції та пам'яті, що сприяє розвитку симптомів посттравматичного стресового розладу (ПТСР). У цьому дослідженні було оцінено поведінкові та нейробіохімічні ефекти комплексу магнію та вітаміну B6 у мишачій моделі ПТСР, індукованій неминучими електричними ударами по лапах. Самців мишей C57BL/6J випадковим чином поділили на три групи: Контроль, Стрес (вплив електрошоку) та Стрес+Mg-B6 (електрошок із подальшим введенням комплексу Mg-B6 з наростаючою дозою від 100 до 200 мг/кг маси тіла). Для оцінки розвитку ПТСР-подібних симптомів застосовували низку поведінкових тестів, а саме тест аверсивного контексту, тест «відкрите поле», тест світло-темрява, піднятий хрестоподібний лабіринт, сплеш-тест і тест підвішування за хвіста. Миші, які зазнали електричних ударів, продемонстрували значно триваліший час завмирання порівняно з контрольною групою, що вказує на посилену страхову пам'ять. Споживання препарату у групі Стрес+Mg-B6 призвело до зниження локомоторної активності та скорочення часу, проведеного у відкритих зонах як у тесті «відкрите поле», так і в піднятому хрестоподібному лабіринті, що свідчить про тимчасове поведінкове гальмування. Комплекс Mg-B6 також зменшував грумінг у сплеш-тесті, що може свідчити про нижчу мотивацію або пригнічення стрес-індукованого збудження, а не про депресивноподібні симптоми. Електричний шок спричинив зниження рівня загальних лейкоцитів, але підвищення активності мієлопероксидази в плазмі крові; ці зміни не модулювалися комплексом Mg-B6. Не було виявлено значних змін у активності параоксонази, рівнях IL-1β у крові або рівнях ліпідних пероксидів у корі головного мозку в жодній з експериментальних груп. У цілому дослідження свідчить, що високі дози Mg-B6 можуть модулювати нейроповедінкові реакції, пов'язані зі стресом, які, очевидно, тимчасово пригнічують дослідницьку та мотиваційну поведінку, можливо, завдяки інгібувальному впливу магнію на нейрональну збудливість.

Ключові слова: посттравматичний стресовий розлад; модель стресу електрошоком; магній; вітамін B_6; оксидативний стрес; запалення