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Effects of Glycine Supplementation on Behavioral and Biochemical Parameters in a Mouse Model of Post-Traumatic Stress Disorder

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Abstract

Glycine is an amino acid that is involved in neurotransmission and glutathione synthesis. However, the effects of glycine treatment on stress-related behavior and biochemical changes in experimental animal models remain insufficiently understood. This study investigated the effects of oral glycine treatment on behavior and some biochemical parameters in a mouse model of post-traumatic stress disorder (PTSD). A sequential dose-escalation design was used. Glycine was administered at 300 mg/kg during the first 14 days and then the dose was increased to 600 mg/kg for a further 14 days. Mice were exposed to footshock to induce PTSD sympthoms and were theated with glycine orally in a form of gummy. Behavior was assessed using the elevated plus maze (EPM), light-dark box (LDB), open field (OF), splash, and tail suspension tests (TST). Glycine treatment reduced immobility in the TST compared with the untreated stress group. Measures of anxiety-like behavior did not show a consistent improvement following glycine treatment. Footshock exposure was associated with increased peripheral leukocyte counts, which remained elevated in mice that were treated with glycine. Paraoxonase (PON) activity in blood plasma was higher in mice that were exposed to glycine treatment, however, myeloperoxidase (MPO) activity remained unchanged. Reduced glutathione (GSH) levels and the GSH/GSSG ratio were decreased in mice exposed to stress. Our findings suggest that glycine may improve behavioural coping and modulate circulating antioxidant and lipid-protective mechanisms, as indicated by

increased plasma PON1 activity, but did not alleviate anxiety-like behaviour or restore cortical glutathione homeostasis in stressed mice. Investigation of glycinergic and glutamatergic neurotransmission may help clarify the mechanisms underlying these effects.

Keywords: electric footshock, glycine, inflammation, mice, oxidative stress, post-traumatic stress disorder

Abbreviations: PTSD, post-traumatic stress disorder; ROS, reactive oxygen species; HPA axis, hypothalamic-pituitary-adrenal axis; SAM axis, sympatho-medullo-adrenal axis; CNS, central nervous system; EPM, elevated plus maze; LDB, light-dark box; OF, open field; TST, tail suspension test; PON, paraoxonase; MPO, myeloperoxidase; NMDA, N-methyl-D-aspartate.

1. Introduction

Traumatic stress is associated with an increased risk of mood disorders such as post-traumatic stress disorder (PTSD), anxiety disorders, and depression that affect health and therefore quality of life (Wild et al. 2016). Previous data provides an understanding of the primary mechanisms of stress response system that include activation of two key systems such as the hypothalamic-pituitary-adrenal (HPA) axis and the sympatho-medullo-adrenal (SAM) axis (Del Campo et al. 2011; DeMorrow 2018; Erickson et al. 2003). However, the consequences of trauma may extend beyond psychiatric symptoms and may involve persistent changes not only in major stress-response systems, but also in immune regulation, and redox balance (Alotiby 2024). Preclinical rodent models were established to understand the underlying biological basis of the mechanisms that link traumatic experiences to behavioral and physiological changes (Verbitsky et al. 2020). Animal models of disorders caused by traumatic stress indicated potential therapeutic targets and could help to evaluate the efficacy of pharmacological interventions. Indeed, previous studies showed that D-cycloserine improved fear extinction in rats exposed to single prolonged stress. This data evidenced a potential role for N-methyl-D-aspartate (NMDA)-dependent synaptic plasticity in the regulation of fear learning (Yamamoto et al. 2008). Treatment by FAAH inhibitor URB597 of rats exposed to electric shock led to improved fear extinction (Fidelman et al. 2018). The effects were dependent by CB1-receptor and suggested the role of endocannabinoid signaling (Fidelman et al. 2018). Other studies have demonstrated beneficial effects on anxiety of ketamine treatment in mice after footshock (Zhang et al. 2015).

Glycine is the simplest amino acid. Glycine acts as a neurotransmitter, stimulating and inhibiting brain cells, and in turn, affects cognition, mood and appetite (Sajid et al. 2024). It was previously shown that glycine treatment extends the lifespan of *C. elegans* by 33% (Edwards et al. 2015). Moreover, lifespan extending effect of glycine treatment was shown in rats by approximately 20% (Brind et al. 2011) and mice by 6% (Miller et al. 2019). The anti-inflammatory effects of glycine have been observed in rodents (Alarcon-Aguilar et al. 2008). The study by Sajid and colleagues (2024) showed that glycine significantly improves anxiety and depression like symptoms in stressed rats. (Sajid et al. 2024). The study by Dolu (2007) investigated the effects of single dose glycine injection in mice using the elevated plus maze and reported reduced time spent in the open arms at intermediate and high doses (Dolu 2007). It was previously shown that glycine acts as an inhibitory neurotransmitter via glycine receptors in the central nervous system (CNS) (Dutertre et al. 2012).

Moreover, glycine functions as a co-agonist of NMDA receptors (Shimomura et al. 2015). Glycine also supports glutathione synthesis and in this way enhances antioxidant defence (McCarty et al. 2018). Despite these observations, the effects of glycine treatment at different dosed and duration in the context of traumatic stress remain insufficiently characterized. Therefore, the present study investigated the behavioral and biochemical effects of glycine treatment in mice exposed to traumatic stress. A distinctive feature of the study was its sequential dose-escalation design where lower doses of glycine was used for the first two weeks, after which its effects were evaluated before the dose was doubled for a further two weeks of treatment. This design allowed us to examine the effects of two dose over the course of different duration of treatment. In addition, glycine was administered orally in a form of gummy to avoid the additional stress caused by other administration methods.

2. Materials and methods

C57BL/6J laboratory mice were used in the study. The animals were housed under a 12-hour light/dark cycle (6:00 a.m./6:00 p.m.) at a temperature of 22 ± 2°C and a humidity of 50-60%. One-month-old animals were separated by sex, and only males were used for this study. All mice were fed a standard rodent diet, "KombyKorm" (PF Vita, Ukraine). All animals had free access to food and water. At four months of age, the animals were randomly divided to three groups: a control group and two experimental groups, with 9 mice per group (27 mice in total). Control group, with no exposure either to stress or treatment; Stress group, exposed to electric foot shocks; and Stress + Glycine group, exposed to electric foot shocks and treated orally with glycine (Fig. 1).

Stress induction. On Days 0 and 1, mice in the Stress and Stress + Glycine groups were subjected to electric foot-shock stress in an individual chamber. Each 7-min session included 15 foot shocks delivered at 10-s intervals. The detailed procedure for electrical stress induction has been described in our previous work (Strilbytska et al. 2025). Following each session, animals were returned to their home cages. Control mice were placed in the same chamber for the same duration but were not exposed to electrical stimulation.

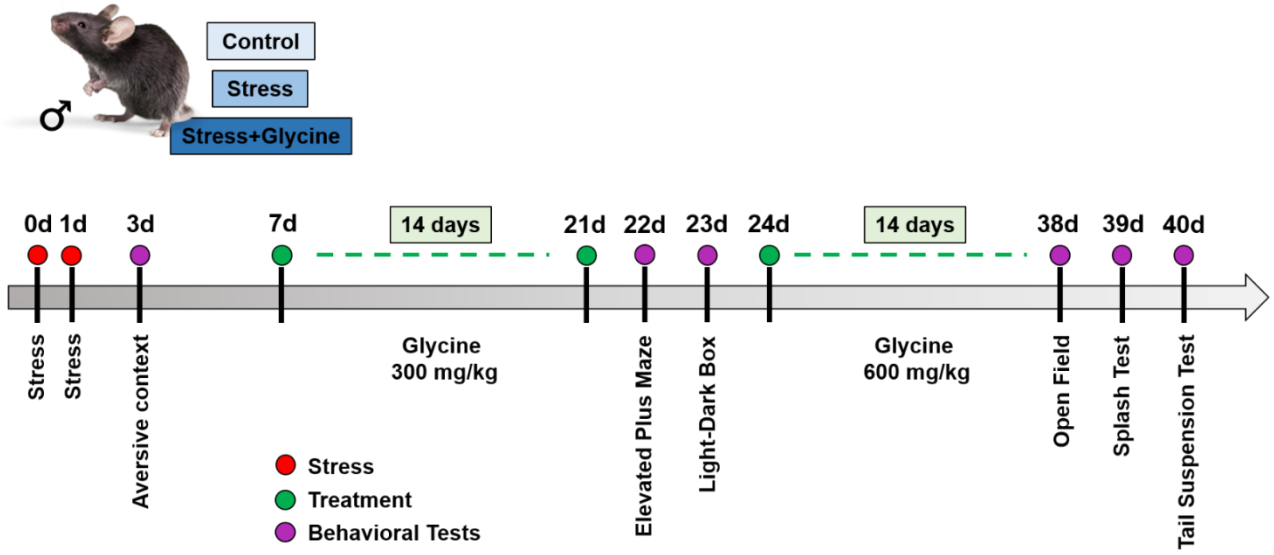

Fig. 1. Experimental design.

Aversive context test. On Day 3, mice were re-exposed to the same chamber used for stress induction without electric shock. Frequency and duration of freezing, defined as the absence of movement except for respiration, was used as an indicator of contextual fear memory (Verbitsky et al. 2020; Strilbytska et al. 2025).

Glycine treatment. From Days 7 to 21, mice in the Stress + Glycine group received glycine at 300 mg/kg body weight daily. From Days 24 to 38, the dose was increased to 600 mg/kg body weight daily. Glycine was administered orally as standardized gummies to minimize the stress associated with conventional gavage. The gummy mixture was prepared by dissolving sugar (60 g) in 100 mL of water following boiling. Infant milk formula (10 g), gelatin (6 g), and vanila flavoring (4 mL) were added after cooling the mixture. After cooling, glycine was also added at a concentration of 27.12 mg/mL. Placebo gummies were prepared identically but without glycine. Control and Stress groups were fed by placebo gummies. The mixtures were transferred into silicone molds (0.5 mL per well) and placed to the fridge until solidified. Gummies were divided into standardized portions providing glycine doses of 300 or 600 mg/kg body weight. Each mouse received one gummy daily at the same time and was monitored individually until the entire portion was consumed (Lelong et al. 2026).

All behavioral testing procedures were conducted as previously described (Strilbytska et al. 2025).

Elevated plus maze test. Mice were placed individually in the maze for 5 min following a 1-min habituation period. Plus-shaped apparatus was elevated 40 cm above the floor and had two opposite open arms and two opposite closed arms (each 5 cm wide and 30 cm long), with 15 cm high walls on the closed arms. The number of entries and time spent in the open arms were recorded manualy using pre-recorded video (Bouguiyoud et al. 2022). Anxiety index and general activity was calculated as was described previously (Cohen et al. 2013).

Light-dark box test. The apparatus had a light and a dark compartment separated by a wall with a small opening. Mice were placed in the illuminated compartment and allowed to explore both compartments for 7 min (Bourin and Hascoët 2013). The time spent in the light compartment and the number of transitions between compartments were recorded.

Open field test. Each mouse was placed in the center of a open-field arena and allowed to explore for 10 min. Locomotor activity, time spent in the central part of the arena and distance traveled were analyzed from recorded video using ToxTrac software. (Rodriguez et al. 2018; Seibenhener and Wooten 2015).

Splash-test. A 10% sucrose solution was sprayed on to the dorsal coat of each mouse, and grooming behavior was recorded for 5 min. Grooming latency, duration, and frequency were evaluated (Bouguiyoud et al. 2022).

Tail suspension test. Mice were suspended by the tail 50 cm above the floor for 6 min. Immobility time, defined as passive motionless hanging, was recorded (Bouguiyoud et al. 2022).

Sleep test. Mouse activity was monitored continuously with passive infrared sensors mounted 220 mm above the cage floor. Animals were placed in cages with minimal bedding, and the cage layout was adjusted to prevent the animal from being hidden from the sensor. Recordings were performed for a 5 days under a 12 h light/12 h dark cycle. Food and water were available throughout the recording period. Routine animal checks were conducted while avoiding unnecessary movement

of the cages. The activity signal was collected automatically in 10-s intervals and exported as csv files for further analysis (Brown et al. 2020).

At the end of the experiment, blood and brain cerebral cortex tissue were collected from the mice. The mice were anesthetized with carbon dioxide. Blood was collected from the retro-orbital sinus into heparin-containing tubes using heparin-rinsed glass capillaries. To obtain plasma, a portion of the blood was centrifuged (1500 × g, 15 min, 4°C) and the supernatant (blood plasma) was then collected and frozen in liquid nitrogen for subsequent storage at -80°C. Another portion of the collected blood was used to count leukocytes in a Goryaev chamber under a light microscope at ×1.000 magnification (Sorochynska et al. 2018). Following blood sampling, mice were euthanized by cervical dislocation.

Myeloperoxidase activity was determined in blood plasma by the change in solution color intensity at a wavelength of 450 nm. The reaction is based on the ability of myeloperoxidase to oxidize 3,3',5,5'-tetramethylbenzidine to brown-colored oxybenzidine in the presence of hydrogen peroxide (Yadav et al. 2014). Paraoxonase activity was determined in blood plasma using p-nitrophenyl acetate as the substrate, and the formation of p-nitrophenol was recorded on a spectrophotometer at a wavelength of 405 nm (Vatashchuk et al. 2022).

Glutamate measurement in mice cerebral cortex was performed using the method we described earlier (Demianchuk et al. 2025). Homogenization of the samples was done in 0,5M $HClO_4$ in a 1:10 (w:v) ratio on ice. Next, homogenates were centrifuged at 13000 g, 4°C for 15 min. The resulting supernatants were neutralized with 2 M KOH and centrifuged again under the same conditions. The final supernatants were collected and stored on ice. The determination was performed in a quartz cuvette at a wavelength of 340 nm using a spectrophotometer DeNovix DS-11. The mixture used for the determination contained 0.5 M glycine-hydrazine buffer (pH 9.5) with 2 mM NAD^+, 2 U/mL glutamate dehydrogenase (final concentrations are specified) and 50 µL of neutralized supernatant. The total volume of the reaction mixture was 1.25 mL. For the calibration curve, a standard glutamate solution with concentrations ranging from 10 to 250 µM was used.

Total and oxidized glutathione were determined in cerebral cortex using the enzymatic method described by Tietze (Tietze 1969). Tissue samples were homogenized in 5% trichloroacetic acid containing 0.01N HCl and centrifuged at 17000 g for 15 minutes to remove precipitated proteins. The supernatant was subjected to repeated extraction with diethyl ether five times and the aqueous (lower) phase was used to determine glutathione. To determine oxidized glutathione (GSSG), an aliquot of the extract was treated with N-ethylmaleimide (NEM; final concentration 0.02 M) for 40-60 minutes at 25°C. Excess NEM was subsequently removed by repeated extraction with diethyl ether 10 times, and the aqueous (lower) phase was collected again. Glutathione was quantified using an enzymatic reaction containing 0.6 µmol of 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB), 10 µg of glutathione reductase, and 0.2 µmol of NADPH in a phosphate-EDTA buffer (pH 7.5). The reaction was initiated by the addition of NADPH, and the increase in absorbance at 412 nm was recorded spectrophotometrically. A blank reagent was used to correct for background reduction of DTNB by the NADPH-glutathione reductase system. Total glutathione represented the combined concentration of GSH and GSSG, while GSSG was determined after treatment of the samples with NEM.

Statistical Analysis. The required sample size was calculated using sample size calculator (https://wnarifin.github.io/ssc/ssanimal.html). The normality of the datasets was tested using the

Shapiro-Wilk normality test (Table 1 and 2). The statistical differences among groups were evaluated using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test. Differences were considered statistically significant at $p < 0.05$. Statistical analysis and visualization were performed using GraphPad Prism version 8.0.2 for Windows (GraphPad Software, Boston, MA, USA).

3. Results and discussion

Glycine is a key precursor to glutathione which is a powerful antioxidant, and other important metabolites including creatine, heme, and purines (McCarty et al. 2018). That is why glycine supplementation can help restore glutathione levels and decrease oxidative stress (Ruparell et al 2024). This is particularly important in cases of traumatic stress when excessive production of free radicals can damage biomolecules. Glycine is capable to maintain glutathione levels in cells during oxidative stress and has anti-inflammatory effects (McCarty et al. 2018). This is why glycine is considered a potential neuroprotector and immunomodulator. Glycine also acts as an inhibitory neurotransmitter in the brain (Béchade et al. 1994). Based on these observations, our study aimed to test whether glycine treatment alters animal behavior following stress exposure and whether it modulates biochemical parameters associated with oxidative stress in mice.

Fear memory was assessed using the aversive context test after foot-shock exposure. Mice that were exposed to foot-shock showed increased freezing frequency and duration compared with control mice (Fig. 2A,B). After the initial 14-day treatment period with glycine at 300 mg/kg behavioral changes to stress exposure and glycine treatment were evaluated using validated behavioral tests including elevated plus maze (EPM) test and light-dark box (LDB) test. In the EPM test, it is generally expected that an anxious animal will spend less time in the open arms and more time in the closed arms (Cohen et al. 2013). In our study EPM test showed that the number of entries into the open arms was 2.0-fold and 3.6-fold lower in the Stress and Stress + Glycine groups, respectively, compared with the Control group (Fig. 2C). The Stress + Glycine group spent 4.2-fold less time in the open arms than the Control group (Fig. 2E). The anxiety index was higher in both the Stress and Stress + Glycine groups compared with the Control group that confirms an anxiety-like behavior (Fig. 2F). At the same time, general activity in the EPM was not affected by either stress or glycine treatment (Fig. 2D). This may suggest that the reduced open-arm exploration was not due to changes in overall locomotor activity. Thus, 300 mg/kg glycine treatment for 14 days did not alleviate the anxiety-like response induced by footshock.

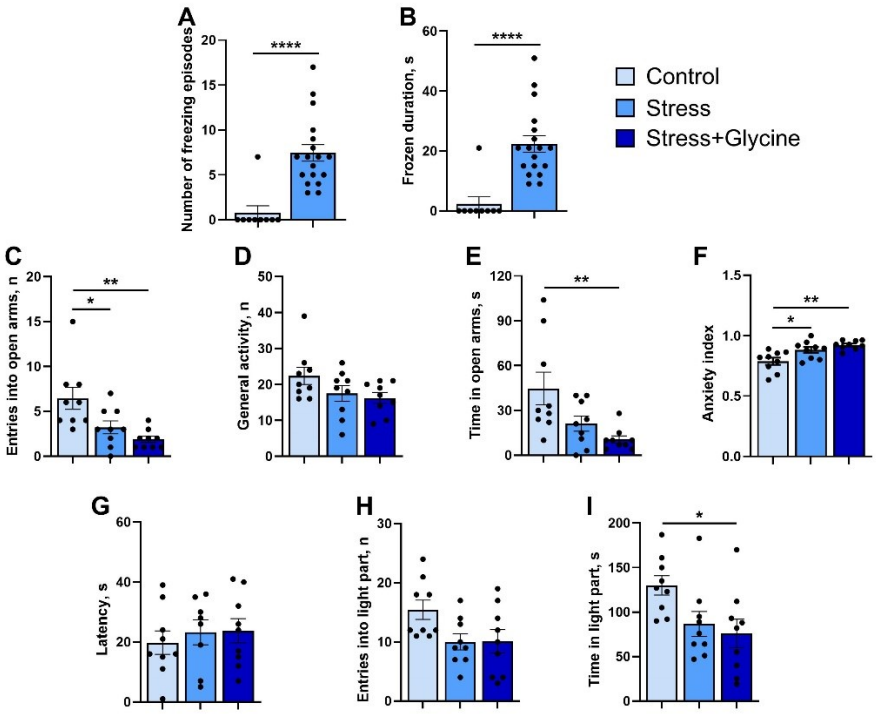

Fig. 2. Effects of 300 mg/kg glycine treatment for 14 days on behavior after stress exposure. Aversive context test: (A) number of freezing episodes and (B) total immobility time measured after stress exposure and before glycine treatment. Elevated plus maze test after 14 days of glycine treatment: (C) number of entries into the open arms, (D) total activity, (E) time spent in the open arms, and (F) anxiety index. Light-dark box test after 14 days of glycine treatment: (G) latency to the first entry into the light compartment, (H) number of entries into the light compartment, and (I) time spent in the light compartment. Data are presented as mean ± SEM; n = 9 per group. Asterisks indicate statistical significance: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

Next, a light-dark box (LDB) test which also measures a natural fear of bright light test was performed. Latency time to the first entry and number of entries to the light part of LDB were the same in three groups of mice (Fig. 2G,H). However, mice in the Stress + Glycine group spent 1.7-fold less time in the light compartment than mice in the Control group (Fig. 2I). Based on the results of EPM and LDB tests, the 300 mg/kg daily glycine treatment for 14 days did not decrease anxious behavior induced by fooshock.

Previous studies showed that the beneficial behavioral effects of glycine may need prolonged treatment and may become significant only after several weeks of treatment (Wang et al 2025; Nowak et al. 2000). In a mouse model with impaired glycine metabolism, oral administration of glycine at 1.3 g/kg/day was continued for at least 21 days before behavioral assessment (Wang et al 2025). These findings suggest that the effects of glycine may depend not only on dose but also on the duration of exposure. Therefore, after the initial 14-day treatment period, we increased the glycine dose and extended the treatment period to determine whether prolonged and higher glycine exposure could influence behavior in response to footshock. Considering the fact that repeated exposure to the same behavioral tests can induce habituation and influence behavior (Rodgers et al. 1993) the open-field (OF), splash tests and tail suspension test (TST) were used after second round of glycine treatment to evaluate anxiety- and depressive-like behavior in mice. No significant differences were observed between the Control, Stress, and Stress + Glycine groups for the

parameters assessed in OF test including distance traveled, speed of movement and time spent in the inner area (Fig. 3A-C). Similarly, experimental interventions did not influence grooming time, latency to first grooming and grooming events that were evaluated in Splash test (Fig. 3D-F).

Tail Suspension Test (TST) is widely used to evaluate passive coping behavior (Bouguiyoud et al. 2022). The Stress group showed a ~3-fold higher total immobility time than the Control group. Mice of Stress + Glycine group showed 2.7-fold reduced immobilization time as compared to Stress group (Fig. 3G). Moreover, Stress + Glycine group did not differ from the Control group in the immobility time. While latency period to first immobilization was unchanged under experimental interventions, we observed a tendency to lower latency in mice of Stress group and the same latency in mice of Stress + Glycine group as compared to the Control (Fig. 3H). In addition, was 2.6-fold lower number of immobility episodes in the Stress + Glycine group than in the Stress group and was at the same level as Control group (Fig. 3I). Thus, glycine treatment prevented the increase in immobility induced by traumatic stress. Our strategy is based on the assumption that the effects of glycine depend on duration and cumulative dose. Immobility in this test is an indicator of the balance between active escape behavior and passive coping during acute inescapable stress (Bouguiyoud et al. 2022). One possible explanation for the effect of glycine is its involvement in NMDA receptor-mediated neurotransmission. Glycine acts as a co-agonist at NMDA receptors, and prolonged glycine treatment may induce adaptive changes in glutamatergic signaling (Pupkiewicz et al. 2015). Glycine binds to the GluN1 subunit of NMDA receptors and is required for their activation in the presence of glutamate (Yu et al. 2018). NMDA receptor signaling regulates Ca^{2+}-dependent pathways involved in synaptic plasticity and neuronal function (Danysz and Parsons 1998; Paoletti et al., 2013). To further investigate the possible role of glycine in excitatory neurotransmission, we measured glutamate levels in cerebral cortex of mice. However, glutamate levels did not differ between groups (Fig. 5D). At the same time, glycine can also activate postsynaptic glycine receptors chloride channels that mediate inhibitory neurotransmission (Lynch et al., 2017). Thus, glycine may influence the balance between excitatory and inhibitory neurotransmission. However, these mechanisms were not directly examined in our study and remain possible explanations for the observed reduction in immobility in TST test caused by stress.

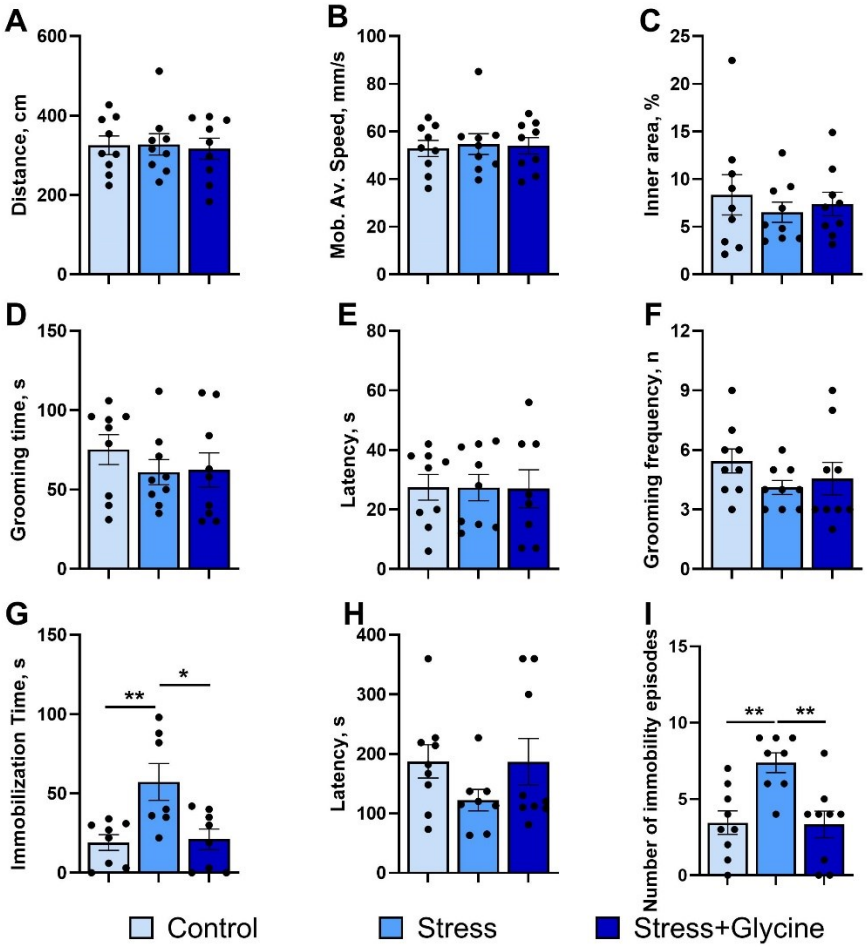

Fig. 3. Behavioral parameters after a total of 28 days of glycine treatment, with the dose increased to 600 mg/kg. Open-field test (OFT) parameters included (A) distance traveled, (B) speed of movement, and (C) time spent in the central area. Splash test parameters included (D) grooming time, (E) latency to the first grooming episode, and (F) the number of grooming episodes. Tail suspension test parameters included (G) total immobility time, (H) latency to the first immobility episode, and (I) the number of immobility episodes. Data are presented as mean ± SEM; n = 9 per group.

It was previously shown that glycine administration increased Non-Rapid Eye Movement sleep (NREM) and reduced waking assocociated with decreased activity of orexin neurons in mice (Hondo et al. 2011). Glycine has also been shown to promote NREM sleep through NMDA receptor in the suprachiasmatic nucleus (Kawai et al. 2015). This may indicate that glycinergic signaling can influence both sleep regulation and circadian control. Therefore, measuring of daytime activity was performed to determine whether chronic glycine treatment affected the daily activity after traumatic stress. No significant differences in daily activity were detected between groups. However, the Stress + Glycine group showed a tendency to slightly higher daily activity compared with the Stress group (Fig. 4A,B). An important limitation of the daily activity assessment was the absence of control group data. The lack of control data prevented a complete comparison of activity levels between the experimental groups.

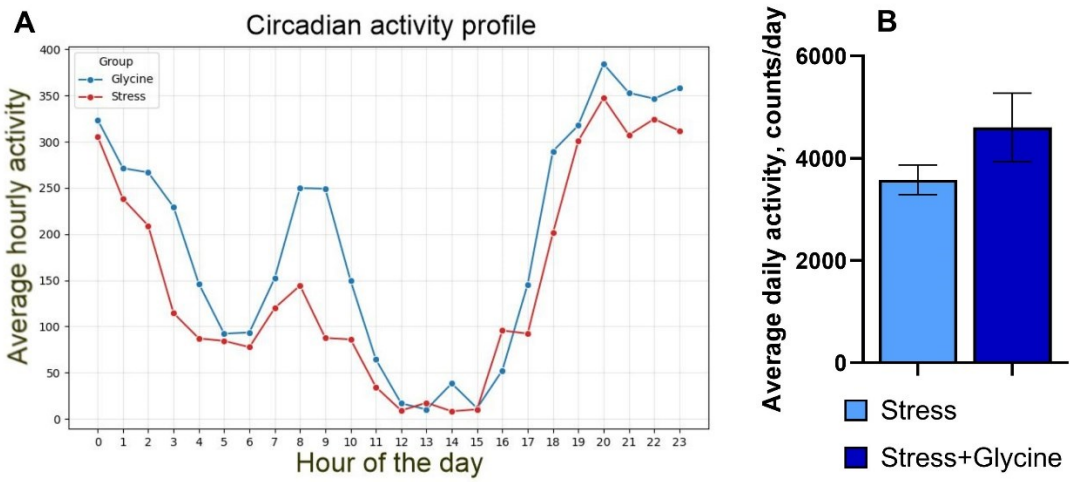

Fig. 4. Effects of oral glycine supplementation on daily activity patterns in mice. (A) Circadian activity profile. (B) Total daily activity. Data are presented as mean ± SEM.

The total leukocyte count increased by 31.0% in the Stress group and by 32.7% in the Stress + Glycine group compared with the Control group (Fig. 5A). This effect may indicate that leukocytosis induced by stress was not corrected by glycine treatment. Glycine treatment in mice was associated with a 36.8% higher paraoxonase activity compared with the Control group and a 36.4% higher compared with the Stress group (Fig. 5B). PON1 is a liver-derived, HDL-associated enzyme involved in the hydrolysis of lipid peroxides and other substrates (Marsillach et al. 2010). Reduced PON1 activity is generally associated with oxidative stress and inflammation (Marsillach et al. 2010). At the same time, PON1 may contribute to antioxidant defense via reduction of lipid oxidation (Marsillach et al. 2010). Moreover, PON1 can also modulate macrophage inflammatory responses including the production of pro-inflammatory cytokines (Aharoni et al. 2013). Myeloperoxidase activity in the blood plasma of mice was not affected by either foot shock exposure or glycine treatment (Fig. 5C). MPO is mainly associated with neutrophil and monocyte inflammatory activity and contributes to oxidative processes through the generation of reactive oxygen species (ROS) (Arnhold et al. 2020). While MPO promotes the formation of ROS, PON1 helps protect lipids from oxidation. The increase in PON activity and unchanged MPO activity after glycine treatment may suggests that glycine may influence antioxidant defense without substantially altering oxidative processes in general. To test this hypothesis we measured both reduced and oxidized glutathione levels in cerebral cortex of mice. GSSG levels remained unchanged, whereas GSH levels were 2-fold lower in both the Stress and Stress + Glycine groups compared with the Control group (Fig. 5E,F). Consequently, the GSH/GSSG ratio was also reduced in both groups that were exposed to stress and glycine treatment did not restore GSH level (Fig. 5G). GSH depletion may indicate increased utilization of this antioxidant to neutralize ROS or impaired glutathione synthesis. Although glycine is a substrate for glutathione synthesis, its supplementation did not restore cortical GSH levels under the conditions of our study.

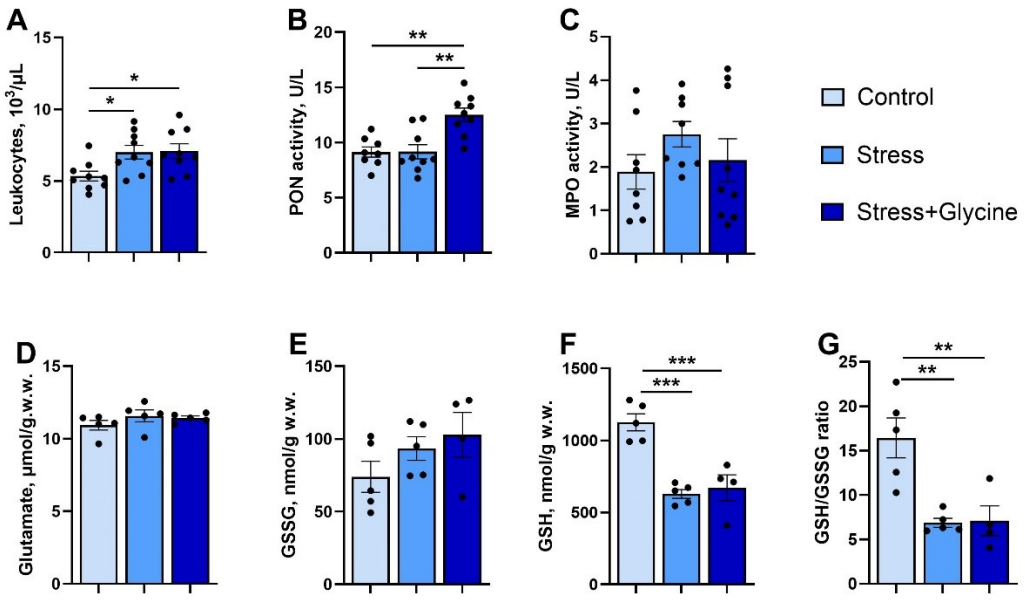

Fig. 5. Effects of glycine treatment on hematological and biochemical parameters in mice. (A) Blood leukocyte count. (B) Plasma paraoxonase-1 (PON1) activity. (C) Plasma myeloperoxidase (MPO) activity. (D) Glutamate concentration in the cerebral cortex. (E) Reduced glutathione (GSH) concentration in the cerebral cortex. (F) Oxidized glutathione (GSSG) concentration in the cerebral cortex. (G) GSH/GSSG ratio in the cerebral cortex. Data are presented as mean ± SEM; n = 5-9 per group.

4. Conclusions

In this study, we investigated the effects of oral glycine treatment in a mouse model of traumatic stress caused by footshock. We used a sequential dose-escalation design, with the dose increased from 300 to 600 mg/kg after the first two weeks of treatment. Glycine was administered in gummy to minimize additional stress associated with treatment. While glycine had no anxiolytic effect, the findings suggest that glycine at higher dose and prolonged treatment may modulate behavioral coping. Increased PON1 activity under glycine treatment may suggest about changes in circulating antioxidant and lipid-protective mechanisms. However, cortical GSH depletion and the reduced GSH/GSSG ratio in mice exposed to stress indicate that supplementation did not restore glutathione homeostasis under the conditions tested. Future studies should examine dose- and time-dependent effects of glycine on NMDA and glycine receptor signaling, glutathione synthesis, and stress-related neuroinflammatory pathways.

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Author contributions. Conceptualization: OS. Methodology, data collection, figures, tables, and referencing: VH, MV, VB, VD, ML, VB, OD. Writing – original draft preparation: OS, MV. Writing – review and editing: OS.

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Data availability.

Some original data reported in this study is available in the DataSet repository at the following link: https://dataset.cnu.edu.ua/records/nreen-mds56

The original data generated in this study are also available in the Zenodo repository at the following link: https://doi.org/10.5281/zenodo.23499583

Declarations.

Conflict of interest. The authors declare no competing interests.

Research involving human participants and/or animals. All animal procedures 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 study was conducted in accordance with the ARRIVE Guidelines for reporting animal research (https://arriveguidelines.org/arrive-guidelines).

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Гліцин – це амінокислота, яка бере участь у нейротрансмісії та синтезі глутатіону. Однак вплив лікування гліцином на поведінку, пов'язану зі стресом, та біохімічні зміни в експериментальних тваринних моделях досі недостатньо вивчений. У цьому дослідженні вивчався вплив перорального прийому гліцину на поведінку та деякі біохімічні показники в мишачій моделі посттравматичного стресового розладу (ПТСР). Застосовано послідовний дизайн дослідження з поступовим підвищенням дози. Гліцин вводили у дозі 300 мг/кг протягом перших 14 днів, а потім дозу збільшували до 600 мг/кг на наступні 14 днів. Мишей піддавали електрошоку на лапки для індукції симптомів ПТСР, після чого вони споживали гліцин у вигляді желе. Поведінку оцінювали за допомогою тестів підвищений плюс-лабіринт, світло-темна коробка, відкрите поле, сплеш-тест та підвішування за хвіст. Споживання гліцину призводило до зменшення тривалості нерухомості в тесті підвішування за хвоста порівняно з групою стресу, що не отримувала гліцин. Показники тривожної поведінки не проявляли стійкого полегшення після введення гліцину. Вплив електрошоку на лапки супроводжувався підвищенням кількості лейкоцитів, яка залишалася підвищеною у мишей, що отримували гліцин. Активність параоксонази (PON) у плазмі крові була вищою у мишей, які отримували гліцин, проте активність мієлопероксидази (MPO) залишилася незмінною. Рівень відновленого глутатіону (GSH) та співвідношення GSH/GSSG були знижені у мишей, що зазнали стресу. Наші результати свідчать про те, що гліцин може послаблювати інтенсивність поведінкових проявів стресу та впливати на деякі біохімічні шляхи, залучені до стресової реакції. Дослідження гліцинової та глутаматергічної нейротрансмісії може допомогти з'ясувати механізми, що лежать в основі цих ефектів.

Ключові слова: посттравматичний стресовий розлад, електричний футшок, миші, гліцин, оксидативний стрес, запалення

Supplementary Table 1: Results of the Shapiro-Wilk normality test for behavioral test data in mice. **EPM**—elevated plus maze test; **LD**—light-dark box; **TST**—tail suspension test; **ST**—splash-test**; OFT**—open field test**.**

Tests	Groups	Shapiro-Wilk normality test		
		W	p-value	Passed normality test?
EPM entries in open arms	**Control**	0.8068	0.0244	No
	Stress	0.9625	0.8241	Yes
	Stress+Glycine	0.8249	0.0391	No
EPM time in open arms	**Control**	0.8426	0.0617	Yes
	Stress	0.9210	0.4008	Yes
	Stress+Glycine	0.7909	0.0161	No
EPM entries in closed arms	**Control**	0.8871	0.1864	Yes
	Stress	0.9789	0.9584	Yes
	Stress+Glycine	0.9075	0.2991	Yes
EPM time in closed arms	**Control**	0.8426	0.0617	Yes
	Stress	0.9210	0.4008	Yes
	Stress+Glycine	0.7909	0.0161	No
LD latency	**Control**	0.9536	0.7299	Yes
	Stress	0.5169	<0.0001	No
	Stress+Glycine	0.9444	0.6287	Yes
LD entries into light part	**Control**	0.8332	0.0484	No
	Stress	0.9661	0.8592	Yes
	Stress+Glycine	0.9309	0.4896	Yes
LD time in light part	**Control**	0.9587	0.7844	Yes
	Stress	0.8397	0.0573	Yes

	Stress+Glycine	0.9398	0.5801	Yes
TST immobilization time	**Control**	0.7491	0.0053	No
	Stress	0.8914	0.2412	Yes
	Stress+Glycine	0.9191	0.3846	Yes
TST latency	**Control**	0.9363	0.5438	Yes
	Stress	0.8724	0.1592	Yes
	Stress+Glycine	0.7442	0.0046	No
TST number of immobilization	**Control**	0.9797	0.9629	Yes
	Stress	0.8480	0.0909	Yes
	Stress+Glycine	0.8881	0.1908	Yes
ST grooming Time	**Control**	0.8451	0.0658	No
	Stress	0.8990	0.2462	Yes
	Stress+Glycine	0.8639	0.1058	Yes
ST grooming evemts	**Control**	0.9520	0.7117	Yes
	Stress	0.8889	0.1942	Yes
	Stress+Glycine	0.8306	0.0453	No
ST latency	**Control**	0.8905	0.2018	Yes
	Stress	0.8398	0.0574	Yes
	Stress+Glycine	0.9191	0.4225	Yes
OFT distance	**Control**	0.9524	0.7159	Yes
	Stress	0.8782	0.1503	Yes
	Stress+Glycine	0.9045	0.2794	Yes
OFT speed	**Control**	0.9544	0.7382	No
	Stress	0.8501	0.0748	Yes
	Stress+Glycine	0.9236	0.4232	Yes
OFT inner area	**Control**	0.8690	0.1200	Yes
	Stress	0.8823	0.1659	Yes
	Stress+Glycine	0.9198	0.3907	Yes
OFT defecations	**Control**	0.9393	0.5746	Yes
	Stress	0.9165	0.3644	Yes
	Stress+Glycine	0.9263	0.4470	Yes

Supplementary Table 2: Results of the Shapiro-Wilk normality test for biochemical parameters in mice.

Parameters	**Groups**	**Shapiro-Wilk normality test**		
		W	**p-value**	**Passed normality test?**
Paraoxonase	**Control**	0.9898	0.9948	Yes
	Stress	0.8782	0.1501	Yes
	Stress+Glycine	0.9881	0.9929	Yes
Myeloperoxidase	**Control**	0.8940	0.2547	Yes
	Stress	0.8916	0.2423	Yes

	Stress+Glycine	0.8207	0.0351	No
Leukocytes	**Control**	0.9349	0.5290	Yes
	Stress	0.9535	0.7285	Yes
	Stress+Glycine	0.9388	0.5694	Yes