doi: 10.4149/gpb_2023022

Early-life maternal deprivation in rats increases sensitivity to the subsequent stressors: a pilot study

Maria Komelkova[1,2,3], Boris Yushkov[2], Stanislav Fedorov[1], Roman Ibragimov[2], Pavel Platkovskiy[1], Desheng Hu[3], Shanshan Luo[4] and Alexey Sarapultsev[1,2,3]

[1] *Russian-Chinese Education and Research Center of System Pathology, South Ural State University, Chelyabinsk, Russia*
[2] *Institute of Immunology and Physiology, Ural Branch of the Russian Academy of Science, Ekaterinburg, Russia*
[3] *South Ural State Medical University, Chelyabinsk, Russia*
[3] *Department of Integrated Traditional Chinese and Western Medicine, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China*
[4] *Institute of Hematology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China*

Abstract. Early life experiences, particularly maternal deprivation (MD), have long-lasting implications on emotional and cognitive development. Using Wistar rats as a model, this study explores the impact of MD followed by predator stress exposure (PSS) to simulate aspects of post-traumatic stress disorder (PTSD). A cohort of 41 male rat pups underwent MD from postnatal days 2 to 14, followed by PSS at day 90. Female rat pups were not included in the experiment. Behavior was subsequently assessed using the Elevated Plus Maze test 14 days post-PSS. While MD led to subtle changes such as decreased activity and increased anxiety-like behavior, PSS induced pronounced anxiogenic effects. Notably, PSS after MD resulted in decreased basal corticosterone levels, mirroring conditions observed in PTSD. The findings suggest that although MD itself does not induce significant behavioral changes, it predisposes individuals to heightened sensitivity to subsequent stressors. This study underscores the utility of a two-stage PSS model for more accurately reflecting the complexities inherent in stress-related disorders, including PTSD.

Key words: Cortisol — Early life stress — Maternal deprivation — Post-traumatic stress disorder — PTSD rats — Sensitivity

Abbreviations: ACTH, adrenocorticotropic hormone; BDNF, brain-derived neurotrophic factor; CRF, corticotropin-releasing factor; ELS, early life stress; EPM, elevated plus maze; MD, maternal deprivation; PSS, predator scent stress; PTSD, posttraumatic stress disorder; SAP, stretched attend posture.

Introduction

The first period of life in mammals plays a crucial role in their emotional and cognitive development, shaping their behavioral and physiological responses at later stages of ontogenesis. Maternal behaviors, such as licking/grooming and nursing, inhibit the secretion of adrenocorticotropic hormone (ACTH) and corticosterone, ensuring the maintenance of low and stable levels of corticosterone, which are necessary for proper brain development (Rosenfeld et al. 1992). Consequently, adverse events in early life, particularly changes in the maternal-offspring relationship, can cause acute disturbances and produce various detrimental effects (Lo Coco et al. 1989; Vetulani 2013). These acute and long-lasting effects arise from repeated activation of stress mediators, such as glucocorticoids and catecholamines, and were described by McEwen (2004) as an allostatic load.

Correspondence to: Maria Komelkova, Russian-Chinese Education and Research Center of System Pathology, South Ural State University, 454080 Chelyabinsk, Russia
E-mail: komelkovamv@susu.ru

Maternal deprivation (MD), a paradigm used to mimic the transient loss of maternal care, can lead to the removal of the inhibitory regulation that mothers exert on the hypothalamic-pituitary-adrenal (HPA) axis of offspring (Hofer 1994; Miragaia et al. 2018, Nishi et al. 2020). As a result, MD increases basal and stress-induced plasma concentrations of ACTH and corticotropin-releasing factor (CRF) (Ladd et al. 1996), and alters brain-derived neurotrophic factor (BDNF) expression and processing in the striatum, hippocampus, and ventral tegmental area (Ladd et al. 1996; Lippmann et al. 2007; Nishi et al. 2020). Recent literature suggests that the impact of MD on offspring extends beyond these disturbances, causing changes in other endocrine axes, including the hypothalamic-pituitary-gonadal (estrogen, progesterone, and testosterone) and hypothalamic-pituitary-thyroid axes, as well as secretions of growth hormone, prolactin, and oxytocin (Strüber et al. 2013; Myers et al. 2014).

It is not surprising that the literature documents associations between early life stress (ELS) exposures, including MD, and the subsequent development of various pathologies later in life. Several epidemiological studies have revealed that ELS is associated with clear increases in posttraumatic stress disorder (PTSD), mood and anxiety disorders, and substance use disorders (Anda et al. 2006; Cabrera et al. 2007; Fritch et al. 2010; Green et al. 2010; Scott et al. 2010). Furthermore, ELSs have been associated with an increased risk of not only psychiatric disorders but also a variety of medical disorders, including ischemic heart disease, cancer, chronic lung disease, skeletal fractures, autoimmune disorders, and liver disease (Nemeroff et al. 2016; Pervanidou et al. 2020).

However, it has been suggested that factors increasing the risk of exposure to adverse events later in life may differ from those that sensitize individuals to later stressors (Koenen et al. 2007; Manukhina et al. 2021). Therefore, the present pilot study aimed to reveal the effects of MD on subsequent predator stress exposure used as a model of experimental PTSD (Manukhina et al. 2020, 2021; Tseilikman et al. 2020; Ullmann et al. 2020). Based on data from the literature, we hypothesized that maternally deprived rats would display more pronounced anxiety and/or depressive-like behaviors on behavioral tests and reveal detectable changes in hormone levels and blood parameters. The study was conducted exclusively on male rodents to minimize physiological variability that could be associated with the estrous cycle in female rodents.

Materials and Methods

Animals and ethical permissions

Experiments were conducted using 41 Wistar rats, including 8 female mother rats and 33 male rat pups. On average, each litter contained 8 pups born on the same day. Female rat pups were not included in the experiment. The discrepancy in the animal numbers between the groups was a result of the limited quantity of animals received and our previous experience with the PSS model (Manukhina et al. 2020, 2021; Tseilikman et al. 2020; Ullmann et al. 2020). We elected to increase the sample size for the unfamiliar maternal deprivation model to better account for potential variances. Given the rapid maturation of these early-age animals, even a single day can significantly affect the studied parameters; hence our careful selection of rat pups born on the same day.

All animal experiments adhered to the requirements of the Council for International Organizations of Medical Sciences (CIOMS) and the International Council for Laboratory Animal Science (ICLAS), as described in the "International Guiding Principles for Biomedical Research involving Animals" (2012). The study protocol was approved by the Committee for Bioethics and Humane Treatment of Laboratory Animals at South Ural State University, Russia (Project 0425-2018-0011 from 17 May 2018, protocol number 27/521).

Animals were housed in an animal facility at a constant temperature (23 ± 1°C) and maintained on a 12:12 hour light-dark cycle (lights turned on at 8:00) with relative humidity (40–50%). Water and granulated forage (Sniff, Soest, Germany) were provided *ad libitum*. The handling of all animals was identical, ensuring consistent treatment across the study.

Experimental models

To mimic the conditions of ELS, we used the MD experimental model (Barreau et al. 2004). Primiparous pregnant rats were housed individually in standard polypropylene cages with wood chip bedding. Food and water were freely available. After birth, the rat pups were randomly assigned to the MD group or the mother-raised control group.

MD was performed daily for three consecutive hours (from 9:00 to 12:00 am), during which time the pups were removed from their home cage and kept in temperature-controlled cages at 28 ± 1°C. During MD, the pups were individually isolated. This procedure was applied between postnatal days 2 and 14. The control pups were left undisturbed with their dam. From days 15 to 22, all control and MD pups were kept with their dam. Weaning from the mother occurred on day 22, after which the offspring were separated by sex and housed in cages of 5–6 individuals until the end of the experiment. Females were excluded from the study.

On postnatal day 90, the animals were divided into 4 groups:

1. Control group – intact animals ($n = 5$);
2. MD group – animals subjected to ELS ($n = 12$);

MATERNAL DEPRIVATION	KEEPING PUPS+DAM	KEEPING ANIMALS IN STANDARD CONDITIONS	EXPOSITION TO THE PSS	REST	SUCROSE TEST	EPM	EUTHANASIA
2-14 day	15-22 day	23-90 day	91-100 day	100-112 day	113 day	114 day	115 day

Figure 1. Experimental design.

3. PSS group – animals subjected to repeated exposure to predator scent stress (PSS; $n = 5$);
4. MD+PSS group – animals subjected to repeated exposure to PSS in the background of ELS ($n = 11$).

A paradigm of repeated exposure to PSS was carried out as previously described in earlier studies (Manukhina et al. 2020, 2021; Tseilikman et al. 2020; Ullmann et al. 2020), where rats were exposed to the smell of urine marks of an adult domestic cat, poured into Petri dishes (100 ml each) and covered with medical gauze. For 10 days, PSS and MD+PSS rats were exposed daily for 10 min to PSS. For 10 days, all control and MD rats were exposed daily for 10 min to sham PSS (tap water). At 14 days after PSS or sham PSS, the behavior of the rats was tested and then euthanized for biochemical and hematological analyzes.

The experimental design is illustrated in Figure 1.

On the 115th day of the study, the animals were euthanized. They were decapitated, and their blood was collected. Blood samples were collected in two types of tubes (with K3-EDTA as an anticoagulant and without anticoagulants). Clotted blood was centrifuged at 3000 rpm for 15 min at 4°C, and the serum was separated. Serum aliquots were stored at −80°C until analysis.

Behavioral assessment

Sucrose Preference Test: To assess depression-like behavioral traits, we used a sucrose solution preference test ("Anhedonia"), which measured the amount of water and sucrose solution consumed by the animals (Liu et al. 2018). The test was conducted on the 113th day from the beginning of the experiment. Before the test, the animals were housed individually in cages. The animals were then provided with two preweighed 50 ml drinkers, one with water and the other with a 1% sucrose solution. After 24 hours, the drinkers were removed and weighed again. We calculated the sucrose solution preference index using the following formula:

$$Sucrose\ index = \frac{Volume\ of\ sucrose\ solution\ drunk}{Volume\ of\ sucrose\ solution\ drunk + Volume\ of\ water\ drunk)}$$

Elevated Plus Maze (EPM) Test: We conducted behavioral evaluations using the 3D animal tracking system "EthoStudio" in the EPM (Kulikov et al. 2014). We analyzed the following behaviors: number of entries into the closed arms, number of entries into and time spent on the open arms and closed arms, total number of entries, freezing, grooming, and the number of stretch attend postures (protected and unprotected), as described by Cruz, Frei, and Graeff (Cruz et al. 1994). The EPM test was conducted on the 114th day from the beginning of the experiment.

Laboratory studies

Corticosterone levels: We determined corticosterone levels using standard commercial kits from "Cloud-Clone Corp" (USA) through enzyme immunoassay.

Hematological analyses: We studied animal blood using an 18-parameter Celly 70 hematologic analyzer (Biocode-Hycel, France). Blood was collected in specially designed plastic tubes containing K3-EDTA as an anticoagulant. Subsequently, the following blood counts were automatically calculated: WBC, the absolute number of leukocytes (10^3/μl); Lym, the absolute number of lymphocytes (10^3/μl); Mid, the absolute number of middle cells (10^3/μl); Grn, the absolute number of granulocytes (10^3/μl); Lym%, the relative content of lymphocytes (%); Mid%, the relative content of middle cells (%); Grn%, relative content of granulocytes (%); RBC, the absolute number of erythrocytes (10^6/μl); Hb, hemoglobin content (g/dl); Hct, hematocrit (%); MCV, the average volume of erythrocytes (fl); MCH, the average content of hemoglobin in the erythrocyte (pg); MCHC, the average concentration of hemoglobin in the erythrocyte (g/dl); RDW, distribution of erythrocytes by size (%); Plt#, platelet count (10^3/μl); Pct, thrombocyte (%); MPV, mean platelet volume (fl); PDW, platelet size distribution (%).

Statistical analysis

Statistical calculations and transformations were performed in the RStudio programming environment (Allaire 2012) using the programming language R (Ihaka and Gentleman 1996). Data were tested for normality with the Shapiro-Wilk test, for equality of variances with the Levene test. To test the hypothesis of no difference between groups, two-way analysis of variance (Two-way ANOVA) or Two-way ANOVA with Aligned Rank Transform (ART) was used in the case of nonnormal distribution of the data. Tukey's test was used for repeated measures between groups after univariate analysis of variance, and contrast tests were used after the ART Two-way ANOVA.

Results

Effects of MD and PSS on rat behavior

Behavioral indicators are presented in Table 1. The analysis, conducted using a two-way ANOVA, revealed the contribution of maternal deprivation to the number of freezing ($F_{1.25} = 8.82$; $p = 0.006$), protected stretched attend posture ($F_{1.24} = 8.27$; $p = 0.008$), and sucrose consumption ($F_{1.25} = 5.16$; $p = 0.03$). Maternal deprivation in PSS-affected animals (MD+PSS group) reduced the number of freezing by 2.6 times compared to PSS animals ($\Delta 0.95 \in [-11.3,-3.9]$; $p < 0.0001$). Simultaneously, in animals of the MD+PSS group, a six-fold increase in protected stretched attend posture and a two-fold increase in sucrose consumption were observed compared to animals in the PSS group ($\Delta 0.95 \in [0.8,7.7]$; $p = 0.01$ and $\Delta 0.95 \in [1.4,9.8]$; $p = 0.005$, respectively).

It should be noted that MD did not cause any significant detectable changes in rat behavior. However, the time spent by MD rats in the open arms of the EPM tended to decrease by 37.4%, the number of entries in the open and closed arms, and the total number of entries compared to the control decreased by 64.3%, 36.5%, and 40.9%, respectively. The number of grooming acts and the total grooming time tended to decrease by 45.8% and 39.9% compared to the control animals. Additionally, MD rats showed a tendency to increase the number and total time of freezing compared to control animals.

The two-way ANOVA revealed that PSS significantly affected the number of freezing ($F_{1.29} = 17.97$; $p < 0.0001$). The number of freezing increased 6.7 times in animals exposed to PSS (PSS group) compared to the control ($\Delta 0.95 \in [6.1,14.9]$; $p < 0.0001$) and 3.4 times compared to the MD group ($\Delta 0.95 \in [-12.2,-4.9]$; $p < 0.0001$). Also, in PSS animals, there was a tendency to decrease in time spent in open arms (by 70.9%) and the number of entries into both open arms by 140% compared to control group animals. The increase in closed arms by 48.7% and the decrease in total grooming time by 41.3% and the number of grooming acts by 41.7% were observed after PSS.

The two-way ANOVA showed that the two factors studied, MD and PSS, affected the number of freezing not only separately but also when they interacted ($F_{1.29} = 25.23$; $p < 0.0001$). The interaction between factors was revealed in total stretched attend posture (SAP) ($F_{1.29} = 9.2$; $p = 0.006$) and sucrose consumption ($F_{1.29} = 8.78$; $p = 0.007$). In animals of the MD+PSS group, compared to the PSS group, total SAP increased 7 times ($\Delta 0.95 \in [-15.5,-9.4]$; $p = 0.02$) and sucrose consumption increased 1.9 times ($\Delta 0.95 \in [0.2,4.6]$; $p = 0.005$).

Table 1. Behavioral indicators

	Control ($n = 5$)	MD ($n = 12$)	PSS ($n = 5$)	MD+PSS ($n = 11$)
Opened time (s)	104.4 ± 75.5	65.4 ± 48.8	30.4 ± 26.5	30.5 ± 14.9
Closed time (s)	495.6 ± 75.5	534.6 ± 48.8	569.6 ± 26.5	569.5 ± 14.9
% open time	7.6 ± 4.6	9.5 ± 8.2	0.0 ± 0.0	2.1 ± 1.1
Open entries	1.4 ± 0.7	0.5 ± 0.1	0.0 ± 0.0	0.5 ± 0.2
Closed entries	7.4 ± 1.4	4.7 ± 1.1	3.8 ± 1.5	5.2 ± 1.0
Total entries	8.8 ± 1.1	5.2 ± 1.2	3.8 ± 1.5	5.7 ± 1.2
% Open entries	16.7 ± 8.4	14.6 ± 8.1	0.0 ± 0.0[1]	9.6 ± 4.8
Protected SAP	2.8 ± 0.6	3.6 ± 0.8	0.8 ± 0.4	5.0 ± 0.5[3]
Unprotected SAP	4.75 ± 1.0	1.4 ± 0.5	0.3 ± 0.2[1]	2.1 ± 0.5
Total SAP	7.0 ± 0.8	4.9 ± 1.2	1.0 ± 0.6	7.0 ± 0.9
% Protected SAP	40.8 ± 11.2	76.8 ± 9.5	83.3 ± 10.5	73.5 ± 5.7
Number of freezing	1.8 ± 0.4	3.6 ± 0.6	12.2 ± 2.0[1,2]	4.6 ± 0.5[3]
Total time of freezing (s)	31.4 ± 9.6	80.7 ± 23.6	143.4 ± 59.7	64.7 ± 11.5
Number of grooming acts	2.4 ± 0.6	1.3 ± 0.4	1.4 ± 0.9	1.8 ± 0.5
The total grooming time (s)	21.8 ± 6.3	13.1 ± 3.7	12.8 ± 9.5	21.2 ± 7.8
Anxiety index	0.82 ± 0.1	0.87 ± 0.08	0.97 ± 0.02	0.92 ± 0.03
Sucrose consumption (ml)	8.6 ± 0.9	9.1 ± 0.9	7.8 ± 2.4	11.2 ± 0.8[3]
Water consumption (ml)	0.2 ± 0.2	0.8 ± 0.2	0.8 ± 0.5	0.5 ± 0.2
Sucrose consumption index	1.0 ± 0.0	0.9 ± 0.0	0.9 ± 0.1	1.0 ± 0.0

[1] differences from control animals; [2] differences from MD rats; [3] differences from PSS rats. Control, intact animals; MD, animals subjected to ELS; PSS, animals subjected to repeated exposure to predator scent stress; MD+PSS, animals subjected to repeated exposure to predator scent stress in the background of ELS.

Biochemical and hematological analyzes

Laboratory studies revealed differences in corticosterone levels (Fig. 2). The two-way ANOVA showed that both MD ($F_{1.29}$ = 12.54; p = 0.002) and PSS ($F_{1.29}$=5.6; p = 0.03) factors could influence corticosterone levels separately, and combined ($F_{1.29}$ = 9.92; p = 0.004). The level of corticosterone in the MD+PSS group was the lowest among all groups: significantly lower than in the control group ($\Delta 0.95 \in [-630,-33]$; p = 0.03), 1.85 times lower than in the PSS group ($\Delta 0.95 \in [-213,-810]$; p = 0.0004), and 1.5 times lower than in the MD group ($\Delta 0.95 \in [-519,-72]$; p = 0.007).

Hematological analysis revealed a change in two blood parameters: the average volume of erythrocytes and the absolute number of granulocytes. The two-way ANOVA showed a pronounced effect of MD on blood granulocyte count ($F_{1.29}$ = 5.78, p = 0.03). There was a decrease in the level of granulocytes in the MD group compared to the control ($\Delta 0.95 \in [-0.8,-0.1]$; p = 0.04) (Fig. 3).

A two-factor ANOVA revealed the contribution of both PSS ($F_{1.14}$ = 6.27, p = 0.03) and the effect of the interaction between the studied factors on the average erythrocyte volume ($F_{1.14}$ = 5.97, p = 0.03). Animals in the MD+PSS group exhibited a higher mean erythrocyte volume compared to the MD group ($\Delta 0.95 \in [0.3,3.4]$; p = 0.02) (Fig. 4).

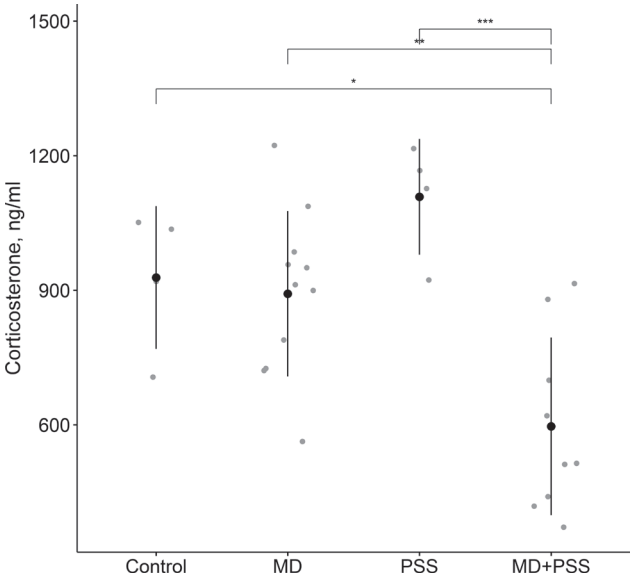

Figure 2. Corticosterone levels (mean ± SD) in experimental animals. Schemes follow the same formatting. Control, intact animals; MD, animals subjected to ELS; PSS, animals subjected to repeated exposure to predator scent stress; MD+PSS, animals subjected to repeated exposure to predator scent stress in the background of ELS. * $p < 0.01$, ** $p < 0.001$, *** $p < 0.0001$.

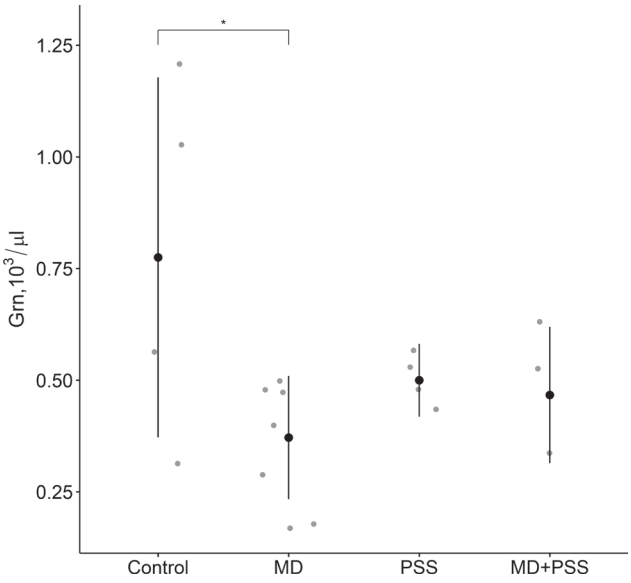

Figure 3. Number of granulocytes in the blood of experimental animals (mean ± SD). * $p < 0.01$. Grn, the absolute number of granulocytes. For more abbreviations, see Figure 2.

Discussion

The present study aimed to investigate whether transient loss of maternal care would affect the development of parameters related to PTSD in animals subjected to PSS, a paradigm used to mimic PTSD (Manukhina et al. 2020, 2021; Tseilik-

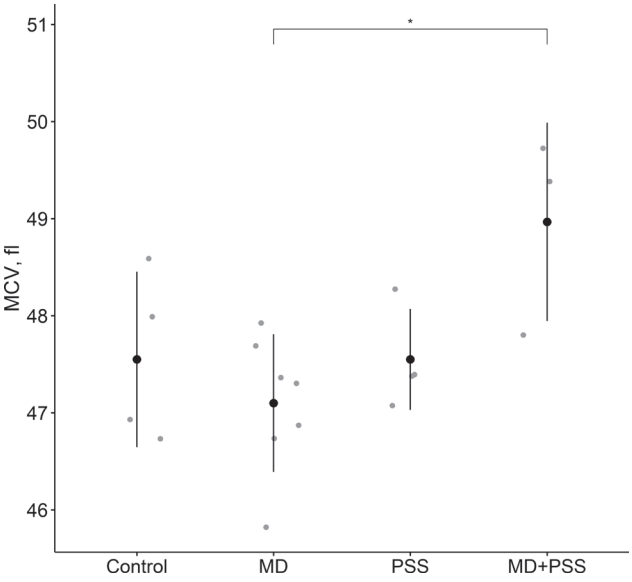

Figure 4. The average erythrocyte volume in experimental animals (mean ± SD). * $p < 0.01$. MCV, the average volume of erythrocytes. For more abbreviations, see Figure 2.

man et al. 2020; Ullmann et al. 2020). Male Wistar rat pups underwent repeated maternal separation during postnatal days 2–14. After 90 days, the rats were exposed to a PSS model consisting of exposure to predator odor stimulus. The behavior of the rats was evaluated on the EPM test on day 14 after PSS exposure.

EPM is a widely used rodent model for evaluating anxiety-related behavior, based on the natural preference of these animals for dark and protected places. Anxiety is measured by the proportion of test time spent in the closed arms of the maze, without coming into contact with the open areas (Acero-Castillo et al. 2021).

The study revealed several long-lasting effects of both experimental interventions (MD and PSS). Anxiety-like behavior increased and exploratory activity decreased in all groups. Moreover, the basal corticosterone level in plasma decreased in rats subjected to PSS after MD, paralleling the effects observed in patients with PTSD (Zoladz et al. 2012, 2015; Wilson et al. 2014). These results are consistent with previous studies (Cruz et al. 1994; de Oliveira Soares et al. 2014) and suggest that, in rats, an early stress experience such as MD can aggravate some effects of exposure to a stressor during adulthood.

According to the results, MD led to only slight behavioral effects, which manifested as a general decrease in activity and impulsivity, and increased anxiety-like behavior (evaluated by the total number of entries, open arms entries and time spent in (Cruz et al. 1994; de Oliveira Soares et al. 2014), and the immobility time (Walf and Frye 2007). MD also resulted in decreases in unprotected SAP and grooming, which are indicative of increased approach avoidance conflict and/or risk assessment behavior (Fernández Espejo 1997; Rodgers and Dalvi 1997; Horii-Hayashi et al. 2021). The data on the effects of MD in the literature are contradictory; while several studies reported lasting changes in behavioral reactivity (Troakes et al. 2009), others did not replicate that, reporting no alterations or even opposite effects (e.g., lower anxiety or lower depressive-like behavior) (Eklund and Arborelius 2006). Therefore, the present study's results reflect mainly those of Estanislau and Morato (2005), who also found that maternal separation does not cause strong behavioral effects. Furthermore, hematologic and biochemical studies did not reveal any specific changes.

Exposure to cat odor (PSS) produced clear anxiogenic responses in both the social interaction and EPM tests, suggesting a generalization of the anxiety response in both place and time (Zangrossi and File 1992). PSS revealed signs of anxiety-related behavior and general decline in activity, and resulted in a decrease in the percentage of open arms time (Cruz et al. 1994), the total number of stretches, and the number of unprotected stretches, which are indicative of risk assessment and/or exploratory behavior (Rodgers and Cole 1993; Rodgers and Dalvi 1997; Sestakova et al. 2013; Horii-Hayashi et al. 2021). The significant increase in freezing, indicating fearful/non-exploratory behavior (Rodgers and Cole 1993) also corresponded to increased anxiety (Walf and Frye 2007). This finding broadly supports the work of other studies in this area linking experimental PSS rat models with increased anxiety and reduced exploratory activity (Adamec et al. 2004; de Paula et al. 2005; Diehl et al. 2007; Albrechet-Souza and Gilpin 2019; Wu et al. 2019; Blount et al 2023). After PSS, the rats expectedly reduced sucrose consumption by 1%. This finding is consistent with that of Papp et al. (2016), who found that chronic stress led to reduced ingestion of 1% sucrose solution. Reduced low-concentration sucrose consumption has also been reported when other models of chronic stress are used (Willner et al. 1987; He et al. 2020; Wang et al. 2020). Anhedonia, in this context, can be defined as low reward sensitivity (Rygula et al. 2005). It should be noted that the concentration of sucrose solutions used can strongly affect the level of its ingestion (Acero-Castillo et al 2021; Markov 2022).

Thus, PSS after MD led to a decrease in basal corticosterone levels in animals, a condition similar to those observed in PTSD. According to the literature, low levels of cortisol (corticosterone in rats) could be due to partial primary adrenal insufficiency, HPA axis underactivity, increased negative feedback sensitivity and/or changes in glucocorticoid metabolism (Yehuda and Seckl 2011; Sarapultsev et al. 2020). Furthermore, it is known that in PTSD, low plasma levels of cortisol occur particularly in the context of ELSs, physical or sexual abuse, implying a degree of developmental programming, perhaps both of lower cortisol and vulnerability to psychopathology (Yehuda and Seckl 2011). The latter fully corresponds to our findings, as the MD model was established to mimic the adverse effects of early life. This also agrees with the studies by Zoladz et al. (2012, 2015) which revealed that rats exposed to a cat in conjunction with chronic social instability exhibited reduced basal glucocorticoid levels, increased glucocorticoid suppression, increased anxiety, and robust fear memory. The identified discrepancy with studies by Rodgers et al. (1999) can be explained by the fact that in our studies the negative correlations between SAP and corticosterone were detected in animals after MD, which provides the basis for the development of a pathological process resembling PTSD and whose reactions have already been changed, but not in naïve animals. Thus, Korte and De Boer (2003) have shown that animal emotions (fear) and behavior could be enhanced by prior stressor exposure and that the neural mechanisms involved in that fear-potentiated plus-maze behavior (state anxiety) as compared to spontaneous plus-maze behavior (trait anxiety) are quite different.

After PSS, MD rats increased sucrose consumption. This finding is contrary to previous studies which have suggested that rats subjected to these chronic stress models showed

reduced sucrose consumption (Katz and Hersh 1981; Willner et al. 1987; Sur and Lee 2022). However, in several studies (Matthews et al. 1995; Strekalova et al. 2006; Henningsen et al. 2013), increases in sucrose consumption and preference in stressed rats were also revealed. These effects can be interpreted as a manifestation of stress-induced diabetes mellitus and a hypercompensatory 'prohedonic' response to stress (Strekalova et al. 2022). They may also be associated with hypersecretion of corticotropin-releasing factor (CRF) and vasopressin in the hypothalamus and hypophysis, which provokes behavioral invigoration, a stronger consumption response, and stress-induced thirst (Strekalova et al. 2006, 2022). In line with this, according to Coplan et al. (1996), nonhuman primates with variable foraging mothers (a model for ELS) also had elevated CRF and decreased cortisol levels in adulthood, a picture that is close to PTSD (Bremner et al. 1997).

Finally, we can conclude that although MD does not cause pronounced behavioral effects (Estanislau and Morato 2005), it gives grounds for the development of complications in the future, increasing sensitivity to subsequent stressful effects (Agorastos et al. 2019). This also supports evidence from a meta-analysis of rodent studies by Schuler et al. (2022), which confirmed that early life stress (MD) creates a vulnerable phenotype (Schuler et al. 2022), which depends on genetic variations at specific genes that moderate HPA axis and brain function, and manifests itself only when sufficiently triggered (Lee et al. 2018, Schuler et al. 2022).

In summary, the observed changes in the present study can be understood through the concept of the 'training reaction.' This concept refers to a general nonspecific adaptive reaction that arises in response to weak stimuli of different qualities. According to Garkavi, this reaction leads to neither suppression nor significant stimulation of protective subsystems (Garkavi et al. 1998) (Fig. 5).

The MD variant used in this study, which involved weaning rat pups from their mother alone, should not be considered a pathological impact but rather a physiological one. This MD variant can be seen as a natural acceleration of the rat pups' transition to independent life when they leave their mothers. Generally, the 'training reaction' is characterized by increased secretion of glucocorticoids (within the upper half of normal) that results in a mild antiinflammatory effect without causing immunosuppression.

Thus, the present study's findings suggest that while MD may not cause pronounced behavioral effects, it can set the stage for future complications by increasing sensitivity to subsequent stressful effects. This supports the notion that ELS can create a vulnerable phenotype that depends on genetic variations at specific genes that moderate HPA axis and brain function and manifests only when sufficiently triggered. The concept of the 'training reaction' helps explain these findings and the complex relationship between ELS and later vulnerability to stress-related disorders.

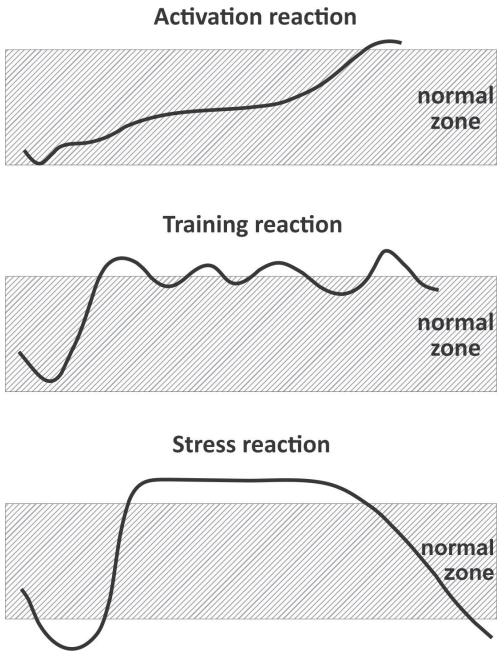

Figure 5. Various reactions of the body to frequentative weak stimuli (Garkavi et al. 1998).

The subsequent exposure to PSS initiates an 'activation reaction,' which is a general nonspecific adaptive reaction to medium stimuli, including olfactory stress. The biological purpose of this reaction is to increase the activity of the regulatory and protective systems within the body. The 'activation reaction' is characterized by a predominance of moderate excitation in the central nervous system and reduced secretion of glucocorticoids.

Animals that have experienced the 'activation reaction' due to MD display a weaker response to olfactory stress. This suggests a protective effect that may help the animals better adapt to the stressor. The observed changes in the MD+PSS group compared to the PSS group, such as an increase in unprotected SAP, total SAP, and sucrose consumption, as well as a decrease in the number of freezing instances and glucocorticoid levels, are consistent with the characteristics of an 'activation reaction.'

In conclusion, the study demonstrates that ELS, such as MD, can lead to an 'activation reaction' that enables animals to exhibit a weaker and potentially more adaptive response to subsequent stressors like PSS. This protective effect may contribute to an increased resilience against the development of stress-related disorders in later life.

Study limitations

It is important to emphasize that this study was conducted on the relatively small number of animals, which limited

the reliability of the data obtained for various parameters, particularly in the biochemical and hematological domains. This constraint restricts our ability to confidently confirm one concept over another, such as the development of training responses in MD and the subsequent activation response in PSS according to Garkavy et al. (1998), or the perception of MD as the basis for the formation of a sensitive phenotype according to Schuler et al. (2022).

To establish a clearer understanding of the effects of maternal deprivation, additional experiments are required. Future studies should focus on the differences between single and multiple exposures in early periods of ontogeny, as the development of the training response necessitates multiple exposures. Furthermore, investigating the differences in response to stronger stressors, compared with olfactory stress (e.g., immobilization stress), would provide further insights into the impact of ELS on later stress sensitivity.

Lastly, a significant limitation of this study is the exclusive use of male rats and not females. This is particularly relevant given that the prevalence of PTSD in women is twice as high as in men, even though there are few gender differences in the experience of traumatic events (Olff 2017). Inclusion of female rats in future studies could provide valuable insights into sex-specific responses to ELS and its potential role in the development of PTSD.

Conclusions and future perspectives

The present study aimed to investigate the effects of MD on rat behavior and anticipate pronounced anxiety-related changes in hormone levels and blood parameters. While we did not observe significant changes in most measures, our findings provide important insights and implications in two key areas.

Firstly, our study contributes to the understanding that early life experiences can influence an individual's sensitivity to subsequent stressors (Koe et al. 2009; Lewis and Olive 2014; Syed and Nemeroff 2017; Diaz-Chávez et al. 2020; Ochi and Dwivedi 2023). By examining the effects of ELS and subsequent stress exposure, we shed light on the potential vulnerability of specific groups. This knowledge can guide healthcare professionals in providing tailored interventions and early intervention strategies to mitigate the long-term consequences of stress and improve mental health outcomes.

Secondly, our study demonstrates the relevance of two-stage experimental models of PTSD, which involve both ELS experiences and subsequent exposure to pronounced stressors (Yehuda et al. 1990; Yehuda and Antelman 1993). This model better mimics the characteristics of PTSD, characterized by increased anxiety and lowered glucocorticoid levels. By utilizing such models, researchers can gain a deeper understanding of the complex mechanisms underlying PTSD and develop more effective diagnostic and therapeutic strategies.

Furthermore, our findings have implications for the optimization of treatment algorithms for stress-related disorders (Mathur and Sutton 2017). Understanding the intricate relationship between ELS and later vulnerability allows healthcare professionals to tailor therapeutic strategies based on an individual's history. This personalized approach enhances treatment effectiveness and improves patient outcomes by addressing the specific needs and challenges faced by each individual. Additionally, our research supports the development of preventive measures to mitigate complications and adverse mental consequences in individuals exposed to stress (Smith and Pollak 2020). By identifying predictors of negative outcomes, targeted interventions and preventive strategies can be implemented early on, reducing the burden of stress-related disorders and promoting overall population mental health.

In conclusion, our study's findings contribute to a better understanding of the complex nature of stress-related disorders and pave the way for the development of more effective strategies for their diagnosis, treatment, and prevention. By considering the impact of ELS and subsequent stress exposure, researchers and healthcare professionals can make significant strides towards improving the well-being of individuals and promoting mental health. This protective effect may contribute to increased resilience against the development of stress-related disorders, such as PTSD, later in life.

Author contributions. Conceptualization, MK and AS; Data curation, MK, BY; Investigation, MK, SD, PP, IK; Methodology, BY, DH, SL; Project administration, MK; Resources, MK and AS; Software, IK; Supervision, BY; Visualization, IK; Roles/Writing – original draft, MK and AS; Writing – review & editing, DH, BY and SL.

Institutional review board statement. The animal study protocol was approved by the Committee for Bioethics and Humane Treatment of Laboratory Animals at South Ural State University, Russia (Project 0425-2018-0011 from 17 May 2018, protocol number 27/521).

Data availability statement. The data sets generated and/or analyzed during the present study are available from the corresponding author on reasonable request.

Acknowledgments. The authors would like to acknowledge Maxim Lapshin (South Ural State University) for help with the experiments.

Conflicts of interest. The authors declare no conflict of interest.

References

Acero-Castillo MC, Ardila-Figueroa MC, Botelho de Oliveira S (2021): Anhedonic type behavior and anxiety profile of wistar-

uis rats subjected to chronic social isolation. Front. Behav. Neurosci. **15,** 663761
https://doi.org/10.3389/fnbeh.2021.663761

Adamec R, Walling S, Burton P (2004): Long-lasting, selective, anxiogenic effects of feline predator stress in mice. Physiol. Behav. **83,** 401-410
https://doi.org/10.1016/j.physbeh.2004.08.029

Agorastos A, Pervanidou P, Chrousos GP, Baker DG (2019): Developmental trajectories of early life stress and trauma: a narrative review on neurobiological aspects beyond stress system dysregulation. Front. Psychiatry **10,** 118
https://doi.org/10.3389/fpsyt.2019.00118

Albrechet-Souza L, Gilpin NW (2019): The predator odor avoidance model of post-traumatic stress disorder in rats. Behav. Pharmacol. **30,** 105-114
https://doi.org/10.1097/FBP.0000000000000460

Allaire J (2012): RStudio: integrated development environment for R. Boston

Anda RF, Felitti VJ, Bremner JD, Walker JD, Whitfield C, Perry BD, Dube SR, Giles WH (2006): The enduring effects of abuse and related adverse experiences in childhood. A convergence of evidence from neurobiology and epidemiology. Eur. Arch. Psychiatry Clin. Neurosci. **256,** 174-186
https://doi.org/10.1007/s00406-005-0624-4

Barreau F, Ferrier L, Fioramonti J, Bueno L (2004): Neonatal maternal deprivation triggers long term alterations in colonic epithelial barrier and mucosal immunity in rats. Gut **53,** 501-506
https://doi.org/10.1136/gut.2003.024174

Blount HL, Dee J, Wu L, Schwendt M, Knackstedt LA (2022): Stress resilience-associated behaviors following predator scent stress are accompanied by upregulated nucleus accumbens mGlu5 transcription in female Sprague Dawley rats. Behav. Brain Res. **436,** 114090
https://doi.org/10.1016/j.bbr.2022.114090

Bremner JD, Licinio J, Darnell A, Krystal JH, Owens MJ, Southwick SM, Nemeroff CB, Charney DS (1997): Elevated CSF corticotropin-releasing factor concentrations in posttraumatic stress disorder. Am. J. Psychiatry **154,** 624-629
https://doi.org/10.1176/ajp.154.5.624

Cabrera OA, Hoge CW, Bliese PD, Castro CA, Messer SC (2007): Childhood adversity and combat as predictors of depression and post-traumatic stress in deployed troops. Am. J. Prev. Med. **33,** 77-82
https://doi.org/10.1016/j.amepre.2007.03.019

Coplan JD, Andrews MW, Rosenblum LA, Owens MJ, Friedman S, Gorman JM, Nemeroff CB (1996): Persistent elevations of cerebrospinal fluid concentrations of corticotropin-releasing factor in adult nonhuman primates exposed to early-life stressors: implications for the pathophysiology of mood and anxiety disorders. Proc. Natl. Acad. Sci. USA **93,** 1619-1623
https://doi.org/10.1073/pnas.93.4.1619

Cruz AP, Frei F, Graeff FG (1994): Ethopharmacological analysis of rat behavior on the elevated plus-maze. Pharmacol. Biochem. Behav. **49,** 171-176
https://doi.org/10.1016/0091-3057(94)90472-3

de Oliveira Soares R, Marcellino de Oliveira L, Almeida SS (2014): Effects of environmental stimulation during different periods of central nervous system development in malnourished rats subjected to the elevated plus maze. Psychology & Neuroscience **7,** 521-529
https://doi.org/10.3922/j.psns.2014.4.11

de Paula HM, Gouveia A Jr, de Almeida MV, Hoshino K (2005): Anxiety levels and wild running susceptibility in rats: assessment with elevated plus maze test and predator odor exposure. Behav. Processes **68,**135-144
https://doi.org/10.1016/j.beproc.2004.12.003

Diaz-Chávez A, Lajud N, Roque A, Cheng JP, Meléndez-Herrera E, Valdéz-Alarcón JJ, Bondi CO, Kline AE (2020): Early life stress increases vulnerability to the sequelae of pediatric mild traumatic brain injury. Exp. Neurol. **329,** 113318
https://doi.org/10.1016/j.expneurol.2020.113318

Diehl LA, Silveira PP, Leite MC, Crema LM, Portella AK, Billodre MN, Nunes E, Henriques TP, Fidelix-da-Silva LB, Heis MD, et al. (2007): Long lasting sex-specific effects upon behavior and S100b levels after maternal separation and exposure to a model of post-traumatic stress disorder in rats. Brain Res. **1144,** 107-116
https://doi.org/10.1016/j.brainres.2007.01.084

Eklund MB, Arborelius L (2006): Twice daily long maternal separations in Wistar rats decreases anxiety-like behaviour in females but does not affect males. Behav. Brain Res. **172,** 278-285
https://doi.org/10.1016/j.bbr.2006.05.015

Estanislau C, Morato S (2005): Prenatal stress produces more behavioral alterations than maternal separation in the elevated plus-maze and in the elevated T-maze. Behav. Brain Res. **163,** 70-77
https://doi.org/10.1016/j.bbr.2005.04.003

Fernández Espejo E (1997): Structure of the mouse behaviour on the elevated plus-maze test of anxiety. Behav. Brain Res. **86,** 105-112
https://doi.org/10.1016/S0166-4328(96)02245-0

Fritch AM, Mishkind M, Reger MA, Gahm GA (2010): The impact of childhood abuse and combat-related trauma on postdeployment adjustment. J. Trauma Stress **23,** 248-254
https://doi.org/10.1002/jts.20520

Garkavi LKh, Kvakina EB, Kuzmenko TS, Shikchlyarova AI (1998): Anti-stressor Reactions and Activation Therapy. Yekaterinburg, Filantrop (in Russian)

Green JG, McLaughlin KA, Berglund PA, Gruber MJ, Sampson NA, Zaslavsky AM, Kessler RC (2010): Childhood adversities and adult psychiatric disorders in the national comorbidity survey replication I: associations with first onset of DSM-IV disorders. Arch. Gen. Psychiatry **67,** 113-123
https://doi.org/10.1001/archgenpsychiatry.2009.186

He LW, Zeng L, Tian N, Li Y, He T, Tan DM, Zhang Q, Tan Y (2020): Optimization of food deprivation and sucrose preference test in SD rat model undergoing chronic unpredictable mild stress. Animal Model Exp. Med. **3,** 69-78
https://doi.org/10.1002/ame2.12107

Henningsen K, Woldbye DP, Wiborg O (2013): Electroconvulsive stimulation reverses anhedonia and cognitive impairments in rats exposed to chronic mild stress. Eur. Neuropsychopharmacol. **23,** 1789-1794
https://doi.org/10.1016/j.euroneuro.2013.03.011

Hofer MA (1994): Hidden regulators in attachment, separation, and loss. Monogr. Soc. Res. Child Dev. **59,** 192-207
https://doi.org/10.1111/j.1540-5834.1994.tb01285.x

Horii-Hayashi N, Nomoto K, Endo N, Yamanaka A, Kikusui T, Nishi M (2021): Hypothalamic perifornical urocortin-3 neurons modulate defensive responses to a potential threat stimulus. Science **24,** 101908 https://doi.org/10.1016/j.isci.2020.101908

Ihaka R, Gentleman R (1996): R: a language for data analysis and graphics. J. Comput. Graph Stat. **5,** 299-314 https://doi.org/10.1080/10618600.1996.10474713

Katz RJ, Hersh S (1981): Amitriptyline and scopolamine in an animal model of depression. Neurosci. Biobehav. Rev. **5,** 265-271 https://doi.org/10.1016/0149-7634(81)90008-7

Koe AS, Jones NC, Salzberg MR (2009): Early life stress as an influence on limbic epilepsy: an hypothesis whose time has come? Front. Behav. Neurosci. **3,** 24 https://doi.org/10.3389/neuro.08.024.2009

Koenen KC, Moffitt TE, Poulton R, Martin J, Caspi A (2007): Early childhood factors associated with the development of post-traumatic stress disorder: results from a longitudinal birth cohort. Psychol. Med. **37,** 181-192 https://doi.org/10.1017/S0033291706009019

Korte SM, De Boer SF (2003): A robust animal model of state anxiety: fear-potentiated behaviour in the elevated plus-maze. Eur. J. Pharmacol. **463,** 163-175 https://doi.org/10.1016/S0014-2999(03)01279-2

Kulikov VA, Khotskin NV, Nikitin SV, Lankin VS, Kulikov AV, Trapezov OV (2014): Application of 3-D imaging sensor for tracking minipigs in the open field test. J. Neurosci. Methods **235,** 219-225 https://doi.org/10.1016/j.jneumeth.2014.07.012

Ladd CO, Owens MJ, Nemeroff CB (1996): Persistent changes in corticotropin-releasing factor neuronal systems induced by maternal deprivation. Endocrinology **137,** 1212-1218 https://doi.org/10.1210/endo.137.4.8625891

Lee RS, Oswald LM, Wand GS (2018): Early life stress as a predictor of co-occurring alcohol use disorder and post-traumatic stress disorder. Alcohol. Res. **39,** 147-159

Lewis CR, Olive MF (2014): Early-life stress interactions with the epigenome: potential mechanisms driving vulnerability toward psychiatric illness. Behav. Pharmacol. **25,** 341-351 https://doi.org/10.1097/FBP.0000000000000057

Lippmann M, Bress A, Nemeroff CB, Plotsky PM, Monteggia LM (2007): Long-term behavioural and molecular alterations associated with maternal separation in rats. Eur. J. Neurosci. **25,** 3091-3098 https://doi.org/10.1111/j.1460-9568.2007.05522.x

Liu MY, Yin CY, Zhu LJ, Zhu XH, Xu C, Luo CX, Chen H, Zhu DY, Zhou QG (2018): Sucrose preference test for measurement of stress-induced anhedonia in mice. Nat. Protoc. **13,** 1686-1698 https://doi.org/10.1038/s41596-018-0011-z

Lo Coco F, Basso G, di Celle PF, Tassinari A, Pasqualetti D, De Cuia MR, Putti MC, Del Poeta G, Ponzetto C, Saglio G (1989): Molecular characterization of Ph‘ + hybrid acute leukemia. Leuk. Res. **13,** 1061-1067 https://doi.org/10.1016/0145-2126(89)90151-3

Manukhina EB, Tseilikman VE, Karpenko MN, Pestereva NS, Tseilikman OB, Komelkova MV, Kondashevskaya MV, Goryacheva AV, Lapshin MS, Platkovskii PO, et al. (2020). Intermittent hypoxic conditioning alleviates post-traumatic stress disorder-induced damage and dysfunction of rat visceral organs and brain. Int. J. Mol. Sci. **21,** 345 https://doi.org/10.3390/ijms21010345

Manukhina EB, Tseilikman VE, Komelkova MV, Lapshin MS, Goryacheva AV, Kondashevskaya MV, Mkhitarov VA, Lazuko SS, Tseilikman OB, Sarapultsev AP, et al. (2021): Cardiac injury in rats with experimental posttraumatic stress disorder and mechanisms of its limitation in experimental posttraumatic stress disorder-resistant rats. J. Appl. Physiol. **130,** 759-771 https://doi.org/10.1152/japplphysiol.00694.2019

Markov DD (2022): Sucrose preference test as a measure of anhedonic behavior in a chronic unpredictable mild stress model of depression: outstanding issues. Brain Sci. **12,** 1287 https://doi.org/10.3390/brainsci12101287

Mathur S, Sutton J (2017): Personalized medicine could transform healthcare. Biomed. Rep **7,** 3-5 https://doi.org/10.3892/br.2017.922

Matthews K, Forbes N, Reid IC (1995): Sucrose consumption as a hedonic measure following chronic unpredictable mild stress. Physiol. Behav. **57,** 241-248 https://doi.org/10.1016/0031-9384(94)00286-E

McEwen BS (2003): Early life influences on life-long patterns of behavior and health. Ment. Retard. Dev. Disabil. Res. Rev. **9,** 149-154 https://doi.org/10.1002/mrdd.10074

Miragaia AS, de Oliveira Wertheimer GS, Consoli AC, Cabbia R, Longo BM, Girardi CEN, Suchecki D (2018): Maternal deprivation increases anxiety- and depressive-like behaviors in an age-dependent fashion and reduces neuropeptide y expression in the amygdala and hippocampus of male and female young adult rats. Front. Behav. Neurosci. **12,** 159 https://doi.org/10.3389/fnbeh.2018.00159

Myers AJ, Williams L, Gatt JM, McAuley-Clark EZ, Dobson-Stone C, Schofield PR, Nemeroff CB (2014): Variation in the oxytocin receptor gene is associated with increased risk for anxiety, stress and depression in individuals with a history of exposure to early life stress. J. Psychiatr. Res. **59,** 93-100 https://doi.org/10.1016/j.jpsychires.2014.08.021

Nemeroff CB (2016): Paradise lost: the neurobiological and clinical consequences of child abuse and neglect. Neuron **89,** 892-909 https://doi.org/10.1016/j.neuron.2016.01.019

Nishi M (2020): Effects of early-life stress on the brain and behaviors: Implications of early maternal separation in rodents. Int. J. Mol. Sci. **21,** 7212 https://doi.org/10.3390/ijms21197212

Ochi S, Dwivedi Y (2023): Dissecting early life stress-induced adolescent depression through epigenomic approach. Mol. Psychiatry **28,** 141-153 https://doi.org/10.1038/s41380-022-01907-x

Olff M (2017): Sex and gender differences in post-traumatic stress disorder: An update. Eur. J. Psychotraumatol. **8,** 1351204 https://doi.org/10.1080/20008198.2017.1351204

Papp M, Gruca P, Lason-Tyburkiewicz M, Willner P (2016): Antidepressant, anxiolytic and procognitive effects of rivastigmine and donepezil in the chronic mild stress model in rats. Psychopharmacology (Berl.) **233,** 1235-1243 https://doi.org/10.1007/s00213-016-4206-0

Pervanidou P, Makris G, Chrousos G, Agorastos A (2020): Early life stress and pediatric posttraumatic stress disorder. Brain Sci. **10,** 169
https://doi.org/10.3390/brainsci10030169

Rodgers RJ, Cole JC (1993): Anxiety enhancement in the murine elevated plus maze by immediate prior exposure to social stressors. Physiol. Behav. **53,** 383-388
https://doi.org/10.1016/0031-9384(93)90222-2

Rodgers RJ, Dalvi A (1997): Anxiety, defence and the elevated plus-maze. Neurosci. Biobehav. Rev. **21,** 801-810
https://doi.org/10.1016/S0149-7634(96)00058-9

Rodgers RJ, Haller J, Holmes A, Halasz J, Walton TJ, Brain PF (1999): Corticosterone response to the plus-maze: high correlation with risk assessment in rats and mice. Physiol. Behav. **68,** 47-53
https://doi.org/10.1016/S0031-9384(99)00140-7

Rosenfeld P, Suchecki D, Levine S (1992): Multifactorial regulation of the hypothalamic-pituitary-adrenal axis during development. Neurosci. Biobehav. Rev. **16,** 553-568
https://doi.org/10.1016/S0149-7634(05)80196-4

Rygula R, Abumaria N, Flügge G, Fuchs E, Rüther E, Havemann-Reinecke U (2005): Anhedonia and motivational deficits in rats: impact of chronic social stress. Behav. Brain Res. **162,** 127-134
https://doi.org/10.1016/j.bbr.2005.03.009

Sarapultsev A, Sarapultsev P, Dremencov E, Komelkova M, Tseilikman O, Tseilikman V (2020): Low glucocorticoids in stress-related disorders: the role of inflammation. Stress **23,** 651-661
https://doi.org/10.1080/10253890.2020.1766020

Schuler H, Bonapersona V, Joëls M, Sarabdjitsingh RA (2022): Effects of early life adversity on immediate early gene expression: Systematic review and 3-level meta-analysis of rodent studies. PLoS One **17,** e0253406
https://doi.org/10.1371/journal.pone.0253406

Scott KM, Smith DR, Ellis PM (2010): Prospectively ascertained child maltreatment and its association with DSM-IV mental disorders in young adults. Arch. Gen. Psychiatry **67,** 712-719
https://doi.org/10.1001/archgenpsychiatry.2010.71

Sestakova N, Puzserova A, Kluknavsky M, Bernatova I (2013): Determination of motor activity and anxiety-related behaviour in rodents: methodological aspects and role of nitric oxide. Interdiscip. Toxicol. **6,** 126-135
https://doi.org/10.2478/intox-2013-0020

Smith KE, Pollak SD (2020): Early life stress and development: potential mechanisms for adverse outcomes. J. Neurodev. Disord. **12,** 34
https://doi.org/10.1186/s11689-020-09337-y

Strekalova T, Gorenkova N, Schunk E, Dolgov O, Bartsch D (2006): Selective effects of citalopram in a mouse model of stress-induced anhedonia with a control for chronic stress. Behav. Pharmacol. **17,** 271-287
https://doi.org/10.1097/00008877-200605000-00008

Strekalova T, Liu Y, Kiselev D, Khairuddin S, Chiu JLY, Lam J, Chan YS, Pavlov D, Proshin A, Lesch KP, Anthony DC, Lim LW (2022): Chronic mild stress paradigm as a rat model of depression: facts, artifacts, and future perspectives. Psychopharmacology (Berl.) **239,** 663-693
https://doi.org/10.1007/s00213-021-05982-w

Strüber N, Strüber D, Roth G (2014): Impact of early adversity on glucocorticoid regulation and later mental disorders. Neurosci. Biobehav. Rev. **38,** 17-37
https://doi.org/10.1016/j.neubiorev.2013.10.015

Sur B, Lee B (2022): Luteolin reduces fear, anxiety, and depression in rats with post-traumatic stress disorder. Anim. Cells Syst. (Seoul) **26,** 174-182
https://doi.org/10.1080/19768354.2022.2104925

Syed SA, Nemeroff CB (2017): Early life stress, mood, and anxiety disorders. Chronic Stress (Thousand Oaks) **1,** 2470547017694461
https://doi.org/10.1177/2470547017694461

Troakes C, Ingram CD (2009): Anxiety behaviour of the male rat on the elevated plus maze: associated regional increase in c-fos mRNA expression and modulation by early maternal separation. Stress **12,** 362-369
https://doi.org/10.1080/10253890802506391

Tseilikman V, Komelkova M, Lapshin M, Alliluev A, Tseilikman O, Karpenko M, Pestereva N, Manukhina E, Downey HF, Kondashevskaya M, et al. (2020): High and low anxiety phenotypes in a rat model of complex post-traumatic stress disorder are associated with different alterations in regional brain monoamine neurotransmission. Psychoneuroendocrinology **117,** 104691
https://doi.org/10.1016/j.psyneuen.2020.104691

Ullmann E, Chrousos G, Perry SW, Wong ML, Licinio J, Bornstein SR, Tseilikman O, Komelkova M, Lapshin MS, Vasilyeva M, et al. (2020): Offensive behavior, striatal glutamate metabolites, and limbic-hypothalamic-pituitary-adrenal responses to stress in chronic anxiety. Int. J. Mol. Sci. **21,** 7440
https://doi.org/10.3390/ijms21207440

Vetulani J (2013): Early maternal separation: a rodent model of depression and a prevailing human condition. Pharmacol. Rep. **65,** 1451-1461
https://doi.org/10.1016/S1734-1140(13)71505-6

Walf AA, Frye CA (2007): The use of the elevated plus maze as an assay of anxiety-related behavior in rodents. Nat. Protoc. **2,** 322-328
https://doi.org/10.1038/nprot.2007.44

Wang H, Xiao L, Wang H, Wang G (2020): Involvement of chronic unpredictable mild stress-induced hippocampal LRP1 up-regulation in microtubule instability and depressive-like behavior in a depressive-like adult male rat model. Physiol. Behav. **215,** 112749
https://doi.org/10.1016/j.physbeh.2019.112749

Willner P, Towell A, Sampson D, Sophokleous S, Muscat R (1987): Reduction of sucrose preference by chronic unpredictable mild stress, and its restoration by a tricyclic antidepressant. Psychopharmacology (Berl.) **93,** 358-364
https://doi.org/10.1007/BF00187257

Wilson CB, Ebenezer PJ, McLaughlin LD, Francis J (2014): Predator exposure/psychosocial stress animal model of post-traumatic stress disorder modulates neurotransmitters in the rat hippocampus and prefrontal cortex. PLoS One **9,** e89104
https://doi.org/10.1371/journal.pone.0089104

Wu YP, Gao HY, Ouyang SH, Kurihara H, He RR, Li YF (2019): Predator stress-induced depression is associated with inhibition of hippocampal neurogenesis in adult male mice. Neural. Regen. Res. **14,** 298-305

https://doi.org/10.4103/1673-5374.244792
Yehuda R, Southwick SM, Nussbaum G, Wahby V, Giller EL Jr, Mason JW (1990): Low urinary cortisol excretion in patients with posttraumatic stress disorder. J. Nerv. Ment. Dis. **178,** 366-369 https://doi.org/10.1097/00005053-199006000-00004
Yehuda R, Antelman SM (1993): Criteria for rationally evaluating animal models of posttraumatic stress disorder. Biol. Psychiatry **33,** 479-486 https://doi.org/10.1016/0006-3223(93)90001-T
Yehuda R, Seckl J. (2011): Minireview: Stress-related psychiatric disorders with low cortisol levels: a metabolic hypothesis. Endocrinology **152,** 4496-4503 https://doi.org/10.1210/en.2011-1218
Zangrossi H Jr., File SE (1992): Behavioral consequences in animal tests of anxiety and exploration of exposure to cat odor. Brain Res. Bull. **29,** 381-388 https://doi.org/10.1016/0361-9230(92)90072-6
Zoladz PR, Fleshner M, Diamond DM (2012): Psychosocial animal model of PTSD produces a long-lasting traumatic memory, an increase in general anxiety and PTSD-like glucocorticoid abnormalities. Psychoneuroendocrinology **37,** 1531-1545 https://doi.org/10.1016/j.psyneuen.2012.02.007
Zoladz PR, Park CR, Fleshner M, Diamond DM (2015): Psychosocial predator-based animal model of PTSD produces physiological and behavioral sequelae and a traumatic memory four months following stress onset. Physiol. Behav. **147,** 183-192 https://doi.org/10.1016/j.physbeh.2015.04.032

Received: April 25, 2023
Final version accepted: July 24, 2023