Journal of Neural Transmission (2025) 132:1313–1333
https://doi.org/10.1007/s00702-025-02974-4

PSYCHIATRY AND PRECLINICAL PSYCHIATRIC STUDIES - ORIGINAL ARTICLE

From stress to anhedonia: differential gene expression, behavioural and biochemical modulations in resilient versus susceptible mice in an ultrasound model of juvenile depression

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Received: 17 December 2024 / Accepted: 21 June 2025 / Published online: 3 July 2025

Abstract

Juvenile depression is an increasingly recognized mental health condition, distinct from adult depression. 'Emotional stress', i.e., adverse experience of primarily psychological nature, is a risk factor of particular importance for juvenile depression. Like adults, adolescents display variable susceptibility to depression precipitated by stress, the nature of which is poorly understood. We employed the 3-week ultrasound (US) stress in juvenile C57BL/6 mice to compare behavioral and molecular features of susceptible and resilient to depressive-like syndrome subsets of animals. Mice were exposed to alternating frequencies of 20–25 kHz and 25–45 kHz, an established model of 'emotional stress'. In the sucrose test, mice were categorized as anhedonic (stress-susceptible) or non-anhedonic (stress-resilient), upon their sucrose preference that decreased below control values in some but not all animals. Parameters of emotionality, social and locomotor behaviors, learning, serum corticosterone levels, brain gene expression of pro-inflammatory cytokines, and malondialdehyde (MDA) concentrations were studied. In comparison with controls, susceptible mice exhibited prolonged floating behavior in the swim test, increased anxiety-like and dominant-type social behaviors, elevated corticosterone plasma levels, increased brain expression of cytokines interleukin-1 β (*Il-1β*) and tumor necrosis factor (*Tnf*), reduced expression of proteolipid protein 1 (*Plp1*) and myelin-associated glycoprotein (*Mag*). These changes were not found in resilient mice. Brain MDA concentrations similarly increased in both groups. Hence, the ultrasound stress model appears to replicate several behavioral features relevant to juvenile depression in mice, suggesting its potential as a tool for investigating various aspects of adolescent depression. Additionally, it introduces the stratification of animals into 'resilient' and 'susceptible' subgroups, which may contribute to a better understanding of the mechanisms associated with stress resilience during adolescence.

Keywords Juvenile depression · Resilience · Ultrasound stress · Pro-inflammatory cytokines · Emotionality · Mice

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Introduction

The high incidence of Major Depressive Disorder (MDD) and associated psychiatric pathologies in adolescents is a serious medicinal and social problem (Baune et al. 2012; Shorey et al. 2022). To date, it has become a pressing social and medical issue that affects not only the patients but also their social environment and the wider community (Racine et al. 2021; Wind et al. 2020; Souza et al. 2023; Madigan et al. 2023). The recent COVID-19 pandemic associated with social isolation, anxiogenic environmental factors, and ensuing exhaustion of the healthcare system became serious pre-requisites of increased MDD prevalence in children, adolescents, and youth worldwide (Oliveira et al. 2022; Werling et al. 2022; Maggu et al. 2023; Witteveen et al. 2023).

So-called 'emotional stress', i.e. a state of distress that is primarily caused by psychological trauma and enhanced cognitive processing of negative information (Chrousos and Gold 1992; Simonov 1997) plays an important role as a risk factor for MDD and has even greater contribution in disease for adolescents compared to adults (Nardi et al. 2013; LeMoult et al. 2020; Willinger et al. 2022a). Among these 'emotional stressors' are sexual and emotional abuse, parental neglect and social rejection, family member death, and other traumatic psychological experiences (Beck 1979; Baram et al. 2012; Rice 2019; Willinger et al. 2022b). At the same time, clinical practice points to a large variability in the vulnerability and resistance to mood disorders including depression among individuals with a clinical history of psychological stress (Lesch and Mössner 2006; Feder et al. 2009).

Unveiling molecular and physiological mechanisms of resilience versus susceptibility to MDD-like syndrome associated with stress can be of great help in understanding the nature of this disease that in youth has quite distinguished clinical manifestations (Locher et al. 2017). Understanding these mechanisms can help uncover targets for reliable pharmacotherapy of adolescent depression, a lack of which has become a serious issue. Perhaps the most striking and devastating feature of MDD in youth and adolescents is a high risk of suicidal attempts (Janiaud et al. 2017; Locher et al. 2017) that can be further increased by the use of classic antidepressants, such as SSRIs (Bridge et al. 2007; Li et al. 2022). Adolescent depressed patients poorly respond to standard antidepressant therapies (Kronenberg et al. 2007; Sher 2011; Hankin et al. 2015), and only a few antidepressants are officially approved for clinical use (Cipriani et al. 2016). Furthermore, given the high placebo vehicle response rate and the associated suicide risk with standard MDD pharmacotherapy in young MDD patients, the use of this therapy is currently under question (Spielmans et al. 2020; Meister et al. 2020; Masi 2022). Altogether, the current situation with the clinical management of MDD in adolescents necessitates mechanistic studies addressing the biological basis of depression in adolescents that up to date is largely unknown.

Recently, a phenomenon of individual 'resilience' and 'susceptibility' to depression precipitated by stress became a focus of many researchers using animal models of this disease. Earlier studies have shown that under stress conditions, rodents, like humans, display remarkable inter-individual variability in the development of depressive-like syndrome (Strekalova et al. 2004). Since then, the number of depression paradigms that stratify 'resilient' and 'susceptible' subsets of animals has sharply grown (Steimer and Driscoll 2005; Krishnan et al. 2007; Jakovcevski et al. 2008; Schmidt et al. 2010; Taliaz et al. 2011; Duclot and Kabbaj 2013; Der-Avakian et al. 2014; Cline et al. 2015; Theilmann et al. 2016; Rao and Androulakis 2020; Scherholz et al. 2020; Labaka et al. 2021; Strekalova et al. 2022, 2023; Nestler and Russo 2024).

A stratification of stressed animals upon their individual stress-susceptibility is considered by many researchers to be beneficial for mechanistic studies of adult depression, while potential bias of this stratification are discussed in the literature (Berrio and Kalliokoski 2023; Robinson 2025). E.g., studies that applied social defeat stress, predation stress, chronic social instability stress, and administration of glucocorticoids in adult rodents, have reported marked differences in molecular, biochemical, and physiological profiles of rodents that are distinct in their individual response to stress and susceptibility to MDD-like syndrome (Strekalova et al. 2011, 2022, 2023; y Palacios et al. 2011; Palmfeldt et al. 2016; Nestler and Russo 2024). Specifically, 'resilient' and 'susceptible' cohorts of stressed rodents show distinct responses to psychostimulants and brain expression of dopamine D2 receptor, turnover and binding ability of beta-adrenergic receptor (Cao et al. 2010), altered neuroanatomical features and interactions between the hippocampus and prefrontal cortex (y Palacios et al. 2011; Bessa et al. 2013; Kafetzopoulos et al. 2018). Susceptible rodents revealed reduced brain expression of brain derived neurotrophic factor (BDNF), vascular endothelial factor, and other neuroplasticity markers (Bergström et al. 2007; Jayatissa et al. 2010; Taliaz et al. 2011; Sun et al. 2017), increased expression of immediate early genes in the medial prefrontal cortex (Palmfeldt et al. 2016), elevated secretion of corticotrophin releasing hormone and urocortin 2 (Kolasa et al. 2014), aberrant expression of serotonin transporter (SERT)-related miRNA regulatory mechanisms in the mesocortical circuit (Zurawek et al. 2019), compromised brain expression of somatostatin and prolactin receptors (Faron-Górecka et al. 2016), glucocorticoid and cannabinoid receptors

(McLaughlin et al. 2013; Sun et al. 2017) and altered hippocampal expression of 5-HT1A receptor and its epigenetic regulation (Gorinski et al. 2019; Zurawek et al. 2019), as well as a decrease of the activities of catalase (CAT) and superoxide dismutase (SOD) and accumulation of malondialdehyde (MDA) in the brain (Cline et al. 2015). Susceptible and resilient subsets of mice displayed differences in general proteomic changes in the hippocampus (Bisgaard et al. 2007; Cline et al. 2015; Strekalova et al. 2023), including alterations in mitochondrial and metabolic processes (Tang et al. 2019; Strekalova et al. 2023).

A recent gene expression profiling study of the hippocampus of chronically stressed mice showed mirror changes in the expression of genes encoding mitochondrial enzymes in susceptible versus resilient subsets of mice. Resilient groups revealed an over-expression of mitochondrial complexes NADH-dehydrogenase, succinate dehydrogenase, cytochrome-bc1, cytochrome c-oxidase, F-type and V-type ATPases, and inorganic pyrophosphatase that was decreased in anhedonic mice (Strekalova et al. 2023). Thus, studies of the last decade using a comparison of 'resilient' versus 'susceptible' cohorts of mice and rats elucidated a large portion of the neurobiological basis for these distinct profiles of response to stress. However, the literature addressing this question with animal models of adolescent depression is lacking.

Notably, the majority of the findings delineating the neurobiology of stress- resilience, and susceptibility were obtained in stress models that may not always employ ethologically relevant procedures (Willner 2005; Antoniuk et al. 2019; Cathomas et al. 2019; Strekalova et al. 2022). This is also true for scare animal models, mimicking juvenile depression that often employ artificial manipulations including the use of genetically selected strains of rodents (Coppens et al. 2011; Steyn et al. 2018). In recent years there has been a move toward animal models of adolescent depression that mimic psychological stress (Nakatake et al. 2020). However, only a few examples of such an approach can be currently found in the literature. Meanwhile, the effects of 'emotional stress' in these mechanistic studies are strikingly different from the effects of physical stressors (Nakatake et al. 2020) keeping with clinical observations suggesting a critical role of emotional stress in the etiology of MDD in adolescents (Hankin et al. 2015; Yang et al. 2015; LeMoult et al. 2020).

As has been discussed above, the etiological relevance of stressors exploited, i.e. application of paradigms mimicking 'emotional stress' is of particular importance while modeling MDD, and especially in recapitulating this disease in adolescents. While mimicking 'emotional stress' in rodents has been challenging (Insel 2007; Borsini 2012; Robinson et al. 2018), recent publications suggest the usefulness of animal models that are based on naturalistic stressors in this context (Antoniuk et al. 2019; Nakatake et al. 2020). One of them is a recently established ultrasound stress (US) (Morozova et al. 2016; Sambon et al. 2020; de Munter et al. 2021; Strekalova et al. 2024). In this paradigm, mice are subjected to negative (20–25 kHz) and neutral (25–45 kHz) emotional ultrasound signals that alternate in an unescapable manner, over 21 days. As mice naturally emit sounds in the range of 20–25 kHz in life-threatening situations, such as maternal separation, predation, social defeat, or pain (Gorlova et al. 2023), their continuous exposure to these signals results in depressive-like and anxiety-like behaviors and numerous changes associated with MDD, including hormonal and metabolic changes, neuroinflammation, and oxidative stress (Strekalova et al. 2018, 2024; Pavlov et al. 2019, 2023; Gorlova et al. 2019, 2023; Costa-Nunes et al. 2020; Sambon et al. 2020; de Munter et al. 2021). These abnormalities can be counteracted by chronic administration of the classic antidepressant treatment (Morozova et al. 2016), antioxidants (Costa-Nunes et al. 2020; Sambon et al. 2020; de Munter et al. 2021), including omega-3 fatty acids food supplements (Strekalova et al. 2024).

Our recent study was carried out in juvenile mice suggesting the feasibility of the induction of a depressive-like syndrome in the US model (Strekalova et al. 2024). In this experiment, a subset of 1-month-old mice displayed a decrease in sucrose intake and preference below the lowest control values, a putative indicator of anhedonia, while other animals did not show such changes. Based on this, though, arbitrarily applied criterion, US mice were considered as 'susceptible' or 'resilient' and displayed distinct phenotypes (Strekalova et al. 2024). Feeding with a supplement containing omega-3 fatty acids composition decreased the proportion of 'susceptible' animals. Here, we sought to compare behavioral, physiological, and molecular features of 'stress-resilient' versus 'stress-susceptible' subsets of juvenile mice exposed to the ultrasound model of 'emotional stress' and stratified in to two subgroups according to their hedonic status evaluated in the sucrose test.

We aimed to investigate whether behavioral changes induced by chronic US in 'susceptible' juvenile mice are reminiscent of those in adolescent depressed patients and are accompanied by distinct physiological, hormonal, and molecular changes from those in the 'resilient' subgroup of mice. Specifically, mice were studied for helplessness in the swim test, anxiety-like behavior in the dark light and open field paradigms, social interactions, and novelty exploration. Hippocampus-dependent performance was studied in the marble test and contextual fear conditioning models. Given previously demonstrated brain overexpression of pro-inflammatory cytokines interleukin-1β (*Il-1β*) and tumor necrosis factor (*Tnf*), as well as glycogen

synthase kinase-3β (*Gsk-3β*), a marker of distress (Gorlova et al. 2019; Sambon et al. 2020; Strekalova et al. 2024) and increases of MDA brain concentrations (de Munter et al. 2021) in the adult mice exposed to US, we measured these readouts in the hippocampus and prefrontal cortex (PFC) in current study. In addition, given the important role of hepatic oxidative stress in the regulation of behavior in various mouse models of neuropsychiatric conditions (Campbell et al. 2005; Couch et al. 2013; Svirin et al. 2021), we also examined concentrations of MDA in the liver. Plasma CORT levels were examined as well. Finally, given the ongoing development of the mouse nervous systems and previously demonstrated effects of stress on myelination (Hueston et al. 2017; Svirin et al. 2021), we sought to compare gene expression of major myelination factors, proteolipid protein 1 (*Plp1*) and myelin-associated glycoprotein (*Mag*) in the brain of 'susceptible' and 'resilient' mice. We hypothesized that potential differences in their expression associated with brain plasticity can render the differences in their stress response and susceptibility to MDD-like syndrome. Overall, the susceptible cohort of mice displayed marketable differences in the majority but not all evaluated read-outs. Considering the potential limitations and biases discussed regarding the stratification of animals in sucrose tests (Robinson et al. 2018; Berrio and Kalliokoski 2023), it might be yet suggested that there are distinct neurobiological foundations for 'resilience' and 'susceptibility' to juvenile MDD-like syndrome in employed ultrasound model.

Materials and methods

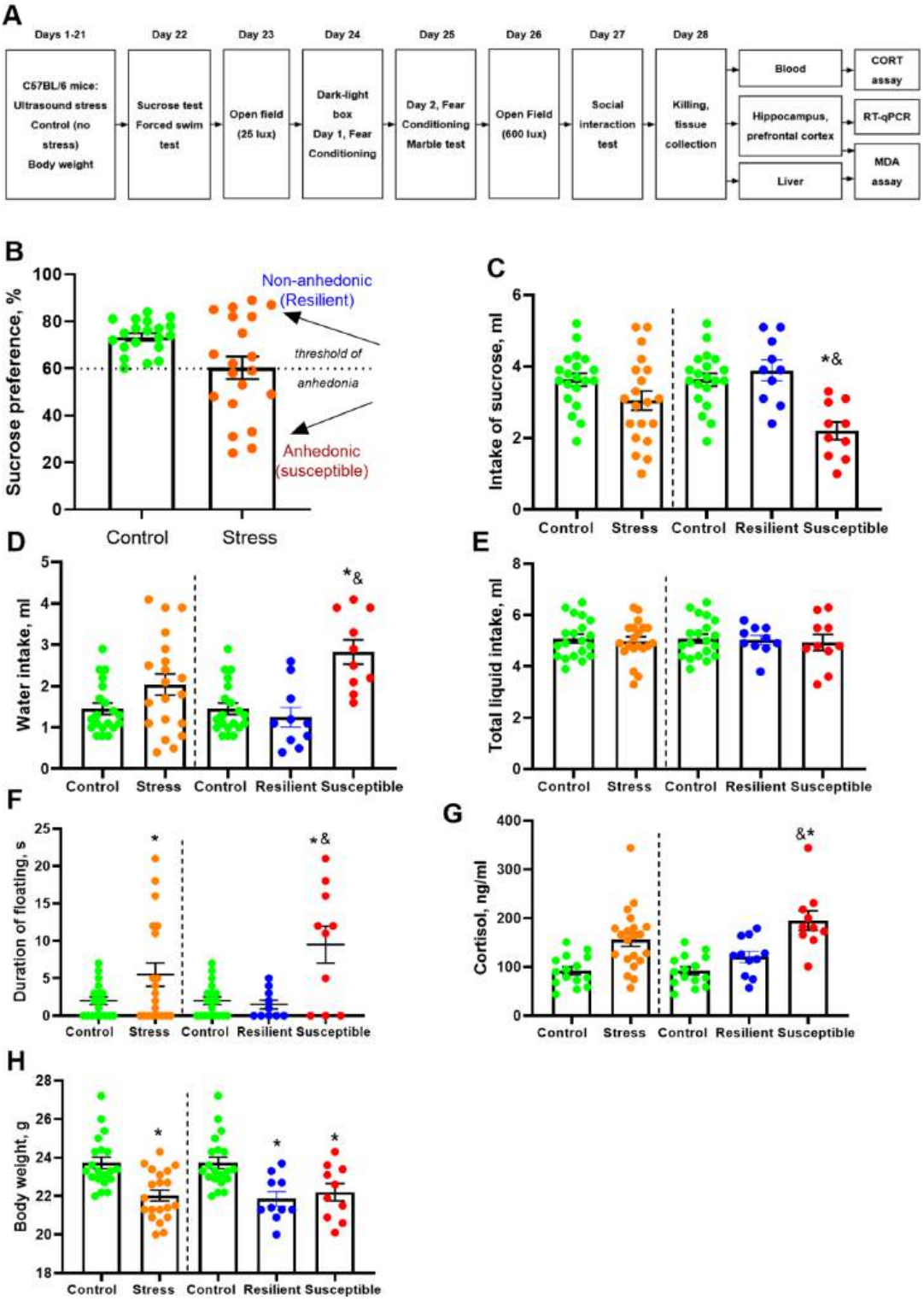
Experimental animals and conditions

US experiment was performed using male C57BL/6J ($n=40$) mice of the age of about 6 weeks originating from the Charles River certified supplier (Janvier, France). 4-week-old male CD1 mice ($n=40$) provided by the same supplier were used as counter-partners for the social interaction test. Mice were single-housed, under standard laboratory conditions with food and water provided ad libitum; light on at 9:00, light out at 19:00. All behavioral experiments were carried out during the dark phase of the light/dark cycle. Mice were allowed to acclimatize for 14 days before the start of experiments. All protocols complied with Directive 2010/63/EU of 22 September 2010,2010/63/EU. Experiments were carried out under the approval of the local veterinarian committees (Comité en Expérimentation Animale de l'Université Claude Bernard Lyon 1; CEEA-55), Ethical Committee of MUMC, Maastricht University (iMETC MUMC and METC Zuyderland Zuid). the MSMU #11-18-2018/2019 on animal care and welfare and were compliant with ARRIVE guidelines (https://arriveguidelines.org/sites/arrive/files/documents/ARRIVE-20French-guidelines). All efforts were undertaken to minimize the potential discomfort of animals used.

Fig. 1 Sequence of experimental procedures and effects of stress on the hedonic state, helplessness and physiological measures. (**A**) Schematic of the study flow and the use of in vivo and in vitro procedures. (**B**) Sucrose preference was significantly decreased in the US-stressed group compared to non-stressed group, in a subgroup of mice (one-way ANOVA and Tukey test, $*p<0.05$). According to the criterion of 60% preference for sucrose solution over water (see the text), the group of stressed mice split into susceptible and resilient to anhedonia subgroups. There was a significant difference (**C**) in the sucrose intake and (**D**) in water intake (one-way ANOVA, $*p<0.05$), (**E**) but not in total liquid intake. Susceptible and resilient groups showed significant difference in sucrose and water intake (Tukey test, $*p<0.05$). (**F**) We found significant group difference in the duration of floating behavior in the swim test (one-way ANOVA, $*p<0.05$), where susceptible animals showed significantly longer floating behaviour than resilient mice (Tukey test, $*p<0.05$). (**G**) There was a significant difference in CORT plasma levels between the groups (one-way ANOVA); susceptible animals displayed significantly higher CORT levels than resilient mice (Tukey test, $*p<0.05$). (**H**) There was a significant difference in body weight between the groups (one-way ANOVA, $*p<0.05$). No significant group differences were found between susceptible and resilient subsets of mice (Tukey test) suggesting that their behaviors was not compromised by this factor. $*p<0.05$, one-way ANOVA with Tukey's test or unpaired t -test. Data is presented as mean ± SEM

Study flow

First, control and stress groups of mice were balanced upon the body weight, of twenty animals each (Fig. 1A). The US group of mice was exposed to a 21-day ultrasound of unpredictably alternating frequencies as described elsewhere (Gorlova et al. 2019; de Munter et al. 2021; Strekalova et al. 2024, *see below*). The control group was housed in a separate laboratory room. Body weight was monitored weekly throughout the 21-day experiment. After the termination of US, on Day 22, all mice were investigated in a sucrose test for hedonic state and in the forced swim test, for helplessness. On Day 23, an open field experiment with modest illumination strength (25 lx) and a novel cage test were performed. Dark-light box anxiety test was carried out on Day 24 and was followed by a training session in the fear conditioning paradigm. A test for recall of fear memory was carried out on Day 25; thereafter mice were studied in the marble test. On day 26, all animals were examined in the open field with strong illumination intensity (600 lx). The social interaction test was performed on day 27. On day 28, all mice were killed, and their hippocampi and PFC were isolated from dissected brains for RT-qPCR and MDA assays. The liver was dissected for MDA determination, blood was collected for a cortisol ELISA assay.



Ultrasound stress

US procedure was carried out as described elsewhere (Strekalova et al. 2018; Pavlov et al. 2019, 2023). For a 21-day period, ultrasound radiation of average intensity of 50 ± 5 dB and variable frequencies in a 20–45 Hz range was constantly delivered within a laboratory environment to experimental groups of mice using a random schedule of alternating frequencies via a commercially available device (Weitech, Wavre, Belgium). The range of ultrasound stimulation frequency was alternated every 10 min between frequencies 20–25 kHz, 25–40 kHz, and 40–45 kHz. The shape

of the ultrasound signal was fluctuating, thus, mimicking the natural ultrasonic vocalizations of mice (Costantini and D'amato 2006). The selectivity of the adverse effects of low-frequency ultrasound during the radiation period versus the potential general negative effects of a constant white noise accompanying the procedure described here was demonstrated previously (Morozova et al. 2016).

Sucrose test

During the sucrose test, mice were given, for 8 h, a free choice between two bottles, one with 0.8% sucrose solution and another with tap water. To prevent possible effects of side preference in drinking behavior, the position of the bottles was switched after 4 h. No previous food or water deprivation was applied before the test; other details of the protocol were used as described elsewhere (Strekalova 2008, 2023; Strekalova and Steinbusch 2010; Strekalova et al. 2022, 2024). The consumption of water and sucrose solution was estimated simultaneously in control and experimental groups by weighing the bottles. The preference for sucrose was calculated as a percentage of consumed sucrose solution of the total amount of liquid drunk:

$$Sucrose\ Preference = 100 \times \frac{Volume\,(Sucrose\ solution)}{Volume\,(Sucrose\ solution) + Volume\ (Water)}$$

According to the results of the test, animals were stratified to 'anhedonic'(susceptible) and 'non-anhedonic' (resilient), taking the minimal value of a sucrose preference in control vehicle-treated mice as a criterion of occurrence of hedonic deficit, as previously established (Strekalova et al. 2004, 2022, 2024) that in the current study was ≤60% (Fig. 1B).

Swim test

Following the termination of the sucrose test, mice were studied in the forced swim test. Animals were introduced to a transparent pool (20 cm x 35 cm x 15 cm) filled with warm water (30C°, height 9.5 cm) lit by red light for 1 min. The duration of floating behavior, defined as an absence of directed movements of animals' heads and bodies, was estimated as described elsewhere (Strekalova et al. 2004, 2005).

Novel cage test

In this test, a mouse was placed in a clear plastic cage (14 × 21 × 27 cm) with a small amount of fresh litter. For 5 min, the number of rears was counted under red light as described elsewhere (Strekalova et al. 2004).

Dark-light box

The apparatus consisted of a dark chamber and an illuminated chamber (600 lx). Mice were introduced to the dark compartment and were allowed to move freely between the two chambers. Latency to exit to the lit compartment, time spent therein, and the number of exits to the lit box were recorded for 5 min as described elsewhere (Strekalova and Steinbusch 2010).

Open field test

The open field test was carried out in a square box (45 × 45 × 45 cm) that was illuminated with a white light of subtle intensity (25 lx), or strong intensity (600 lx) as described elsewhere (Strekalova et al. 2005). Each animal was placed near the wall and its behavior was recorded for a 5 min period. Using previously validated methods with the Ethovision program (Ethovision Program 6.95, Noldus, Wageningen, the Netherlands) total number of crossed sectors (2 × 2 cm each), the duration of freezing, and the number of grooming episodes were scored as described elsewhere (Malatynska et al. 2012; Strekalova et al. 2021).

Social interaction test

The social interaction test was carried out in a square box (20 × 20 × 15 cm) that was illuminated with white light of subtle intensity (5 lx), CD1 juvenile mice were used for social interaction. Several parameters of social interaction were scored as described elsewhere (Strekalova et al. 2004, 2021; Veniaminova et al. 2017), for 8 min. Pairs of mice of each line were simultaneously placed in a plastic observation cage and allowed to interact. During this test, animals were analyzed for the following behavior, crawl-over behavior, as well as body-body, nose-anal, and nose-nose contacts, tail rattling, and attacking behavior. The crawl-over behavior was defined as climbing over the head of another animal. "Nose-nose" contacts were defined as maintaining vibrissae for longer than 1 s. "Nose-anal" contacts were defined as an examination of the anogenital area of another mouse. "Body-body" contacts of the dominant type were defined as climbing over the body of another animal. For each type of social contact, the latency, duration, and a number of behavioral events were scored. Additionally, for each mouse, the total duration of interactions of the dominant type was calculated as a sum of the duration of the following behavior, crawl-over behavior, attacking behavior, and tail rattling.

Fear conditioning paradigm

This test was used to assess hippocampus-dependent contextual memory, it was carried out as described previously (Vignisse et al. 2017; Strekalova et al. 2018). The apparatus (Technosmart, Rome, Italy) consisted of a transparent plastic cubicle (25 × 25 × 50 cm) with a stainless-steel grid floor (33 rods, 2 mm in diameter). For the training session on day 1, each mouse was introduced into the apparatus for a 2 min period and a single alternating electric current (AC, 50 Hz; 0.5 mA, 1 s, Evolocus LLC, Tarrytown, NY, USA) was delivered. Immediately after the delivery of the current, the mouse was returned back in the home cage. On day 2, a recall test was carried out 24 h later as previously described. Therefore, freezing behavior was scored by visual observation during a 3-minute period. The occurrence of freezing behavior was assessed every 10 s for 180 s; each 10 s score was assigned to a freezing or non-freezing period, and the percentage of time spent in freezing was calculated.

Marble test

Marble test or food-displacement test was performed to assess hippocampus-dependent behavior as described elsewhere (Strekalova and Steinbusch 2010; Veniaminova et al. 2017). All experimental groups were tested for a 90-minute time period for pellet displacement in a marble test. A tendency to displace small objects, for example, small stones or food pellets; from a tube inside the cage, is species-specific in mice and has been demonstrated to depend on an intact hippocampal formation (Deacon et al. 2002). Using a paper tube (internal diameter 4 cm, length 10 cm), filled with 20 food pellets and placed in the middle of a cage (21 cm× 27 cm × 14 cm), the latency to displace the first food pellet and time required for 50% and 100% tube emptying were assessed in mice.

Killing of mice and tissue and blood collection

Mice were terminally anesthetized by CO_2 and isoflurane as described elsewhere (Couch et al. 2013; Anthony et al. 2024). Blood collection was performed transcardially, blood was stored in heparinized vials prior to centrifugation (1500 x g, 15 min, 4 °C); plasma was removed and immediately stored at −20 °C until use. Thereafter, mice were perfused with ice-cold NaCl isotonic solution, the brain and liver were removed, hippocampus, prefrontal cortex (PFC), and liver were dissected and frozen immediately on dry ice and stored at -80 C until use.

ELISA CORT assay

To study the concentration of cortisol in blood plasma, a mouse enzyme-linked immunosorbent assay (ELISA) was performed using Invitrogen™ Cortisol Competitive ELISA Kit (Thermo Fisher Scientific, MA, USA) according to the manufacturer's instructions. The microwell absorbance was measured at 450 nm with Synergy 4 Hybrid Multi-Mode Microplate Reader (BioTek, Thermo Fisher Scientific, MA, USA) as described elsewhere (Couch et al. 2013).

Quantitative real-time PCR

Total mRNA was isolated from each sample with RNeasy Lipid Tissue Mini Kit (Qiagen, Hilden, Germany). During first-strand cDNA synthesis, 1 µg total RNA was converted into cDNA using QuantiTect Reverse Transcription Kit (Qiagen, Hilden, Germany). qRT-PCR was performed using the SYBR Green master mix (Bio-Rad Laboratories, Philadelphia, PA, USA) and the ProFlex PCR system (Thermo Fisher Scientific, MA, USA). qRT-PCR was performed in a 10 µl reaction volume containing a SYBR Green master mix (5 µl), RNase-free water (3 µl), specific forward and reverse primers used at the concentration 20 pmol/µl (1 µl), cDNA (1 µl). Glyceraldehyde-3-phosphate dehydrogenase (*Gapdh*) was selected as a reference gene since in previous experiments it was observed relatively low variability in its brain expression (Gorlova et al. 2019). The initial denaturation step for qRT-PCR was at 95 °C for 4 min followed by 40 cycles of denaturation at 95 °C for 20 s, annealing was at 54 °C for 90 s. The PCR efficiency was assessed using Linreg, melting point for each PCR was run for each PCR. Sequences of all primers used are listed in Table S1 (*see Supplementary file*). All samples were run in triplicate. Data were normalized to *Gapdh* mRNA expression and calculated as relative-fold changes compared to control vehicle-treated mice, as described elsewhere (Pavlov et al. 2019, 2023; Gorlova et al. 2019; Strekalova et al. 2024).

Malondialdehyde assay

Brain malondialdehyde content was measured following Abcam ab118970 kit instructions (Abcam, Eugene, OR, USA). Briefly, the tissue of the hippocampi and PFC was merged in equal proportions in the cryostat, washed in cold PBS, homogenized in lysis solution, and then centrifuged at 13,000 g for 10 min. TBA reagent was added to the supernatant and, after incubation at 95 °C for 60 min and cooling to room temperature on ice bath, the supernatant was analyzed at 532 nm in a 96-well microplate as described elsewhere (Vignisse et al. 2017; Pavlov et al. 2019). Previous studies revealed similar increases in MDA content in the

hippocampus and PFC of US-exposed mice (Costa-Nunes et al. 2020; de Munter et al. 2021). A similar protocol was employed for the evaluation of MDA content in the liver (Veniaminova et al. 2020).

Statistical analysis

Behavioral, RT-qPCR, ELISA, and univariate metabolite data were analyzed with GraphPad Prism 6.00 software (San Diego, CA, USA) with one-way ANOVA. Post-hoc Tukey's multiple comparisons test was used in case of significant factor interaction; the exact Fisher test was used to analyze qualitative data. For two group comparisons, an unpaired two-tailed t-test was used. The significance level was set at $p<0.05$. All results are presented as Mean ± SEM.

Results

Depressive-like behaviors and physiological changes induced by US exposure

Sucrose test

Sucrose preference was significantly decreased in the US-stressed group compared to the non-stressed group ($p=0.0139$, t=2,581, df=38, two-tailed t-test; Fig. 1B). As the minimal sucrose preference value in the control group was 60%, this value was taken as the criterion of anhedonia. According to this criterion, the group of stressed mice split into anhedonic (susceptible) and non-anhedonic (resilient) subgroups. There was a significant group difference in the sucrose intake and in water intake (F=6,214, $p=0.0002$ and F=14.61, $p<0.0001$, respectively, one-way ANOVA, Fig. 1C, D), but not in total liquid intake (F=0,1325, $p=0.8763$; Fig. 1E). Susceptible and resilient groups showed significant difference in sucrose and water intake ($p<0.0001$; Tukey test, Fig. 1D); this parameter was significantly lower in susceptible mice than in control and resilient groups (both $p<0.0001$), no group differences were shown between control and resilient groups ($p=0.7879$). There was no significant difference in sucrose solution intake, water intake, and total liquid intake between the control and stress groups (t=1.826, df=38, $p=0.0757$, t=1.997, df=38, $p=0.531$ and t=0.4323, df=38, $p=0.668$, respectively, Fig. 1C-E).

Swim test

We found significant group difference in the duration of floating behavior in the swim test (F=11.72, $p=0.0001$, one-way ANOVA), where susceptible animals showed significantly longer floating behavior than control and resilient mice ($p=0.002$ and $p=0.0006$; respectively, Tukey test, Fig. 1F). No significant differences were revealed between control and resilient mice ($p=0.9530$, Fig. 1F). The duration of floating behavior was significantly longer in the stress group than in control mice (t=2,107, df=37, $p=0.420$, Fig. 1F).

Cortisol levels in blood plasma

Cortisol (CORT) blood level was significantly affected by the stress. There was a significant difference in CORT plasma levels between the groups (F=6.067, $p=0.0083$, one-way ANOVA); susceptible animals displayed significantly higher CORT levels than control mice ($p<0.0001$, Tukey test, Fig. 1G) and than resilient animals ($p=0.0016$). The latter group did not differ from the controls in this measure ($p=0.2547$). Two-tailed t-test revealed a significant increase in CORT levels in the stress group in comparison to controls (t=3.586, df=34, $p=0.001$, Fig. 1G).

Changes in body weight

There was a significant difference in body weight between the groups (F=8.362, $p=0.0001$, one-way ANOVA); both susceptible and resilient subsets of mice showed a significant decrease in body weight in comparison to control mice ($p=0.0139$ and $p=0.0024$, Tukey test, respectively, Fig. 1H). No significant group differences were found between susceptible and resilient subsets of mice ($p=0.8346$) suggesting that their behaviors were not compromised by this factor (Fig. 1H). There was no significant difference in body weight measured by the end of stress, between control and stress groups (t=4.085, df=38, $p=0.0002$, two-tailed t-test, Fig. 1H).

Changes in emotionality, locomotion, and hippocampus-dependent performance

Dark-light box

For the latency to exit recorded in this test, a one-way ANOVA revealed a significant difference between groups (F=3.453, $p=0.0422$). The latency to exit was longer in the susceptible group than in the control group ($p=0.0348$, Tukey's test. Figure 2A) and was not different between resilient and control groups ($p=0.598$). Two-tailed t-test demonstrated significant group differences in this parameter between stress and control animals (t=2.719, df=38, $p=0.0205$). There was a strong trend to a significant difference in the time spent in the lit box (F=2.691, $p=0.0608$, Fig. 2B); susceptible mice showed a significant reduction in this measure in

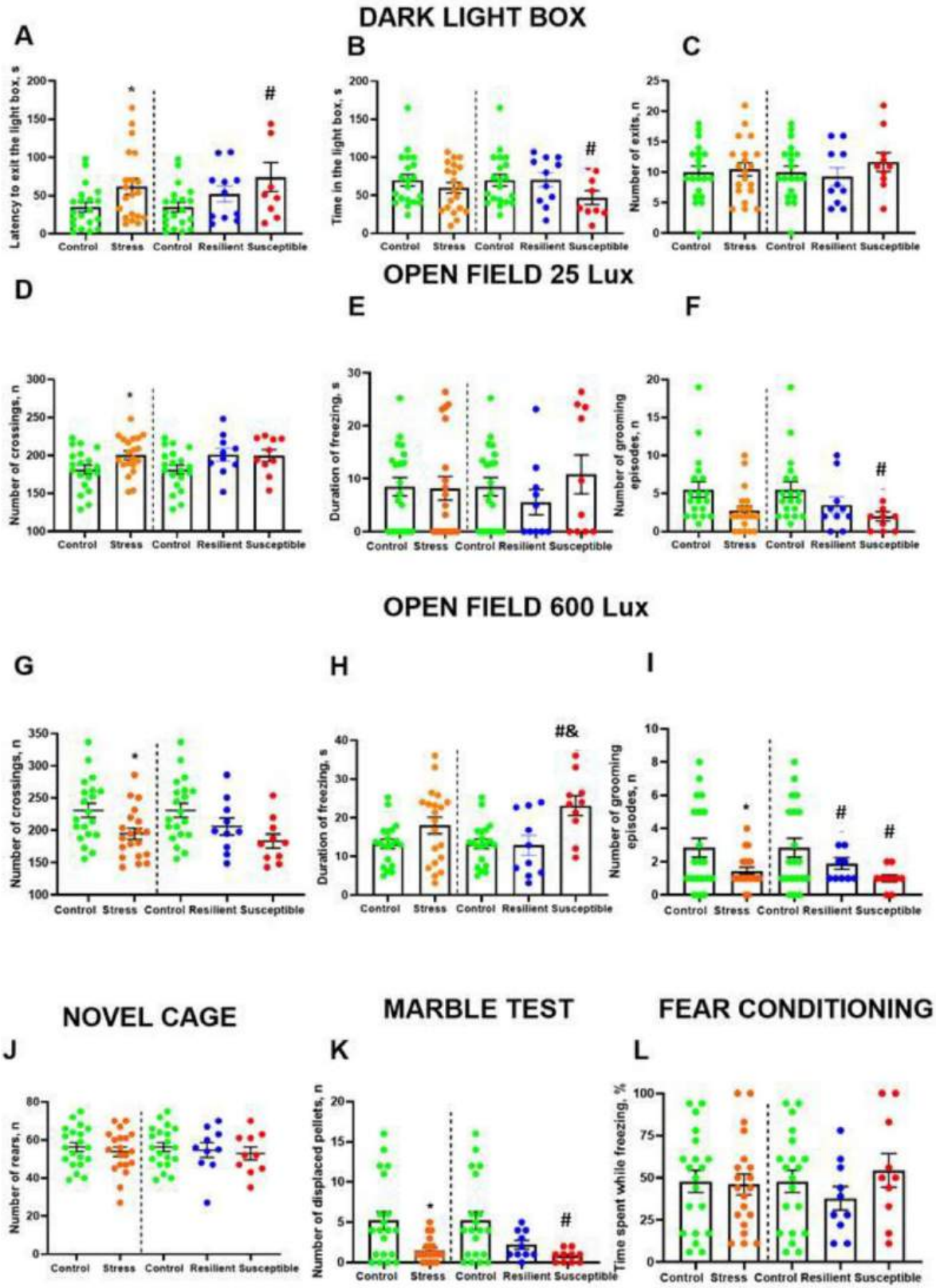

DARK LIGHT BOX
A
Latency to exit the light box, s
B
Time in the light box, s
C
Number of exits, n
OPEN FIELD 25 Lux
D
Number of crossings, n
E
Duration of freezing, s
F
Number of grooming episodes, n
OPEN FIELD 600 Lux
G
Number of crossings, n
H
Duration of freezing, s
I
Number of grooming episodes, n
NOVEL CAGE
J
Number of rears, n
MARBLE TEST
K
Number of displaced pellets, n
FEAR CONDITIONING
L
Time spent while freezing, %
Control
Stress
Resilient
Susceptible

Fig. 2 Changes in behavioral measures for emotionality, locomotion and hippocampus-dependent performance in US-exposed mice. In the dark-light box (**A**) the latency of exit to lit box was significantly different between the groups. The latency to exit was longer in the susceptible group than in the control group (**B**) there was no significant group differences in the time spent in the lit box and (**C**) no significant changes in the number of exits to the light box. In the open field test lit 25 lx, (**D**) there was significant increase in the total number of crossings in stress vs. control group. No significant group differences were found between control, resilient and susceptible mice (**E**) There was no group differences in the duration of freezing behavior (**F**) the number of grooming episodes was significantly different between groups, susceptible mice had significantly less grooming episodes than control group (*$p<0.05$). In the open field test lit 600 lx, (**G**) there was significant decrease in the total number of crossings in stress vs. control group. No significant group differences between control, resilient and susceptible mice were shown (**H**) There were group differences in the duration of freezing behavior. The duration of freezing was significantly longer in susceptible group than in control group (*$p<0.05$). (**I**) In the novel cage test, no significant group differences were found. (**K**) One-way ANOVA revealed significant group differences and a significant reduction in a number of displaced pellets in the susceptible group in comparison to controls that was not shown for resilient mice (*$p<0.05$). (**L**) In the novel fear conditioning test, no significant group differences were found by one-way ANOVA, Tukey's test and t-test. Data is presented as mean ± SEM

comparison to controls ($p=0.044$, Tukey test) that was not demonstrated by resilient mice ($p=0.9120$). Stress and control groups were not statistically different in this measure (t=0.922, df=38, $p=0.2876$). We found no statistical group differences in the number of exits (F=0.7048, $p=0.5007$). The stress and control group showed no statistical differences (t=0.2512, df=38, $p=0.8951$, Fig. 2C). No other group differences were found.

Open field test

One-way ANOVA did not reveal a significant difference in the number of sectors crossed in the open field lit with modest lighting of 25 lx between the groups (F=0.07383, $p=0.9290$, Fig. 2D). However, t-test revealed an increase in this measure in the stressed in comparison to control mice (t=2.424, df=38, $p=0.0327$, two-tailed t-test). No other group differences were found. There was no significant group difference in the duration of freezing under employed conditions (F=2.674, $p=0.0823$, Fig. 2E). No group differences were found in this parameter. Stress and control groups did not significantly differ in the duration of freezing behavior (t=0.809, df=38, $p=0.3348$). We found significant group differences in the number of episodes of grooming between the groups (F=2.780, $p=0.0413$, one-way ANOVA). The number of grooming events was reduced in the susceptible group compared to the control group ($p=0.0495$, Tukey's test, Fig. 2F). There was no significant difference between the control and the resilient groups in this measure ($p=0.3481$) and between the resilient and the susceptible groups ($p=0.6377$, Fig. 2F).

In the open field lit with bright lighting of 600 lx, one-way ANOVA did not reveal a significant difference in the number of sectors crossed (F=1.982, $p=0.1208$), while t-test revealed a decrease in this measure in the stressed mice in comparison to the control mice (t=2.504, df=38, $p=0.018$, Fig. 2G). No other significant group differences were found. In this test, there was a significant group difference in the duration of freezing (F=7.864, $p=0.0014$, Fig. 2E). The susceptible group showed longer freezing behavior than the control and the resilient groups ($p=0.0020$ and $p=0.0058$, respectively, Tukey's test, Fig. 2H); resilient mice did not differ in this measure from controls ($p=0.6549$, Fig. 2H). There was no significant difference between stress and control groups (t=1.975, df=38, $p=0.0531$). In the brightly illuminated open field (lit with 600 lx), one-way ANOVA showed no significant difference in the number of grooming episodes between groups (F=1.357, $p=0.2687$, Fig. 2I). However, susceptible mice displayed a significantly lower number of grooming episodes than controls ($p=0.0451$) that was not shown by resilient animals ($p=0.259$, Fig. 2I). There was a significant difference between stress and control groups in this parameter (t=2.124, df=38, $p=0.0412$, two-tailed t-test, Fig. 2L).

Novel cage test

In the novel cage test, no significant group differences were found by one-way ANOVA (F=0.02124, $p=0.8849$; Fig. 2J). Stress and control groups did not reveal significant differences (t=0.8154, df=38, $p=0.3419$). No other significant group differences were found.

Marble test

One-way ANOVA revealed significant group differences (F=3.742, $p=0.0256$) and a significant reduction in a number of displaced pellets in the susceptible group in comparison to controls ($p=0.0409$, Tukey test) that was not shown for resilient mice ($p=0.0173$; Fig. 2K). This measure was significantly lower in stress mice than in control animals (t=2.298, df=38, $p=0.0276$, two-tailed t-test). No other significant group differences were found.

Contextual fear conditioning

In the fear conditioning test, no significant group differences were found by one-way ANOVA (F=0.01724, $p=0.8249$; Fig. 2L), and no by two-tailed test (t=0.8416, df=36, $p=0.402$). No other significant group differences were found.

Alterations in social behaviors

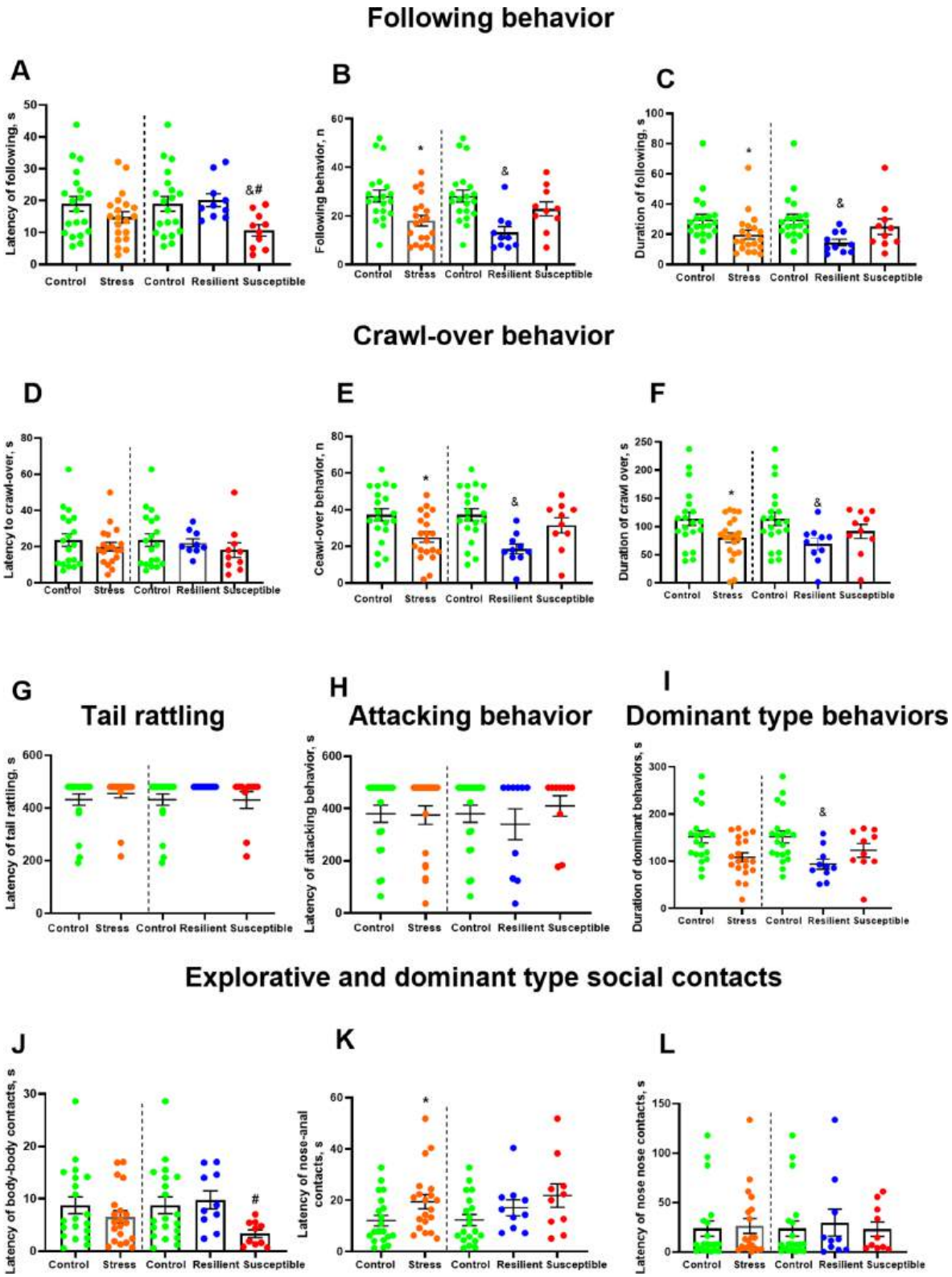
In the social interaction test, there was a significant group difference in the latency to the following behavior (F = 3.975, $p = 0.0273$, one-way ANOVA). The susceptible group had a significantly shorter latency to following than control and resilient mice ($p = 0.0416$, and $p = 0.0462$, respectively, Tukey test, Fig. 3A). Stress group was not significantly different from the control group in this measure (t = 1.648, df = 38, $p = 0.1562$, two-tailed t-test). In the number of following episodes, there was a significant group difference (F = 7.611, $p = 0.016$). Resilient animals showed fewer episodes of following than control, but not susceptible group ($p = 0.0011$ and $p = 0.3625$, respectively, Fig. 3B). The latter group was not significantly different from controls in this parameter ($p = 0.3625$). The stress group had significantly fewer following episodes than controls (t = 1.447, df = 38, $p = 0.038$). We found significant group differences in the duration of the following behavior (F = 4.040, $P = 0.0259$, Fig. 3C). This measure was significantly lower in resilient mice than in control animals ($p = 0.0194$) and was not different from the susceptible group ($p = 0.2240$), nor from controls ($p = 0.6466$). The stress group showed a significant reduction in the duration of following behavior in comparison to control mice (t = 2.235, df = 38, $p = 0.0314$).

As for crawl-over behavior, one-way ANOVA did not reveal group differences in the latency of this behavior (F = 0.5901, $p = 0.5597$). There were no significant differences between stressed and control animals (t = 0.8601, df = 36, $p = 0.394$, two-tailed t-test, Fig. 3D). The number of crawl-over episodes was significantly different between the groups (F = 6.908, $p = 0.028$). This parameter was significantly lower in resilient mice than in control animals ($p = 0.0019$, Tukey test, Fig. 3E) but not in the susceptible group ($p = 0.0810$). Susceptible mice did not reveal statistical differences in the number of episodes of crawl-over from controls ($p = 0.4886$). One-way ANOVA demonstrated a significant group difference in the duration of crawl-over behavior (F = 3.95, $p = 0.0482$). The duration of this behavior was significantly shorter in resilient mice than in control mice ($p = 0.0410$, Fig. 3F) and was not different from that of the susceptible group ($p = 0.4264$), which did not show differences in this measure from the controls ($p = 0.5208$). The stress group was significantly different from the control group in this parameter (t = 2.838, df = 38, $p = 0.0072$, two-tailed t-test).

There were no significant group differences in the latency of tail rattling behavior (F = 1.241, $p = 0.3010$, one-way ANOVA). Stress and control mice did not significantly differ in this measure (t = 0.8688, df = 38, $p = 0.3904$). However, the Fisher test revealed a significant difference in the number of mice displaying this behavior between resilient and control groups ($p = 0.0163$, Tukey test) and a trend to such difference between susceptible and resilient mice ($p = 0.10$, Fig. 3G). Remarkably, no episodes of tail-rattling behavior were recorded in the latter group of mice. For the susceptible group of mice, no difference from the control group was found ($p = 0.32$, exact Fisher test).

One-way ANOVA did not show significant group differences in the latency of attacking behavior (F = 0.5324, $p = 0.5916$). Two-tail t-test did not reveal significant differences between stress and control groups (t = 0.1039, df = 38, $p = 0.9178$, Fig. 3H). The duration of dominant behaviors was significantly different between the groups (F = 4.763, $p = 0.0144$, one-way ANOVA). There was a reduction in the duration of dominant type behavior in the resilient group in comparison to control, mice ($p = 0.0117$, Tukey test, Fig. 3I), no such differences were shown for susceptible mice ($p = 0.3885$). Resilient and susceptible groups did not differ in this measure ($p = 0.2992$). Stress and control groups were significantly different from each other in the duration of dominant type behavior (t = 2.758, df = 38, $p = 0.0089$, two-tailed t-test, Fig. 3I). We found that the latency of body-body contacts of the dominant type was significantly different between the groups (F = 3.724, $p = 0.0336$, one-way ANOVA). This parameter was significantly shorter in susceptible mice than in controls ($p = 0.0480$, Fig. 3J); there was a strong trend toward this difference from resilient mice ($p = 0.566$). The latter group of animals did not show significant differences in this parameter ($p = 0.8932$). The stress group was not different from control mice in the latency of body-body interactions (t = 1.112, df = 38, $p = 0.2731$, Fig. 3J).

The latency of nose-anal contacts was not significantly different between the groups (F = 2.750, $p = 0.0770$, one-way ANOVA). We found a strong trend to an increase of this measure in susceptible animals in comparison to control mice ($p = 0.0680$, Tukey test, Fig. 3K). The latency of nose-anal contacts was not significantly different between this group and resilient mice ($p = 0.6038$) and between the latter group and controls ($p = 0.4672$). Stressed mice demonstrated a significant increase in the latency of nose-anal interaction in comparison to control mice (t = 2.027, df = 38, $p = 0.0499$, two-tailed t-test, Fig. 3K). There were no differences in nose-nose interactions found between the groups (F = 0.1182, $p = 0.8889$, one-way ANOVA). Two-tailed t-test did not reveal significant group differences in this parameter (t = 0.2484, df = 38, $p = 0.8051$, Fig. 3L).



Molecular markers of stress and inflammation

Gene expression of markers of cellular distress and inflammation

The relative mRNA expression of *Tnf* was significantly different between the groups both for the PFC and the hippocampus ($F=5.478$, $p=0.008$, $F=3.756$, $p=0.0327$, respectively, one-way ANOVA) and was significantly higher

Fig. 3 US-induced alterations in social behaviour in susceptible and resilient to stress mice. In the social interaction test, **(A)** there were no significant changes in the latency of following behavior while susceptible mice had a reduction of this measure in comparison with control and resilient groups ($p=0.0011$, $p=0.0194$, $p=0.8654$, respectively, one-way ANOVA). Rresilient mice showed significantly **(B)** fewer number of episodes of following behavior, as well as **(C)** a decreased duration of following. No significant changes were observed in the same parameters in susceptible mice (one-way ANOVA). As for crawl-over behavior, **(D)** there were no significant changes in the latency of crawl-over behavior in comparison to control, **(E)** but there was group difference in the number of episodes of crawl-over behavior; resilient mice had significant decrease of this measure in comparison to control. **(F)** We found group difference in the duration of episodes of crawl-over behavior; resilient mice had significant decrease of this parameter in comparison to control (one-way ANOVA). No significant changes were observed in the crawl-over behavior were revealed in susceptible mice. **(G)** There were no significant changes in the latency of tail rattling behavior between the groups, but resilient mice had a strong trend to an increase of this measure. **(H)** No group differences were found for attacking behavior. **(I)** There were significant changes observed in the total duration of dominant like behaviors (one-way ANOVA), resilient but not susceptible mice displayed a significant decrease in comparison to control group. **(J)** The latency of dominant-type body-body interactions was different between the groups; we found a reduction of this measure in susceptible mice in comparison to controls. The latency of nose-anal **(K)** and nose-nose **(L)** contacts were not different between the groups. *$p<0.05$, one-way ANOVA with Tukey's test or unpaired t -test. Data is presented as mean ± SEM

in susceptible mice than in control group (PFC: $p=0.0069$; hippocampus: $p=0.0457$, Tukey test, Fig. 4A). No such changes were observed in resilient mice for *Tnf* expression (PFC: $p=0.2219$; hippocampus: $p=0.1467$, Fig. 4A). Stress group had significantly higher *Tnf* e mRNA levels in the PFC and the hippocampus than control animals (t=2.912, df=38, $p=0.006$ and t=2.694, df=38, $p=0.0104$, respectively, two-tailed t-test, Fig. 4A).

Il-1β relative mRNA expression in the PFC and hippocampus was significantly different between the groups (F=9.149, $p=0.0006$ and F=4.292, $p=0.0211$, respectively, one-way ANOVA); it was increased in both brain areas of susceptible mice in comparison to control mice (PFC: $p=0.0006$; hippocampus: $p=0.0170$, Tukey test, Fig. 4B), and no such changes were observed for resilient mice (PFC: $p=0.0560$; hippocampus: $p=0.3574$, one-way ANOVA. Figure 4B). *Il-1β* expression was not significantly different between susceptible and resilient groups ($p=0.288$), and significantly different between control and stress groups (t=3,831, df=38, $p=0.0005$, two-tailed t-test, Fig. 4B).

Gsk-3β mRNA expression was not significantly different between the groups (F=0.1231, $p=0.3038$, one-way ANOVA) and was not changed in either the PFC or the hippocampus in both susceptible and resilient mice in comparison to controls (PFC: $p=0.2948$; hippocampus: $p=0.1921$ and PFC: $p=0.6551$; hippocampus: $p=0.3220$, respectively, Tukey test, Fig. 4C). Two-tailed t-test revealed no significant differences in *Gsk-3β*mRNA concentrations for the PFC (t=1,454, df=38, $p=0.151$) or for the hippocampus (t=1.989, df=38, $p=0.0539$).

Two-way ANOVA demonstrated significant group differences in the expression of *Plp-1* mRNA in the PFC, but not in the hippocampus (F=5.279, $p=0.0094$ and F=0.412, $p=0.6652$, respectively, one-way ANOVA). In the PFC, this measure was significantly lower in susceptible mice than in controls ($p=0.0096$, Tukey test, Fig. 4D), while in the hippocampus, no such differences were found ($p=0.7776$). No significant differences from control were observed in *Plp-1* expression in the PFC and hippocampus of resilient mice (PFC: $p=0.1632$; hippocampus: $p=0.7086$). The expression of *Plp-1* in the PFC was significantly lower in the stress group than in control animals and unchanged in the hippocampus (t=2.999, df=40, $p=0.00046$ and t=0.9183, df=38, $p=0.3643$, respectively, two-tailed t-test).

There was a significant group difference in *Mag*mRNA expression in the PFC (F=3.710, $p=0.340$) but not in the hippocampus (F=2.532, $p=0.0937$, one-way ANOVA). *Mag*mRNA expression was significantly decreased in the PFC of susceptible mice ($p=0.0390$, Tukey test, Fig. 4E) but not in the hippocampus ($p=0.8397$); no significant changes were observed in resilient mice (PFC: $p=0.2011$; hippocampus: $p=0.0774$). In the PFC, the expression of *Mag* was significantly lower in stress animals than in the control group (t=2.610, df=38, $p=0.0129$, two-tailed t-test). No differences between these groups were found for *Mag*mRNA expression in the hippocampus (t=1,740, df=37, $p=0.0901$, two-tailed t-test).

MDA content in the brain and liver

MDA concentration was significantly different between the groups (F=9.439, $p=0.0009$, one-way ANOVA) and was significantly increased both in the brain of resilient and susceptible mice ($p=0.0229$ and $p=0.0008$, Tukey test, one-way ANOVA, Fig. 4F). Control and stress group significantly differed in this measure (t=4,061, df=26, $p=0.0004$, two-tailed t-test). As for MDA levels in the liver, there were no significant group differences observed (F=2.113, $p=0.1419$), and no changes in this parameter in both resilient and susceptible mice compared to the control group $p=0.5888$ and $p=0.5132$, respectively, Fig. 4F). Two-group comparison revealed no significant differences in hepatic MDA concentrations (t=0,06844, df=26, $p=0.9460$).

Discussion

The present study suggests the potential of the US paradigm of 'emotional stress' in juvenile C57BL6 mice to recapitulate the key features of depressive disorder in adolescents,

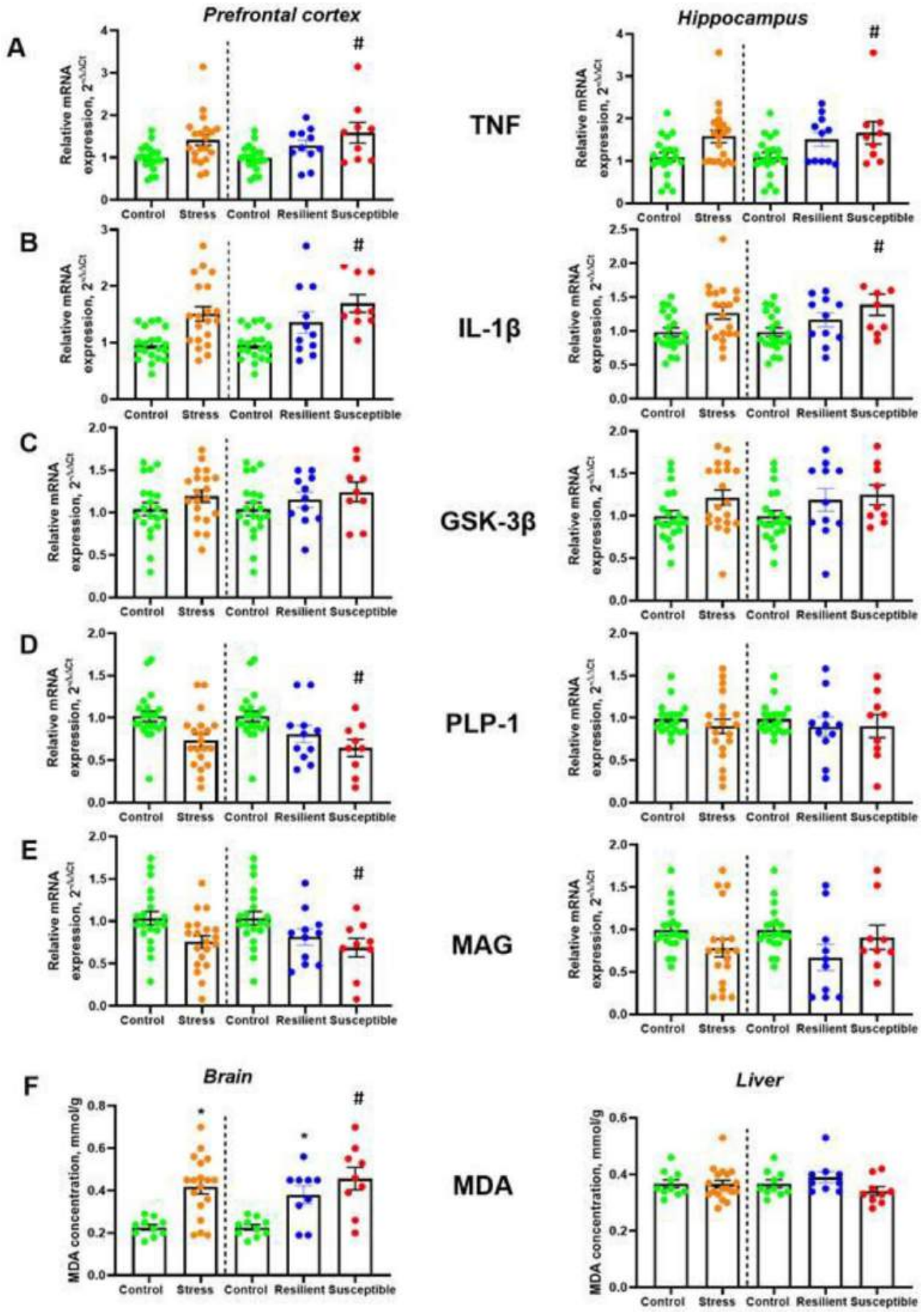

Prefrontal cortex
Hippocampus
A
TNF
B
IL-1β
C
GSK-3β
D
PLP-1
E
MAG
F
Brain
Liver
MDA
Relative mRNA expression, 2^-ΔΔCt
MDA concentration, mmol/g
Control
Stress
Resilient
Susceptible
#
*

Fig. 4 Molecular markers of stress-related readouts, parameters of neuroinflammation, myelination and oxidative stress in susceptible and resilient mice. The relative mRNA expression of (**A**) *Tnf* and (**B**) *Il-1β* was significantly increased both in the PFC and the hippocampus in susceptible mice, but not in resilient mice. (**C**) *Gsk-3β* mRNA expression was not significantly changed in either the PFC or the hippocampus in both experimental groups. The relative mRNA expression of (**D**) *Plp-1* and (**E**) *Mag* was significantly decreased in the PFC of susceptible mice, but not in resilient mice, no significant changes were present in the hippocampus. (**F**) The concentration of MDA was significantly increased in the brain of resilient mice but was even more increased in the brain of susceptible mice, there were no significant changes in the livers of both experimental groups. $*p < 0.05$, one-way ANOVA with Tukey's test or unpaired t -test. Data is presented as mean ± SEM

as well as to model a phenomenon of inter-individual variability in susceptibility to MDD-like syndrome precipitated by stress. The substantial behavioral, hormonal, and molecular alterations induced by US exposure were found to be distinct in resilient versus susceptible subsets of mice that were stratified to these subsets by previously established criteria of anhedonic behavior, a key symptom of MDD. In particular, we noted increases in helplessness and anxiety-like behaviors, altered locomotion, and shortened grooming behaviors in susceptible, but not in resilient mice. In comparison to the resilient group, susceptible mice revealed increases in dominant-type behavior, while the former group had opposing changes in comparison to non-stressed mice. Notably, novelty exploration, social exploration, and contextual learning were not affected by US stress; susceptible mice displayed only minor deficits in hippocampus-dependent performance that were likely due to their elevated anxiety. Susceptible animals showed pronounced increases in CORT plasma level, brain expression of pro-inflammatory cytokines, and significantly decreased expression of myelination factors, while in the resilient group no significant changes in these parameters were found. The decrease in body weight and increase in MDA brain levels were similar between the two groups.

Behavioral changes found in a subset of susceptible mice in the current study are in keeping with clinical observations of adolescent depressed patients. Adolescent patients with depression can experience fear and anxiety, stress-induced unrest, and helplessness. Similarly, US-exposed juvenile mice in our experiments displayed increased floating behavior in the swim test, and locomotor hyperactivity in a modestly illuminated open field. They showed prolonged freezing behavior, a sign of fear and anxiety in brightly illuminated open field, and reduced self-grooming behavior, suggesting a state of discomfort. All these changes were shown by susceptible but not resilient animals. At the same time, all US-exposed mice showed normal contextual fear conditioning learning. Indeed, cognitive impairments are known to be not typical for MDD in youth and adolescents (Chaudhury et al. 2015; Rice 2019; Zwolińska et al. 2023). Here, susceptible mice showed only minimal deficits in the marble paradigm, a test for hippocampus-dependent performance, as they had a slowed down pellet displacement behavior at the first 5 min which was likely due to their increased anxiety-like behaviors. Their overall performance in the marble test was unaltered, unlike the performance of adult stressed mice in this model (Strekalova and Steinbusch 2010).

Indeed, the susceptible group has exhibited signs of elevated anxiety-like behavior in the dark-light box and brightly illuminated open field in the present study. Unlike previous stress experiments stratifying adult C57Bl6 mice to resilient and susceptible subgroups upon a manifestation of hedonic deficit in the sucrose test (Strekalova et al. 2004, 2023; Cline et al. 2015), this study on juvenile mice did not reveal disruptive effects of stress on long-term contextual learning, social exploration and exploration of a new environment in the novel cage. At the same time, stress experiments on adult mice of the same strain revealed elevated anxiety in both resilient and susceptible subgroups (Strekalova et al. 2004, 2006, 2022; Cline et al. 2015).

Our present results are in agreement with previous findings in the US model. By applying the US paradigm in juvenile mice, our previous study demonstrated the anti-anxiety effects of the omega-3-containing food supplement whose administration counteracted the induction of an MDD-like syndrome and anhedonia, accompanied by heightened anxiety-like behavior (Strekalova et al. 2024).

Of interest, the current study in juvenile mice reveals hyperactivity in stressed animals under conditions of modest lighting, while under bright lighting conditions, there was an inhibition of locomotor behavior, which is in line with the reported changes in adult mice that were exposed to chronic stress (Strekalova et al. 2004, 2005). Freezing behavior was increased in susceptible mice under bright but not modest lighting; this group of mice also showed inhibited grooming behavior, regardless of illumination strength used in the open field model. No changes in grooming behavior or freezing behavior were reported in adult stressed mice in the US model (Gorlova et al. 2020; de Munter et al. 2021). It is of note that the difference in stress paradigms, employed in the adolescent and adult mice, may have also played a role in the modulation of stress-induced behavioral responses.

Besides, accumulated evidence suggests that MDD symptoms in youth are often manifested by problems in social interactions, such as expression of anger or aggression towards other individuals. Our observations in the social interaction test are in line with these clinical abnormalities that showed reduced latency of contacts of the dominant type in susceptible mice. Surprisingly, resilient mice revealed significant reductions in manifestations of

dominant-like and aggressive behaviors, such as following, crawl-over behavior, and tail rattling. These behaviors were not altered in the susceptible subgroup. These data illustrate a complex relationship between social behavior and the vulnerability to stress-related depressive syndrome.

It has to be noted that a lack of differences between susceptible and resilient subgroups of mice in locomotion, body weight, and general drinking behavior in the sucrose test allows us to rule out possible confounds in the evaluation of investigated here behavioral changes of US-exposed mice.

Demonstrated here behavioral alterations in stressed mice were accompanied by increases in the CORT plasma levels and the expression of pro-inflammatory cytokines, notably *Il-1β* and *Tnf*, aligning with previous clinical reports on the role of these changes in adolescent depressed patients (Lamers et al. 2019; Colasanto et al. 2020; Ferencova et al. 2022) and with our previous observations on adult US-exposed mice (Gorlova et al. 2019; Pavlov et al. 2020; de Munter et al., 2021). Notably, we found significant increases in brain expression of pro-inflammatory cytokines in a subset of susceptible but not resilient mice which led us to hypothesize that these molecular changes underpin the reported here abnormalities of the susceptible subset of mice.

Ultrasound-stressed adult mice were previously shown to display elevated plasma corticosterone levels (Pavlov et al. 2019), activated hippocampal microglia, upregulated production of pro-inflammatory cytokines *Il-1β* and *Il-6* locally both in the brain and plasma (Costa-Nunes et al. 2020; de Munter et al. 2021; Pavlov et al. 2023) and increased concentration of a marker of oxidative stress, protein carbonyl in the brain (Gorlova et al. 2019; Sambon et al. 2020). However, performed here stratification of stressed mice to resilient and susceptible subgroups in the US experiment revealed that these changes have occurred at the expense of susceptible mice.

The stress-induced rise in MDA levels appeared to be similar in both subsets of stressed animals suggesting that while oxidative stress and production of pro-inflammatory cytokines are interrelated, this relationship can be rather complex and follow distinct dynamics with respect to the termination of stress procedure. Besides, gene expression of *Gsk-3β*, the molecular hub of cellular distress was unaltered in the brain of stressed mice, conflicting with previous findings in the US model (Gorlova et al. 2019; Pavlov et al. 2020). This can be explained by the substantially longer stress-free period prior to sacrifice used in the current study as it can be a case for unchanged hepatic MDA levels, whose increases were reported in other chronic stress studies in which mice were killed within much shorter post-stress time window than in our study (Sambon et al. 2020; de Munter et al. 2021).

Generally, overlapping molecular and hormonal changes in adult and juvenile mice exposed to the US model may suggest the validity of the employed here paradigm as a model of adolescent depression. They also warrant further more extensive studies addressing potential differences in the neurobiology of MDD between adults and youth. Studies of the last decade using high-throughput metabolomics or proteomics approaches on 'resilient' and 'susceptible' cohorts of adult rodents elucidated a large portion of the neurobiological basis for their distinct physiological and behavioral profiles of stress response (Henningsen et al. 2012; Palmfeldt et al. 2016; Akimoto et al. 2019; Zhang et al. 2021; Strekalova et al. 2023). These stratification studies provide evidence that stress susceptibility and resilience are likely to be underpinned by distinctive molecular mechanisms (Christiansen et al. 2012; Li et al. 2014; Nieto-Gonzalez et al. 2015; Remus et al. 2015; Sun et al. 2017; Yu et al. 2020). However, no such attempts were reported with regard to animal models of adolescent depression, while the issue of the inter-individual variability in response to stress is now well-established in the literature (Antoniuk et al. 2019; Strekalova et al. 2022; Nestlet and Russo, 2024).

At the same time, one has to bear in mind that major depression is an extremely complex disorder, and modeling this psychiatric condition in non-human animals continues to present significant challenges (Insel 2007; Borsini 2012; Robinson 2025). For example, the human sweet taste test, which attempts to measure the form of sucrose preference in humans, failed to find a deficit in depressed patients (Robinson et al. 2018).

While our findings are keeping with the outcome from other stress paradigms, further studies are warranted to explore the validity of this model, as the sucrose test and chronic stress paradigm have been criticized for decades for inconsistent behavioral outcomes and limited reproducibility (Phillips and Barr 1997; Nielsen et al. 2000; Berrio and Kalliokoski 2023; Robinson 2025). Our prior research has indicated that such inconsistencies in mice may, in part, result from methodological variations of sucrose test and behavioural tests that are sensitive to stress-induced hyperlocolotion (Strekalova 2008; Strekalova and Steinbusch 2010, 2011; Strekalova et al. 2011, 2022; Strekalova 2023;). It is therefore essential to explore the neurobiological basis of the interconnected changes reported here - particularly in sucrose preference and other MDD-related behavioral, hormonal, and molecular markers of a depressive-like state. A key question remains whether the differences observed between 'resilient' and 'susceptible' animals genuinely reflect depressive-like phenotypes or merely represent natural variability in stress response. Although antidepressant treatment has been shown to reverse stress-induced reductions in sucrose preference and related changes (Papp et al.

2016; Morozova et al. 2016; Strekalova 2023), scepticism persists regarding the translational validity of the sucrose preference test (Forbes et al. 1996; Hatcher et al. 1997; Phillips and Barr 1997; Weiss 1997; Berrio and Kalliokoski 2023; Sanchez et al. 2025; Robinson 2025). In particular, it is crucial to consider the possibility that the classification of animals into 'anhedonic' and 'non-anhedonic' groups based on control thresholds may introduce bias. Future studies employing high-throughput methodologies within the ultrasound stress model may help clarify these issues and identify novel biomarkers and therapeutic targets relevant to adolescent depression.

Acknowledgements We thank Prof. Gregor Berger for valuable discussions and Mr. Andrey Nedorubov for important technical support.

Author contributions Co-author contributions to the paper was following: TS, SW, AL, RC – conceptualization; TS, RC, AL, JM - Data curation; AG, AB, AL, EK- Formal analysis; TS, SW, EG, JM - Funding acquisition; AG, EK, DR, AB, ES, AL - Investigation; TS, RC - Methodology; BS, TS, SW, RC - Project administration; JM, TS, DA, AL, RC - Resources; BS -Software; TS, SW, JM, RC - Supervision; BSh, AG, AB - Validation; AB, AG, EK, AL -Visualization; TS, AB, AG, RC -Writing, original draft; JM, SW, EG, EK, BS, AL - Writing - review & editing.

Funding This study was supported by Research Russia-Switzerland Grant-2020 (all experimental work was carried out by January 2022) to SW and TS and PhytoAPP EU framework (2021–2025, to TS). The PhytoAPP project has received funding from the European Union's HORIZON 2020 research and innovation program under the Marie Skłodowvska-Curie grant agreement 101007642. Animal procedures werefunded by the State Task of the Laboratory of Genetic Technologies and Genome Editing for Biomedicine and Animal Health - FZWG-2021-0016. The sponsors had no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report and in the decision to submit the article for publication.

Data availability All available data is provided with the article or with attached supplementary materials.

Declarations

Conflict of interest The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. All experimental data acquisition and analysis were accomplished by December 2021-January 2022.

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