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RESEARCH ARTICLE

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Long-term effects of stress resilience: Hippocampal neuroinflammation and behavioral approach in male rats

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Abstract

Resilience to stress is the ability to quickly adapt to adversity. There is evidence that exposure to prolonged stress triggers neuroinflammation what produces individual differences in stress vulnerability. However, the relationship between stress resilience, neuroinflammation, and depressive-like behaviors remains unknown. The aim of this study was to analyze the long-term effects of social defeat stress (SDS) on neuroinflammation in the hippocampus and depressive-like behaviors. Male rats were subjected to the SDS paradigm. Social interaction was analyzed 1 and 2 weeks after ending the SDS to determine which animals were susceptible or resilient to stress. Neuroinflammation markers glial fibrillary acidic protein, ionized calcium-binding adaptor molecule 1, and elevated membrane permeability in astrocytes and microglia, as well as depressive-like behaviors in the sucrose preference test and forced swim test were evaluated in all rats. One week after SDS, resilient rats increased their sucrose preference, and time spent in the floating behavior decreased in the forced swim test compared to susceptible rats. Surprisingly, resilient rats became susceptible to stress, and presented neuroinflammation 2 weeks after SDS. These findings suggest that SDS-induced hippocampal neuroinflammation persists in post-stress stages, regardless of whether rats were initially resilient or not. Our study opens a new approach to understanding the neurobiology of stress resilience.

KEYWORDS

depression, hemichannels, hippocampus, neuroinflammation, resilience, RRID:AB_2827276, RRID:AB_839504, RRID:RGD_10395233, RRID:RGD_2308852, stress

Abbreviations: ACSF, artificial cerebral spinal fluid; ANOVA, analysis of variance; BSA, bovine serum albumin; CD45, cluster of differentiation 45; CRF, corticotropin-releasing factor; DAPI, 4,6 diamidino-2-phenylindole; DG, dentate gyrus; EPM, elevated plus-maze; EtBr, ethidium bromide; FST, forced swim test; GCs, glucocorticoids; GFAP, glial fibrillary acidic protein; GRs, glucocorticoid receptors; HPA, hypothalamic pituitary adrenal; Iba-1, ionized calcium-binding adaptor molecule 1; IL-1β, interleukin-1 beta; IL-6, interleukin-6; MDD, major depressive disorder; mPFC, medial prefrontal cortex; NAc, nucleus accumbens; OF, open field; PBS, phosphate-buffered saline; PND, post-natal day; RFI, relative fluorescence intensity; *SD*, standard deviation; SDS, social defeat stress; SI, social interaction; SNS, sympathetic nervous system; SPT, sucrose preference test; TNF-α, tumor necrosis factor-alpha; VEGF, vascular endothelial growth factor.

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1 | INTRODUCTION

Stress is defined as a nonspecific biological response of an organism to any real or perceived threat from the environment that affects its homeostasis (Selye, 1936, 1956). The main biological purpose of stress responses is to restore homeostasis and adapt to environmental threats or stressors (McEwen & Akil, 2020; Taborsky et al., 2020). Stress may even be positive (eustress), when animals adapt to environmental threats (Tafet & Bernardini, 2003). A more recent perspective on the concept of stress relates to the idea of "allostasis," defined as the adaptive process of preserving stability in response to stressful conditions (McEwen & Akil, 2020). When the energy cost of adaptation (allostatic load) is too high, stress induces negative health consequences and maladaptive responses (McEwen & Akil, 2020). In this sense, two sub-concepts would underlie allostasis: Susceptibility and resilience to negative stress or distress (Karatsoreos & McEwen, 2011).

It has been reported that chronic exposure to stressful life events is a key risk factor for major depressive disorder (MDD), a neuropsychiatric illness with high social and economic impact worldwide (Han & Nestler, 2017; Kessler et al., 1994; Myers et al., 2017). However, there are some people who do not get sick or develop a neuropsychiatric disorder such as MDD, despite having been exposed to distress. These types of people are called "resilient individuals" (Han & Nestler, 2017; Hoge et al., 2007; Kessler et al., 1994), where resilience is defined as the ability to "rebound" or adapt quickly from adversity when one's ability to function has been impaired to some degree (Karatsoreos & McEwen, 2011; Wood & Valentino, 2017).

Social defeat stress (SDS) is a psychosocial stress model that entirely relies on social conflicts and interactions within conspecific animals and closely mimics the etiology of depression (Pfau & Russo, 2016). Several effects on the immune system have been reported for rodents exposed to SDS. The most important effects are increases in central and peripheral cytokine levels, microglial activation, surges in circulating leukocytes, and monocyte recruitment to the brain (Lehmann et al., 2016; Weber et al., 2017). These inflammatory statuses play a key role in the development of resilience and susceptibility to stress (Finnell & Wood, 2018). Thus, exposure to acute stress triggers a fast and brief increase in pro-inflammatory cytokines in brain areas that modulate stress responses, including the hippocampus (Patel et al., 2018). There is a close interrelationship between hippocampal GRs, the hypothalamic pituitary adrenal (HPA) axis, pro-inflammatory cytokines, and neuroinflammation (Kim et al., 2016). Importantly, reduced hippocampal activation and neurogenesis along with hormonal and inflammatory processes have been implicated in stress-related disorders (Kim et al., 2016). However, the precise mechanisms remain incompletely defined. In addition, the hippocampus is particularly sensitive to stress hormones and responds to them through changes in structure, neurochemistry, and excitability (Conrad et al., 2017).

To further understand the influence of distress on MDD, it is critical to pinpoint when an individual was exposed to distress for the first time during their life span. If the first exposure occurred early at perinatal ages, the neuroinflammation response is likely to affect the ontogeny of central nervous system (Hantsoo et al., 2019) and the behavioral outcome could be irreversible. Whereas, if the first exposure to distress occurs during adulthood, behavioral alterations are likely to be reversible despite the transient neuroinflammatory response. Such evidence suggests that distress and stress-related disorders like MDD are associated with neuroinflammation (Liu, Wang, et al., 2017; Rohleder, 2019). In line with this, peripheral inflammatory biomarkers have been observed to increase in patients with MDD compared to healthy control subjects (Kim et al., 2016).

Despite the above findings, few studies have focused on the long-term effects of social distress. Consequently, the aim of this study was to test the hypothesis that long-term effects of stress susceptibility are related with hippocampal neuroinflammation and depressive-like behaviors when compared to the resilient behavioral phenotype. We found that 1 week after the SDS protocol, rats that were susceptible to stress showed hippocampal neuroinflammation and depressive-like behaviors compared to resilient rats. However, resilience to stress was lost 2 weeks after SDS, as reflected by persistent hippocampal neuroinflammation and depressive-like behavior.

Significance

Resilience to stress is the ability to quickly adapt to environmental demands. In this study, we wonder how long does stress resilience last? To answer this question, the effects of social stress on neuroinflammation and depressive-like behavior were evaluated in male rats. Animals that were resilient to stress up to 1 week after the stress period displayed neuroinflammation and depressive-like behaviors comparable with susceptible rats 2 weeks after social stress. Our findings suggest that it is required to find mechanisms to stimulate stress resilience to prevent the development of stress-related disorders, such as depression.

2 | MATERIALS AND METHODS

2.1 | Animals

Male *Sprague-Dawley* rats (Rattus norvegicus, Crl:CD (SD). RGD Cat# 10395233, RRID:RGD_10395233), bred in our own laboratory, about 350 g and 55 days old at the start of the experiment were used as subjects and adult male *Long–Evans* rats (Rattus norvegicus, Crl:LE. RGD Cat# 2308852, RRID:RGD_2308852) weighing between 700 and 850 g were utilized as aggressors in the SDS paradigm. *Sprague-Dawley* and *Long–Evans* rats were commercially acquired (Charles River Laboratories, Wilmington, USA). All rats were maintained under a 12-hr light–dark cycle (lights on at 8:00 a.m.) and provided with food (Prolab RMH 3000, LabDiet®, MO, USA) and water *ad*

libitum, except when specified. All manipulations and experiments were performed in the light phase. *Sprague-Dawley* rats were housed in transparent polycarbonate cages (45 × 23 × 15 cm) with stainless steel lids and sawdust bedding, while Long–Evans rats were housed in transparent polypropylene cages (42 × 33 × 30 cm) that served as the home cage and maintained under standard laboratory conditions of humidity (55 ± 5%) and temperature (22 ± 1°C) and housed in groups of two per cage and not subjected to any type of stress only were exposed to sounds of cleaning the cages three times 1 week and room traffic.

All animals were adapted to standard conditions for 1 week before commencement of the social defeat procedure. Social defeats took place in an adjacent room, while non-defeated animals were separated from animals under the SDS protocol and kept in a different room in our vivarium. Body weights were measured daily during the stress protocol.

2.2 | Experimental design

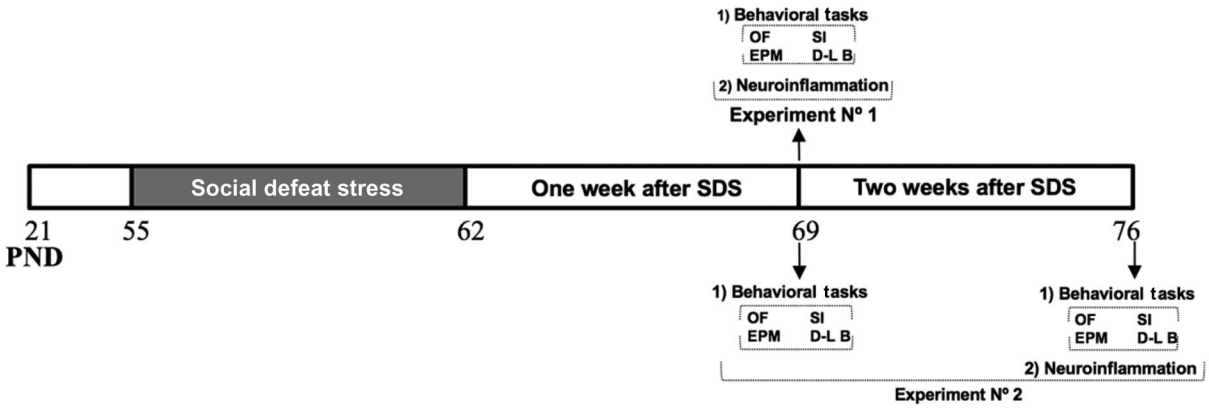
Scheme 1 shows the timeline of the experimental design. Social interaction, anxiety, depressive-like behaviors, neuroinflammation, and stress markers were assessed 1 week (Experiment 1) and 2 weeks (Experiment 2) after the SDS protocol. Different sets of animals were used in each experiment (Table 1). Two experimental groups were used in Experiment 1 (non-SDS, $n = 10$; SDS, $n = 10$) and Experiment 2 (non-SDS, $n = 10$; SDS, $n = 16$). Rats that were susceptible to SDS engaged less in social interaction and consumed less sucrose preference in comparison with non-defeated rats. Five susceptible and resilient rats were obtained in Experiment 1, as well as seven susceptible rats and nine resilient rats were obtained in Experiment 2 (Table 1). The behavioral tasks were carried out 1 week after the SDS protocol in each experiment (Scheme 1), then the results of susceptible and resilient rats had $n = 12$ and $n = 14$, respectively. The results of Experiment 1 for neuroinflammation and stress markers had $n = 5$ in the groups of susceptible and resilient rats. The results of Experiment 2 for neuroinflammation had $n = 5$ in the groups of susceptible and resilient rats, and the results for stress markers had $n = 7$ in the group of susceptible rats and $n = 9$ in the group of resilient rats (Table 1).

2.3 | Social defeat stress

In this study, the same resident-intruder paradigm published in Iturra-Mena et al. (2019) was used. In each session of SDS, a *Sprague-Dawley* rat (intruder) was placed into the home cage of an unfamiliar male *Long–Evans* rat (resident) classified as highly aggressive (Iturra-Mena et al., 2019). This protocol was repeated for seven consecutive days, with one 30-min SDS session each day. Subordination or defeat in the intruder was determined when the resident rat assumed a supine position for approximately 3 s. After a defeat episode, the Long–Evans resident rat was placed inside a wire mesh (20 × 15 cm) enclosure the session, such that no further physical contact occurs with the *Sprague-Dawley* rat (intruder), while allowing visual, auditory, and olfactory detection for the remainder of the 30-min session. After each SDS session, Sprague-Dawley rats returned to their original home cage for the rest of the day.

2.4 | Experiments

Two experiments were performed to evaluate the long-term effects of the SDS protocol on locomotor activity, social behavior, as well as anxiety- and depressive-like behaviors (Scheme 1). Experiment N° 1 was designed to evaluate the effects of SDS on behavioral assays



SCHEME 1 Timeline of the experimental design. Two experiments were carried out using two different sets of animals. Male *Sprague-Dawley* rats were subjected to the social defeat stress paradigm and a non-defeated group was left undisturbed. In Experiment 1, 1 week after completion of the social defeat stress protocol, locomotor activity was established in the open field (OF), anxiety-like behaviors and social interaction were evaluated on an elevated plus-maze (EPM) and the social preference-avoidance test (SI), respectively. Depressive-like behaviors (D-L B) were assessed using the sucrose preference test and forced swim test. After behavioral assays, two markers of neuroinflammation were measured in the hippocampus, GFAP and Iba-1. In Experiment N° 2, the same behavioral tests were carried out 1 and 2 weeks after social defeat stress. Neuroinflammation was evaluated 2 weeks after the stress protocol ended

TABLE 1 Number of animals used in each experiment

	One week after SDS			Two weeks after SDS		
	Non-SDS	Resilient	Susceptible	Non-SDS	Resilient	Susceptible
Number of rats/Experiment 1	10	5	5			
Number of rats/Experiment 2		9	7	10	9	7
Number of rats /group	10	14	12	10	9	7

1 week after completing the stress protocol. Experiment N° 2 was designed to determine the effects of SDS on behavioral experiments 1 and 2 weeks after applying the SDS protocol (Scheme 1). After performing the behavioral assays, two of neuroinflammation markers were measured in the hippocampus, anti-ionized calcium-binding adapter molecule 1 (Iba-1) and anti-glial fibrillary acidic protein (GFAP). Ten *Sprague-Dawley* rats were used in each experiment (non-SDS, $n = 10$; SDS, $n = 10$).

2.5 | Behavioral testing

Prior to the experiments, rats were habituated to the testing room for 30 min on 3 successive days. Habituation and behavioral examinations were carried out in a soundproof and temperature-controlled (22 ± 1℃) room. *Sprague-Dawley* rats were naive to the all behavioral tests.

Rat behavior was recorded with a webcam (WideCam 1050, Genius, Taiwan, China) and videos were automatically analyzed using EthoVision® XT version 15 (Noldus, Wageningen, The Netherlands). After each trial, all mazes were cleaned with a 5% ethanol solution.

2.6 | Social preference-avoidance test

A social interaction paradigm was used to test social behavior in *Sprague-Dawley* rats (Iturra-Mena et al., 2019). This test consists of two sessions. In the first session, rats were placed in an open field (70 × 70 × 54 cm), which contained an empty transparent perforated chamber (25 × 15 cm) in a designated interaction zone. This chamber was placed in the middle of one side of the open field. The rats were free to explore for 5 min and time spent in the interaction zone was measured ("non-social target" stage). In the second session, a novel conspecific (male *Sprague-Dawley* of comparable age and weight) was placed inside the perforated chamber. The experimental animal was then allowed to explore the maze for 15 min ("social target" stage). Time spent in the interaction zone with and without social targets was determined. Representative heat maps track rats in each experimental group were made using EthoVision® XT. In the social target stage, defeated rats that had interaction times two standard deviations below the mean of non-defeated rats were classified as susceptible. Rats that engaged in social interaction two standard deviations below the mean of non-defeated rats were classified as resilient (Iturra-Mena et al., 2019). The experimenter was blind to group conditions.

2.7 | Open field test

Locomotor activity was assessed using the open field test (Walker et al., 2020). In brief, each animal was placed in the center of a black Plexiglass cage (70 × 70 × 40 cm) for 5 min. The background noise level in the open field was 40 dB SPL and the arena was illuminated to 300 lux.

The time spent in the central zone and perimeter were analyzed in the video recordings, and the highest speeds were calculated. The arena was divided into 16 equal squares. The central zone was defined within the four central squares and the rest of the squares corresponded to the border zone or perimeter. Entry to a zone was defined as having occurred when the animal placed all four limbs onto the center or periphery.

2.8 | Elevated-plus maze test

Anxiety-like behavior was evaluated using an elevated plus-maze paradigm. Each rat was placed individually in an elevated plus-maze, consisting of two closed arms (60 × 15 × 20 cm each), two open arms (60 × 15 cm each), and a central platform (15 × 15 cm) arranged so that the two arms of each type were opposite to each other. The maze was elevated 100 cm above the floor. The lighting was 210 lux in the closed arms and 300 lux in the open arms. At the beginning of the 5-min test, rats were located at the center of the maze, facing an open arm. Entry into an arm was defined as having occurred when the animal placed all four limbs onto the arm floor. Frequency of entries and time spent in the closed arms were used as measures of anxiety-like behaviors.

2.9 | Sucrose preference test

Depressive-like behavior was evaluated using the sucrose preference test, which assesses the inability to experience pleasure in rodents. The *Sprague-Dawley* rats were first trained for 3 days to drink sweet liquid (5% sucrose) as previously published (Iturra-Mena et al., 2019). Then, animals were water deprived for 12 hr before the test. During the test, the rats were allowed to choose between two bottles for 12 hr, one containing a 5% sucrose solution and the other containing only water. The amount of liquid consumed by the rats was measured, and the percentage of preference of sucrose solution in relation to the neutral liquid was calculated.

2.10 | Forced swim test

Low mood or dysthymia is a core symptom of major depression that was evaluated in rats using the FST (Wang et al., 2017). *Sprague-Dawley* rats were individually immersed for 5 min on a see-through Plexiglas cylinder (46 cm height; 25 cm in diameter), filled with 30 cm of water at 25℃. Behavior was recorded and later manually scored using EthoVision® XT. Three types of behavior were assessed: Floating, climbing, and swimming. Floating behavior was defined as minimal movements needed for the rat to keep its head above water and maintaining a vertical position of at least 10° from the surface.

2.11 | Neuroinflammation

2.11.1 | Hippocampal slice preparation

Acute hippocampal slices were obtained from animals as previously described (Gajardo-Gómez et al., 2017). In brief, rats were decapitated, the brain was rapidly removed through craniotomy and placed in ice-cold (<4℃) artificial cerebral spinal fluid (ACSF) containing (in mM): 125 NaCl, 2.5 KCl, 25 glucose, 25 $NaHCO_3$, 1.25 NaH_2PO_4, 2 $CaCl_2$, and 1 $MgCl_2$, adjusted to pH 7.4, gassed with 95% O_2/5% CO_2. Transversal brain slices (300 μm) were cut using a vibratome (Leica, VT 1000GS; Leica, Wetzlar, Germany) and then incubated in regular ACSF solution for 1 hr at room temperature (21–24℃) before being used.

2.11.2 | Permeability of glial cell membrane and detection of glial reactivity

Hippocampal slices were incubated in 5 μM ethidium bromide (EtBr) (Sigma-Aldrich, St. Louis, MO, USA) for 10 min in a chamber oxygenated by a bubbling gas mixture (95% O2 and 5% CO2) into ACSF, pH 7.4. Slices were then washed five times with ACSF, fixed at room temperature with 4% paraformaldehyde for 30 min, and rinsed extensively with 1× phosphate-buffered saline (PBS). To quantify dye uptake in a cell type, slices were then processed for immunofluorescence detection of a specific molecular marker for the cell type of interest. Hippocampal slices were incubated for 1 hr at room temperature in blocking solution (50 mM NH_4Cl, 0.01%Triton X-100, 1% BSA in PBS). Then, the slices were incubated overnight at 4℃ with either polyclonal rabbit anti-Iba-1 antibody (1:500, Wako Cat# 019-19741, RRID:AB_839504) or monoclonal mouse anti-GFAP antibody (1:300, Sigma-Aldrich Cat# SAB5201104, RRID:AB_2827276) to detect microglia or astrocytes, respectively (Table 2). The appropriate secondary antibodies goat anti-rabbit or goat anti-mouse Cy2 conjugated AffiniPure IgGs (H+L) (Jackson Immuno Research, Indianapolis, IN, USA) were used at 1:300 (for details of antibody, see Table 1). After washing, the slices were transferred to a glass slide and coverslips were mounted using DAPI Fluoromount-G™ (Electron Microscopy Sciences, Hatfield, PA, USA) for image collection. Stacks of consecutive images were taken with a 603 objective at 250 nm interval with three lasers (405, 488, and 561 nm), and *Z* projections were reconstructed with a Nikon C1 Plus confocal microscope with a 40× oil immersion objective. The fluorescence intensity of each antigen and EtBr uptake ratio in cells of non-SDS and SDS were compared using ImageJ 1.47 software (Wayne Rasband, National Institute of Health, USA) and Adobe Photoshop 6.0. (Adobe Systems Incorporated, CA, USA) to calculate corrected total cell fluorescence as described previously (Maturana et al., 2017; Orellana et al., 2015). The corrected total cell fluorescence is represented as relative fluorescence intensity (RFI) = integrated density − (area of selected cell × mean fluorescence of background readings). The total number (*n*) of cells counted for each group was approximately 60 and at least three fields were selected in every slice.

2.12 | Stress markers

2.12.1 | Body weight gain

As a physiological marker of stress, *Sprague-Dawley* rats were weighed daily. Rats were removed at 10.00 hr from their home cage by hand and transferred to another cage on a digital scale to be weighed. The experimenters who applied the SDS protocol were different from those who conducted the handling procedure. This procedure was applied to all *Sprague-Dawley* rats from weaning until the end of the experiment. A scale (model WLC2/A1, Radwag, Poland) was used for this purpose.

2.12.2 | Adrenal glands

Adrenal glands were obtained after euthanasia and their weights were calculated based on the last weight measurements to assess the physiological stress response. Adrenal gland weights were

TABLE 2 Primary antibodies used in the neuroinflammatory experiments

Antibody	Host	Immunogen description	Source, catalog No.	Dilution used
Anti-Iba-1	Rabbit polyclonal	Raised against synthetic peptide corresponding to C-terminal of Iba1	Wako Cat# 019-19741 RRID:AB_839504	1:500, IF
Anti-GFAP	Mouse monoclonal	Generated against purified GFAP from pig spinal cord	Sigma-Aldrich Cat# SAB5201104 RRID:AB_2827276	1:300, IF

obtained using the following formula: Wet weight of adrenal glands (mg) × 100/body weight (g) (Ulrich-Lai et al., 2006).

2.13 | Statistical analyses

All variables met the criteria for homoscedasticity (Levene's test) and normal distributions (Shapiro–Wilk test) were thus analyzed with parametric statistics. The statistical tests used in each experiment are shown in Table 3

In Experiment N° 1, one-way ANOVA was used to compare between non-defeated, resilient, and susceptible rats in the maximum speed, depressive-like behaviors, neuroinflammation experiment, body weight change, and adrenal weight. Interaction zone times in the social interaction test, time spent in the open field test, anxiety-like behaviors were analyzed with a two-way ANOVA, considering social stress and type of behavior as factors (non-social vs. social targets in the social interaction test, center vs. perimeter in the open field test; open vs. closed arms in the elevated-plus maze).

In Experiment N° 2, one-way ANOVA was used to compare between non-defeated, resilient, and susceptible rats in the neuroinflammation experiment, body weight change, and adrenal weight. Social interaction and depressive-like behaviors were analyzed with repeated measures two-way ANOVA. The factors were social target and weeks after SDS. Time spent in the interaction zone was the dependent variable. A three-way ANOVA analyzed locomotor activity and anxiety-like behaviors based on monitoring 1 and 2 weeks after SDS. The factors used in the analysis were (a) the behavioral phenotype in response to social stress (resilient vs. susceptible), (b) time after SDS (1 vs. 2 weeks), and (c) rat position inside the open field (center vs. perimeter) or elevated-plus maze (open vs. closed arms). The dependent variables were time spent and frequency of entries.

Bonferroni post hoc test for multiple comparisons was used to analyze all results since the criteria of normality and homoscedasticity were met in all variables.

Statistical analyses were performed using Prism 8 (GraphPad Software Inc., La Jolla, CA, USA) and IBM SPSS® (IBM Corp, New York, NY, USA). A probability level of 0.05 or less was accepted as significant. Results were expressed as mean ± standard deviation (*SD*).

3 | RESULTS

3.1 | Experiment N° 1: One week after social stress

3.1.1 | Social interaction

We studied social behavior to determine which rats were resilient and susceptible to the SDS paradigm (Iturra-Mena et al., 2019). Two-way ANOVA analysis showed a significant main effect of social target ($F_{(2,33)} = 81.1$, $p < 0.001$) and SDS ($F_{(1,33)} = 37.4$, $p < 0.001$) on time spent in the interaction zone (Figure 1a). A subsequent post hoc analysis showed that when socially defeated rats were classified according to the interaction times during the social target stage, two groups of rats emerged: A group resilient to stress (blue bar, Figure 1a), since the recorded social interaction was comparable to the mean of non-defeated rats (mean ± *SD*; non-defeated rats = 278.1 ± 161.6 s; resilient rats = 295.6 ± 211.4 s), and another group of rats susceptible to stress, given that their interaction times were two standard deviations below the mean of the non-defeated rat group (red bar, Figure 1a). In the social target stage, resilient rats spent significantly longer in the interaction zone compared to susceptible rats ($t = 11.3$; $df = 66$; $p < 0.001$). Representative heat maps shown in Figure 1b reveal that resilient rats spend more time in the interaction zone compared to susceptible rats.

3.1.2 | Locomotor activity and anxiety-like behaviors

The effect of the stress protocol on locomotor activity was determined to evaluate its impact on the performance of rats during the behavioral assessments. Social stress did not affect locomotor activity after 1 week of SDS, as measured by the maximum speed ($F_{(2,33)} = 0.3$; $p = 0.774$) at which rats explored the open field (Figure 2a).

TABLE 3 Statistical tests used in each experiment

	Experiment 1		Experiment 1		
	One-way ANOVA	Two-way ANOVA	One-way ANOVA	Two-way ANOVA	Three-way ANOVA
Locomotor activity	X				X
Social interaction		X		X	
Anxiety-like behaviors		X			X
Depressive-like behaviors	X			X	
Neuroinflammation	X		X		
Body weight	X		X		
Adrenal weight	X		X		

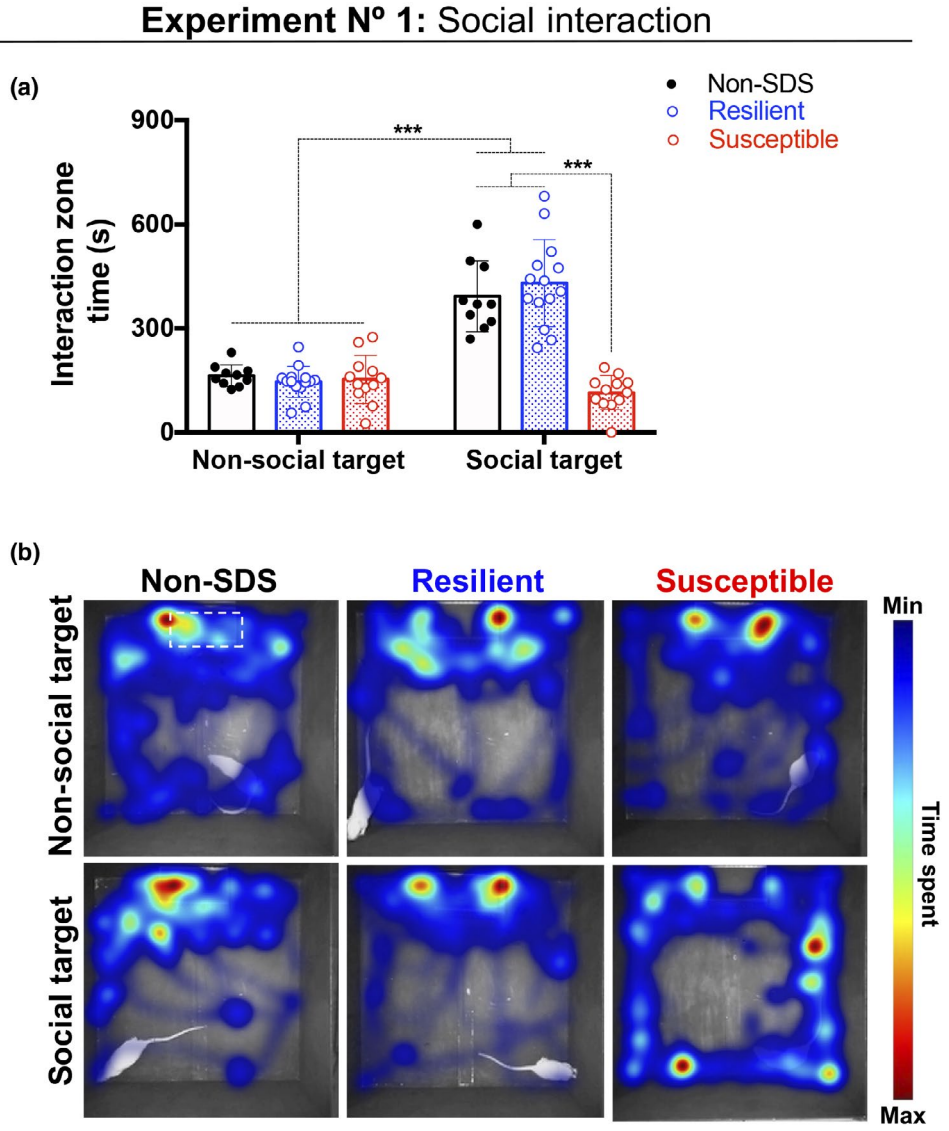


FIGURE 1 Social preference-avoidance test results for Experiment Nº 1. (a) Time spent interacting with an empty transparent perforated chamber (non-social target) and a novel rat (social target) after social defeat stress (SDS). Social interaction times for non-stressed (black circles), resilient rats (blue circles), and susceptible rats (red circles). Resilient rats spent significantly more time engaged in social interaction than susceptible rats (**$p < 0.01$). (b) Representative heat maps are shown for non-stressed, resilient, and susceptible rat tracking

The amygdala is a brain area that regulates anxiety and it is vulnerable to being altered by distress (Roozendaal et al., 2009). Anxiety is assessed by thigmotaxis behavior in the open field and elevated plus maze (Pérez et al., 2013). While SDS does not have an anxiogenic effect in rats at the end of stress periods (Liu, Zhou, et al., 2017), its long-term effects are not known. SDS did not affect the time spent in the center zone and border zone of the open field 1 week after the SDS protocol. Non-defeated, resilient, and susceptible rats, spent significantly less time in the center zone and more time in the perimeter ($F_{(1,33)} = 2{,}889$, $p < 0.001$), respectively (Figure 2b).

The frequency of entries into the open arms was significantly less compared to closed arms in both non-defeated, resilient, and susceptible rats 1 week after the stress period ($F_{(1,33)} = 128.2$, $p < 0.001$) (Figure 2c). Both non-stressed and stressed rats spent significantly less time in the open arms than in the closed arms ($F_{(1,33)} = 145.6$, $p < 0.001$) (Figure 2d).

3.1.3 | Sucrose preference test

Anhedonia or the loss of the tendency to feel pleasure, which is a core symptom of major depression, was evaluated in rats through the sucrose preference test (SPT; Iturra-Mena et al., 2019). A one-way ANOVA analysis showed a significant main effect of SDS on sucrose preference ($F_{(2,33)} = 62.8$, $p < 0.001$) (Figure 3a). A subsequent post hoc analysis showed that susceptible rats significantly decreased sucrose preference compared to resilient ($t = 7.6$; $df = 33$;

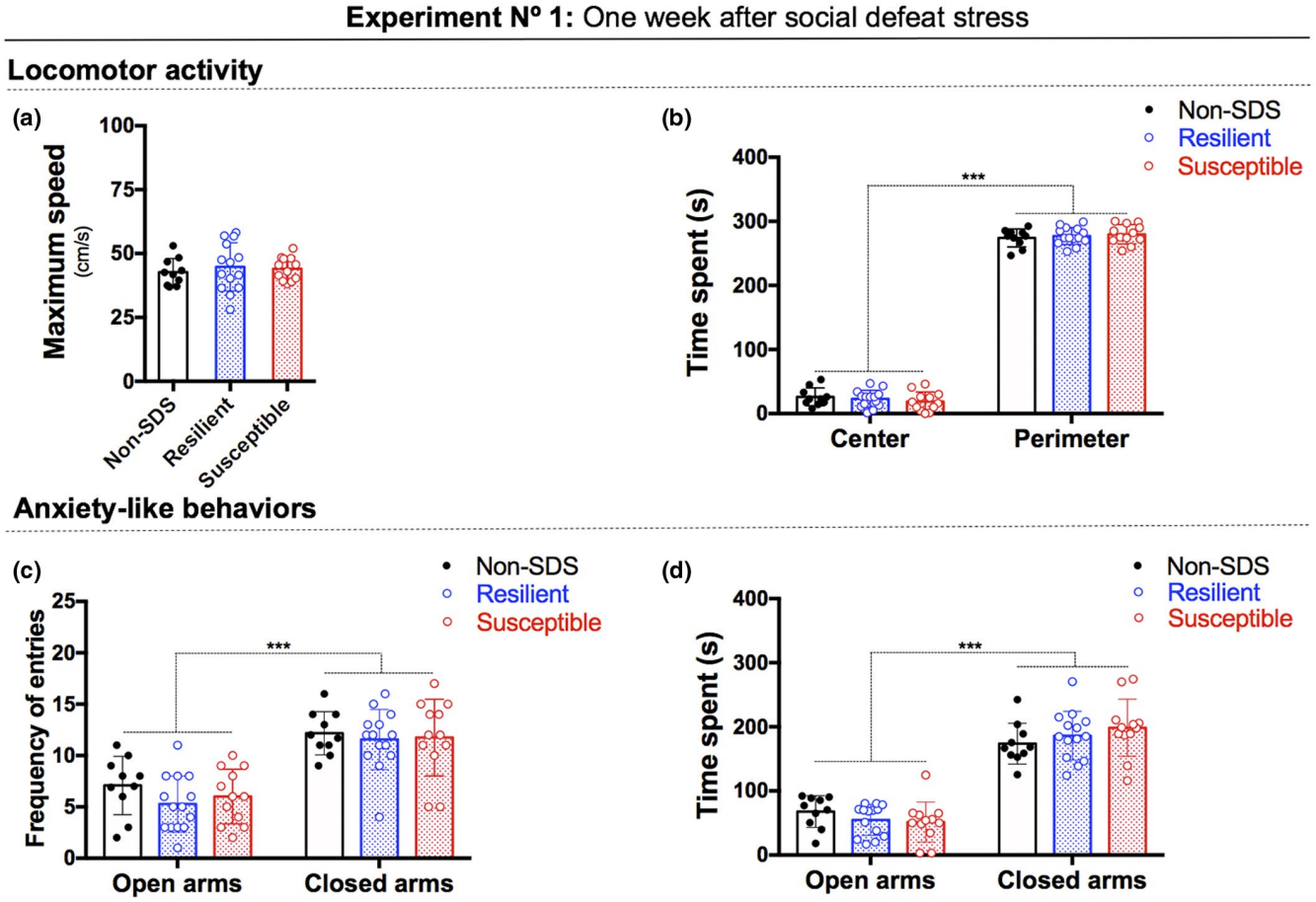


FIGURE 2 Locomotor activity and anxiety-like behaviors in the Experiment N° 1. (a) Shows maximum speed of travel in the open field. There were no significant differences between non-stressed (black circles), resilient (blue circles), and susceptible (red circles) rats. Thus, exposure to social defeat stress (SDS) had no effect on locomotor activity. (b) Shows time spent in the center and border zone of the open field, respectively. Non-defeated (black circles), resilient (blue circles), and susceptible (red circles) rats spent significantly less time in the center zone and more time in the border zone (*$p < 0.001$). There were no significant differences between experimental groups. Figures c and d show anxiety-like behaviors. Non-defeated (black circles), resilient (blue circles), and susceptible (red circles) rats entered fewer times (c) and spent significantly less time (d) in the open arms compared to the time spent in the closed arm (***$p < 0.001$). Thus, exposure to SDS had no effect on anxiety-like behaviors

$p < 0.001$) and non-defeated rats ($t = 10.9$; $df = 33$; $p < 0.001$) (Figure 3a). Sucrose preference was significantly lower in resilient rats than in non-defeated rats ($t = 4.0$; $df = 33$; $p < 0.01$) (Figure 3a).

3.1.4 | Forced swim test

Another core symptom of major depression is low mood or dysthymia, which was evaluated in rats through the forced swim test (FST; Walker et al., 2020). A one-way ANOVA analysis of the forced swim test revealed a significant main effect of SDS on time spent floating ($F_{(2,33)} = 21.0$, $p < 0.001$) and climbing ($F_{(2,33)} = 29.9$, $p < 0.001$) in the forced swimming test (Figure 3b–d). Subsequent post hoc analysis showed that susceptible rats significantly increased the floating time compared to non-stressed ($t = 6.4$; $df = 33$; $p < 0.001$) and resilient rats ($t = 4.0$; $df = 33$; $p < 0.001$) (Figure 3b). Conversely, susceptible rats spent less time in climbing behavior than non-stressed ($t = 7.7$; $df = 33$; $p < 0.001$) and resilient rats ($t = 3.6$; $df = 33$; $p < 0.01$) (Figure 3c). There were no differences between non-defeated, resilient, and susceptible rats in swimming behavior ($F_{(2,33)} = 3.3$, $p = 0.053$) (Figure 3d).

3.1.5 | SDS induces astrocytes and microglial activation in hippocampal slices

Previous studies have been shown that distress triggers neuroinflammation (Calcia et al., 2016), which is potentially associated with the pathophysiology of MDD (Fekri et al., 2020; Kim & Won, 2017). In this regard, astrocytes and microglia have been well recognized to mediate the neuroinflammatory process in the brain (Orellana et al., 2015). Upstream from the neuroinflammatory response, different neuroinflammatory conditions have shown elevated membrane permeability of glial cells non-selective channels called hemichannels and reactive astrocytes and microglia show higher reactivity for GFAP and Iba-1, respectively (Orellana et al., 2015). In order to

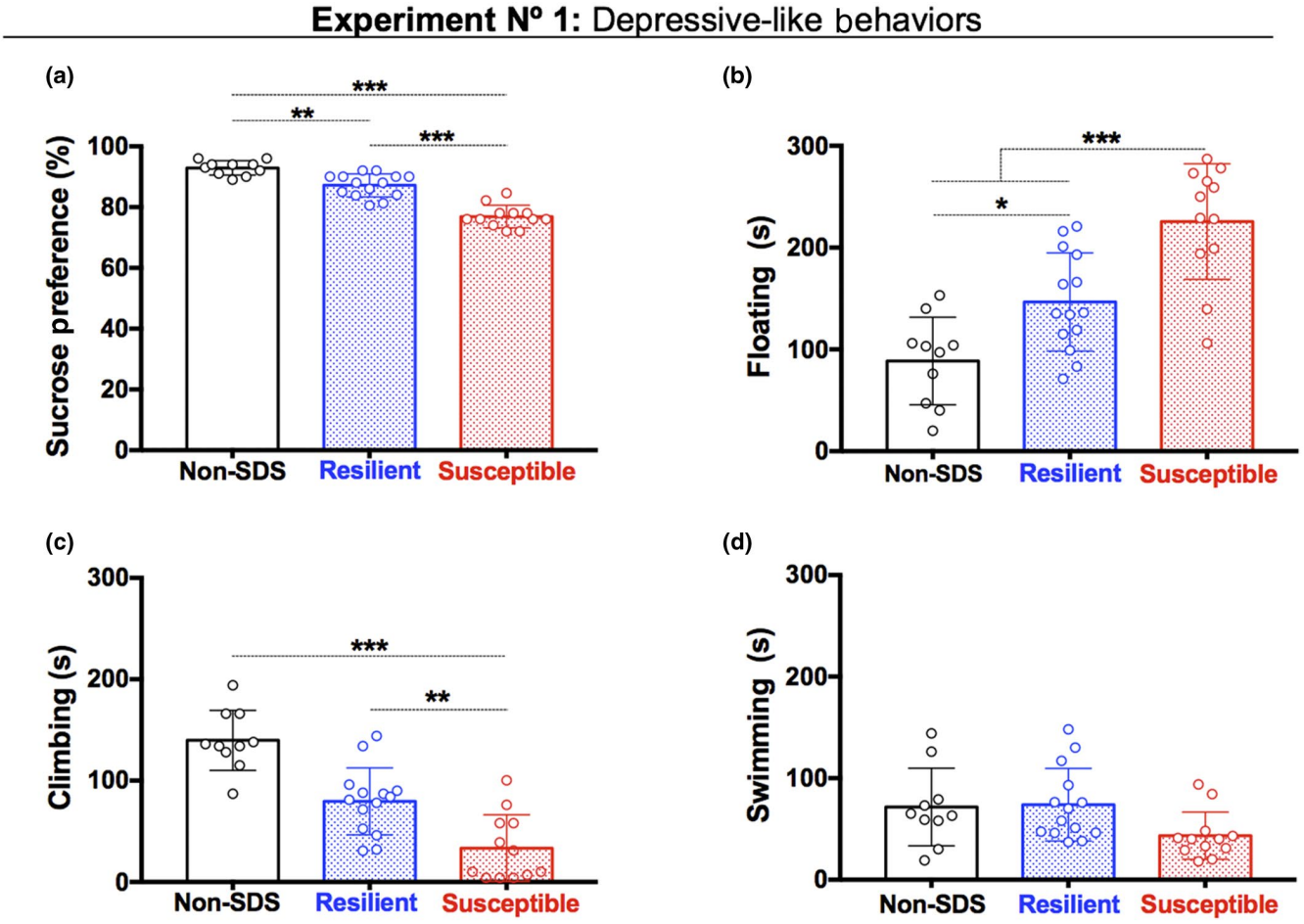


FIGURE 3 Depressive-like behaviors 1 week after social defeat stress. (a) There were significant differences between resilient and susceptible rats in the sucrose preference test, and the animals of both experimental groups compared with the group of non-social defeat stress (non-SDS). Floating (b), climbing (c), and swimming (d) behavior in the forced swim test. Susceptible rats spent significantly more time floating and less time climbing than the resilient and non-defeated rats (**$p < 0.01$, ***$p < 0.001$). There were no significant differences between animals of all experimental groups in the time used for swimming behavior

determine whether SDS induces neuroinflammation, we evaluated the above parameters in the socially defeated and non-defeated rats.

Immunoreactivity for GFAP (astrocytes) and Iba-1 (microglia) positive cells in the dentate gyrus (DG) was significantly higher in susceptible rats than in resilient and non-defeated rats (Figure 4a,b). A main effect of SDS on GFAP fluorescence ($F_{(2,14)} = 118.1$, $p < 0.001$) (Figure 4d). GFAP fluorescence quantification was increased in susceptible rats ($t = 15.0$; $df = 14$; $p < 0.001$) as well as in resilient rats ($t = 3.0$; $df = 14$; $p < 0.05$) compared with non-defeated rats (Figure 4d), indicating that astrocytes presented a reactive phenotype characteristic of a neuroinflammatory condition (Giaume et al., 2021). Since the cell membrane of control glial cells is impermeable to EtBr but reactive glial cells present hemichannels with high open probability that permeabilize the membrane to ions and small molecules, in numerous studies it has been possible to evidence changes in membrane permeability using EtBr. This dye fluoresces upon intercalation with nucleic acids that are abundant in the cell nucleus. Consequently, increases in cell membrane permeability are reflected by increases in fluorescence intensity of cell nuclei (Giaume et al., 2021). Therefore, we decided to use this approach to evaluate the consequences of neuroinflammation on the cell membrane of glial cells. A one-way ANOVA analysis revealed a significant main effect of SDS on EtBr uptake in the hippocampal slices ($F_{(2,14)} = 54.2$, $p < 0.001$) (Figure 4c). We found that astrocytes presented a significant increase in EtBr uptake in the hippocampal slices of susceptible rats ($t = 10.1$; $df = 14$; $p < 0.001$) compared to non-defeated rats (Figure 4c). In line with this, the EtBr uptake increased significantly more in astrocytes of the DG from susceptible compared to resilient rats ($t = 7.4$; $df = 14$; $p < 0.001$) (Figure 4c).

We also analyzed membrane permeability of microglial cells recognized by their Iba-1 reactivity. We observed a main effect of SDS on EtBr uptake ($F_{(2,14)} = 418.2$, $p < 0.001$) (Figure 4e). EtBr uptake significantly increased in both resilient ($t = 12.5$; $df = 14$; $p < 0.001$) and susceptible rats ($t = 29.0$; $df = 14$; $p < 0.001$) compared to non-defeated rats (Figure 4e), being significantly higher in resilient than in susceptible rats ($t = 15.2$; $df = 14$; $p < 0.001$) (Figure 4e). A main effect of SDS on Iba-1 immunoreactivity ($F_{(2,14)} = 35.4$, $p < 0.001$) (Figure 4f). Interestingly, Iba-1 immunoreactivity increased

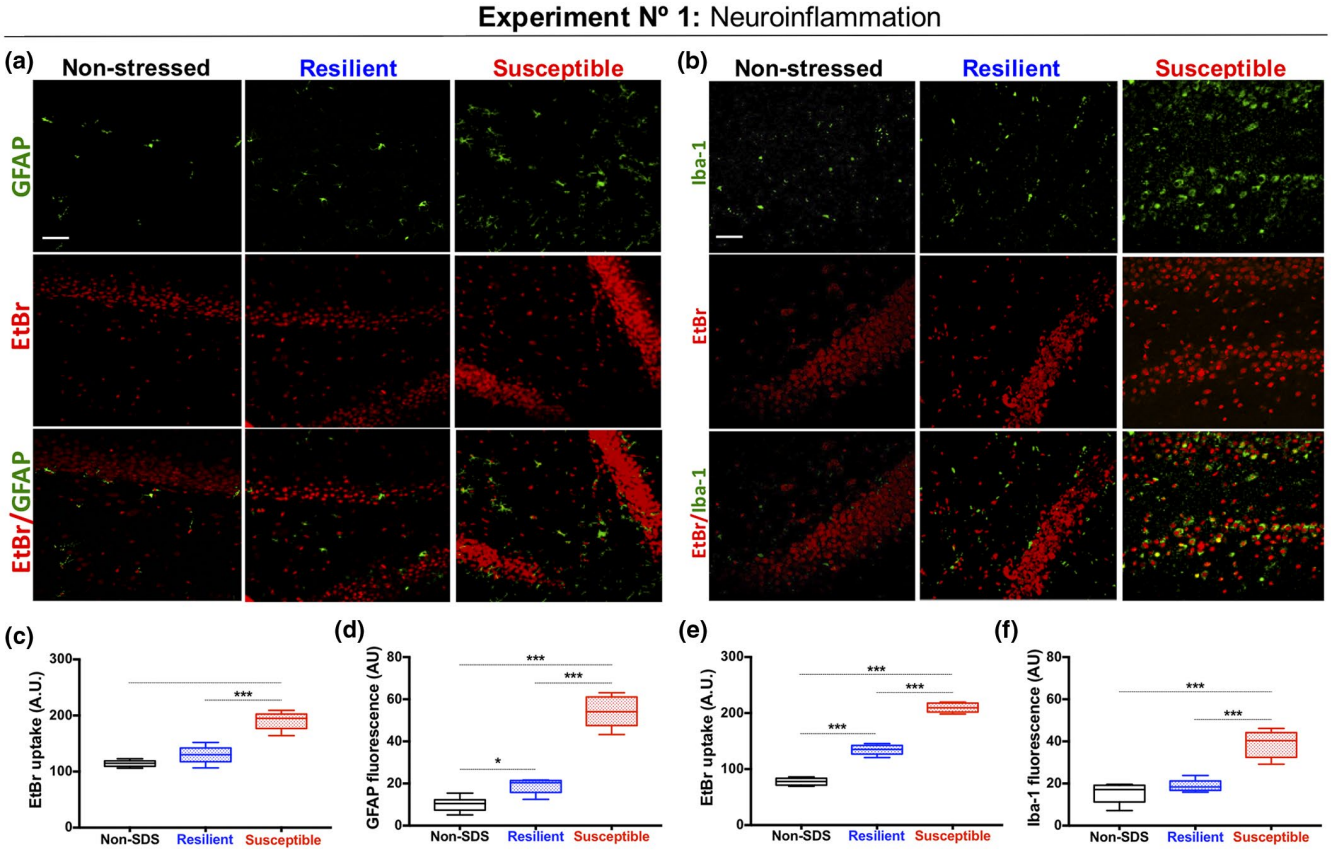


FIGURE 4 Astrocyte and microglial activation 1 week after social defeat stress. (a) Representative photomicrographs of brain sections stained for GFAP (green) and EtBr uptake (red) from non-stressed, resilient, and susceptible hippocampal slices. The merged image shows GFAP-positive astrocytic cell bodies and processes. (b) Representative photomicrographs of Iba-1-immunopositive microglia (green) with EtBr uptake (red) staining in the dentate gyrus of non-defeated, resilient, and susceptible rats. Bar graphs showing quantification of EtBr uptake (c and e), GFAP (c), and Iba-1 (f) levels following immunofluorescence analysis. All averaged data were obtained from $n = 40$ cells and six slices for each condition. $^{*}p < 0.05$, $^{***}p < 0.001$ when data were compared with non-stressed rats. Images of hippocampal slices were taken with a 40× objective. Scale bar: 50 μm. A.U., arbitrary units. Each value corresponds to median with its minimum and maximum

significantly more in the DG of susceptible rats compared to resilient ($t = 6.4$; $df = 14$; $p < 0.001$) and non-stressed rats ($t = 8.1$; $df = 14$; $p < 0.001$) (Figure 4f).

3.2 | Experiment N° 2: One week and 2 weeks after social stress

3.2.1 | Social interaction

The social preference-avoidance test was affected in socially defeated rats when effects were compared 1 and 2 weeks after the stress period (Figure 5). The repeated measures two-way ANOVA analysis showed a non-significant time x group interaction ($F_{(1,18)} = 0.2$, $p = 0.645$) for non-defeated rats (Figure 5a). Non-defeated rats spent more time in the social interaction zone during the social target stage than in non-social target stage 1 week ($t = 8.3$; $df = 36$; $p < 0.001$) and 2 weeks after SDS ($t = 7.9$; $df = 36$; $p < 0.001$) (Figure 5a).

A significant time × group interaction for the resilient rats was evident ($F_{(1,14)} = 37.6$, $p < 0.001$) (Figure 5b). A main effect of the time point was found ($F_{(1,14)} = 27.6$, $p < 0.001$). A subsequent post hoc analysis showed that 2 weeks after the SDS protocol, the time spent in the interaction zone decreased in resilient rats compared to 1 week after SDS ($t = 7.8$; $df = 28$; $p < 0.001$) (Figure 5b).

Representative heat maps indicated that resilient rats spent less time in the interaction zone 2 weeks after the SDS period compared to 1 week after SDS (Figure 5c).

3.2.2 | Locomotor activity and anxiety-like behaviors

Two-way ANOVA analysis showed that social stress did not affect locomotor activity after the SDS protocol ($F_{(1,23)} = 3.4$, $p = 0.079$) (Figure 6a). Three-way ANOVA analysis showed that SDS did not affect the time spent in the center zone and border zone in the open field ($F_{(2,100)} = 0.1$; $p = 0.905$) (Figure 6a). Non-defeated, resilient, and susceptible rats spent significantly less time in the center zone

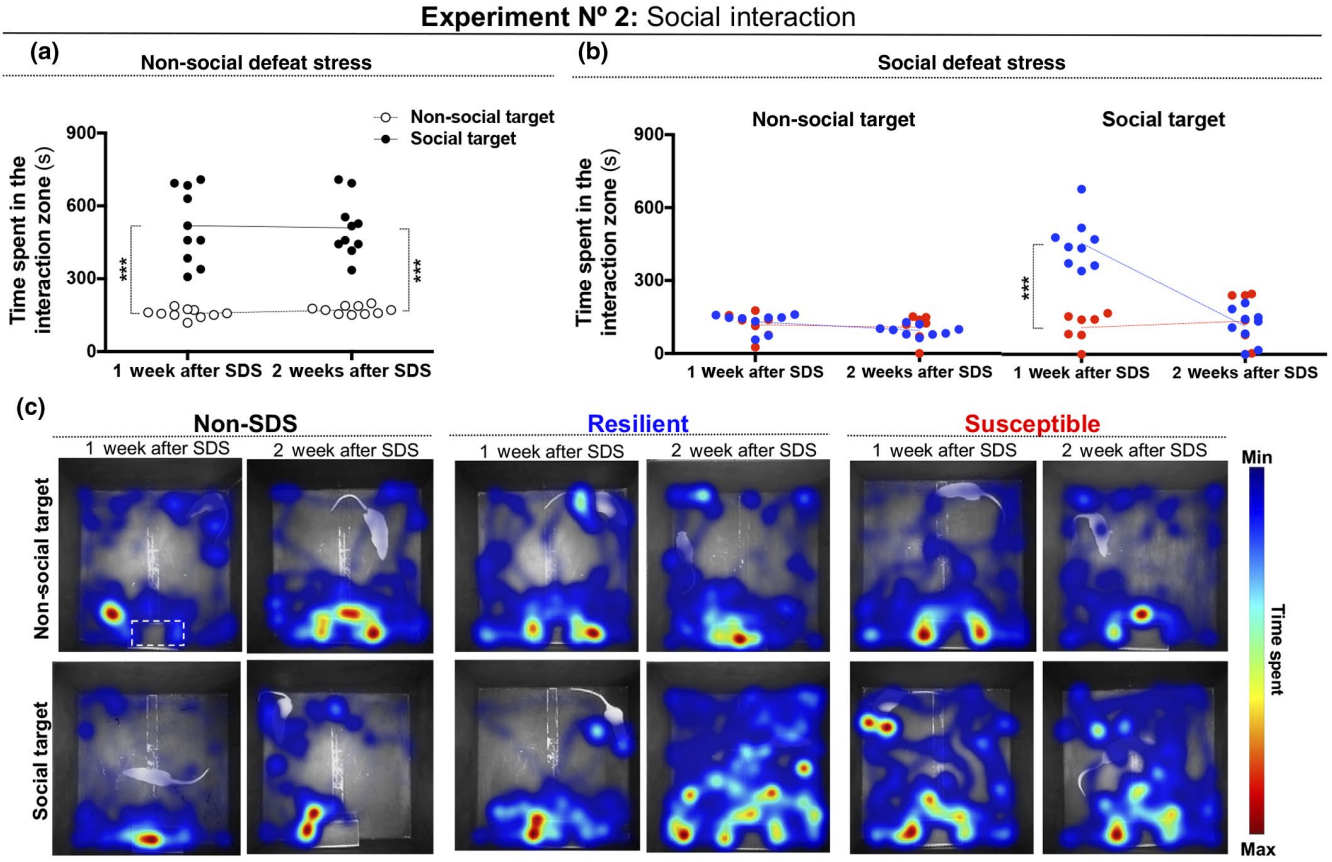


FIGURE 5 Social behavior in the Experiment N° 2. Time spent interacting with an empty transparent perforated chamber (non-social target) and a novel rat (social target), 1 week and 2 weeks after social defeat stress (SDS), for the non-defeated stress (a) and social defeat stress (b) experimental groups. Resilient rats spent significantly more time engaged in social interaction than susceptible rats 1 week after SDS, while there were no significant differences between resilient and susceptible rats after 2 weeks of SDS. (c) Representative heat maps are shown for non-defeated, resilient, and susceptible rat tracking

and more time in the perimeter 1 week and 2 weeks after SDS ($F_{(2,100)} = 15{,}267.9$; $p < 0.001$) (Figure 6b).

Three-way ANOVA analysis showed that 2 weeks after SDS the frequency of entries into the open arms was significantly less compared to entries into closed arms 1 week after SDS in non-defeated, resilient, and susceptible rats ($F_{(1,104)} = 116.4$; $p < 0.001$) (Figure 6c). Three-way ANOVA analysis showed that non-defeat, resilient, and susceptible rats spent significantly less time in the open arms and more time in the closed arms 1 week and 2 weeks after SDS ($F_{(1,104)} = 519.9$; $p < 0.001$) (Figure 6d).

3.2.3 | Sucrose preference test

The repeated measures two-way ANOVA analysis showed a significant time x group interaction ($F_{(2,46)} = 10.9$, $p < 0.001$) (Figure 7a). A subsequent post hoc analysis showed that sucrose preference decreased in resilient rats 2 weeks after SDS protocol compared to 1 week after SDS ($t = 5.7$; $df = 46$; $p < 0.001$) (Figure 7a). Sucrose preference decreased in susceptible rats compared to non-defeated rats 1 ($t = 10.1$; $df = 46$; $p < 0.001$) and 2 weeks ($t = 11.1$; $df = 46$; $p < 0.001$) after SDS (Figure 7a).

3.2.4 | Forced swim test

The repeated measures two-way ANOVA analysis showed a significant time × group interaction for floating behavior ($F_{(2,46)} = 6.1$, $p < 0.01$) and climbing ($F_{(2,23)} = 14.7$, $p < 0.001$) (Figure 7b,c). A subsequent post hoc analysis showed that floating behavior significantly increased in resilient rats 2 weeks after the SDS protocol compared to 1 week after SDS ($F_{(1,46)} = 4.1$, $p < 0.05$) (Figure 7b), whereas climbing behavior significantly decreased in resilient rats after 2 weeks of SDS ($F_{(1,23)} = 12.5$, $p < 0.01$) (Figure 7c). Swimming behavior did not change between first and second week after SDS ($F_{(1,46)} = 0.2$, $p = 0.657$) (Figure 7d).

3.2.5 | SDS induces astrocytes and microglial activation in hippocampal slices

The SDS paradigm is a common preclinical model that has been used to study susceptibility and resilience to stress (Pryce & Fuchs, 2017). In addition, this protocol increases the inflammatory pattern (Weber et al., 2017). Immunoreactivity for GFAP (astrocytes) and Iba-1 (microglia) positive cells in the DG was significantly higher in resilient

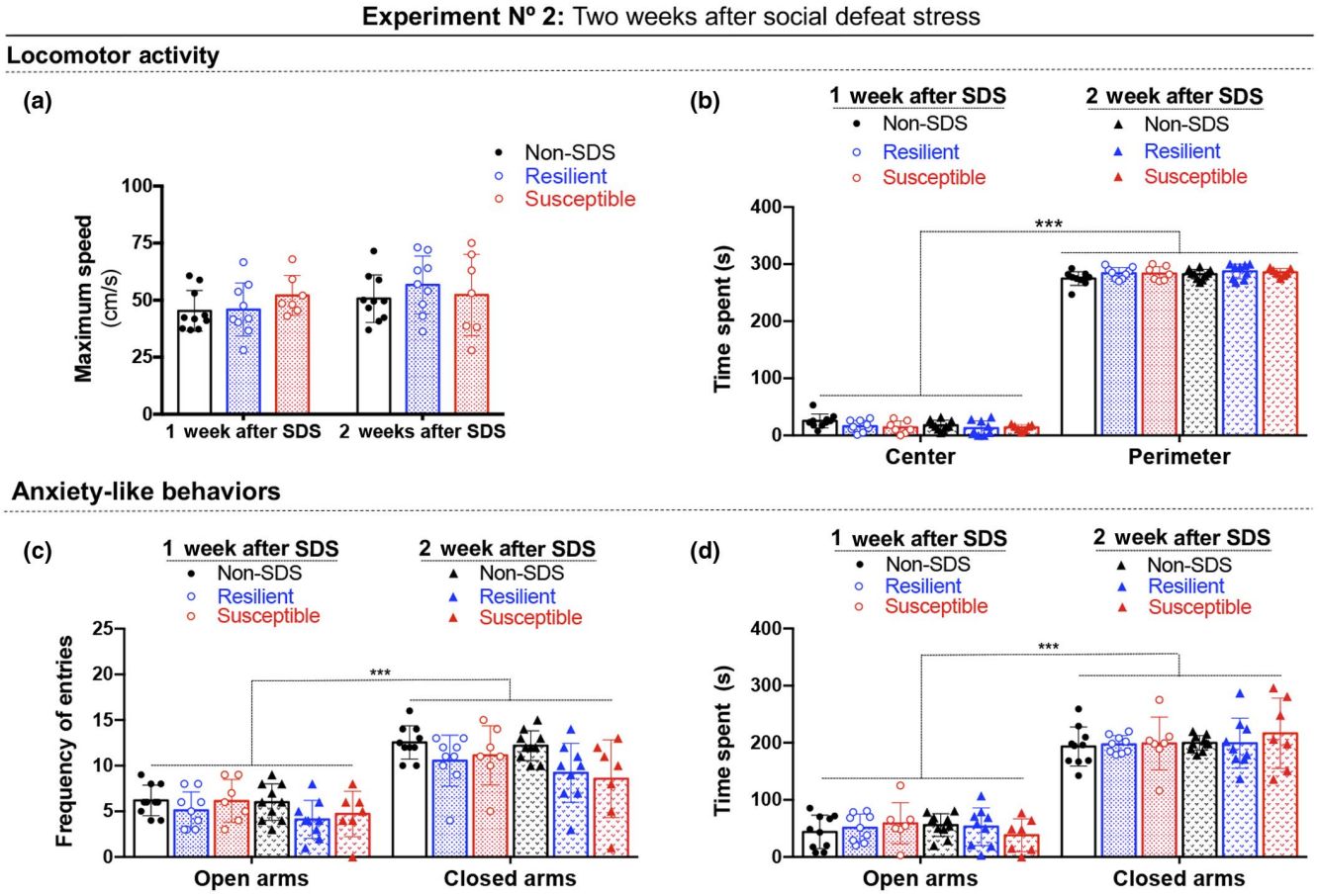


FIGURE 6 Locomotor activity and anxiety-like behaviors in the Experiment N° 2. (a) Shows maximum speed of travel in the open field. There were no significant differences between experimental groups. Thus, exposure to social defeat stress (SDS) had no effect on locomotor activity. (b) Shows time spent in the center and border zone of the open field, respectively. Non-defeated and stressed rats spent significantly less time in the center zone and more time in the border zone (*$p < 0.001$). There were no significant differences between experimental groups. Figures c and d show anxiety-like behaviors. Non-stressed and stressed rats entered less times (c) and spent significantly less time (d) in the open arms compared with time spent in the closed arm (***$p < 0.001$). Thus, exposure to SDS had no effect on anxiety-like behaviors

and susceptible rats than in non-stressed rats (Figure 8a-b). Along similar lines, one-way ANOVA analysis revealed a significant main effect of SDS on EtBr uptake in the hippocampal slices ($F_{(2,14)} = 267.6$, $p < 0.001$) (Figure 8c). EtBr uptake of astrocytes was observed to be significantly higher in the hippocampal slices of resilient ($t = 14.4$; $df = 14$; $p < 0.001$) and susceptible rats ($t = 22.5$; $df = 14$; $p < 0.001$) compared to non-defeated rats (Figure 9c). Moreover, EtBr uptake increased significantly in the DG of susceptible rats compared to resilient rats ($t = 7.4$; $df = 14$; $p < 0.001$) (Figure 8c).

A main effect of SDS on GFAP fluorescence in the hippocampal slices ($F_{(2,14)} = 43.4$, $p < 0.001$) (Figure 8c). GFAP fluorescence intensity increased in resilient ($t = 6.3$; $df = 14$; $p < 0.001$) and susceptible rats ($t = 8.9$; $df = 14$; $p < 0.001$) compared with non-stressed rats (Figure 8d). Therefore, analyzed the microglia activation by immunofluorescence. The one-way ANOVA analysis revealed a significant main effect of SDS on EtBr uptake ($F_{(2,14)} = 50.4$, $p < 0.001$) (Figure 8e). EtBr uptake significantly increased in both resilient ($t = 6.0$; $df = 14$; $p < 0.001$) and susceptible rats ($t = 10.0$; $df = 14$; $p < 0.001$) as compared to non-defeated rats (Figure 8e). There was a significant difference in EtBr uptake between resilient and susceptible rats ($t = 3.7$; $df = 14$; $p < 0.01$) (Figure 8e). Moreover, one-way ANOVA analysis revealed a significant main effect of SDS on EtBr uptake ($F_{(2,14)} = 207$, $p < 0.001$) (Figure 8f). Iba-1 immunofluorescence reactivity significantly increased in the DG of resilient ($t = 12.9$; $df = 14$; $p < 0.001$) and susceptible ($t = 19.8$; $df = 14$; $p < 0.001$) rats compared to non-defeated rats (Figure 8f). There was a significant difference in Iba-1 immunofluorescence between resilient and susceptible rats ($t = 6.7$; $df = 14$; $p < 0.001$) (Figure 8f).

3.3 | Long-term effects of social stress on stress markers

Since stress reduces weight gain of and induces hypertrophy of the adrenal glands (Iturra-Mena et al., 2019; Negrón-Oyarzo et al., 2015), we evaluated these parameters in our animal model in order to validate it. We found that 1 week after the stress period, rats exposed to SDS gained less weight ($t = 6.5$; $df = 18$; $p < 0.001$) and showed

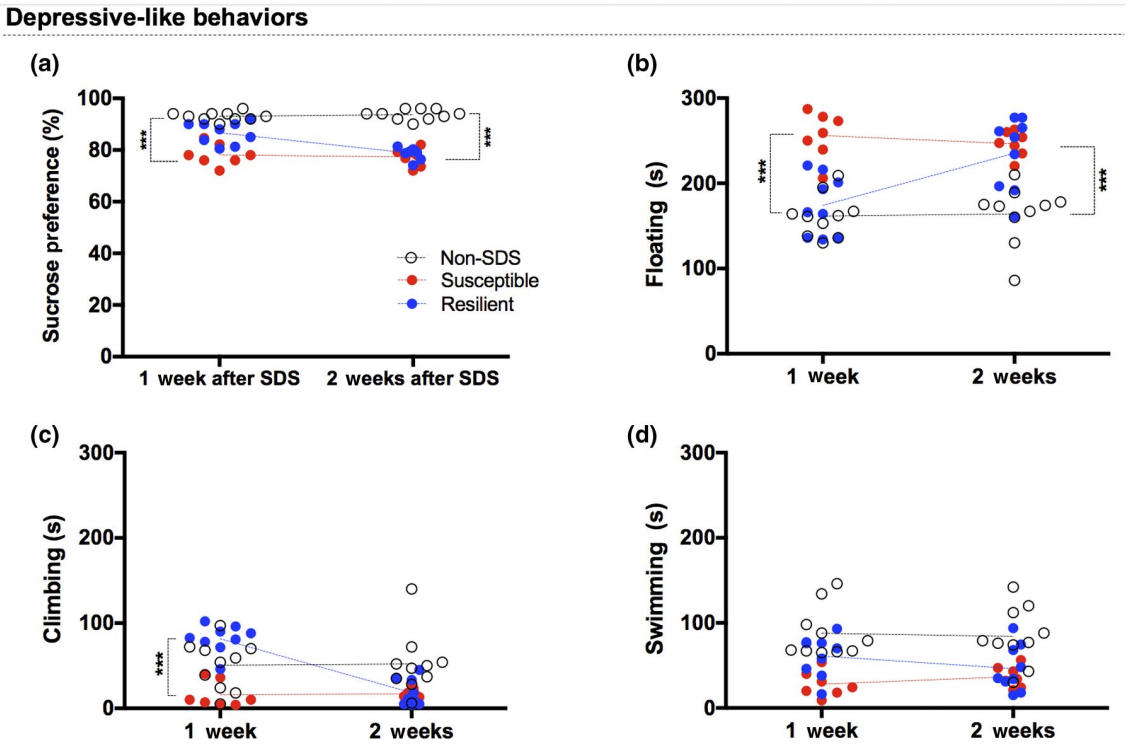


FIGURE 7 Depressive-like behaviors in the Experiment N° 2. (a) There were significant differences between non-defeated and resilient rats compared to susceptible rats in the sucrose preference test 1 week after social defeat stress (SDS). However, resilient and susceptible rats showed a significant decrease in sucrose preference compared to non-defeated rats after 2 weeks of SDS. Floating (b), climbing (c), and swimming (d) behavior in the forced swim test. Susceptible rats spent significantly more time floating than the resilient and non-stressed rats (**$p < 0.01$, ***$p < 0.001$). There were no significant differences between animals of all experimental groups in the climbing and swimming behavior

adrenal gland hypertrophy ($t = 6.4$; $df = 18$; $p < 0.001$) compared to non-stressed rats (Figure 9a,b). Two weeks after the SDS, rats gained less weight ($t = 6.5$; $df = 18$; $p < 0.001$) and showed adrenal gland hypertrophy ($t = 6.4$; $df = 18$; $p < 0.001$) compared to non-stressed rats (Figure 9c,d), indicating that our stress protocol promotes phenotypic changes that are characteristics of stress response.

4 | DISCUSSION

The present study investigated long-term effects of social stress on neuroinflammation and depressive-like behaviors. Rats that were resilient to stress up to 1 week after the stress period displayed neuroinflammation in the hippocampus and depressive-like behaviors comparable with susceptible rats 2 weeks after social stress. These results suggest that resilience to stress in rats lasts at least 2 weeks after the stress protocol has ended. Consistent with the latter, rats exposed to the SDS had significantly lower body weight and hypertrophy of the adrenal glands relative to non-defeated control animals 1 and 2 weeks after the SDS ended, indicating that the effects of SDS persist at physiological levels for up to 2 weeks after the stress phase.

In the social interaction test, a group of defeated rats were sociable and resilient to stress up to 1 week after the end of the SDS, while another group of rats was susceptible to stress and decreased their socialization (Figure 1) in the absence of any general changes in locomotor activity and anxiety (Figure 2). It has been shown that social behavior is a natural reinforcer in mammals, such as feeding and sex, which are associated with pleasure (O'Connell & Hofmann, 2011). The reward system modulates social behavior and the nucleus accumbens (NAc), which is part of this system, is highly vulnerable to distress (Alkire et al., 2018; Christoffel et al., 2011; Francis et al., 2015; Muir et al., 2018). In line with this, it has been shown that during social behavior, the power of high gamma oscillations in the NAc decreases in susceptible rats compared to resilient ones (Iturra-Mena et al., 2019). Gamma oscillations are involved in reward processing, so the functional deterioration of the NAc is related to anhedonia or lack of motivation to feel pleasure (Lega et al., 2011; Schlaepfer et al., 2008; van der Meer & Redish, 2009). In our study, anhedonia was expressed in the behavior of susceptible rats through a decrease in the interest to feel pleasure from socializing with a conspecific (social reinforcer) (Figure 1a), as well as less preference to drink the sweet solution in the sucrose preference test (Figure 3a).

Susceptible rats that showed anhedonic behavior in the social interaction test (Figure 1a) and sucrose preference test (Figure 3a) also displayed an increase in floating behavior in the FST compared to resilient rats (Figure 3b). When rodents are subjected to the FST, they try to adapt to this stressful event by means of the stress responses fight or flight (Herman, 2018). However, floating behavior or immobility in the FST is not always related to depressive-like behavior

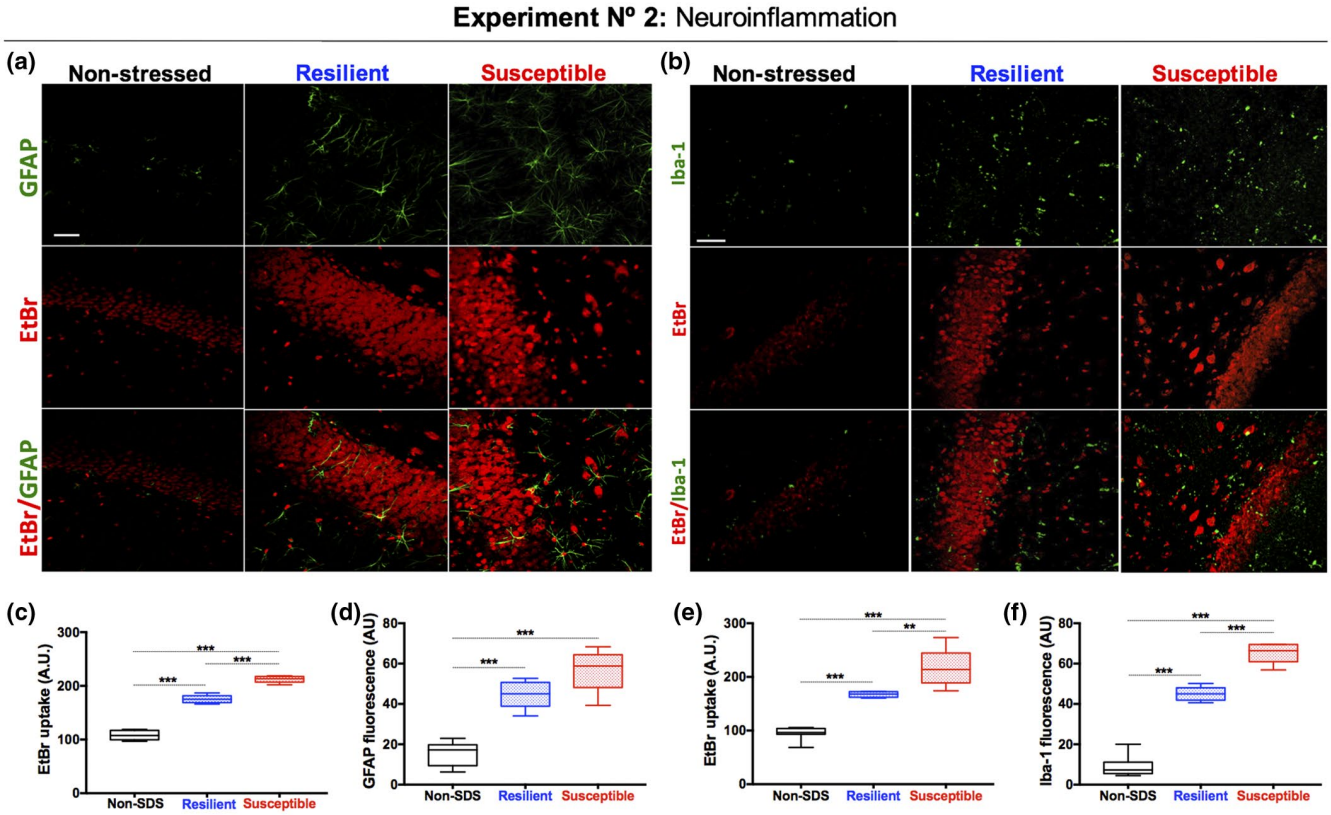


FIGURE 8 Astrocyte and microglial activation 2 weeks after social defeat stress. (a) Representative photomicrographs of brain section stained for GFAP (green) and EtBr uptake (red) from non-defeated, resilient, and susceptible hippocampal slices. The merged image shows GFAP-positive astrocytic cell bodies and processes. (b) Representative photomicrographs of Iba-1-immunopositive microglia (green) with EtBr uptake (red) staining in the dentate gyrus of non-defeated, resilient, and susceptible rats. Bar graphs showing quantification of EtBr uptake (c and e), GFAP (c), and Iba-1 (f) levels following immunofluorescence analysis. All averaged data were obtained from $n = 40$ cells and six slices for each condition. $^{*}p < 0.05$, $^{***}p < 0.001$ when data were compared with non-stressed rats. Images of hippocampal slices were taken with a 40× objective. Scale bar: 50 μm. A.U., arbitrary units. Each value corresponds to median with its minimum and maximum

such as low mood or dysthymia (Wang et al., 2017), it can also be related to survival behavior (de Kloet & Molendijk, 2016; Molendijk & de Kloet, 2015, 2019). In our study, the same susceptible rats that resorted to more floating activity also exhibited depressive-like behaviors (Figure 3), suggesting that increased floating behavior was related to low mood in susceptible rats. Conversely, resilient rats showed an increase in climbing activity behavior, which is related to active coping with stress (Figure 3c). Stress responses and coping to stress are regulated by the HPA axis and the limbic structures that regulate their activity, such as the hippocampus (McEwen & Akil, 2020), which is particularly vulnerable to stress. The hippocampus contains a high number of receptors for glucocorticoids, which may make it sensitive to changes in glucocorticoid levels (Conrad et al., 2017). In addition, the hippocampus is one of the few brain regions whereby neurogenesis continues beyond development into adulthood, so that the changes in hippocampal functions can have profound effects on the synaptic plasticity (Hei et al., 2019).

In our study, susceptible rats showed elevated neuroinflammatory markers in the hippocampus when compared to resilient rats (Figure 4). This observation suggests that neuroinflammation could impair the control of the hippocampus over the HPA axis, which in turn induces maladaptive responses to stress. The latter, in our study, was deemed to be reflected by susceptible rats' passive coping strategy of floating, which was not observed in resilient and non-defeated rats (Figure 3b). On the other hand, it has been shown that the hippocampus is involved in social behavior through its projections from the ventral CA1 to the NAc (Okuyama et al., 2016). This neural circuit regulates the consolidation of social memory, which is essential for social interaction. Therefore, neuroinflammation in the hippocampus observed in susceptible rats could have impaired their socialization, as shown in Figure 4.

Surprisingly, rats that were resilient to stress 1 week after the SDS protocol decreased in their socialization 2 weeks after the SDS protocol ended (Figure 5b), which was also when their depressive-like behaviors ended (Figure 7a) without locomotor activity or anxiety alterations (Figure 6a,b). Neuroinflammatory markers in the hippocampus after 2 weeks of the stress period were found in rats that had shown the resilient phenotype 1 week after the SDS (Figure 8). The hippocampus plays a key role in the modulation of HPA axis activity and stress responses, as well as in social behavior due to its direct connection with the NAc (Okuyama et al., 2016). Consequently, hippocampal impairments can trigger a reduction in

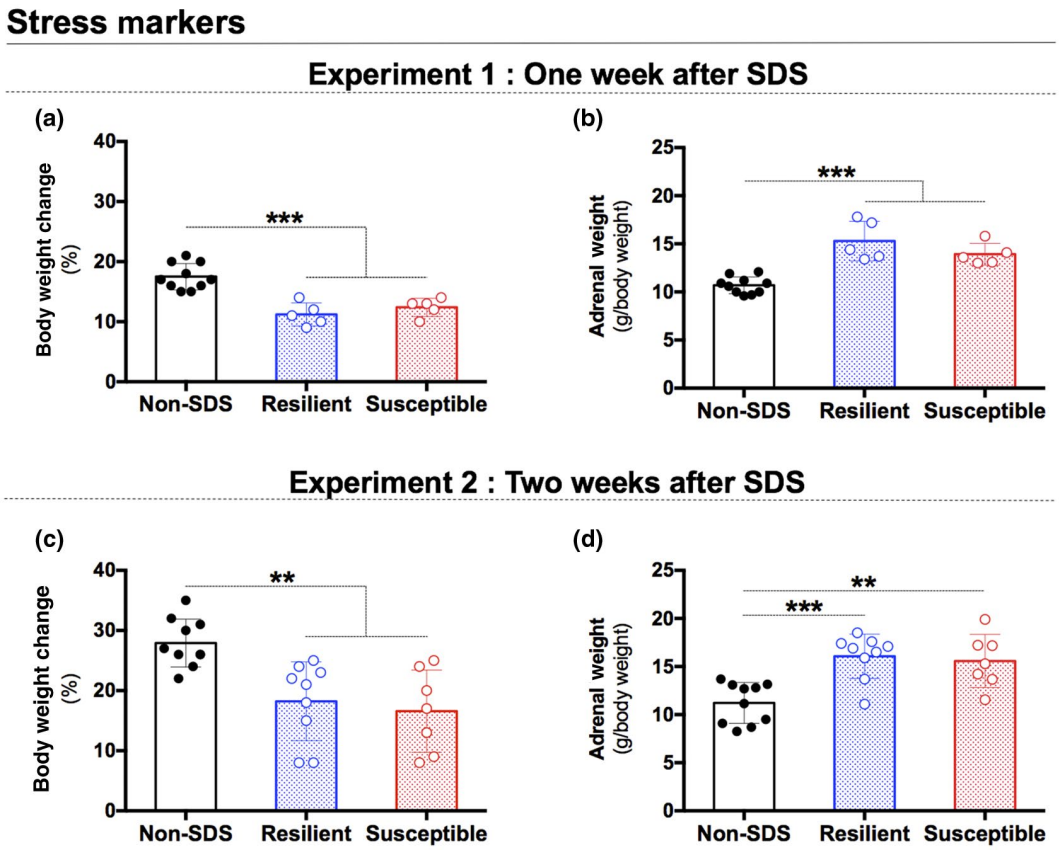


FIGURE 9 Long-term effects of social defeat stress (SDS) on stress markers. (a) There were significant differences in body weight gains between experimental groups. All rats exposed to SDS gained significantly less weight after 1 (***$p < 0.001$) and 2 weeks (***$p < 0.01$) of the stress protocol. (b) Adrenal weights of non-defeated and stressed animals were measured after SDS. There were significant increases in adrenal weight after 1 (***$p < 0.001$) and 2 weeks (***$p < 0.001$) of the stress period than non-defeated rats. Thus, rats that were exposed to SDS still showed the effects of distress at the physiological level after 1 and 2 weeks after the stress protocol ended

socialization and depressive-like behaviors such as anhedonia and low mood, as can be seen in Figures 5 and 7.

If the results between resilient and non-defeated rats are compared, it is possible to see that there is a difference between behavior and neuroinflammation in the hippocampus. For instance, 1 week after the SDS protocol, stress resilience was observed in the behavior of the rats, but was not seen at the level of neuroinflammation in the hippocampus. In other words, rats that showed resilience to stress in their social behavior (Figure 1) and depressive-like behavior (Figure 3) had increased neuroinflammation in the hippocampus, whereas non-defeated control animals did not (Figure 4). These results suggest that neuroinflammation in the hippocampus induced by social stress continues to develop after the stress period, even if rats show resilience or susceptibility to stress at the behavioral level. The only difference between resilient and susceptible rats regarding neuroinflammation is that neuroinflammation development in the hippocampus was slower in resilient rats compared to susceptible rats.

The mechanisms by which social stress induces brain changes and behavior are unknown. To this end, it is important to understand that stress sensitive processes promote neuroinflammation. Accordingly, in the mice model, McKim et al. (2016) found that mice exposed to repeated social defeat showed a pro-inflammatory profile along with neurogenesis deficits in the hippocampus compared to non-stressed controls. Moreover, Weber et al. (2017) observed increased hippocampal cytokine and growth factor gene expression (IL-1β, TNF-α, IL-6, VEGF), enhanced microglial Iba-1 immunoreactivity in the DG, and an increase in DG CD45-positive cells in defeated mice, suggesting recruitment of peripheral monocytes to the brain. On the other hand, using SDS paradigm, a validated animal model of psychosocial stress, we found that resilience to stress was lost 2 weeks after SDS, providing insights on neuroinflammation as long-term consequence of the depressive-like behaviors.

Social stressors activate the (HPA) axis resulting in GC release. One of the main molecular elements of the HPA axis is corticotropin-releasing factor (CRF), which is a neuropeptide that is part of a peptide family including urocortin 1–3, urotensin 1–3, and sauvagine (Backström & Winberg, 2013). It has been previously shown that cells of hippocampal slices treated with dexamethasone, a synthetic glucocorticoid, present elevated membrane permeability via activation of connexin and pannexin hemichannels, which are two non-selective channel types (Maturana et al., 2017). This response

is associated with inflammasome activation, which generates pro-inflammatory cytokines and occurs in a time- and cell-dependent manner, being mast cells the first responders followed by microglia, astrocytes, oligodendrocytes, and neurons (Maturana et al., 2017). Interestingly, a similar response was also elicited in hippocampal slices treated with UNC-II, a ligand of the CRF receptors, and both dexamethasone- and UNC-II-induced responses were prevented by the inhibition of the CRF receptor with antalarmin (Maturana et al., 2017). The involvement of CRF interneurons has been proposed with their cell body located in the CA1 region of the hippocampus (Chen et al., 2012; Hooper & Maguire, 2016), whereas CRF receptor 1 mainly resides on dendritic spines of pyramidal cells. CRF interneurons are activated by "psychological" stress and CRF release, leading to the activation of principal neurons (Chen et al., 2012). Although it remains to be determined whether GCs induce CRF release in these interneurons as they do in CRF neurons of the hypothalamus (Sorrells et al., 2009), it can be proposed that a mechanism similar to that described above could explain the increase in membrane permeability found in microglia and astrocytes of rats subjected to SDS. It should be highlighted that other stressors, such as restrain stress, also increase cell membrane permeability in microglia and astrocytes (Orellana et al., 2015). Notably, acute and chronic restrain stress increases membrane permeability of microglia mainly due to greater pannexin1 channel activity. In astrocytes, on the other hand, acute stress is mostly explained by an increase in connexin43 hemichannel activity, where chronic stress leads to elevated membrane permeability via the activation of both pannexin 1 channels and Cx43 hemichannels (Orellana et al., 2015). Whether the distinct involvement of these two non-selective channels in microglia and astrocytes could explain the slower unfolding of post-stress neuroinflammation in resilient rats compared to susceptible rats remains to be studied. Since in other neuroinflammatory conditions such as Alzheimer´s disease the absence of Cx43 in astrocytes or inhibition of hemichannels has been shown to drastically reduce the neuronal dysfunctions (Giaume et al., 2021), it is possible that such experimental paradigms might also be effective in preventing the transition from resilient to susceptible in the animal model described here. The fact that hemichannels have been described as novel molecular target to prevent of significantly reduce neuroinflammation is in line with the resistance to antidepressant medication accompanied by increased inflammation (Adzic et al., 2018). Moreover, activation of the inflammasome has been shown to mediate chronic mild stress-induced depression via neuroinflammation (Zhang et al., 2015) and activation hemichannels are upstream of inflammasome activation (Giaume et al., 2021).

In conclusion, while the neurobiological basis of stress resilience remains to be fully elucidated, this study showed that stress resilience in rats, measured through hippocampal neuroinflammation and depressive-like behaviors, disappears 2 weeks after the end of the SDS protocol. Undoubtedly, more experiments are needed to fully understand the long-term effects of social stress on neuroinflammation in other limbic areas that modulate stress responses, such as the amygdaloid complex and medial prefrontal cortex. Another limitation of this study is that female rats were not included and it is necessary to explore sex of the subjects as a biological variable in the results that we have found. Despite this, our study opens a new way to understand the neurobiology of stress resilience.

DECLARATION OF TRANSPARENCY

The authors, reviewers and editors affirm that in accordance to the policies set by the *Journal of Neuroscience Research*, this manuscript presents an accurate and transparent account of the study being reported and that all critical details describing the methods and results are present.

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ETHICS STATEMENT

Animal maintenance and experimentation were in strict agreement with animal care standards outlined by the National Institutes of Health (USA) guidelines and approved by the Institutional Animal Ethics Committee of the Universidad de Valparaíso (Anillo de Ciencia y Tecnología Grant N° ATC 1403). Efforts were made to reduce the number of rats used and their suffering.

CONFLICT OF INTEREST

None of the authors have any conflict of interest to disclose.

AUTHOR CONTRIBUTIONS

Conceptualization, I.D.B.-T., J.C.S., and A.D.-S.; *Performed the Experiments*, I.D.B.-T. and P.F.; *Writing – Original Draft*, I.D.B.-T., J.C.S., and A.D.-S.; *Writing – Review & Editing*, I.D.B.-T., J.C.S., and A.D.S.; *Supervision*, A.D.-S.

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All data presented in this manuscript can be accessed by contacting the corresponding author.

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