Ethanol intake in male mice exposed to social defeat: Environmental enrichment potentiates resilience

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ABSTRACT

Large preclinical evidence shows that exposure to social defeat (SD) increases vulnerability to drug abuse, increasing the consumption of ethanol. However, not all subjects are equally affected by the changes induced by stress. Previous reports have evidenced that the resilient phenotype to depressive-like behaviors after SD is associated with the resistant phenotype to cocaine-increased rewarding effects and the smaller neuro-inflammatory response. The aim of the present study was to further clarify whether the resilient profile to depressive-like behavior also predicts a protection against the increase in ethanol intake induced by SD. The neuroinflammatory profile was studied after the end of the oral ethanol self-administration (SA) procedure, measuring levels of the pro-inflammatory cytokine IL-6 and the chemokine CX3CL1 or fractalkine in the striatum and prefrontal cortex. Previous studies have shown that environmental enrichment (EE) is an effective mecha-nism to dimish the detrimental effects of social stress. In a second study, we aimed to evaluate if EE housing before exposure to SD could potentiate resilience. Our results showed that mice with a phenotype susceptible to SD-induced depressive-like behaviors showed increased ethanol consumption and increased neuroinflammatory signaling. In contrast, despite the lack of effect on depressive-like behaviors, defeated mice previously housed under EE conditions did not show an increase in ethanol SA or an increase in immune response. To sum up, the resilient phenotype to SD develops at different levels, such as depressive-like behaviors, ethanol consumption and the neuroinflammatory response. Our results also point to the protective role of EE in potentiating resilience to SD effects.

1. Introduction

We are continuously exposed to different types of stress throughout our life, and stress produced by social interaction is the most common type of stress in human beings (Montagud-Romero et al., 2018). Numerous studies have shown that exposure to social stress is associated with an increase in drug use, such as cocaine (Ferrer-Pérez et al., 2018; Reguilón et al., 2017; Rodríguez-Arias et al., 2016, 2017), MDMA (García-Pardo et al., 2015) or alcohol (Beutel et al., 2018; Hwa et al., 2016; Montagud-Romero et al., 2021; Newman et al., 2018a; Reguilón et al., 2020, 2021; Rodríguez-Arias et al., 2016). Regarding alcohol, the studies to date show that both exposure to stress and the way to cope with it should be considered as predictors of alcohol consumption in humans (see review by Newman et al., 2018b). People who consume

alcohol in a negative context, for example to reduce anxiety or stress, are more likely to develop a long-term problematic use and develop a chronic alcohol consumption with the corresponding negative conse-quences that characterize long-term alcohol abuse (e.g. negative social, physical and mental consequences; Newman et al., 2018a; Sinha, 2001). Moreover, exposure to social stress can further increase the likelihood of developing uncontrolled alcohol use or relapse (Adinoff et al., 2017).

The social defeat (SD) model is the most widely used model to study the effects of social stress (Hammels et al., 2015). SD consists of an agonistic encounter between conspecifics of the same species (Miczek et al., 2004), imitating the subordination status of human relationships (Selten et al., 2013). Exposure to SD stress induces profound physio-logical changes and endocrine responses, yielding a significant increase in corticosterone levels (Montagud-Romero et al., 2015; Rodrígue-z-Arias et al., 2017). In addition, it produces modifications in numerous

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Abbreviations

AUD	alcohol use disorder	IL:	infralimbic cortex
BDNF	brain-derived neurotrophic factor	IL-6	interleukin 6
CPP	conditioned place preference	NAc	nucleus accumbens
CRF	corticotrophin-releasing factor	NLRP3	Nod-like receptor pyrin containing 3
CX3CL1	C-X₃-C motif ligand 1 (fractalkine)	PFC	prefrontal cortex
EE	environmental enrichment	PND	postnatal day
ELISA	enzyme-linked immunosorbent assay	PR	progressive ratio
FR1	fixed ratio 1	PrL:	prelimbic cortex
FR3	fixed ratio 3	SA	self-administration
HPA	hypothalamic-pituitary-adrenal	SD	social defeat
		SWR	social withdrawal ratio
		TLR-4	Toll-like receptor 4

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neurotransmitter systems such as the serotonergic, dopaminergic or the GABAergic systems (Montagud-Romero et al., 2018).

Using this procedure, we have previously shown that exposure to four SD episodes either during adolescence or adulthood induced a long-lasting increase of ethanol self-administration (SA) during adulthood (Montagud-Romero et al., 2021; Reguilón et al., 2020, 2021; Rodrí-guez-Arias et al., 2016). Adolescent or adult mice were exposed to four SD episodes on alternating days and, three weeks after the last encounter, we measured oral ethanol SA. Defeated mice showed a delayed increase in ethanol consumption, made more active responses and showed increased motivation for alcohol in the progressive ratio (PR). Our results and other similar results obtained with voluntary ethanol drinking (Hwa et al., 2016; Norman et al., 2015) or indicating increased sensitivity to ethanol –induced conditioned place preference (CPP; Macedo et al., 2018)– confirmed that social stress increases vulnerability to the rewarding effects of alcohol.

Besides increasing alcohol intake, animals exposed to SD also exhibit increased anxiety and depressive-like behaviors, such as social avoid-ance (Blanco-Gandía et al., 2019; Ferrer-Pérez et al., 2019; Patel et al., 2019; Spijker et al., 2020). In the last decade, numerous studies have observed that the behavioral and psychological reactions to SD are not equal. Some animals are more susceptible and develop unhealthy re-sponses, such as increased drug intake, anxiety or depressive-like be-haviors. However, other subjects show resilience to stress and present a more adjusted psychological functioning (Brockhurst et al., 2015; Charney, 2004; Dantzer et al., 2018; Krishnan et al., 2007; Nasca et al., 2019).

Numerous studies have shown that passive, rather than active, coping strategies during SD are linked to stress-induced maladaptive behaviors (Ballestín et al., 2021; Hawley et al., 2010; Russo et al., 2012; Wood and Bhatnagar, 2015). Animals that display passive coping mechanisms seem to be susceptible to physiological effects and psy-chopathology (Hawley et al., 2010; Russo et al., 2012; Wood and Bhatnagar, 2015). However, the stress response does not only involve the coping strategies during stress, but also its physiological processes (Murrough and Russo, 2019). The link between individual differences and the immune system response to stress is now a critical field of research (Ballestín et al., 2021; Hodes et al., 2014; Westfall et al., 2021; Wood et al., 2015). As a general result, these pre-clinical studies report lower immune system responses to depressive-like behaviors induced by social stress in resilient mice, compared to susceptible animals that developed anhedonia or social withdrawal behavior. Moreover, social stressors can modify the brain's reward system function due to the close association between the brain systems that regulate stress and the sys-tems responsible for responses to drugs of abuse (Rodríguez-Arias et al., 2013). In contrast to the numerous reports that focus on predicting the individual response to depression-like stress consequences, only a recent study focused on the resilience and susceptibility to stress-induced enhancement of the cocaine response. We showed that resilient mice to depressive-like behaviors are also resilient to the increased cocaine reward induced by SD and exhibit a less intense neuroinflammatory response (Ballestín et al., 2021).

Although the increase in psychostimulant effects induced by SD has been thoroughly studied in the literature, there are fewer studies regarding the increased ethanol intake, and to our knowledge, only one study has focused on the resilient response to SD. Riga et al. (2020) suggest that resilience to depressive-like behaviors could protect from the development of alcohol use disorder (AUD)-like phenotypes. In their study, rats classified as depression-prone were more vulnerable to alcohol, emulating patterns of alcohol dependence as those seen in in-dividuals with an alcohol use disorder. In this study, animals were exposed to repeated SD, and subsequently isolated for several weeks. Their depression profile was evaluated during isolation, weeks after the last defeat. In addition to social avoidance, cognitive performance was also used to further classify animals into resilient or susceptible to depressive-like behaviors. Although the authors claimed that depression-prone animals showed a more intense pattern of alcohol consumption, their increase in alcohol intake during SA acquisition was not significantly higher. However, they observed a greater response to alcohol reward during the fixed ratio 3 (FR3) and PR schedule. In addition to high motivation toward alcohol, these depression-prone rats showed a tendency toward extinction resistance and relapse facilitation.

The present study was designed to further clarify if the resilient profile to depressive-like behavior also predicts a protection against the increase in ethanol intake induced by SD. The neuroinflammatory pro-file of resilient and susceptible mice were also studied after the end of the oral SA procedure, measuring levels of the pro-inflammatory cyto-kine interleukin 6 (IL-6) and the chemokine C-X3-C motif ligand 1 (CX3CL1) or fractalkine in the striatum and the prefrontal cortex (PFC). Both neuroinflammatory markers were reported to be differentially affected by social stress experiences in resilient or susceptible animals (Ballestín et al., 2021; Reguilón et al., 2020, 2021). To further charac-terize the potentiation of the resilience response, a second experiment took place to evaluate the effect of environmental enrichment (EE) exposure during adolescence, prior to the SD stress. The EE model selected for this work can be considered a basic and modest EE model, which based on the results obtained previously (Giménez-Gómez et al., 2021), we hypothesize is sufficient to stimulate resilience and block the increase in the reinforcing effects of ethanol and the neuroinflammatory response induced by SD.

2. Methodology

2.1. Animals

A total number of 87 adult male C57BL/6 mice (Charles River, France) were delivered to our laboratory at postnatal day (PND) 21. Experimental mice were housed in groups of four in plastic cages (27 × 27 × 14 cm) during the entire experimental procedure. OF1 adult mice (Charles River, France) were used as aggressive opponents (N = 20) and

were individually housed in plastic cages (21 × 32 × 20 cm) for at least one month prior to initiation of the experiments in order to heighten aggression (Rodríguez-Arias et al., 1998). All mice were housed in controlled laboratory conditions: constant temperature and humidity and a reversed light schedule (red light from 8:00 to 20:00). Food and water were available ad libitum to all the mice used in this study, except during behavioral tests. All procedures were conducted in compliance with the guidelines of the European Council Directive 2010/63/UE regulating animal research and were approved by the local ethics committees of the University of Valencia (number 2017-VSC-PEA-00224, on December 11th, 2017).
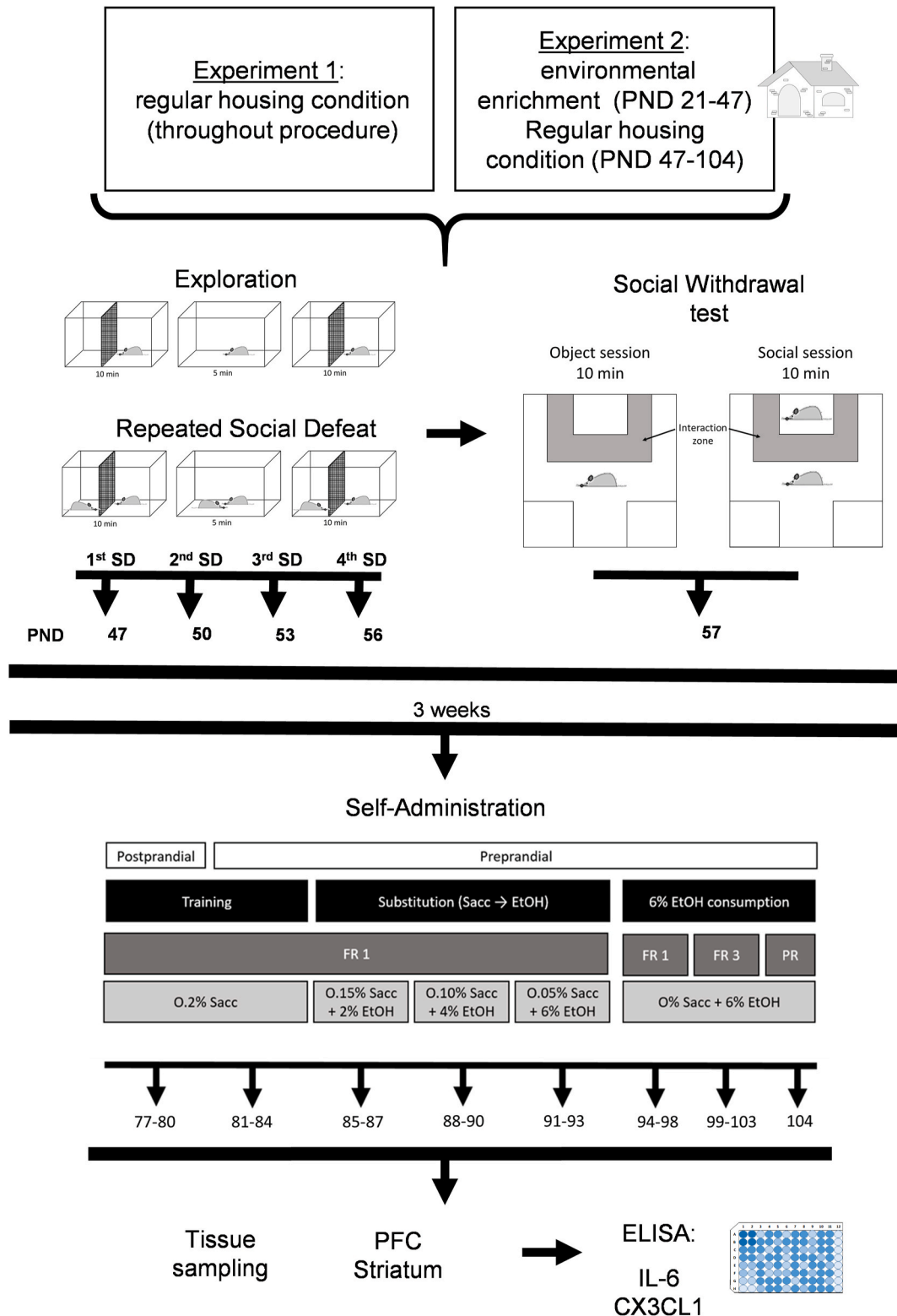

Fig. 1. **Experimental design**.

2.2. Drugs

For the oral SA procedure, absolute ethanol (Merck, Madrid, Spain) was diluted in water using a w/v percentage, i.e. a 6% (w/v) ethanol solution equivalent to a 7.6% (v/v) ethanol solution. Saccharin sodium salt (Sigma, Madrid, Spain) was dissolved in water.

During the SA training phase, a 0.2% (w/v) saccharin solution in water was used. During the SA substitution phases, a mixture of 0.15% saccharin concentration dissolved in water and 2% ethanol was used for the first subphase; in the second subphase, a mixture of 0.10% saccharin solution in water and 4% ethanol was used; and, in the third subphase, a mixture of 0.05% saccharin solution in water and 6% ethanol was used.

2.3. Experimental design

The study consisted of two experiments. The experimental design between the two experiments differs only in the housing condition of the animals. In the first experiment, all the animals were housed in regular condition throughout the study. In the second experiment, all mice were housed in a consistent EE in big cages (59 x 38 × 20 cm) with PVC items such as plastic houses and tubes from PND 21 to 47. The day before the beginning of SD, mice housed in EE were moved to standard housing conditions until the end of the SA procedure, i.e., the animals were only exposed to EE from the onset of adolescence until early adulthood or late adolescence.

All mice were exposed to the SD procedure or exploration from PND 47 to 56 (i.e., during early adulthood or late adolescence). 24 h after the last SD episode, animals performed the Test for Social Interaction to evaluate depressive-like behaviors and were characterized as resilient or susceptible depending on their social withdrawal ratio (SWR). Subsequently, three weeks after the last defeat, the animals initiated the ethanol SA protocol for approximately 28 days. At the end of this test, all the animals were sacrificed to obtain the PFC and striatum for further analysis of the cytokine and chemokine levels.

The experimental design is depicted in Fig. 1.

2.4. Procedure and apparatus

2.4.1. Housing conditions

Male mice in the regular housing condition were housed in groups of four in transparent plastic cages (27 × 27 × 14 cm) with no more enrichment than standard bedding (wood flakes 1–3.35 mm), nesting material (paper strands) and two wooden gnaw sticks (5 x 1 × 1 cm) per cage. Male mice in EE conditions were housed in groups of four in plastic cages (59 x 38 × 20 cm) with standard bedding and nesting material, two wooden gnaw sticks plus additional PVC tunnel (13 × 5.5 cm) and a plastic mouse house (12.5 x 10.5 × 11 cm; Ferrer-Pérez, 2019; Giménez-Gómez et al., 2021).

2.4.2. Procedure of social defeat (SD)

Animals in the stress/defeated groups were exposed to 4 episodes of SD during adulthood, each lasting 25 min and consisting of three phases. The initial phase began by introducing the "intruder" (the experimental animal) into the home cage of the "resident" (the aggressive opponent) for 10 min (Tornatzky and Miczek, 1993). During this initial phase, the intruder was protected from attack, but the wire mesh walls of the cage allowed for social interactions and species-typical threats from the male aggressive resident, thus facilitating instigation and provocation (Covington and Miczek, 2001). In the second phase, the wire mesh was removed from the cage to allow confrontation between the two animals over a 5-min period. Finally, the wire mesh was returned to the cage to separate the two animals once again for another 10 min to allow for social threats by the resident. The non-stressed exploration groups underwent the same protocol, but without the presence of a "resident" mouse in a clean cage. The intruder mice were exposed to a different aggressor mouse during each SD episode. The criterion used to define an

animal as defeated was the adoption of a specific posture signifying defeat, characterized by an upright submissive position, limp forepaws, upwardly angled head, and retracted ears (Miczek et al., 1982; Rodríguez-Arias et al., 1998). A detailed description of these behaviors can be found in Rodríguez-Arias et al., 1998).

2.4.3. Social withdrawal ratio (SWR)

The SWR used was based on the social approach-avoidance test previously described by Berton et al. (2006). The test took place 24 h after the last SD during dark cycle and in a different environment of the confrontation sessions. First, animals were transferred to a quiet, dimly lit room 1 h before the test was initiated. After habituation, each animal was placed in the center of a square arena (white Plexiglas open field, 30 cm on each side and 35 cm high) and its behavior was monitored by video (EthoVision XT 11, 50 fps; camera placed above the arena). Animals were allowed to explore the arena twice, for 600 s in each session, during two different experimental sessions. In the first (object session), an empty perforated Plexiglas cage (10 × 6.5 × 35 cm) was placed in the middle of one wall of the arena. In the second session (social session), an unfamiliar C57BL/6 male mouse was introduced into the cage as a social stimulus. Although it can be argued that the probe mouse used in the social interaction test resembles the aggressor, and that this could foster social aversion, this is unlikely, since previous experiments demonstrate similar amounts of social investigation, irrespective of the strain used (i. e., C57BL/6; Berton et al., 2006). Before each session, the arena was cleaned with 5% alcohol solution to minimize odor cues. Between sessions, the experimental mouse was removed from the arena and returned to its home cage for 2 min.

Locomotion and arena occupancy during object and social sessions were determined using the animals' horizontal positions, determined by commercial video tracking software (EthoVision XT 11, Noldus). Conventional measures of arena occupancy, such as time spent in the interaction zone and corners, were quantified. The former is commonly used as social preference-avoidance score and is calculated by measuring the time spent in a 6.5 cm wide corridor surrounding the restraining cage. Corners were defined as two squares of similar areas on the opposite wall of the arena.

2.4.4. Apparatus and procedures: Oral ethanol self-administration

This procedure is based on that employed by Navarrete et al. (2014). Oral ethanol SA was carried out in 8 modular operant chambers (MED Associated Inc., Georgia, VT, USA). Software package (Cibertec, SA, Spain) controlled stimulus and fluid delivery and recorded operant responses. The chambers were placed inside noise isolation boxes equipped with a chamber light, two nose-poke holes, one receptacle to drop a liquid solution, one syringe pump, one stimulus light and one buzzer. Active nose-pokes delivered 36 μl of fluid combined with a 0.5s stimulus light and a 0.5s buzzer beep, which was followed by a 6s time-out period. Inactive nose-pokes did not produce any consequence.

To evaluate the consequences of SD on the acquisition of oral ethanol SA, animals underwent an experiment carried out in three phases: training, saccharin substitution and 6% ethanol consumption.

2.4.4.1. Training phase (8 days). Two days before the initiation of the experiment, access to the standard diet was restricted to 1h per day. Before the first training session, water was withdrawn for 24h, and food allotment was provided 1h prior to the session to increase the motivation for active nose-poking. During the subsequent three days, water was provided ad libitum, except during the 1h period of food access before beginning each session, in which the water bottle was removed from the cages (postprandial). For the following four days, and for the remainder of the experiment, food access was provided for 1h after the end of each daily session and water was available ad libitum to avoid ethanol consumption due to thirst (preprandial). The food restriction schedule produced weight loss in the mice of around 15% of their free-feeding

weight (Navarrete et al., 2012). Mice were trained to respond to the active nose-poke to receive 36 μl of 0.2% (w/v) saccharin reinforcement.

2.4.4.2. Saccharin substitution (9 days). The saccharin concentration was gradually decreased as the ethanol concentration was gradually increased (Roberts et al., 2001; Samson, 1986). Each solution combination was set up to three consecutive sessions per combination (0.15% Sac −2% ethanol; 0.10% Sac −4% ethanol; 0.05% Sac −6% ethanol).

2.4.4.3. 6% ethanol consumption (11 days). The aim of the last phase was to evaluate the number of active nose-poke responses, the 6% ethanol (w/v) intake and the motivation to drink. This phase began 38 days after the last SD. After each session, the alcohol that remained in the receptacle was collected and measured with a micropipette. To achieve this goal, during the last phase, the number of active responses and ethanol consumption (μl) were measured under a fixed ratio 1 (FR1) for 5 daily consecutive sessions, FR3 (mice have to respond three times on the active nose-poke to achieve one reinforcement) for 5 consecutive daily sessions, and finally, on the day after FR3, a PR session was completed to establish the breaking point for each animal (the maximum number of nose-pokes each animal is able to perform to earn one reinforcement). The response requirement to achieve reinforcements escalated according to the following series: 1-2-3-5-12-18-27-40-60-90-135-200-300-450-675-1000. To evaluate motivation toward ethanol consumption, the breaking point was calculated for each animal as the maximum number of consecutive responses it performed to achieve one reinforcement according to the previous scale. For example, if an animal activated the nose-poke a total of 108 times, this meant that it was able to respond a maximum of 40 times consecutively for one reinforcement. Therefore, the breaking point value for this animal would be 40. All the sessions lasted 1 h, except the PR session, which lasted 2 h (Navarrete et al., 2012, 2014).

2.4.5. Immunoassay analysis (ELISA)
Samples from the striatum and the PFC were obtained 24 h after SA. To obtain tissue samples, mice were sacrificed by cervical dislocation and then decapitated. Brains were rapidly removed and the striatum and PFC dissected with a brain slicer matrix with 1 mm coronal section slice intervals using mouse brain atlas coordinates (Heffner et al., 1980; Paxinos and Franklin, 2001), which were then kept in dry ice until storage at −80 °C. Before IL-6 and CX3CL1 determination, brains were homogenized and prepared following the procedure described by Alfonso-Loeches et al. (2010). Frozen brain cortices were homogenized in 250 mg of tissue/0.5 ml of cold lysis buffer (1% NP-40, 20 mM Tris-HCl pH 8, 130 mM NaCl, 10 mM NaF, 10 μg/ml aprotinin, 10 μg/ml leupeptin, 40 mM DTT, 1 mM Na3VO4, and 10 mM PMSF). Brain homogenates were kept on ice for 30 min and centrifuged at the maximum speed for 15 min; the supernatant was collected, and protein levels were determined by the Bradford assay from ThermoFisher (Ref: 23227).

The concentrations of CX3CL1 and IL-6 in homogenized extracts were measured with commercial enzyme-linked immunosorbent assay (ELISA) kits in 96-well strip plates (Abcam, ab100683, ab100712). We determined CX3CL1 and IL-6 concentration in the striatum and PFC. All reagents and standard dilutions were prepared following the manufacturer's instructions. To determine absorbance, we employed an iMark microplate reader (Bio-RAD) controlled by Microplate Manager 6.2 software. Optical density of plates was read at 450 nm and the final results were calculated using a standard curve following the manufacturer's instructions. Total protein concentrations were determined using the Pierce BCA Protein Assay Kit (ThermoFisher Scientific) to determine the number of nanograms of CX3CL1 and picograms of IL-6. Data are expressed as ng/mg or pg/mg of protein for tissue samples.

2.5. Statistical analysis

Mice were previously classified into resilient and susceptible groups based on the SWR. SWR is calculated by considering the time spent by an experimental mouse in the interaction zone when a social target is present divided by the time it spends in the interaction zone when the target is absent. A ratio equal to 1 means that equal time has been spent in the presence versus absence of a social target. Based on the regular behavior of control C57BL/6 mice, animals with a ratio under 1 are classified as susceptible, while those with a ratio equal to or higher than 1 are classified as resilient (Golden et al., 2011). No statistics were needed to identify separate groups of defeated mice and the criteria described in the statistical analysis section were sufficient to establish separate groups. To study the relationship between the percentages of susceptible mice in non-enriched and enriched mice, the chi-square (χ^2) test was used to evaluate the categorical variables Stress and Housing.

To analyze acquisition of ethanol SA, a two-way ANOVA was performed with one between-subjects variable –Stress with three levels (Control, Resilient and Susceptible; or EE-Control, EE-SD-R and EE-SD-S)– and a within-subjects variable –Days, with five levels of FR1 or FR3–. The effects of SD and treatment on breaking point values and ethanol consumption during PR was analyzed by a two-way ANOVA, with one between-subjects variable –Stress.

The data of the CX3CL1 and IL-6 levels were analyzed using a one-way ANOVA with one between-subjects variable –Stress, with three levels (Control, Resilient and Susceptible; or EE-Control, EE-SD-R and EE-SD-S).

In all the studies, following the ANOVA, Bonferroni post-hoc tests were calculated whenever required. All statistical analyses were performed using SPSS Statistics v.26. Data were expressed as mean ± SEM and a value of $p < 0.05$ was considered statistically significant.

In order to evaluate the differences induced by housing conditions (standard housing and environmental enrichment), we additionally performed a statistical analysis with the variable Housing for the 6 groups. For ethanol SA, we performed a two-way ANOVA with two between-subjects variable –Stress, with three levels (Control, Resilient and Susceptible) and Housing, with two levels (SH and EE)– and for FR1 and FR3, a within-subjects variable –Days, with five levels–. The effects of SD and housing on the breaking point values and the ethanol consumption during PR, as well as the results of striatum protein levels of IL-6 and CX3CL1, were analyzed by a two-way ANOVA, with two between-subjects variables –Stress and Housing.

3. Results

3.1. Resilience to SD under regular housing conditions

3.1.1. Classification between susceptible and resilient mice according to their social withdrawal ratio
Following the SWR calculation criteria, the control group (n = 12) showed a mean SWR higher than 1.

In the defeated group of animals (n = 30), 53.3% of the mice showed a SWR under 1, which classifies them as susceptible mice (n = 14), and the remaining 46.6% of the mice showed a SWR equal to or higher than 1, which classifies them as resilient mice (n = 16).

3.1.2. Susceptible mice showed higher ethanol intake than resilient animals
No differences were found between the animals during training or substitution phases, showing that SD did not induce any learning deficit (data not shown).

The ANOVA for the number of active responses during the FR1 schedule of ethanol SA revealed a significant effect of the variable Days [$F_{(1,39)} = 17.697$; $p < 0.001$] and Stress [$F_{(2,39)} = 4.854$; $p < 0.01$] (Fig. 2a). The post-hoc comparison showed that mice performed fewer active responses on days 1 and 2 compared to the last day ($p < 0.001$ in all cases). Moreover, susceptible mice performed fewer active responses
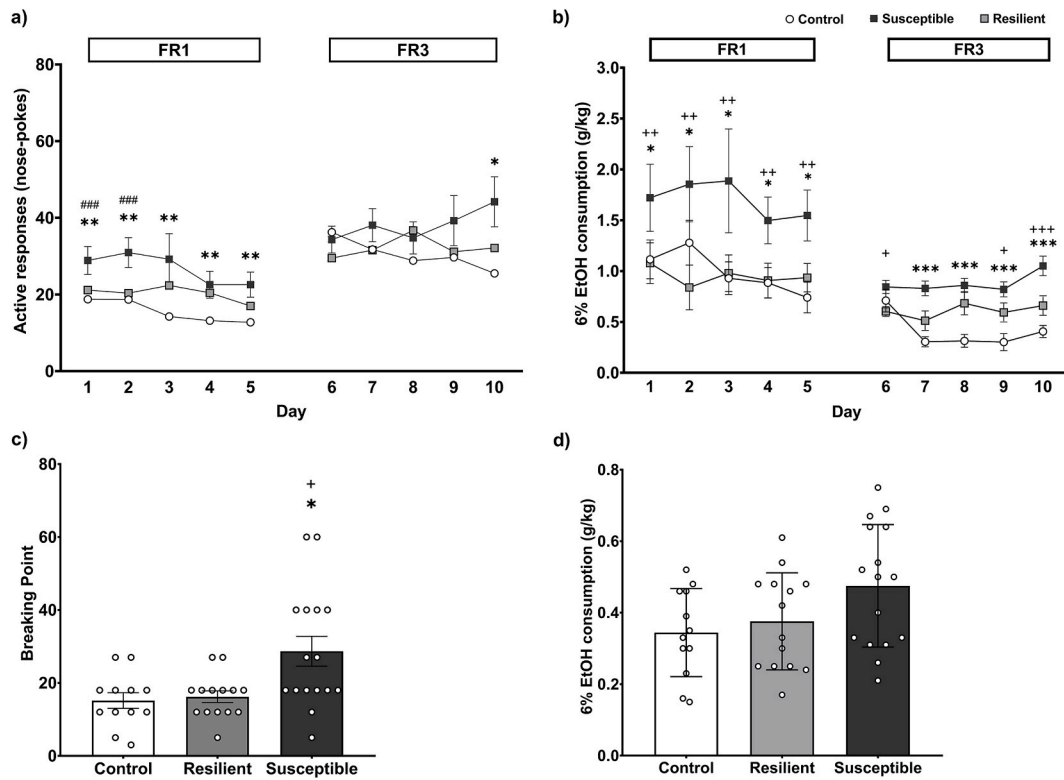
Fig. 2. **Resilient mice showed lower ethanol intake than susceptible animals**. Mice were divided into Control (n = 12); Resilient (n = 14) and Susceptible (n = 16). Defeated mice were characterized as resilient or susceptible depending on their SWR. The dots represent means and the vertical lines ± SEM of (a) the number of active responses and (b) the volume of 6% ethanol consumption during FR1 and FR3. The columns represent the mean and the vertical lines ± SEM of (c) the breaking point values, (d) the volume of 6% ethanol consumption during PR. *p < 0.05, **p < 0.01 ***p < 0.001 significant difference with respect to controls; + p < 0.05, ++ p < 0.01, +++ p < 0.001 significant difference with respect to resilient mice. ###p < 0.001 significant difference with respect to day 5.

than the control group (p < 0.01).

With respect to ethanol consumption, the ANOVA revealed a significant effect of the variable Stress [F(2,35) = 4.650; p = 0.01] (Fig. 2b). The post-hoc comparison showed that the susceptible group consumed ethanol at higher rates than the control (p's < 0.05) and resilient groups (p < 0.01).

During the FR3 schedule, the ANOVA revealed a significant effect of the interaction Days × Stress [F(4,184) = 4.940; p = 0.001] for the number of active responses (Fig. 2a). Susceptible mice showed a higher number of active responses than controls on day 10. Moreover, control animals showed a lower number of active responses on day 10 compared to day 6 (p < 0.05). However, susceptible mice increased the number of active responses on days 6 and 8 compared to day 10 (p < 0.05 and p < 0.01, respectively).

With respect to ethanol consumption, the ANOVA revealed a significant effect of the variable Days [F(4,156) = 5.216; p < 0.001], Stress [F(2,39) = 3.949; p < 0.001] and the interaction Days × Stress [F (8,156) = 3.691; p < 0.001] (Fig. 2b). Susceptible mice consumed significantly more ethanol than controls on days 7–10 (p < 0.001 in all cases) and than resilient mice on days 6 (p < 0.05), 9 (p < 0.05) and 10 (p < 0.001). Moreover, control animals consumed more ethanol on day 6 compared to the rest of the days (p < 0.001 in all cases). Susceptible mice also consumed more ethanol on day 10 compared to the previous days (p < 0.01 with respect to days 6, 7 and 9; p < 0,05 with respect to day 8).

During the PR, the ANOVA for the breaking point values of ethanol SA revealed a significant effect of the variable Stress [F(2,39) = 6.418; p < 0.004] (Fig. 2c). The post-hoc comparison showed that the breaking point values were higher in susceptible mice with respect to control and resilient animals (p < 0.01 in both cases). The ANOVA for ethanol consumption during PR did not reveal a significant effect of the variable

Stress (Fig. 2d).

3.1.3. Susceptible mice showed altered levels of cytokine IL-6 and chemokine CX3CL1

The ANOVA for the striatal IL-6 levels showed an effect of the variable Stress [F(2,37) = 9.957; p < 0.001] (see Fig. 3a). Susceptible mice displayed higher IL-6 levels than the controls (p < 0.001), and resilient animals (p < 0.01). The ANOVA for IL-6 levels in PFC showed an effect of the variable Stress [F(2,37) = 4.283; p < 0.021] (see Fig. 3c). Susceptible mice displayed higher IL-6 levels than control animals (p < 0.05) without differences with resilient animals.

The ANOVA of striatal CX3CL1 levels revealed a significant effect of the variable Stress [F(2,37) = 4.807; p < 0.014] (see Fig. 3b). Striatal CX3CL1 levels were lower in susceptible animals in comparison with controls (p < 0.01). The ANOVA of CX3CL1 levels in PFC also revealed a significant effect of the variable Stress [F(2,37) = 13.037; p < 0.007] (see Fig. 3d). CX3CL1 levels in PFC were lower among all defeated animals (either resilient or susceptible) in comparison with controls (p < 0.05 for resilient and p < 0.001 for susceptible).

3.2. Environmental enrichment effects on resilience to SD

3.2.1. Environmental enrichment did not increase the percentage of resilient mice depending on the social withdrawal ratio

Following the SWR calculation criteria, the control group exposed to EE (n = 14) showed a mean SWR higher than 1.

In the defeated group of animals with EE (n = 31), 51.6% of mice showed a SWR under 1, which classifies them as susceptible mice (n = 16), and the remaining 48.4% of mice showed a SWR equal to or higher than 1, which classifies them as resilient mice (n = 15).

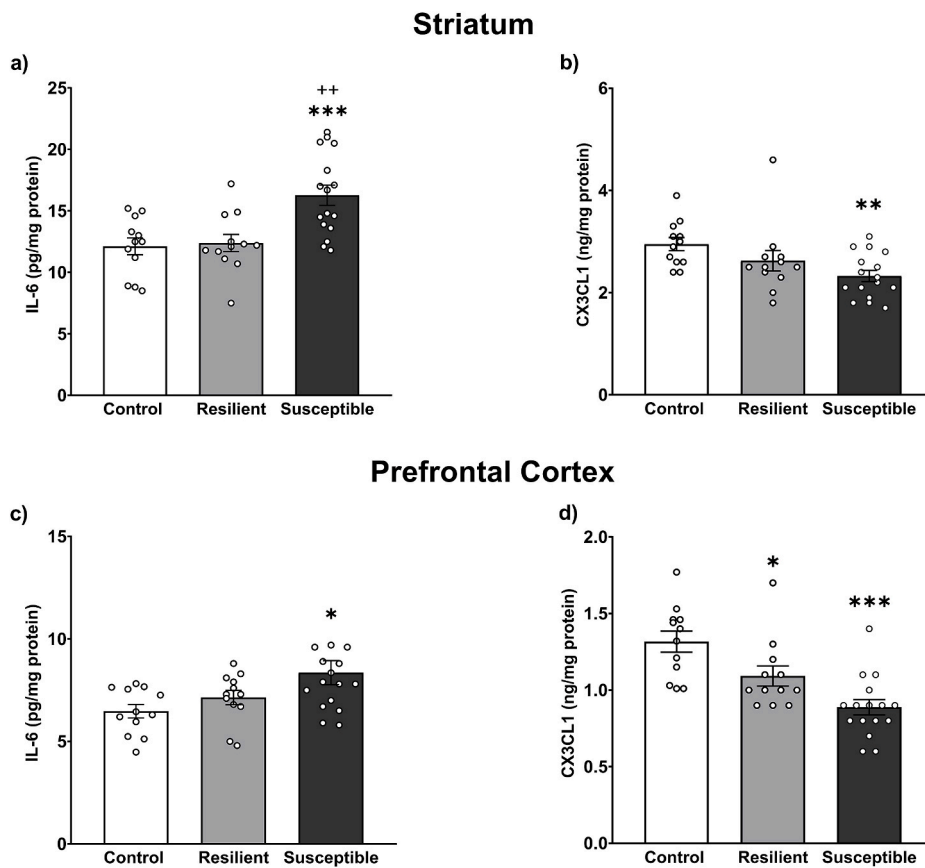The comparison between the percentage of susceptible mice in the

Striatum

Prefrontal Cortex

Fig. 3. Effect of repeated SD on IL-6 and CX3CL1 levels in the striatum and PFC. Bars represent mean pro-inflammatory cytokine IL-6 (in pg/mg) and chemokine CX3CL1 levels (in ng/mg) in the striatum (a and b) and PFC (c and d) and vertical lines $\pm$ SEM. Mice were divided into Control (n = 12); Resilient (n = 14) and Susceptible (n = 16). Defeated mice were characterized as resilient or susceptible depending on their SWR. *p < 0.05, **p < 0.01, ***p < 0.001 significant difference with respect to the control; ++ p < 0.01 significant difference with respect to resilient mice.

non-enriched experiment (53.3%) and the percentage of susceptible mice in the enriched experiment (51.6%) showed no statistical difference ($\chi^2(1) = 0.018$; p = 0.893; Table 1).

3.2.2. Adolescent exposure to environmental enrichment reduces ethanol intake in susceptible animals

No differences were found between the animals during the training and substitution phases, showing that EE and SD did not induce any learning deficit (data not shown).

The ANOVA for the number of active responses during the FR1 schedule of ethanol SA revealed a significant effect of the variable Stress (Fig. 4a), with susceptible mice making more active responses than control animals (p < 0.05). During the FR3 schedule, the ANOVA revealed a significant effect of the variable Days [F(4,168) = 4.215; p < 0.01] (Fig. 4a). Mice performed less active responses on the 10th day compared to the 7th (p < 0.01), 8th (p < 0.01), and 9th (p < 0.05) days.

With respect to ethanol consumption, the ANOVA of the g/kg of ethanol intake during the FR1 and FR3 schedule of ethanol SA did not reveal any significant effect of the variable Days or Stress (Fig. 4b),

meaning that defeated mice, either resilient or susceptible, did not consume more ethanol than non-stressed control animals.

During the PR, the ANOVA for the breaking point values of ethanol SA and for ethanol consumption did not reveal any significant effect of the variable Stress (Fig. 4c and d).

3.2.3. Environmental enrichment diminishes the neuroinflammatory response in susceptible mice

The ANOVA of striatal IL-6 (Fig. 5a) and CX3CL1 (Fig. 5b) levels did not reveal any significant effect of the variable Stress.

3.2.4. Environmental enrichment vs standard housing condition

The ANOVA for the number of active responses during the FR1 schedule of ethanol SA revealed a significant effect of the variable Housing [F(1,78) = 4.451; p < 0.001]. The post-hoc comparison showed that the enriched mice performed higher active responses than the non-enriched mice (p < 0.05). With respect to ethanol consumption, the ANOVA revealed a significant effect of the variable Housing [F(1,75) = 6.050; p < 0.05]. The post-hoc comparison showed that the standard-housed mice consumed ethanol at higher rates than the enriched mice (p < 0.05).

During the FR3 schedule, the ANOVA revealed a significant effect of the interaction Days $\times$ Stress $\times$ Housing [F(8,300) = 2.717; p < 0.01] for the ethanol consumption. The standard housed group consumed significantly more ethanol than the control enriched group on day 6 (p < 0.001). The resilient standard-housed group consumed significantly more ethanol than the resilient enriched group on days 6, 7 (p's < 0.05), 8 (p < 0.001), 9 (p < 0.01) and 10 (p < 0.001). Moreover, the susceptible standard-housed group consumed significantly more ethanol than the susceptible enriched group on days 6, 7, 8, 9 and 10 (p's < 0.001).

During the PR, the ANOVA for the breaking point values of ethanol SA revealed a significant effect of the interaction Stress $\times$ Housing [F

Table 1
Housing condition and classification in the Social Interaction Test of defeated mice.

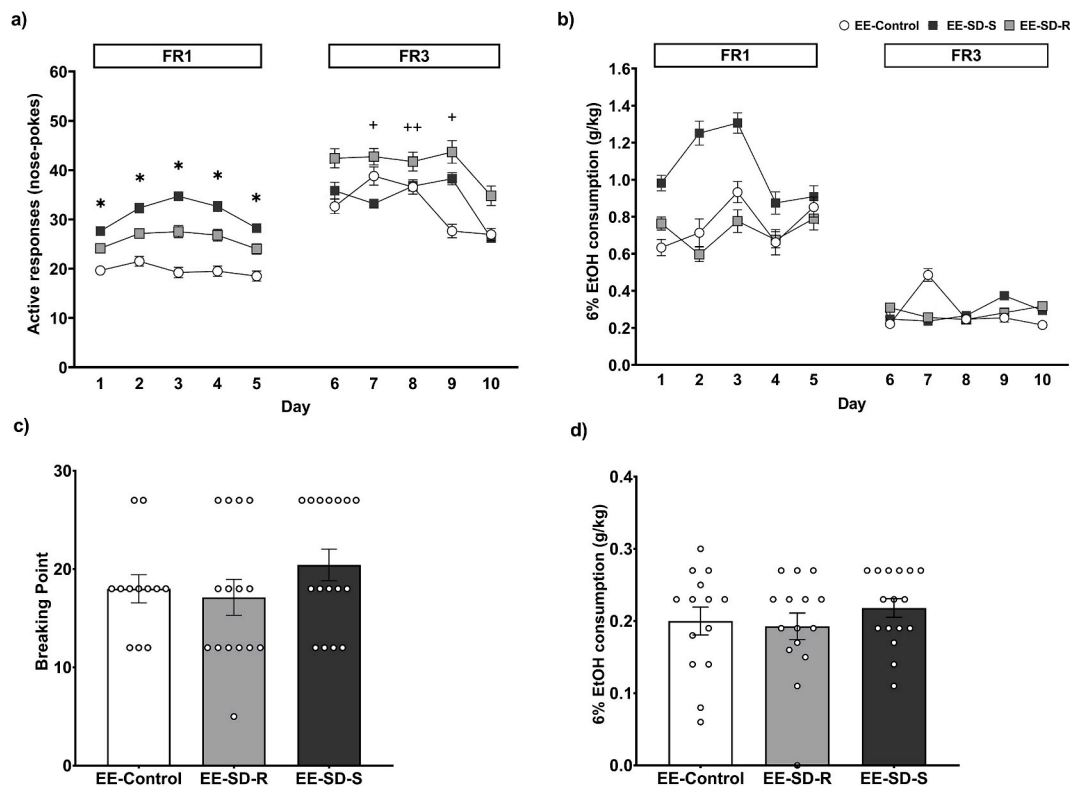|			Housing		Total
			Non-EE	EE	
Stress					
Susceptible mice		n	16	16	32
		%	53.3	51.6	52.5
Resilient mice		n	14	15	29
		%	46.7	48.4	47.5
Total		n	30	31	61
		%	100	100	100

Fig. 4. Environmental enrichment reduces ethanol intake in susceptible animals. Mice were divided into EE-Control (n = 14); EE-SD-R (n = 15) and EE-SD-S (n = 16). Defeated mice were characterized as resilient or susceptible depending on their SWR. The dots represent means and the vertical lines ± SEM of (a) the number of active responses and (b) the volume of 6% ethanol consumption during FR1 and FR3. The columns represent the mean and the vertical lines ± SEM of (c) the breaking point values, (d) the volume of 6% ethanol consumption during PR. *p < 0.05, significant difference with respect to controls; + p < 0.05, ++ p < 0.01 significant difference with respect to the 10th day.
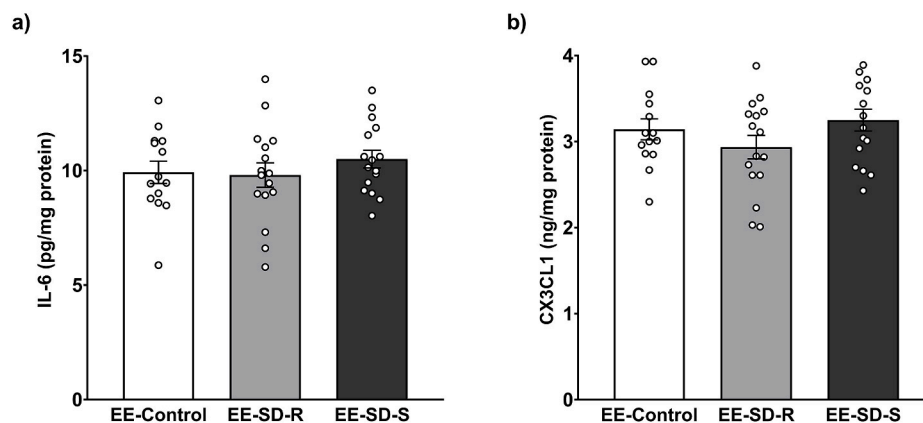
Striatum

Fig. 5. Environmental enrichment reduces levels of the pro-inflammatory cytokine IL-6 and chemokine CX3CL1 in susceptible mice. Bars represent the mean of the striatal IL-6 (a) levels (in ng/kg) and CX3CL1 (b) levels (in pg/mg) and the vertical lines ± SEM. Mice were divided into EE-Control (n = 14); EE-SD-R (n = 15) and EE-SD-S (n = 16). Defeated mice were characterized as resilient or susceptible depending on their SWR.

(2,79) = 3.052; p = 0.05]. The post-hoc comparison showed that the breaking point values were higher in susceptible standard-housed mice with respect to the susceptible enriched animals (p < 0.001). The ANOVA for ethanol consumption during PR revealed a significant effect of the variable Housing [F(1,81) = 65.766; p < 0.001]. The post-hoc comparison showed that enriched mice consumed significantly less ethanol than standard-housed mice (p < 0.001).

The ANOVA for the striatal IL-6 levels showed an effect of the interaction Stress × Housing [F(2,80) = 5.278; p < 0.01]. The ANOVA

revealed that the control standard-housed group displayed higher IL-6 levels than the control enriched group (p < 0.05). The susceptible standard-housed mice showed higher IL-6 levels than the susceptible enriched mice (p < 0.001). In the same line, the resilient standard-housed mice showed higher IL-6 levels than the resilient enriched mice (p < 0.01).

4. Discussion

It is well known that the behavioral and neurobiological effects of social stress are not equally manifested in all individuals. Most of the studies have focused on the particular response to depressive-like behaviors and neuroinflammation (Nasca et al., 2019; Pfau and Russo, 2015). However, few studies have evaluated the resilience/susceptibility response to drug abuse after social stress. We have recently reported that resilient mice to depressive-like behaviors also show a resilient response to the increased cocaine reward induced by SD, which is accompanied by a lower neuroinflammatory response (Ballestín et al., 2021). In the present study, we further confirm that mice presenting a phenotype resistant to depressive-like behaviors are also unaffected by the increased ethanol intake induced by SD. Defeated resilient mice did not show any increase in ethanol intake, conversely to those susceptible, which also showed increased motivation for ethanol in the PR. These resilient mice developed minor neuroinflammatory responses with lower levels of IL-6 and higher levels of CX3CL1 in the PFC and the striatum than their susceptible counterparts. To further unravel the mechanisms of the resilient response, we evaluated the protective role of EE housing during adolescence before exposure to SD. Although EE during adolescence did not increase the percentage of resilient mice to depressive-like behaviors evaluated through the SWR, neither resilient nor susceptible mice increased their oral ethanol SA consumption. Moreover, none of the defeated mice exposed to EE developed any increase in neuroinflammatory markers. One limitation of the study is that the effects produced by SD in female rodents have not been evaluated in this work. Due to the sex differences observed in female mice with respect ethanol intake, it is necessary to perform suitable models of social stress for female rodents to address this issue in the future.

4.1. Resilience to the increase in ethanol intake induced by social defeat

The typical measure to classify animals as resilient or susceptible to SD effects is the SWR. Investigation of a social target is a natural behavior in healthy rodents; therefore, social avoidance is considered a depressive-like behavior. In the social interaction test, performed 24 h after the last SD, susceptible mice are stressed animals that display social avoidance.

Numerous studies have shown that SD induces changes in the reward system, affecting drug intake. With regard to ethanol, exposure to SD increases the conditioned rewarding effects of ethanol using the CPP paradigm (Macedo et al., 2018). Studies of voluntary ethanol consumption have observed increased and escalating consumption of ethanol, as well as an increased motivation to drink alcohol, in defeated animals using the oral SA paradigm (Barchiesi et al., 2021; Montagud-Romero et al., 2021; Norman et al., 2015; Reguilón et al., 2020, 2021; Rodríguez-Arias et al., 2016). Using other paradigms such as the two-bottle choice, an increase in SD-induced escalation of alcohol intake has also been observed (Croft et al., 2005; Deal et al., 2018; Hwa et al., 2016; Newman et al., 2018a).

We have previously reported that animals classified as susceptible to SD-induced depressive-like behaviors were also susceptible to the increased rewarding effects of a subthreshold dose of cocaine three weeks after the last SD (Ballestín et al., 2021). In the present study, we confirmed that the resilient or susceptible phenotype to depressive-like behaviors induced by SD also correlates to the ethanol intake phenotype. In contrast to resilient mice, those classified as susceptible depending on the SWR test showed a higher increase in ethanol consumption and motivation to obtain a reward. There is only one other study evaluating this relation. Riga et al. (2020) evaluated the effects of a long-term SD-induced depressive phenotype, subsequently followed by a period of social isolation, on alcohol-seeking and drinking behaviors in male rats. This study presents significant differences with regards to ours, such as the use of two different social stressors, the SD and social isolation. The authors performed five SD exposures for five consecutive days and the intruder mice were immediately housed in isolation for the rest of the study. On the other hand, mice in our study were subjected to four intermittent sessions of SD and were socially housed throughout the study. It is known that the intensity, duration and number of exposures influence the intensity of subsequent behavioral symptoms and long-term effects on substance abuse (Shimamoto, 2018). Another important difference lies in the criterion to characterize the animals as resilient or susceptible to SD-induced depressive-like behaviors, which was based on social approach-avoidance and the object place recognition tests during the isolation period. Although these authors did not observe any increase in ethanol intake during the SA acquisition and FR1, susceptible rats exhibited a significant increase in alcohol responsiveness during FR3 and a higher motivation to drink alcohol during the PR schedule compared to the control group. Susceptible rats also showed a higher number of extinction sessions and a higher relapse than non-stressed animals. Although no differences in ethanol consumption during FR1 were observed in the work of Riga et al. (2020), susceptible rats performed a higher number of active responses. One possible explanation for the lack of difference in ethanol intake could be the higher ethanol concentrations used (12%). We can hypothesize that SD may change the sensitivity to ethanol preference, with stressed animals being more sensitive to a low ethanol concentration, such as the 6% used in our study. In addition, social isolation is known to induce profound behavioral and neurobiological alterations (Mumtaz et al., 2018). Besides inducing anxiety and depressive-like behaviors in rodents (Amiri et al., 2015), several studies pointed that isolation induces an increase in ethanol consumption in mice and rats (Advani et al., 2007; Evans et al., 2020; Juárez and Vázquez-Cortés, 2003; Lopez et al., 2011; Sanna et al., 2011).

4.2. Susceptible mice showed increased levels of IL-6 and CX3CL1

Numerous studies have shown that ethanol activates the innate immune system by stimulating Toll-like receptor 4 (TLR4) signaling in glial cells, triggering the release of inflammatory mediators and causing neuroinflammation (Alfonso-Loeches et al., 2010; Ibáñez et al., 2019; Montesinos et al., 2016; Pascual et al., 2015). The induction of astrogliosis and microgliosis increases the release of cytokines (IL-1β, IL-6, IL-17, TNF-α) and the production of chemokines (MCP-1, MIP-1α, CX3CL1), causing brain damage in various brain structures such as the PFC, striatum, hippocampus and cerebellum (Alfonso-Loeches et al., 2010; Bachtell et al., 2015; Drew et al., 2015; Fernandez-Lizarbe et al., 2009; Guerri and Pascual, 2019; Pascual et al., 2011, 2018; Vetreno and Crews, 2015). In addition to the neuroinflammatory response, alcohol exposure diminishes cell proliferation, migration, growth and differentiation, even causing cell death (Alfonso-Loeches and Guerri, 2011).

The brain areas analyzed in this study play a crucial role in the addictive cycle. On the one hand, the PFC controls subcortical regions to drive motivated behavior (Koya et al., 2009; West et al., 2014). The PFC consists of subregions that appear to mediate different aspects of the addiction cycle. For example, the prelimbic area (PrL) or dorsal area in rats projects preferentially to the Nucleus Accumbens (NAc) core, and the infralimbic (IL) or ventral area projects preferentially to the NAc shell (Heidbreder and Groenewegen, 2003; Ongür and Price, 2000). PrL appears to play a critical role in cue-elicited drug seeking (Lasseter et al., 2010), and IL appears to be primarily involved in inhibiting drug seeking (Peters et al., 2008). On the other hand, the striatum also consists of subnuclei involved in different stages of the addictive cycle. The ventral striatum (NAc) is associated with incentive salience pathways and salience attribution, i.e., it has been associated with the reinforcing actions of drugs of abuse (Koob, 2015; Koob and Volkow, 2010). While the dorsal striatum is related to habit formation (stimulus-response habit learning), and therefore, is key in the development of habitual compulsive drug use (Koob, 2015; Koob and Volkow, 2010).

Exposure to social stress promotes an increase in the neuroimmune response. Numerous preclinical studies show that SD is accompanied by

the activation of neuroinflammatory events, including microglial activation and increased cytokine production (Calcia et al., 2016; Ferrer-Pérez et al., 2018; Finnell and Wood 2016; Montagud-Romero et al., 2021; Rodríguez-Arias et al., 2017, 2018; Wohleb et al., 2011, 2012, 2014). In addition, SD promotes the deterioration of the blood-brain barrier (BBB), and a decrease in the expression of the tight binding protein claudin-5, laminin and collagen-IV has been observed in the hippocampus and NAc (Menard et al., 2017; Rodríguez-Arias et al., 2017). Using the same procedure to induce SD as in the present study, we have observed that exposure to SD can induce a long-lasting increase in the concentration of pro-inflammatory cytokines such as IL-6 in the PFC, striatum and hippocampus (Ballestín et al., 2021; Ferrer-Pérez et al., 2018) and a significant upregulation of the protein pro-inflammatory markers NFkBp-p65, IL-1β, IL-17 A and COX-2 in the striatum of male mice (Montagud-Romero et al., 2021). An increase of chemokines such as CX3CL1 and CXCL12 in the striatum and PFC of defeated mice has also been observed (Reguilón et al., 2020, 2021), although a decrease in CX3CL1 protein levels in the hippocampus and striatum (Ballestín et al., 2021; Montagud-Romero et al., 2020) has also been described using another strain of mice. Both pro-inflammatory and anti-inflammatory functions for CX3CL1of have been described (Mattison et al., 2013; Sheridan and Murphy, 2013; Zujovic et al., 2000), since the CX3CL1-CX3CR1 signaling has a neuroprotective function and maintains communication between neurons and microglia (Sheridan and Murphy, 2013). CX3CL1 seems to have anti-inflammatory effects mainly (Lyons et al., 2009; Zujovic et al., 2000), and an efficient CX3CL1 signaling between neurons and microglia appears to be critical for the protection of social stress-induced depressive-like behaviors. For example, CX3CR1 KO mice showed an exaggerated HPA axis response to social stress (Winkler et al., 2017).

Moreover, individual differences in the neuroinflammatory mechanisms observed after SD stress have been described. When characterized as susceptible to the depressive-like behaviors induced by SD, these animals showed increased levels of cytokines IL-6, MCP-1 or IL-1β (Hodes et al., 2014; Stewart et al., 2015; Wood et al., 2015), with an increase of anti-inflammatory cytokines IL-4 and IL-10 in resilient rodents (Hodes et al., 2014; Stewart et al., 2015). A recent study observed that exposure to chronic unpredictable mild stress triggered a significant increase in Nod-like receptor pyrin containing 3 (NLRP3) expression only in susceptible mice, but not in resilient mice. These changes were accompanied by altered levels of IL-1β expression (Yang et al., 2021). Increases in both the NLRP3 and IL-1β expressions are associated with the development of depressive-like behaviors (Felger and Lotrich, 2013; Raison and Miller, 2013). Moreover, chronic SD caused a significant decrease in cAMP levels in the NAc neurons of susceptible mice (Zhang et al., 2020), promoting BBB permeability. These results indicate that stress resilience may be associated with reduced pro-inflammatory signaling, and suggest that therapeutic treatment on these pathways could promote stress resilience (Yang et al., 2021). However, only a recent study from our laboratory evaluated if this neuroinflammatory response is also observed in mice susceptible to the increased cocaine reward induced by SD. We observed that these mice exhibited elevated neuroinflammatory levels of the pro-inflammatory cytokine IL-6 and a decrease in the chemokine CX3CL1 in the striatum and hippocampus after being exposed to SD (Ballestín et al., 2021). Moreover, striatal and hippocampal IL-6 levels continued to be elevated more than 5 weeks after the last SD in susceptible mice. In the present study, we have corroborated and extended these results. After oral ethanol SA, susceptible mice showed increased IL-6 levels in the striatum and PFC. In addition, a decreased CX3CL1 was equally observed in both structures after SA in susceptible mice, although resilient animals also showed a decreased CX3CL1 in the PFC. To our knowledge, this is the first study showing that animals susceptible to the increased rewarding effects of ethanol induced by SD showed a long-lasting increase in the neuroinflammatory response.

4.3. EE promotes resilience to the effects of SD on alcohol intake and the neuroinflammatory response

In the second study, mice were housed in an enriched environment during adolescence (PND21), but housed under standard housing conditions from the first SD (PND47) until the end of the experiment. In other words, our objective was to determine the existence of a protective effect of EE on depressive-like behavior and the long-term vulnerability to the rewarding effects of ethanol and the neuroimmune response induced by SD. Our results confirmed the protective effect of EE in ethanol intake and in the neuroinflammatory response induced by SD.

EE has been typically associated with an improved well-being, increased cognitive function and a potentiation of stress resilience, and different models of EE have been used in order to reduce vulnerability to the detrimental effects of SD. However, the results observed in the literature are discrepant. In mice housed in EE and then subjected to 7 days of daily SD, an increase in aggressiveness and anxiety has been described, probably derived from a change in social stability (McQuaid et al., 2013a, 2013b). In these studies, EE not only did not decrease the neuroinflammatory response, but it even increased the corticotropin-releasing factor (CRF) levels in the PFC in both stressed and control mice.

Nevertheless, other studies found that EE is active in diminishing the neurobiological and behavioral effects induced by social stress, indicating that housing conditions may modulate the impact of external stressors. EE reduces acute and chronic stress-induced anxiety-like behaviors and cognitive impairments (Bahi, 2017; Cordner and Tamashiro, 2016; Dandi et al., 2018; Marianno et al., 2017). In addition, animals under EE housing show minor corticosterone increases and neuronal activation after a stressful experience (Branchi et al., 2013; Mesa-Gresa et al., 2016; Reichmann et al., 2013).

In contrast with the previously presented studies, we applied an EE prior to the exposure to SD to determine whether this housing condition during adolescence could potentiate the resilient response to depressive-like behavior and increase ethanol intake induced by this kind of stress. Our results showed that exposure to EE prior to SD does not influence SD-induced depressive-like behavior evaluated by SWR. In this way, we observed the same percentage of resilient and susceptible animals according to this score among those housed in EE when comparing with those from the first experiment housed under standard conditions. However, stressed resilient and susceptible mice housed in EE during adolescence did not show any long-lasting increase in ethanol intake or motivation to get the drug after SD. A recent study of Seo et al. (2021) observed that early exposure to EE is capable of blocking depressive-like behaviors induced by chronic unpredictable stress when animals are housed under standard conditions. In addition, previous housing under EE prevented epigenetic changes induced by this stressor. Although, as in our study, mice were exposed to EE during adolescence, there are important methodological differences between both studies. In Seo's study, mice were housed in EE for a longer period, but more importantly, they used a different type of stressor named chronic unpredictable stress. Finally, we employed mice of the OF1 strain, which are particularly affected by SD due to their high territoriality. All these differences could be responsible for the discrepant results in EE in preventing depression-like behaviors. Despite the lack of a standardized EE model, we consider the model employed in this investigation to be promising. Exposure to EE during adolescence has favored and enhanced adaptive behaviors in the face of subsequent exposure to social stress.

The role of EE in reducing ethanol intake has been widely demonstrated. For example, Rodríguez-Ortega et al. (2018) proved that housing adult mice in EE reduces ethanol binge intake, and likewise, social and environmental enrichment reduced ethanol preference (Holgate et al., 2017). However, there are few studies evaluating the therapeutic potential of EE on the reinforcing and motivational effects of ethanol induced by SD. Bahi (2017) observed that the increased anxiety-like behavior, the increase in ethanol intake and the appearance of

ethanol-induced CPP were buffered by exposure to EE conditions after the stress experience. In a more recent study, EE also proved to counteract social stress effects favoring the extinction of memories associated with ethanol and reducing reinstatement of drug seeking (Bahi and Dreyer, 2020).

EE also modulated the neuroinflammatory response induced by SD in the striatum. We did not observe any changes in IL-6 or CX3CL1 levels in the striatum in any of the groups evaluated. Differently with the first set of animals, susceptible mice housed in EE did not show any increase in IL-6 levels in the striatum, as observed in susceptible animals housed under standard housing conditions. Moreover, CX3CL1 levels also did not decrease in these mice, as it did in susceptible mice in the first study. As we did not observe any differences in the striatum of mice housed under EE conditions, the area most closely related to rewarding behavior, we did not analyze PFC. These results suggest that exposure to EE before SD reduces the impact of long-term social stress on the neuroinflammatory response, acting as a protective factor. There are no similar studies evaluating the neuroinflammatory response of social stress and ethanol consumption applying EE models, but our results are in line with studies evaluating the effect of EE on the neuroinflammatory response of social stress. Attenuations of the increase in IL-6 and IL-β1 in the prefrontal mRNA expression induced by moderate social stress have been observed in animals under EE conditions (McQuaid et al., 2018).

Among the beneficial effects of EE that could account for the protective effect on the increased ethanol intake and the neuroinflammatory response, we should highlight an increase in neurogenesis with an elevated expression of brain-derived neurotrophic factor (BDNF; Novkovic et al., 2015; Schloesser et al., 2010) and an enhanced synaptic and transcriptomic capacity (Hüttenrauch et al., 2016; Zhang et al., 2018). Exposure to EE during adolescence could also change the dynamics of social interaction, sensory processing and the mechanisms underlying baseline stress, with a decrease in CRHR1 genes and an increase in hippocampal CRHR2 observed in male rats housed in EE conditions (Kentner et al., 2018). Among other factors, facilitation in problem-solving ability and oxytocin immunoreactive responsiveness induced by EE in male rats must also be taken into consideration (Neal et al., 2018).

5. Conclusions

To sum up, our results corroborate that SD produces depressive-like behaviors, increased reinforcing and motivational effects of ethanol and induced greater neuroinflammatory response in susceptible mice, contrary to what occurs in resilient animals. The susceptible phenotype for depressive-like behaviors predicts the increased reinforcing and motivational effects of voluntary ethanol consumption and a larger neuroinflammatory response almost 2 months after the last SD exposure. In addition, we demonstrate that EE promotes the development of adaptive responses to social stress, indicating the importance of exposure to complex environments during adolescence.

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CRediT authorship contribution statement

Marina D. Reguilón: Conceptualization, Formal analysis, Investigation, Methodology, Software, Validation, Writing – original draft.
Carmen Ferrer-Pérez: Formal analysis, Investigation, Methodology, Software, Validation, Writing – original draft. **Carmen Manzanedo:** Methodology, Project administration, Resources, Supervision, Writing – review & editing. **José Miñarro:** Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Writing – review & editing. **Marta Rodríguez-Arias:** Conceptualization, Funding acquisition, Methodology, Resources, Supervision, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors have no possible conflict of interest in the carrying out and reporting of this research.

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