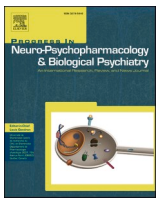
Progress in Neuropsychopharmacology & Biological Psychiatry 119 (2022) 110591

Contents lists available at ScienceDirect

Progress in Neuropsychopharmacology & Biological Psychiatry

journal homepage: www.elsevier.com/locate/pnp



Resilience to social defeat stress in adolescent male mice

Marina D. Reguilón, Raúl Ballestín, José Miñarro, Marta Rodríguez-Arias *

Departamento de Psicobiología, Facultad de Psicología, Universitat de València, Avda. Blasco Ibáñez, 21, 46010, Valencia, Spain

ARTICLE INFO

Keywords:
Social defeat
Susceptibility
Resilience
Neuroinflammation
Cocaine
Ethanol

ABSTRACT

Adverse social experiences during adolescence are associated with the appearance of mental illness in adulthood. Social defeat (SD) is an ethologically valid murine model to study the consequences of social stress. In adolescent mice, SD induces depressive-like behaviors, increased anxiety and potentiates the reinforcing effects of cocaine and alcohol. However, not all mice exposed to SD will be susceptible to these effects. Adult mice resilient to the effects of SD show a consistent phenotype being resilient to depressive-like behaviors and to the increase in cocaine and alcohol consumption. The aim of the present study was to characterize the resilient phenotype to depressive-like behaviors and increase cocaine and ethanol rewarding effects of mice socially defeated during adolescence. To that end, adolescent mice were exposed to repeated SD, and 24 h after the last encounter, they underwent a social interaction test (SIT) in order to evaluate depressive-like behaviors. Cocaine-induced reward conditioning and ethanol intake was evaluated in two different sets of mice 3 weeks after the last SD using cocaine-induced conditioned place preference (CPP) and oral ethanol self-administration (SA). The neuroinflammation response was measured at the end of the experimental procedure by measuring striatal and cortical levels of IL-6 and CX3CL1. The results confirmed that a comparable percentage of adolescent mice develop resilience to depressive-like behaviors to that observed in adult mice. However, increased anxiety was more severe in resilient mice. Likewise, an increased preference for an ineffective dose of cocaine and an increased ethanol consumption was observed in resilient mice compared to controls. The increase in IL-6 and CX3CL1 was mainly observed in the striatum of susceptible mice compared to that of control mice. Our results confirm that, contrary to prior assumptions in adults, responses to SD stress are more complex and singular in adolescents, and caution should be taken for the correct interpretation and translation of those phenotypes.

1. Introduction

Adolescence is a critical period of development characterized, among other behaviors, by an increase in the time spent with peers, a change in the quality of social interaction and frequent appearance of feelings of rejection (Platt et al., 2013; Somerville et al., 2010). Adverse social experiences during adolescence have been strongly associated with the appearance of mental illness in adulthood. Many subjects who have suffered from abuse or have been abandoned by their parents during developmental periods are diagnosed in adulthood with a mental illness such as depression, anxiety or drug addiction (Ho and King, 2021). A recent meta-analysis reported that adverse childhood or adolescent experiences are highly associated with anxiety and depression, costing upwards of billions of dollars annually (Bellis et al., 2019). But the strongest association is found with problematic drug use and interpersonal and self-directed violence (Hughes et al., 2017). Specifically, clinical studies indicate that stressful adolescent experiences increase the risk for substance abuse (Tharp-Taylor et al., 2009; Topper et al., 2011). Due to the close relationship between the brain systems involved in the response to drugs and stress, environmental stressors can produce long-term changes in the brain reinforcement system, inducing the individual to use drugs (Rodríguez-Arias et al., 2013).

Animal models enable the study of the mechanisms through which environmental and psychosocial stressors induce later neuropsychiatric disorders. Social defeat (SD) is considered the most representative

Abbreviations: BP, breaking point; CPP, conditioned place preference; DA, dopamine; ELISA, enzyme-linked immunosorbent assay; EPM, elevated plus maze; FR1, fixed ratio 1; FR3, fixed ratio 3; HPA, hypothalamic- pituitary- adrenal; PFC, prefrontal cortex; PND, postnatal day; Post-C, postconditioning; PR, progressive ratio; Pre-C, preconditioning; SA, self-administration; SD, social defeat; SIT, social interaction test; VTA, ventral tegmental area.

* Corresponding author at: Unidad de Investigación Psicobiología de las Drogodependencias, Departamento de Psicobiología, Facultad de Psicología, Universitat de València, Avda. Blasco Ibáñez, 21, 46010, Valencia, Spain.

E-mail address: marta.rodriguez@uv.es (M. Rodríguez-Arias).

https://doi.org/10.1016/j.pnpbp.2022.110591
Received 26 January 2022; Received in revised form 6 June 2022; Accepted 7 June 2022
Available online 10 June 2022

animal model to study the consequences of social stress (Hammels et al., 2015). SD is an ethologically valid murine model that induces long-term physiological and behavioral changes similar to those seen in depression and anxiety and can mimic the individual differences in the stress response observed in humans (Wang et al., 2021).

SD in adult mice potentiates the reinforcing effects of different drugs, producing an increased intake of cocaine and other psychostimulants on the self-administration (SA) and conditioning of place preference (CPP) paradigms (Ballestín et al., 2021; Covington 3rd et al., 2008; Ferrer-Pérez et al., 2019; Giménez-Gómez et al., 2021; Quadros and Miczek, 2009; Montagud-Romero et al., 2016, 2020; Montagud-Romero et al., 2021; Reguilón et al., 2017; Rodríguez-Arias et al., 2017). A significant increase in alcohol consumption after exposure to SD has also been reported (Montagud-Romero et al., 2021; Reguilón et al., 2020, 2021a; Reguilón et al., 2021b; Rodríguez-Arias et al., 2016). However, the effects of SD during adolescence on subsequent drug abuse or mental health have not been widely investigated.

Neural and behavioral development of rodents is thought to mirror stages of human development (Adriani and Laviola, 2004; Burke and Miczek, 2014). Like adolescent humans, adolescent rodents are highly social, to a greater extent than adult rodents (Do Couto et al., 2009; Yates et al., 2013). Defeated adolescent rats or mice show reduced social behavior, depressive-like behaviors, or increased anxiety similar to what is observed in defeated rodents in adulthood (Huang et al., 2013; Iñiguez et al., 2014; Shimizu et al., 2020). Similar to adults, increases in cocaine SA (Burke and Miczek, 2015), amphetamine-, cocaine-, and alcohol-induced conditioned reinforcement (Burke et al., 2011; Montagud-Romero et al., 2017; Rodríguez-Arias et al., 2017), and oral ethanol SA (Burke and Miczek, 2015; Rodríguez-Arias et al., 2016: Thompson et al., 2020) are observed in socially defeated adolescent rodents.

Nevertheless, not all subjects exposed to stress will develop depressive, anxiety or addictive behaviors. But as in humans, a subset of mice exposed to SD will be susceptible to these effects, developing important disorders such as social inhibition, anhedonia or depressive-like behaviors (Krishnan et al., 2007). However, some rodents will be resilient to these consequences, being able to adaptively cope with stress (Cathomas et al., 2019). We have recently reported that mice resilient to the effects of SD during adulthood show a consistent phenotype; that is, these mice are resilient to the depressive-like behaviors produced by SD, and are also resilient to the reinforcing effects of cocaine and alcohol (Ballestín et al., 2021; Giménez-Gómez et al., 2021; Reguilón et al., 2021b). The response to SD seems to be much more complex in adolescent mice, and this also appears to be the case in the development of an adaptive response to stress. To date, only two studies have evaluated their resilience profile. Both studies reported that only a small proportion of the defeated adolescent mice (between 20 and 30%) were totally susceptible or totally resilient to certain effects of SD. However, these studies did not address the increased susceptibility to drug abuse (Alves-dos-Santos et al., 2020; Vassilev et al., 2021).

The aim of the present study was to characterize the resilient phenotype to depressive-like behaviors and the increased rewarding effects of cocaine and ethanol SA in socially defeated mice during adolescence. To that end, adolescent mice were exposed to repeated SD and, 24 h after the last encounter, underwent a social interaction test (SIT) in order to evaluate depressive-like behavior. Cocaine-induced reward conditioning and ethanol intake was evaluated in two different sets of mice 3 weeks after the last social defeat using cocaine-induced CPP and oral ethanol SA.

Recent studies suggest that the neuroinflammatory response may play an important role in the development of mental illness (Liu et al., 2020a; Soria et al., 2018), as the immune system also regulates the hypothalamic-pituitary-adrenal axis (HPA), thereby modulating the response to a stressful situation (Haroon et al., 2012). It is well known that social stress induces an activation of the immune system with short- and long-term increases in the levels of cytokines and chemokines (Ferle et al., 2020; Ferrer-Pérez et al., 2018; Jiang et al., 2020; Nozaki et al., 2020; Montagud-Romero et al., 2020; Reguilón et al., 2020; Reguilón et al., 2021a). These results have also been confirmed with SD in adolescent mice, which also showed an impairment of integrity in the blood-brain barrier and activation of the microglia (Rodríguez-Arias et al., 2017; Rodríguez-Arias et al., 2018; Zhu et al., 2019). We observed that this neuroinflammatory response is absent in socially defeated mice during adulthood that showed a resilient phenotype to depressive-like behaviors and increased cocaine or ethanol intake (Ballestín et al., 2021; Reguilón et al., 2021b). Therefore, we will also characterize the neuroinflammatory response in defeated adolescent mice after cocaine or ethanol exposure, measuring the IL-6 and CX3CL1 level in the striatum and the prefrontal cortex (PFC).

In summary, we aim to characterize the behavioral response to the conditioned rewarding effects of cocaine and ethanol intake, as well as the neuroinflammatory response in mice with a resilient phenotype to the depressive-like effects induced by social defeat during adolescence.

2. Material and methods

2.1. Subjects

A total number of 77 adolescent male C57BL/6 J mice (Charles River, France) were used in this study. The experimental mice (PND 21) were housed in groups of five 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 a month prior to the 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 (lights off at 08:00 and on at 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 (2019/VSC/PEA/0059 y 2019-VSC-PEA-122).

2.2. Drugs

For CPP, a dose of 1.5 mg/kg of cocaine hydrochloride (Alcaliber laboratory, Spain) was employed and injected intraperitoneally (i.p.). This dose of cocaine was selected based on previous CPP studies showing that doses below 3 mg/kg are sub-threshold (Arenas et al., 2014; Montagud-Romero et al., 2017; Vidal-Infer et al., 2012). Control groups were injected with physiological saline (NaCl 0.9%), which was also used to dissolve the drug. For the oral SA procedure, absolute ethanol (Merck, Madrid, Spain) was dissolved 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 diluted in water.

2.3. Experimental groups and experimental design

In this study, two different sets of mice were employed, all of which were exposed to the SD procedure or exploration from PND 27 to 36. 24 h after the last SD episode, on PND 37, all the mice performed the elevated plus maze (EPM) and the SIT to evaluate depressive-like behaviors. Subsequently, the first set of mice underwent the CPP procedure with 1.5 mg/kg of cocaine on PND 57, after 3 weeks of being undisturbed in their home cages. Mice were characterized as resilient or susceptible depending on their ratios in the SIT. Brain samples were taken at the end of the procedure (PND 65).

Likewise, after performing the SDs, EPM and SIT, the second set of mice initiated the 6% oral ethanol SA protocol on PND 57, 3 weeks after the last defeat, lasting approximately 28 days. During this paradigm, the mice proceeded through the phases of training (7 days), substitution of saccharin for ethanol (10 days), the FR1 (5 days), FR3 (5 days) and PR

(1 day) schedules. At the end of this test, all the mice were sacrificed to obtain the brain samples for further analysis (PND 85).

The experimental design is depicted in Fig. 1.

2.4. Apparatus and procedures

2.4.1. Procedure of social defeat (SD)

Mice in the stress/defeated groups were exposed to 4 episodes of SD during adolescence, each lasting 25 min and consisting of three phases. The initial phase began by introducing the "intruder" (the experimental mice) 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 3rd and Miczek, 2001). In the second phase, the wire mesh was removed from the cage to allow confrontation between the two mice over a 5-min period. Finally, the wire mesh was put back in the cage to separate the two mice once again for a further 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 the cage. The intruder mice were exposed to a different aggressor mouse during each SD episode. The criterion used to define a mouse 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). All agonistic encounters of each SD protocol were videotaped to confirm SD of the intruder mice and to ethologically analyze the threat and attack behaviors (duration and latency) of the resident mice. These behaviors were scored in resident mice and avoidance/flee and defensive/submissive behaviors were evaluated in intruder mice.

2.4.2. Elevated plus maze (EPM)

The EPM test was carried out essentially following the procedure described by Daza-Losada et al. (2009). The maze consisted of two open arms (30 × 5 × 0.25 cm) and two enclosed arms (30 × 5 × 15 cm), and a central platform (5 × 5 cm) elevated 45 cm above floor level. In order to decrease experimental stress, mice were habituated to the experimental room for 1 h prior to testing. At the beginning of each trial, the experimental mice were placed on the central platform facing an open arm and were allowed to explore for 5 min. The behavior displayed by the mice during the test was recorded by an automated tracking system (EthoVision XT 11, Noldus) that tracks the number of entries and time spent in each section of the maze (open arms, closed arms, central platform). The time and percentage of time spent in the open arms were measured to characterize the anxiolytic effects of the SD (Ferrer-Pérez et al., 2018; Rodríguez-Arias et al., 2016).

2.4.3. Social interaction test (SIT)

The social withdrawal ratio 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 the dark cycle and in a different environment from the confrontation sessions. First, mice were transferred to a quiet, dimly lit room 1 h before the test was initiated. After habituation, each mouse was placed in the center of a square arena (white Plexiglas open field, 30 cm each side and 35 cm high) and its behavior was monitored by video (EthoVision XT 11, 50 fps; camera placed above the arena). Mice were allowed to explore the arena twice, for 600 s in each session, during two different experimental sessions. In the first session (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

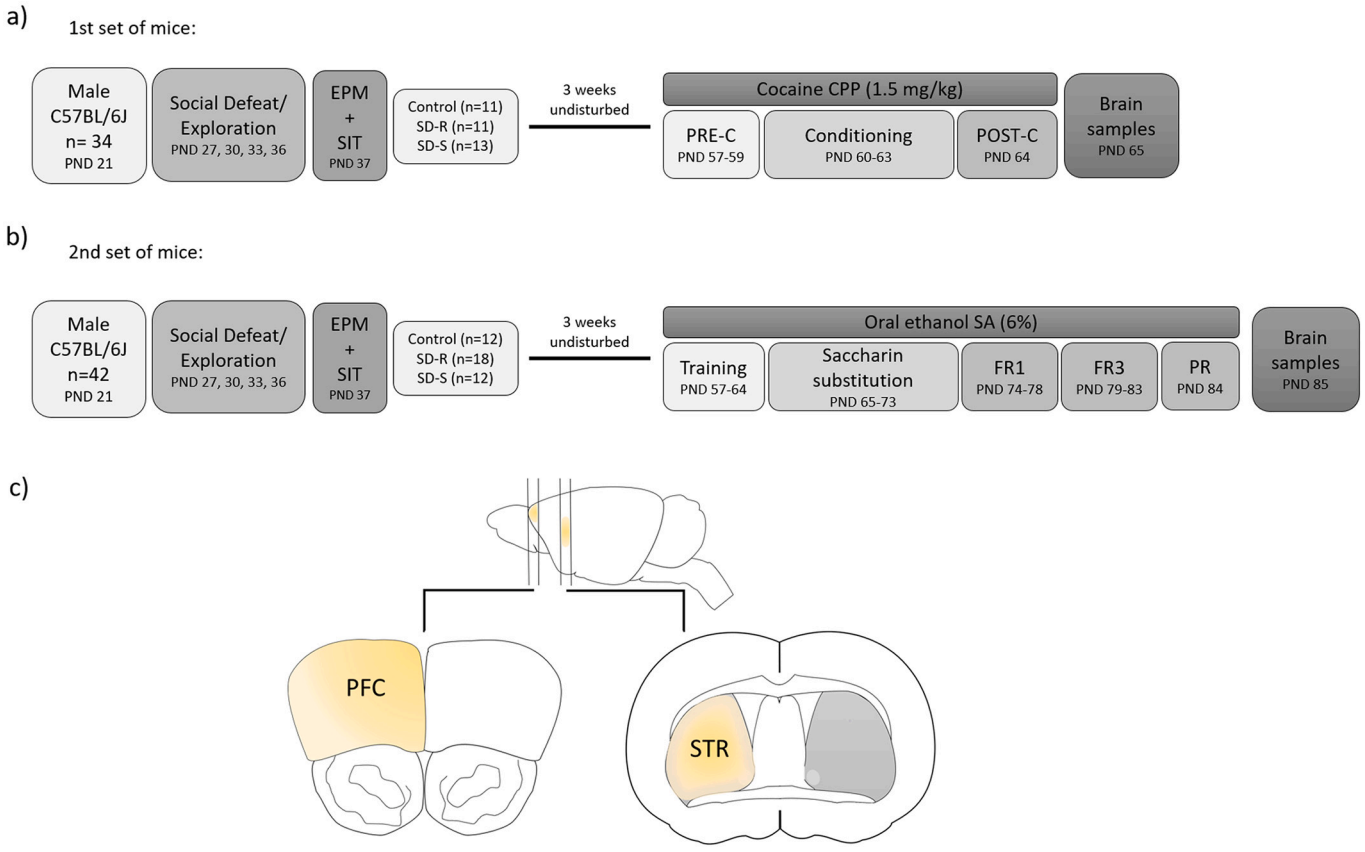


Fig. 1. Experimental design. Experimental protocol of the (a) first and (b) second sets of mice. (c) Diagram of the areas selected for immunoassay analysis. PFC = prefrontal cortex; STR = striatum.

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.

Arena occupancy during object and social sessions was determined using the mice's horizontal position, controlled 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 restrain cage. Corners were defined as two squares of similar areas on the opposite wall of the arena. Social withdrawal ratio 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, mice 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).

2.4.4. Conditioned place preference (CPP)

For place conditioning, we employed eight identical Plexiglas boxes with two compartments of equal size (30.7 × 31.5 × 34.5 cm high) separated by a gray central area (13.8 × 31.5 × 34.5 cm high). The compartments had different colored walls (black vs white) and distinct floor textures (fine grid in the black compartment and wide grid in the white one). Four infrared light beams in each compartment of the box and six in the central area allowed the position of the mice and their crossings from one compartment to the other to be recorded. The equipment was controlled by three computers using MONPRE 2Z software (CIBERTEC, SA, Spain).

Place conditioning, consisting of three phases, was carried out during the dark cycle following a procedure that is unbiased in terms of initial spontaneous preference (Manzanedo et al., 2001). During the first phase -preconditioning (Pre-C)- mice were allowed access to both compartments of the apparatus for 900 s per day on 3 consecutive days. On day 3, the time spent in each compartment was recorded. Mice showing a strong unconditioned aversion (<33% of session time; i.e. 250 s) or preference (>67% of the session time; i.e. 650 s) for any compartment were discarded from the rest of the study. The ANOVA showed no significant differences between the time spent in the drug-paired and vehicle-paired compartments during the Pre-C phase. In the second phase (conditioning), which lasted 4 days, mice were conditioned with 1.5 mg/kg cocaine or saline. During this phase, half of the mice in each group received the drug or vehicle in one compartment, while the other half received it in the other compartment. An injection of physiological saline was administered before confining the mice to the vehicle-paired compartment for 30 min. After an interval of 4 h, the mice received cocaine immediately prior to confinement in the drug-paired compartment for a further 30 min. The central area was made inaccessible by guillotine doors during conditioning. The dose of cocaine used during the conditioning phase was a subthreshold dose (1.5 mg/kg, proven to be ineffective in controls) in order to evaluate increased sensitivity to the conditioned rewarding effects of cocaine. In the third phase -post-conditioning (Post-C)-, which took place on day 8, the guillotine doors separating the two compartments were removed, and the time spent in each compartment by the untreated mice during a 900 s observation period was recorded. The difference in seconds between the time spent in the drug-paired compartment during the Post-C and Pre-C tests is a measure of the degree of conditioning induced by the drug (conditioning score). If this difference is positive, then the drug has induced a preference for the drug-paired compartment, while the opposite indicates an aversion.

2.4.5. Oral ethanol self-administration

This procedure is based on that employed by Navarrete et al. (2014). Oral ethanol SA administration 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 0.5 s stimulus light and a 0.5 s buzzer beep, which was followed by a 6 s time-out period. Inactive nose-pokes did not produce any consequence.

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

2.4.5.1. Training phase (7 days). Two days before the initiation of the experiment, access to the standard diet was restricted to 1 h per day. Before the first training session, water was withdrawn for 24 h, and the food allotment was provided 1 h before the session to increase the motivation for active nose-poking. During the subsequent three days, water was provided ad libitum, except during the 1 h 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 1 h 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.

2.4.5.2. Saccharin substitution (10 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).

2.4.5.3. 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, a fixed ratio 3 (FR3) (mice had 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 progressive ratio (PR) session was completed to establish the breaking point (BP) for each mouse (the maximum number of nose-pokes each mouse 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 BP was calculated for each mouse as the maximum number of consecutive responses performed to achieve one reinforcement according to the previous scale. For example, if a mouse 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 BP value for this mouse would be 40. All the sessions lasted one hour, except the PR session, which lasted two hours.

2.4.6. Immunoassay analysis (ELISA)

Samples from the striatum and the PFC were obtained 24 h after

cocaine CPP and oral ethanol 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; Franklin and Paxinos, 2008, see Fig. 1c). The striatum and PFC 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). 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 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.

Some mice were discarded after measuring concentrations by ELISA due to a lack of signaling, and a few others were considered outliers.

2.5. Statistical analysis

Mice had been previously classified into resilient and susceptible groups based on their social withdrawal ratios. The social withdrawal ratio 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 on 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, mice 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. The data of the time that the experimental mice and their aggressive opponents spent engaged in different behavioral categories during the SD episodes were compared by means of a mixed two-way ANOVA with one between-subject variable Stress, with two levels (Resilient and Susceptible); and one within subject variable Days, with two levels (1st and 4th SD). To evaluate the CPP induced by 1.5 mg/kg of cocaine, the conditioning scores were analyzed with a one-way ANOVA with a between-subjects variable –Stress, with three levels (Control, SD-R and SD-S). To analyze the acquisition of ethanol SA, a two-way ANOVA was performed with one between-subjects variable –Stress with three levels (Control, SD-R and SD-S)– and a within-subjects variable –Days, with five levels of FR1 or FR3. The effects of SD and treatment on BP 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, as well as the EPM, were analyzed using a one-way ANOVA with one between-subjects variable –Stress, with three levels (Control, Resilient and Susceptible). For the data of the ethological analyses of SD and the EPM, all mice were analyzed together (1st and 2nd set) as the encounter, or the test occurred before the initiation of the cocaine CPP or the oral ethanol SA.

In all the studies, following the ANOVA, Bonferroni post-hoc tests were calculated whenever required. Statistical analyses were performed using SPSS Statistics (v.26; IBM, NY, USA) for behavioral data and GraphPad Prism (v8; GraphPad Software Inc., CA, USA) for graph design. Data were expressed as mean ± SEM and a value of $p < 0.05$ was considered statistically significant.

3. Results

3.1. Classification between susceptible and resilient mice according to their social withdrawal ratios

In the first set of experimental mice (Fig. 2a), the Control group ($n = 11$) showed a mean social interaction ratio >1. Of 24 SD mice, 54% had interaction ratio <1 and 46% showed a ratio equal to or higher than 1. We classified mice with an interaction ratio <1 as SD-S mice ($n = 13$) and those with an interaction ratio greater than or equal to one as SD-R mice (n = 11).

In the second set of experimental mice (Fig. 2b), the Control group ($n = 12$) showed a mean ratio higher than 1. In the SD group of mice ($n = 30$), 40% showed a ratio under 1, which classifies them as susceptible (SD-S) mice (n = 12), and the remaining 60% showed a ratio equal to or higher than 1, which classifies them as resilient (SD-R) mice ($n = 18$).

3.2. All defeated adolescent mice increased passive-reactive coping during the 4th social defeat

The ANOVA for the time employed in Avoidance/Flee or Submissive/Defensive behaviors (Table 1) by defeated mice divided into resilient or susceptible according to their SIT scores showed an effect of the variable Days [$F(1,52) = 16.5$; $p < 0.001$] and [$F(1,52) = 9.1$; $p < 0.001$]. Likewise, the ANOVA for the latency to show Avoidance/Flee or Submissive/Defensive behaviors for the first time revealed an effect of the variable Days [$F(1,52) = 16.2$; $p < 0.001$] and [$F(1,52) = 25.9$; $p < 0.001$]. Defeated adolescent mice, classified as either resilient or susceptible, increased the time spent in these behaviors and were quicker to show them during the 4th social defeat ($p < 0.01$ for time spent in Submissive/Defensive behaviors and $p < 0.001$ for the rest of comparisons). The lack of differences depending on the SIT means that susceptible and resilient mice cope similarly with social defeat stress.

The ANOVAs for the time employed in Attack or Threat behaviors (Table 1) by resident mice showed an effect of the variable Days [$F(1,52) = 16.9$; $p < 0.001$] and [$F(1,52) = 8.1$; $p < 0.01$]. Resident mice decreased the time spent in these behaviors during the 4th SD compared to the 1st SD ($p < 0.001$ for time spent in Attack behavior and $p < 0.01$ for the time spent in Threat behavior). The ANOVAs for the latency to perform the first Attack revealed an effect of the interaction Days x Group [$F(1,52) = 4.9$; $p < 0.05$] and an effect of the variable Days [$F(1,52) = 6.7$; $p < 0.05$] for the latency to perform the first Threat behavior. Resident mice threatened faster in the 4th SD in comparison to the 1st SD in all groups ($p < 0.05$). However, resident mice attacked faster during the 4th SD only when attacking the SD-S group ($p < 0.001$).

3.3. Social defeat during adolescence induced anxiogenic effects in resilient mice

One outlier in time spent in the closed arms was removed from the Control group, along with two outliers in time spent in the open arms and in percentage of time in the open arms, and one outlier in the number of entries to the open arms, in total entries and in percentage of entries to the open arms in the SD-S group.

The data of the EPM test are presented in Fig. 3. The ANOVA of the time spent in closed arms [$F(2,72) = 4.5$; $p = 0.014$]; time spent in the open arms [$F(2,72) = 3.9$; $p = 0.023$]; percentage of time spent in the open arms [$F(2,72) = 3.5$; $p = 0.034$]; number of entries into the open arms [$(2,73) = 3.6$; $p = 0.031$], and percentage of entries into the open arms [$(2,73) = 4.4$; $p = 0.015$] revealed a significant effect of the variable Stress. Post-hoc analyses showed that resilient defeated mice

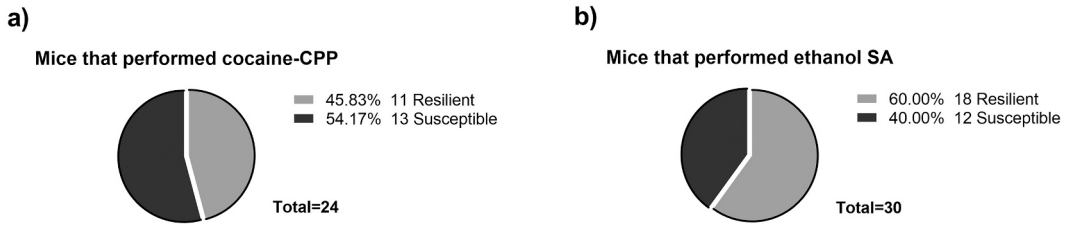


Fig. 2. Percentages of resilient and susceptible mice among groups of mice defeated during adolescence in the two experimental sets. The pie chart represents the percentage of resilient vs susceptible mice after social withdrawal ratio evaluation in the SIT in a) defeated mice that performed cocaine-CPP and b) defeated mice that performed ethanol SA paradigm.

Table 1
Coping behavior of the intruder mice during SD.

			SD-R		SD-S	
			1st SD	4th SD	1st SD	4th SD
Intruder mice	Submissive/ Deffensive	Time (s)	35 ± 4	50 ± 4 **	39 ± 4	52 ± 5 **
		Latency (s)	30 ± 6	11 ± 2 ***	40 ± 8	5 ± 1 ***
	Avoidance/ Flee	Time (s)	74 ± 7	116 ± 12 ***	72 ± 8	101 ± 15 ***
		Latency (s)	17 ± 3	7 ± 2 ***	26 ± 7	4 ± 1 ***
Resident mice	Attack	Time (s)	61 ± 5	38 ± 4 ***	60 ± 6	44 ± 4 ***
		Latency (s)	21 ± 3	19 ± 5	29 ± 6	9 ± 2 ##
	Threat	Time (s)	31 ± 5	17 ± 2 **	30 ± 5	26 ± 3 **
		Latency (s)	23 ± 5	17 ± 5 *	33 ± 10	10 ± 2 *

Results are presented as mean values ±SEM. Mice were divided into resilient (n = 29) and susceptible (n = 25) depending on their SIT scores. Bonferroni post-hoc test *p < 0.05, **p < 0.01, ***p < 0.001 significant difference compared to the 1st SD. ###p < 0.001 significant difference compared to the 1st SD in SD-S group.

according to their SIT scores spent more time in the closed arms, and less time and a lower percentage of time in the open arms than control non-stressed mice (p < 0.05 in all cases). Moreover, a lower number and percentage of entries into the open arms was registered in resilient defeated mice (p < 0.05 in all cases) with respect to the control group.

3.4. Mice considered resilient according to their SIT scores developed a preference for cocaine-induced CPP

The ANOVA for the time spent in the drug-paired compartment (Fig. 4a) showed a significant effect of the variable Days [F(1, 37) =5.2, p = 0.030], and the interaction Days x Stress [F(1,31) =3.3, p = 0.05]. Likewise, the ANOVA of the Conditioning Score (Fig. 4b) showed an effect of the variable Stress [F(2,31) = 4.7; p < 0.016]. Only resilient mice according to their SIT scores developed a preference for this cocaine dose and a significant increase in the time spent in the drug-paired compartment during the Post-C test was observed (p ⟨0,01). Consequently, higher conditioning scores were observed in the resilient mice than control mice (p < 0.01).

In addition, the analysis of the differences between the drug-paired and vehicle-paired compartments during the Pre-C and Post-C phases can be found in Table A of the supplementary material.

3.5. Increased neuroinflammatory response observed following cocaine-induced CPP in susceptible mice compared to control mice

The number of samples in each group was 10 for IL-6 and between 11 and 13 for CX3CL1. For IL6 determination, we lost 2 samples in the striatum of the control group (outliers), and another 2 in the striatum of the SD-R group (one outlier and one due to lack of signaling).

The ANOVA for the Il-6 levels in the striatum (Fig. 5a) showed a significant effect of the variable Stress [F(2,26) = 3.9; p < 0.034]. A higher concentration of Il-6 was observed in the striatum of susceptible mice according to their SIT scores compared to non-stressed control mice (p < 0.05). No differences were observed in the cortex (Fig. 5b).

With respect to fractalkine or CX3CL1 levels, although there were no differences in the striatum (Fig. 5c), a higher concentration was observed in the PFC of both susceptible and resistant mice [F(2,35) = 4.5; p < 0.019] compared to non-stressed controls (p < 0.05 in both cases) (Fig. 5d).

3.6. Resilient mice showed higher ethanol intake than control mice

No differences were found in the active responses or between the different groups during the training or substitution phases, demonstrating that SD did not induce any learning deficits. No differences were found in the body weight of the mice during the FR1, FR3 and PR schedules. Analyses of the acquisition and substitution phases of SA and body weights during the FR1, FR3 and PR schedules can be found on the supplementary material.

The ANOVA for the number of active responses during FR1 schedule (Fig. 6a) did not reveal any significant effects, although there was a tendency toward a Stress effect [F(2,39) = 3.1; p = 0.058). Resilient mice tended to perform more active responses than controls (p < 0.058). With respect to ethanol consumption, the ANOVA revealed a significant effect of the variable Stress [F(2,39) = 4.5; p = 0.018) (Fig. 6b). The post-hoc comparison showed that the resilient mice consumed ethanol at higher rates than the control (p < 0.05) and susceptible mice (p < 0.01).

During the FR3 schedule, the ANOVA of the number of active responses (Fig. 6a), the ANOVA revealed a significant effect of the variable Days [F(4,156) = 4.4; p < 0.002] and Stress [F(2,39) = 6.3; p < 0.004]. All mice made significantly more active responses on days 9 and 10 than on day 6 (p < 0.01 for day 9, and p < 0,05 for day 10). Moreover, resilient mice performed more active responses during the FR3 than non-stressed controls (p < 0.01). With respect to the ethanol consumption, the ANOVA revealed a significant effect of the variable Days [F(4,156) = 16.4; p < 0.001] and Stress [F(2,39) = 6.3; p < 0.004] (Fig. 6b). All mice consumed significantly more ethanol on days 7, 8, 9 and 10 than on day 6 (p < 0.001 in all cases). Moreover, resilient mice consumed more ethanol during the FR3 than non-stressed controls (p < 0.01).

During the PR, the ANOVA for the BP values of ethanol SA revealed a significant effect of the variable Stress [F(2,39) = 4.9; p < 0.012] (Fig. 6c). The post-hoc comparison showed that resilient mice achieved higher BP values than the control group (p < 0.01). The ANOVA for ethanol consumption during PR did not reveal a significant effect of the variable Stress (Fig. 6d).

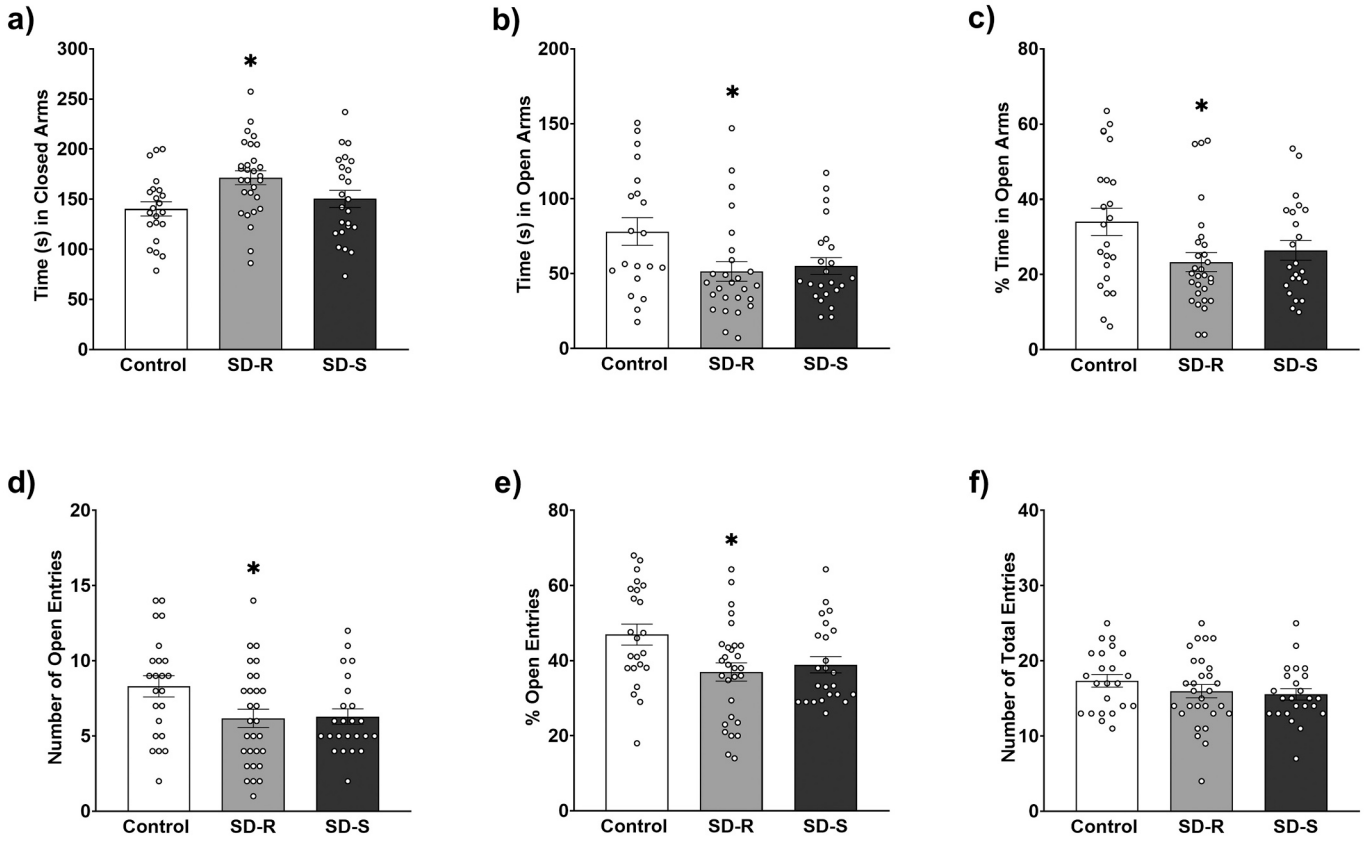


Fig. 3. Long term effects of SD on anxiety-like behavior. Bars represent the mean (± SEM) of (a) time in closed arms in seconds, (b) time in open arms in seconds, (c) percentage of time in open arms, (d) number of open entries, (e) percentage of open entries and (f) number of total entries. Bonferroni post-hoc test * $p < 0.05$, significant difference compared to the control group.

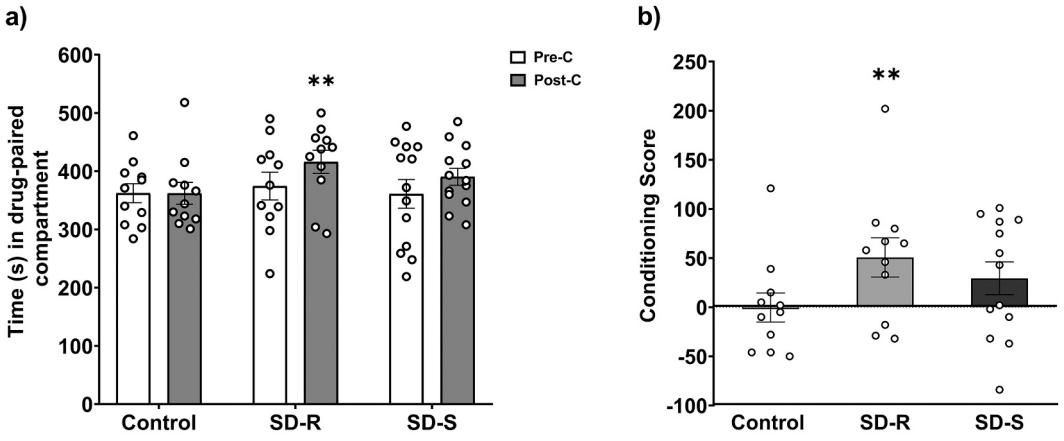


Fig. 4. Resilient mice showed higher preference in cocaine-induced CPP than susceptible mice. Effect of adolescent SD on cocaine-induced CPP. Mice were divided into Control ($n = 11$); Resilient (n = 11) and Susceptible ($n = 13$). Defeated mice were characterized as resilient or susceptible depending on their SIT scores. a) The bars represent the time (in seconds) spent in the drug-paired compartment before conditioning sessions in the pre-conditioning test (Pre—C) (white bars) and after conditioning sessions in the post-conditioning test (Post-C) (gray bars), during which CPP was induced with 1 mg/kg of cocaine. b) The bars represent the conditioning score (difference in seconds between the time spent in the drug-paired compartment after the conditioning sessions and that spent in the same compartment during Pre—C). **$p < 0.01$ significant difference in the time spent in the drug-paired compartment vs Pre-C session or with respect to the control group.

3.7. Increased neuroinflammatory response observed in susceptible mice compared to control mice following oral ethanol self-administration

The number of samples in each group was between 11 and 13 for IL-6 and between 12 and 14 for CX3CL1. For CX3CL1 determination, we lost 3 samples in the SD-R group (three outliers).

The ANOVA for the Il-6 levels in the striatum (Fig. 7a) showed a significant effect of the variable Stress [$F(2,36) = 3.9$; $p < 0.023$]. A higher concentration of Il-6 was observed in the striatum of susceptible mice according to their SIT scores compared to the non-stressed control group ($p < 0.05$). No differences were observed in the PFC (Fig. 7b).

With respect to fractalkine or CX3CL1 levels in the striatum (Fig. 7c) the ANOVA showed a significant effect of the variable Stress [$F(2,33) = 3.8$; $p < 0.03$], as significantly elevated levels were observed in the

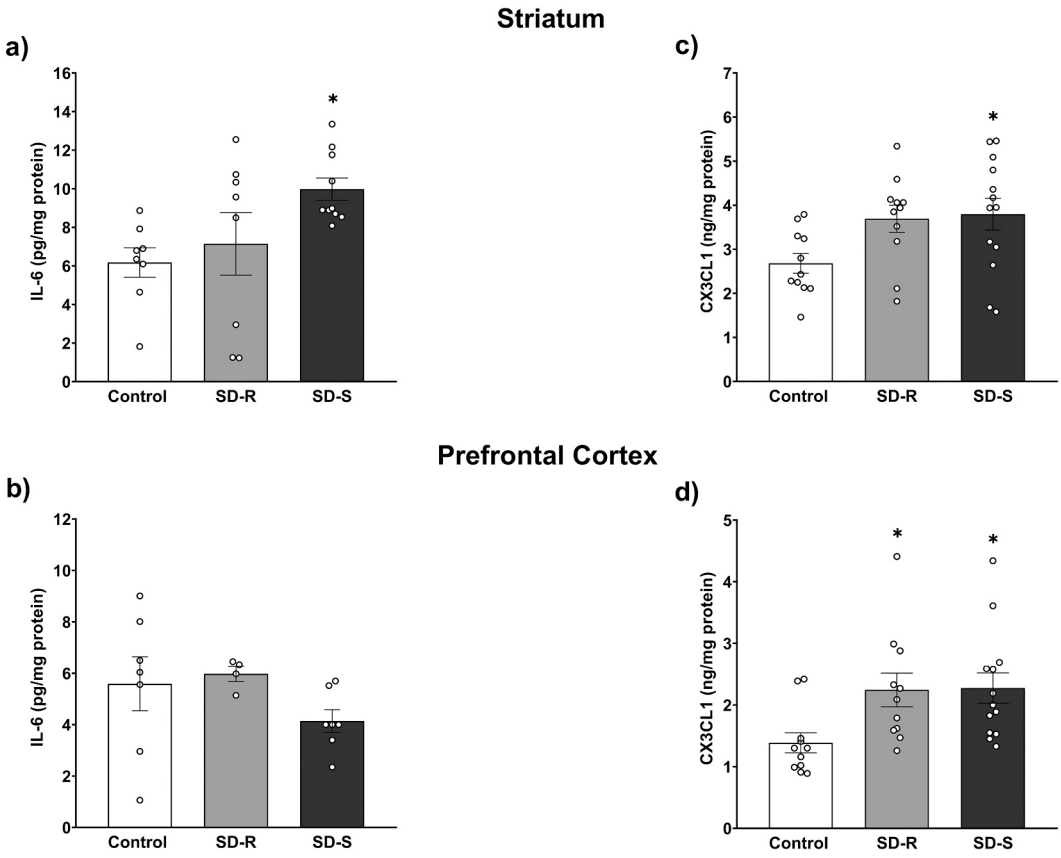


Fig. 5. Susceptible mice showed higher neuroinflammatory markers after cocaine-induced CPP than resilient mice. Effect of repeated SD on striatal and cortical levels of IL-6 and CX3CL1. Bars represent the mean (± SEM) of the striatal (a) and cortical (c) levels (in pg/mg) of the pro-inflammatory cytokine IL-6. Similarly, bars represent the mean (± SEM) of the striatal (b) and cortical (d) levels (in ng/mg) of the pro-inflammatory chemokine CX3CL1 and the vertical lines ± SEM. Bonferroni post-hoc test * $p < 0.05$, significant difference compared to the control group.

susceptible mice compared to the control group ($p < 0.03$). No differences were observed in the PFC (Fig. 7d).

4. Discussion

A recent meta-analysis reported that 62.5% of individuals start to show signs of mental disorders by the age of 25, with a peak at 14.5 years of age (Solmi et al., 2021). Although there is strong evidence linking bullying and later mental illness (McKay et al., 2021), only few adolescents suffering from these traumatic events will develop psychiatric disorders (Dumont and Provost, 1999; Aarestad et al., 2021). Studies suggest that a correct reaction of the body is crucial for an adaptive response to the environment and to avoid stress-related deficits (Cathomas et al., 2019; Dutcher and Creswell, 2018). We already know that a percentage of adult rodents exposed to SD will show a resilient phenotype to social avoidance and to the increase in the rewarding effects of cocaine and ethanol intake (Ballestín et al., 2021; Giménez-Gómez et al., 2021; Reguilón et al., 2021b). However, few studies have been performed to know whether stressed adolescent mice will show a consistent resilient phenotype, as adult mice do. The fact that the developmental process of resilience seems to strengthen over time, together with the increased salient value of social interactions in adolescent mice, indicates that the study of the resilient phenotype in adolescent defeated rodents is highly needed (Sheth et al., 2017; Malhi et al., 2019).

Our results showed that mice defeated during adolescence showed marked differences in their resilient response in comparison with the experience of SD during adulthood. Resilience to the detrimental effects of experiencing SD during adolescence did not develop as a unique phenotype. Mice resilient to depressive-like behavior showed an increased anxiogenic behavior, a higher response to cocaine and higher ethanol intake compared to the control group. However, the increased neuroinflammatory response was present mainly in the mice susceptible to depressive-like behaviors compared to the control group, despite showing a normal response to cocaine and ethanol.

4.1. Adolescent defeated mice showed behavioral flexibility coping with stress

We know that passive coping strategies in response to social stress are associated with more pronounced physiological effects and psychopathology (Hawley et al., 2010; Russo et al., 2012; Wood and Bhatnagar, 2015). Submissive and immobile behavior are considered passive-reactive coping strategies. Meanwhile, active coping is characterized by longer latency to display the defeat posture, fight-back or active escape (Koolhaas et al., 2007; Wood et al., 2010).

We have previously reported that adult mice resilient to the increase in cocaine reward display fewer flee/avoidance and submissive/defensive behaviors during SD than those categorized as susceptible according to their SIT scores (Ballestín et al., 2021; Ródenas-González et al., 2021). In contrast with these results, there were no differences between resilient and susceptible adolescent mice in the way of coping with SD. All defeated adolescent mice showed an increase in the time spent in defensive or flee behaviors in the fourth SD and presented shorter latencies to show these behaviors. The increased time in these behaviors indicates behavioral flexibility to the inescapable SD experience and is characteristically observed only in resilient adult mice. Behavioral flexibility has been associated with emotional resilience, less reactivity of the HPA axis, and increased neuroplasticity (Hawley et al., 2010;

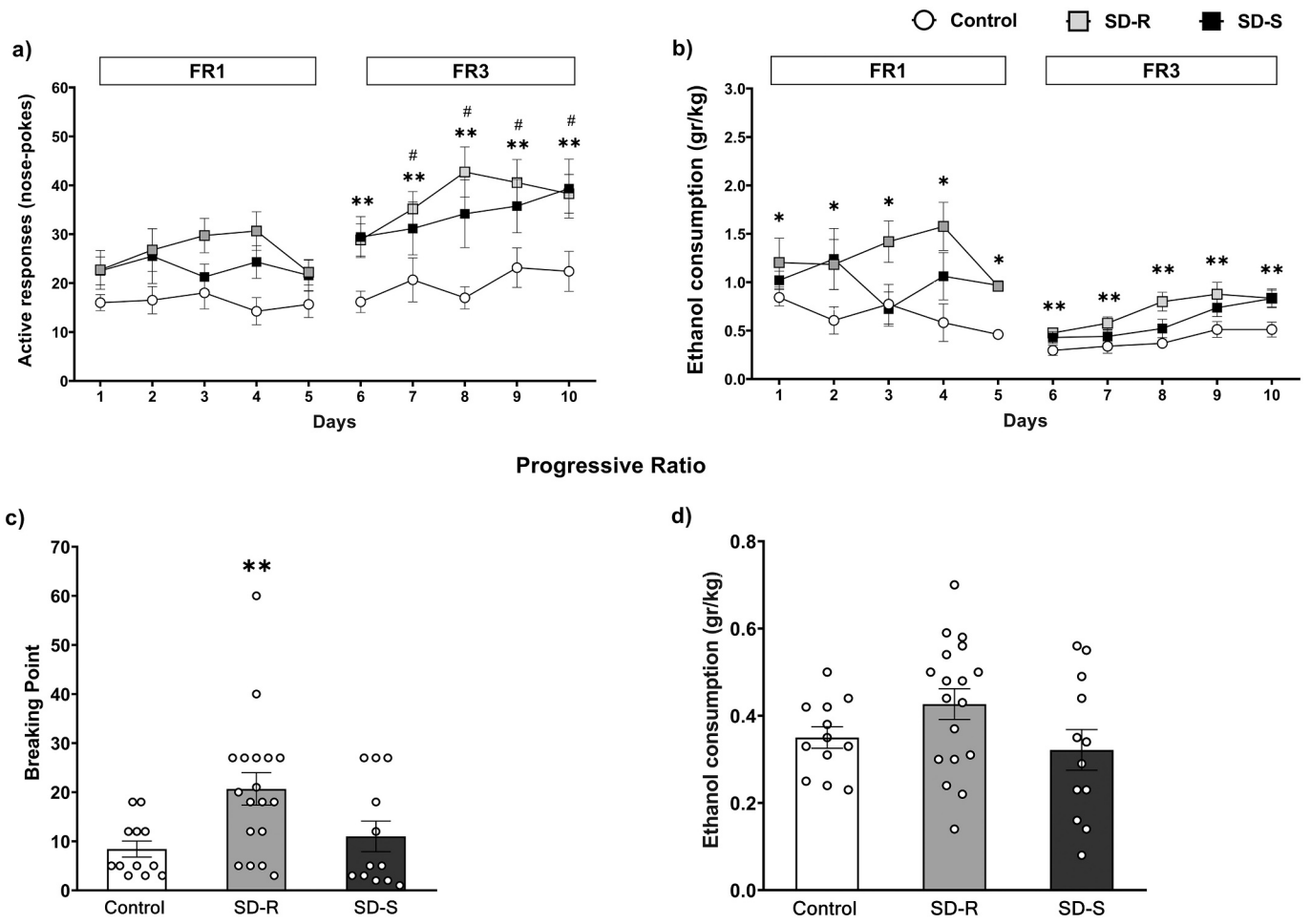


Fig. 6. Resilient mice showed higher ethanol intake than susceptible mice. Mice were divided into Control ($n = 12$); Resilient ($n = 18$) and Susceptible (n = 12). Defeated mice were characterized as resilient or susceptible depending on their SIT. The dots represent means and the vertical lines ± SEM of (a) the number of active responses; (b) the volume of 6% ethanol consumption during FR1 and FR3; (c) the BP values; and (d) the volume of 6% ethanol consumption during PR. *p < 0.05, **p < 0.01 significant difference with respect to controls; #p < 0.05 significant difference with respect to day 6.

Lambert et al., 2014). Therefore, the adolescent response to SD indicates an adequate adaptation to stress.

Despite this adaptive response to SD, the percentage of resilient and susceptible adolescent mice classified according to the SIT, which evaluates depressive-like behaviors, is similar to that observed in adult defeated mice (Giménez-Gómez et al., 2021), with 53% of adolescent defeated mice showing a resilient phenotype to depressive-like behaviors. Albeit with slightly methodological differences, this percentage was also observed in the study of Alves-dos-Santos et al. (2020) and Vassilev et al. (2021) with adolescent defeated mice.

One limitation of the present study is the fact that resilient and susceptible mice were housed together in the same cage throughout the entire procedure. Mice were housed in groups of 4 when they arrived at the laboratory on PND 21 and resilient or susceptible phenotypes to depressive-like behaviors were evaluated 24 h after the last SD on PND 37. Housing resilient and susceptible mice together at that moment would have implied a deeply stressful hierarchical reorganization in each cage, which could affect the behavioral and biochemical results. Therefore, housing resilient and susceptible mice in the same cage must be taken into consideration as a potential variable that could affect the results obtained. Another variable to take into account is the fact that injuries derived from confrontations within the home cage could affect neuroinflammatory markers. However, we find this possibility very unlikely, as the condition of the mice was monitored daily and injuries among adolescent C57BL/6 J strain mice were not observed.

4.2. Anxiogenic response in resilient mice

It has been extensively established that SD induces an acute anxiogenic response (Albrechet-Souza et al., 2017; Ferrer-Pérez et al., 2019; Macedo et al., 2018), an effect that is lacking in resilient mice. We have previously observed that adult mice defeated during adulthood that present a resilient phenotype to depressive-like behaviors and the rewarding effects of cocaine did not present this anxiety increase. In addition, all the environmental or pharmacological treatments that increase the percentage of resilient mice to SD also increase the percentage of mice that did not experience an anxiogenic response (Giménez-Gómez et al., 2021). However, when the SD took place during adolescence, the results are controversial and the experimental protocol used to induce social stress and the time proximity between behavioral tests and stress play a key role in the response observed. Several reports confirmed that after 3 weeks of the last SD there were no observable effects in the EPM in defeated adolescent mice (Rodríguez-Arias et al., 2016; Watt et al., 2009). Alves-dos-Santos et al. (2020) did not observe anxiety-like behaviors in the EPM test when compared to control mice. In that case, the EPM took place in the last days of 10 sessions of chronic social defeat stress and mice were isolated during the entire procedure, which could affect the response. However, in agreement with Iñiguez et al. (2014), we observed that all defeated mice showed an increase in anxiogenic behaviors 24 h after the last encounter, but only resilient mice to depressive-like behavior spent less time and percentage of time in the open arms. Different results were observed by Vassilev et al. (2021) with

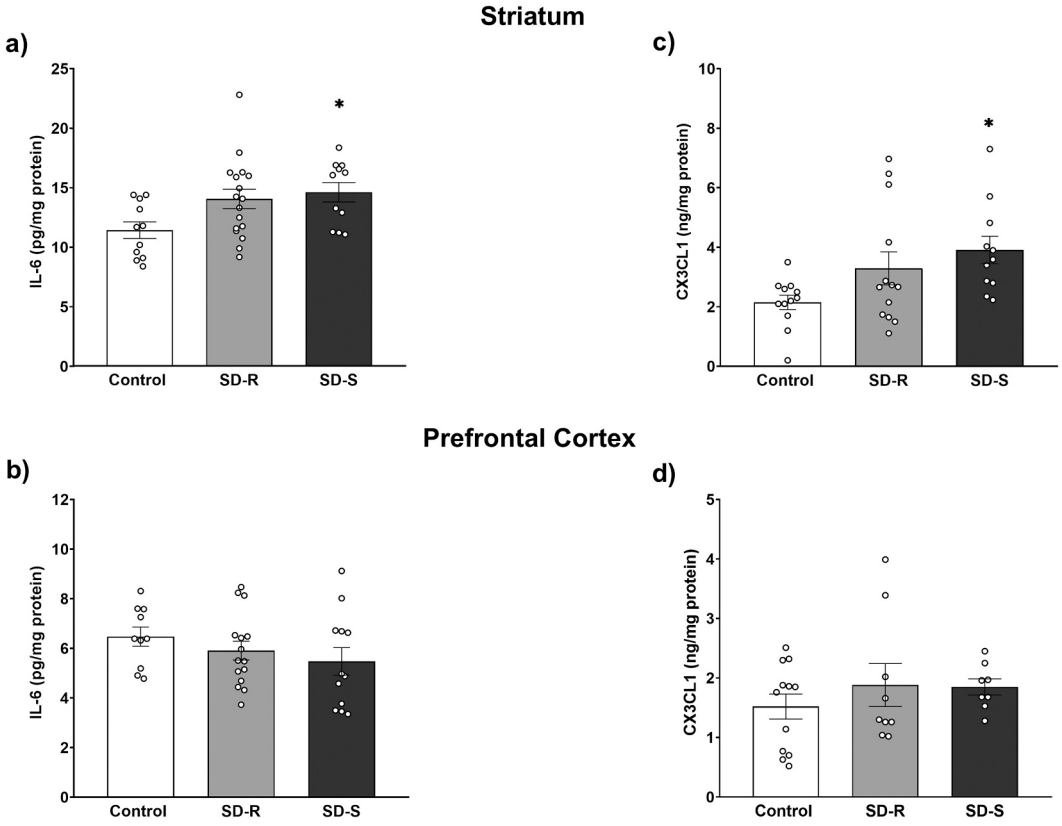


Fig. 7. Susceptible mice showed higher neuroinflammatory markers after oral ethanol self-administration than resilient mice. Bars represent the mean (± SEM) of the striatal (a) and cortical (c) levels (in pg/mg) of the pro-inflammatory cytokine IL-6. Similarly, bars represent the mean (± SEM) of the striatal (b) and cortical (d) levels (in ng/mg) of the pro-inflammatory chemokine CX3CL1 and the vertical lines ± SEM. Bonferroni post-hoc test * $p < 0.05$, significant difference compared to the control group.

no increased anxiety observed in susceptible mice, but surprisingly, in resistant mice an anxiolytic response was observed with an increase in the time spent in the open arms, which the authors suggest may be due to higher propensity for risk-taking-like behaviors in resilient adolescent mice.

Therefore, our results indicate that, differently from the adult response to SD, adolescent mice developed a partial resilient response to anxiety and this was not associated with depressive-like behaviors.

4.3. Increased response to cocaine and ethanol in resilient mice compared to the control group

As we have previously reported for adult mice, a subset of defeated adolescent mice developed a preference for cocaine (Ballestín et al., 2021; Giménez-Gómez et al., 2021). However, different from adult defeated mice, adolescent mice resilient to depressive-like behaviors according to their SIT scores showed a preference for this dose of cocaine. On the other hand, susceptible mice showing social avoidance did not develop a preference for cocaine. However, we should note that a significant preference for the drug-paired compartment compared to the vehicle-paired compartment was observed during the post-conditioning phase in all defeated mice (see Table A in the Supplementary Material). Although SD-S mice did not develop a preference or an increase in the conditioning score, social defeat seems to exert some effect on cocaine preference compared to vehicle administration.

In line with the results observed with cocaine, resilient adolescent mice according to their SIT scores showed higher ethanol intake than control mice. Once again, defeated adolescent mice behave differently from adults. Resilient adult mice according to their SIT scores showed a complete phenotype with no social avoidance and drinking a similar amount of ethanol as non-stressed control mice. On the other hand, defeated adult mice susceptible to social avoidance presented a significantly higher ethanol intake (Reguilón et al., 2021b).

In our defeated adolescent mice, the analysis of the two phenotypes (depressive-like behaviors and response to cocaine or ethanol) showed that, among mice resilient to depressive-like behaviors (with SIT scores superior to 1), 45% did not develop a preference for cocaine (see Table 2). Based on previous studies with this strain of mice, we considered that an increase similar or superior to 60 s was the minimum increase necessary to develop a preference (Ballestín et al., 2021; Giménez-Gómez et al., 2021). Therefore, only 21% of the defeated mice showed resilience to both phenotypes. Similar results were also obtained in the 2nd experiment, with only 33% of resilient mice depending on the SIT drinking ethanol similarly to control mice. In that case, mice that drank ethanol more than two standard deviations from the control mean were considered susceptible. As in the 1st experiment, 20% of the defeated mice showed a complete resilient phenotype. Therefore, there is an inconsistent development of resilience between depressive-like

Table 2
Percentage of resilient and susceptible mice.

	SWR	Cocaine preference		Fully Resilient /Susceptible
Resilient	46%	>60 s (susceptible)	55%	21%
		<60 s (resilient)	45%	
Susceptible	54%	>60 s (susceptible)	38%	21%
		<60 s (resilient)	62%	
	SWR	Ethanol intake (ml/g)		Fully Resilient /Susceptible
Resilient	60%	>7 (susceptible)	67%	20%
		<7 (resilient)	33%	
Susceptible	40%	>7 (susceptible)	58%	23%
		<7 (resilient)	42%	

behaviors and response to drug reward.

4.4. Increased neuroinflammatory response in susceptible mice compared to the control group

Psychological stress induces a series of neuroimmune reactions involving a bidirectional brain-immune signaling that affects mood and behavior (Wohleb et al., 2015). Long-term increments in proinflammatory cytokines such as IL-6 levels after repeated SD have been described in several mice brain areas (Ferrer-Pérez et al., 2018; Montagud-Romero et al., 2020; Montagud-Romero et al., 2021; Reguilón et al., 2021b). In adult mice, resilience to depressive-like behaviors and the increase in cocaine or ethanol reward are associated with a minor neuroinflammatory response. In susceptible mice, an increase in IL-6 levels was observed compared to those of controls or resistant mice shortly after SD or after cocaine or oral ethanol SA (Ballestín et al., 2021; Giménez-Gómez et al., 2021; Reguilón et al., 2021b). These results have also been confirmed in adolescent defeated mice. Although no increased response to cocaine or ethanol was observed in the susceptible mice, an increase in IL-6 levels was observed in the striatum after cocaine-induced CPP and oral ethanol SA, compared to the control group.

With respect to the chemokines response, SD induced changes in CX3CL1 or fractalkine, which seems to depend on the mouse strain used, probably due to their different sensitivity to social stress. Although SD induces increases of striatal CX3CL1 levels in OF1 mice (which are highly territorial) (Reguilón et al., 2020; Reguilón et al., 2021a), in C57BL/6 mice the opposite result was found. Both resilient and susceptible mice, decreases in striatal fractalkine levels were observed immediately after the last SD and even after cocaine-induced CPP (Ballestín et al., 2021). Moreover, after oral ethanol SA, decreases were only observed in susceptible mice (Reguilón et al., 2021b). Surprisingly, compared with the control group, increased levels of CX3CL1 were observed in the striatum of adolescent susceptible C57BL/6 mice following exposure to cocaine or ethanol, and cortical levels were increased in all adolescent defeated mice only following exposure to cocaine. Only few studies have evaluated this chemokine in adolescent mice, but a recent study by Liu et al. (2020b) found increased CX3CL1 expression in the hippocampus of adolescent mice exposed to nicotine during gestation and lactation.

Exposure to social stress during early adolescence produced a permanent alteration of microglia morphology and the induction of an inflammatory episode in the ventral tegmental area (VTA) (Lo Iacono et al., 2018). This inflammatory episode altered the functionality of dopaminergic neurotransmission in the VTA following exposure to a cocaine-CPP in adulthood. The authors of this study concluded that social stress during early life sensitizes the reward pathway and the immune response. In line with this, the inflammatory responses observed in our study may be potentiated by the profound alteration of the immune system produced by social defeat in combination with subsequent exposure to substances of abuse.

4.5. Characteristic development of resilience to SD in adolescent mice

In these experiments, we demonstrated that experiencing SD during adolescence presents specific characteristics. In agreement with the few studies performed in this area, defeated adolescent mice did not develop a general resilient/susceptible phenotype. There is no correlation between the resilience to social avoidance and the increased response to cocaine and ethanol or the neuroinflammatory response. Although, differently from adults, all adolescent mice presented an adaptive coping mechanism with stress, the percentage of resilient/susceptible mice after SD is comparable to that observed in adult mice. Increased anxiogenic behavior, preference for the cocaine-paired compartment or ethanol intake were observed in mice resilient to the development of social avoidance. However, resilience to social avoidance correlated with a minor neuroinflammatory response. These results indicate that the age of exposure to SD affects the development of resilience.

In line with our results, Alves-dos-Santos et al. (2020) observed that defeated adolescents resilient to anhedonia or social avoidance were the most affected mice in terms of both endocrine/physiological outcomes (body weight gain and corticosterone response). Likewise, Vassilev et al. (2021) observed that, in adolescence, SD produces inhibitory control impairment independently from social avoidance. As with ours, all these studies have been performed only in males, which is an important limitation of the present investigation. Marked sex differences in stress responses have been reported in adult rodents and humans (for reviews, see Hodes and Epperson, 2019; and Wellman et al., 2018), but limited data in female rodents during adolescence are available.

Resilience should be considered an active process, which affects both passive and active strategies, in order to achieve the highest adaptation to stress (Russo et al., 2012). Responses on each particular system may develop differently after exposure to stress (Smith, 2019). Our results suggest that SD during adolescence leads to an addiction-prone phenotype in some mice, which manifests itself as resilient during the SIT and presents a normalized neuroinflammatory response.

The response to drugs of abuse is based on their rewarding properties, which depend on the function of the mesocorticolimbic dopaminergic system. Vassilev et al. (2021) observed that SD during adolescence, but not in adulthood, dysregulates the Netrin-1/DCC pathway in the VTA and the nucleus accumbens, which induced changes in dopamine (DA) connectivity in the PFC. These authors observed that, although a reduction in VTA DCC expression was observed in all defeated mice, ectopic growth of mesolimbic DA axons was observed into the medial PFC of resistant mice. This specific adaptation on the dopaminergic system could be related to the increased response to cocaine and ethanol observed in resilient mice.

5. Conclusions

Adolescence is a critical developmental period for later mental illness, and an important period in which to focus intervention strategies. More studies are needed in order to fully evaluate the relationship between bullying and substance use disorders. A recent study showed a bidirectional correlation indicating that individuals who engaged in substance use were more likely to perpetrate cyber aggression than those who did not, a result that suggests a strong relationship between substance use and bullying (Crane et al., 2021).

Our findings illustrate that, contrary to prior assumptions in adults, SD stress responses are more complex and singular in adolescents, and caution should be taken for the correct interpretation and translation of those phenotypes. The Social Interaction Test, considered a depressive-like phenotype, is currently used to classify mice into resilient or susceptible in order to study the neurobiology and molecular aspects of social stress (e.g. Russo et al., 2012). In adolescents, we should not assume that resilience to one phenotype equally develops for others, highlighting the concept of resilience as an active process affected by the person's age at the moment of the stress experience.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.pnpbp.2022.110591.

Funding sources

This work was supported by the following grants: PSI 2017–83,023-R and PID-2020-112672RB-100 by MCIN/AEI/ https://doi.org/10.13039/501100011033 and ERDF A way of making Europe; Instituto de Salud Carlos III, RTA (RD16/0017/0007), Atención primaria, cronicidad y promoción de la salud, RED DE INVESTIGACIÓN EN ATENCIÓN PRIMARIA DE ADICCIONES (RIAPAd) RD21/0009/0005 and Unión Europea, ERDF A way of making Europe. MDR receive the grant PRE2018–084159 funded by MCIN/AEI/ 501,100,011,033 by "ESF Investing in your future".

Ethical statement

The experimental protocol has been approved by an Institutional Review Committee for the use of animal subjects. Procedures involving mice and their care were conducted in conformity with national, regional and local laws and regulations, which are in accordance with European Community Council Directives 2010/63/UE regulating animal research and were approved by the local ethical committees. All the efforts were made to minimize animal suffering and to reduce the number of animals used.

CRediT authorship contribution statement

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

Declaration of Competing Interest

None.

Acknowledgements

We wish to thank Guillem Chuliá for his English language editing.

References

Aarestad, S.H., Harris, A., Einarsen, S.V., Gjengedal, R.G.H., Osnes, K., Hannisdal, M., Hjemdal, O., 2021. Exposure to bullying behaviours, resilience, and return to work self-efficacy in patients on or at risk of sick leave. Ind. Health 59, 180–192. https://doi.org/10.2486/indhealth.2020-0064.

Adriani, W., Laviola, G., 2004. Windows of vulnerability to psychopathology and therapeutic strategy in the adolescent rodent model. Behav. Pharmacol. 15, 341–352. https://doi.org/10.1097/00008877-200409000-00005.

Albrechet-Souza, L., Viola, T.W., Grassi-Oliveira, R., Miczek, K.A., de Almeida, R.M.M., 2017. Corticotropin releasing factor in the bed nucleus of the Stria terminalis in socially defeated and non-stressed mice with a history of chronic alcohol intake. Front. Pharmacol. 8, 762. https://doi.org/10.3389/fphar.2017.00762.

Alfonso-Loeches, S., Pascual-Lucas, M., Blanco, A.M., Sanchez-Vera, I., Guerri, C., 2010. Pivotal role of TLR4 receptors in alcohol-induced neuroinflammation and brain damage. J. Neurosci. 30, 8285–8295. https://doi.org/10.1523/JNEUROSCI.0976-10.2010.

Alves-dos-Santos, L., Resende, L. De S., Chiavegatto, S., 2020. Susceptibility and resilience to chronic social defeat stress in adolescent male mice: no correlation between social avoidance and sucrose preference. Neurobiol. Stress 12, 100221. https://doi.org/10.1016/j.ynstr.2020.100221.

Arenas, M.C., Daza-Losada, M., Vidal-Infer, A., Aguilar, M.A., Miñarro, J., Rodríguez-Arias, M., 2014. Capacity of novelty-induced locomotor activity and the hole-board test to predict sensitivity to the conditioned rewarding effects of cocaine. Physiol. Behav. 133, 152–160. https://doi.org/10.1016/j.physbeh.2014.05.028.

Ballestín, R., Alegre-Zurano, L., Ferrer-Pérez, C., Cantacorps, L., Miñarro, J., Valverde, O., Rodríguez-Arias, M., 2021. Neuroinflammatory and behavioral susceptibility profile of mice exposed to social stress towards cocaine effects. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 105, 110123. https://doi.org/10.1016/j.pnpbp.2020.110123.

Bellis, M.A., Hughes, K., Ford, K., Ramos Rodriguez, G., Sethi, D., Passmore, J., 2019. Life course health consequences and associated annual costs of adverse childhood experiences across Europe and North America: a systematic review and meta-analysis. Lancet Public Health 4, e517–e528. https://doi.org/10.1016/S2468-2667(19)30145-8.

Berton, O., McClung, C.A., Dileone, R.J., Krishnan, V., Renthal, W., Russo, S.J., Graham, D., Tsankova, N.M., Bolanos, C.A., Rios, M., Monteggia, L.M., Self, D.W., Nestler, E.J., 2006. Essential role of BDNF in the mesolimbic dopamine pathway in social defeat stress. Science 311 (5762), 864–868.

Burke, A.R., Miczek, K.A., 2014. Stress in adolescence and drugs of abuse in rodent models: role of dopamine, CRF, and HPA axis. Psychopharmacology (Berlin) 231, 1557–1580. https://doi.org/10.1007/s00213-013-3369-1.

Burke, A.R., Miczek, K.A., 2015. Escalation of cocaine self-administration in adulthood after social defeat of adolescent rats: role of social experience and adaptive coping behavior. Psychopharmacology 232, 3067–3079. https://doi.org/10.1007/s00213-015-3947-5.

Burke, A.R., Watt, M.J., Forster, G.L., 2011. Adolescent social defeat increases adult amphetamine conditioned place preference and alters D2 dopamine receptor expression. Neuroscience 197, 269–279. https://doi.org/10.1016/j.neuroscience.2011.09.008.

Cathomas, F., Murrough, J.W., Nestler, E.J., Han, M.-H., Russo, S.J., 2019. Neurobiology of resilience: Interface between mind and body. Biol. Psychiatry 86, 410–420. https://doi.org/10.1016/j.biopsych.2019.04.011.

Covington 3rd, H.E., Miczek, K.A., 2001. Repeated social-defeat stress, cocaine or morphine. Effects on behavioral sensitization and intravenous cocaine self-administration "binges". Psychopharmacology 158 (4), 388–398. https://doi.org/10.1007/s002130100858.

Covington 3rd, H.E., Tropea, T.F., Rajadhyaksha, A.M., Kosofsky, B.E., Miczek, K.A., 2008. NMDA receptors in the rat VTA: a critical site for social stress to intensify cocaine taking. Psychopharmacology 197 (2), 203–216. https://doi.org/10.1007/s00213-007-1024-4.

Crane, C.A., Wiernik, B.M., Berbary, C.M., Crawford, M., Schlauch, R.C., Easton, C.J., 2021. A meta-analytic review of the relationship between cyber aggression and substance use. Drug Alcohol Depend. 221, 108510 https://doi.org/10.1016/j.drugalcdep.2021.108510.

Daza-Losada, M., Rodríguez-Arias, M., Maldonado, C., Aguilar, M.A., Guerri, C., Miñarro, J., 2009. Acute behavioural and neurotoxic effects of MDMA plus cocaine in adolescent mice. Neurotoxicol. Teratol. 31, 49–59. https://doi.org/10.1016/j.ntt.2008.07.005.

Do Couto, Ribeiro, Aguilar, M.A., Lluch, J., Rodríguez-Arias, M., Miñarro, J., 2009. Social experiences affect reinstatement of cocaine-induced place preference in mice. Psychopharmacology (Berlin) 207, 485–498. https://doi.org/10.1007/s00213-009-1678-1.

Dumont, M., Provost, M.A., 1999. Resilience in adolescents: protective role of social support, coping strategies, Self-esteem, and social activities on experience of stress and depression. J. Youth Adolesc. 28, 343–363. https://doi.org/10.1023/A:1021637011732.

Dutcher, J.M., Creswell, J.D., 2018. The role of brain reward pathways in stress resilience and health. Neurosci. Biobehav. Rev. 95, 559–567. https://doi.org/10.1016/j.neubiorev.2018.10.014.

Ferle, V., Repouskou, A., Aspiotis, G., Raftogianni, A., Chrousos, G., Stylianopoulou, F., Stamatakis, A., 2020. Synergistic effects of early life mild adversity and chronic social defeat on rat brain microglia and cytokines. Physiol. Behav. 215, 112791 https://doi.org/10.1016/j.physbeh.2019.112791.

Ferrer-Pérez, C., Martinez, T.E., Montagud-Romero, S., Ballestín, R., Reguilón, M.D., Miñarro, J., Rodríguez-Arias, M., 2018. Indomethacin blocks the increased conditioned rewarding effects of cocaine induced by repeated social defeat. PLoS One 13, e0209291. https://doi.org/10.1371/journal.pone.0209291.

Ferrer-Pérez, C., Reguilón, M.D., Manzanedo, C., Miñarro, J., Rodríguez-Arias, M., 2019. Social housing conditions modulate the long-lasting increase in cocaine reward induced by intermittent social defeat. Front. Behav. Neurosci. 13, 148. https://doi.org/10.3389/fnbeh.2019.00148.

Franklin, K.B.J., Paxinos, G., 2008. The Mouse Brain in Stereotaxic Coordinates, Compact, 3. ed. Elsevier Academic Press, Amsterdam Heidelberg.

Giménez-Gómez, P., Ballestín, R., Gil de Biedma-Elduayen, L., Vidal, R., Ferrer-Pérez, C., Reguilón, M.D., O'Shea, E., Miñarro, J., Colado, M.I., Rodríguez-Arias, M., 2021. Decreased kynurenine pathway potentiate resilience to social defeat effect on cocaine reward. Neuropharmacology 197, 108753. https://doi.org/10.1016/j.neuropharm.2021.108753.

Golden, S.A., Covington 3rd, H.E., Berton, O., Russo, S.J., 2011. A standardized protocol for repeated social defeat stress in mice. Nat. Protoc. 6 (8), 1183–1191. https://doi.org/10.1038/nprot.2011.361.

Hammels, C., Pishva, E., De Vry, J., van den Hove, D.L.A., Prickaerts, J., van Winkel, R., Selten, J.P., Lesch, K.P., Daskalakis, N.P., Steinbusch, H.W.M., van Os, J., Kenis, G., Rutten, B.P.F., 2015. Defeat stress in rodents: from behavior to molecules. Neurosci. Biobehav. Rev. 59, 111–140. https://doi.org/10.1016/j.neubiorev.2015.10.006.

Haroon, E., Raison, C.L., Miller, A.H., 2012. Psychoneuroimmunology meets Neuropsychopharmacology: translational implications of the impact of inflammation on behavior. Neuropsychopharmacology 37, 137–162. https://doi.org/10.1038/npp.2011.205.

Hawley, D.F., Bardi, M., Everette, A.M., Higgins, T.J., Tu, K.M., Kinsley, C.H., Lambert, K.G., 2010. Neurobiological constituents of active, passive, and variable coping strategies in rats: integration of regional brain neuropeptide Y levels and cardiovascular responses. Stress 13 (2), 172–183. https://doi.org/10.3109/10253890903144621.

Heffner, T.G., Hartman, J.A., Seiden, L.S., 1980. A rapid method for the regional dissection of the rat brain. Pharmacol. Biochem. Behav. 13, 453–456. https://doi.org/10.1016/0091-3057(80)90254-3.

Ho, T.C., King, L.S., 2021. Mechanisms of neuroplasticity linking early adversity to depression: developmental considerations. Transl. Psychiatry 11, 517. https://doi.org/10.1038/s41398-021-01639-6.

Hodes, G.E., Epperson, C.N., 2019. Sex differences in vulnerability and resilience to stress across the life span. Biol. Psychiatry 86 (6), 421–432. https://doi.org/10.1016/j.biopsych.2019.04.028.

Huang, G.-B., Zhao, T., Muna, S.S., Bagalkot, T.R., Jin, H.-M., Chae, H.-J., Chung, Y.-C., 2013. Effects of chronic social defeat stress on behaviour, endoplasmic reticulum proteins and choline acetyltransferase in adolescent mice. Int. J. Neuropsychopharmacol. 16, 1635–1647. https://doi.org/10.1017/S1461145713000060.

Hughes, K., Bellis, M.A., Hardcastle, K.A., Sethi, D., Butchart, A., Mikton, C., Jones, L., Dunne, M.P., 2017. The effect of multiple adverse childhood experiences on health: a systematic review and meta-analysis. Lancet Public Health 2, e356–e366. https://doi.org/10.1016/S2468-2667(17)30118-4.

Iñiguez, S.D., Riggs, L.M., Nieto, S.J., Dayrit, G., Zamora, N.N., Shawhan, K.L., Cruz, B., Warren, B.L., 2014. Social defeat stress induces a depression-like phenotype in adolescent male c57BL/6 mice. Stress 17, 247–255. https://doi.org/10.3109/10253890.2014.910650.

Jiang, N., Lv, J., Wang, H., Huang, H., Wang, Q., Lu, C., Zeng, G., Liu, X., 2020. Ginsenoside Rg1 ameliorates chronic social defeat stress-induced depressive-like behaviors and hippocampal neuroinflammation. Life Sci. 252, 117669 https://doi.org/10.1016/j.lfs.2020.117669.

Koolhaas, J.M., de Boer, S.F., Buwalda, B., van Reenen, K., 2007. Individual variation in coping with stress: a multidimensional approach of ultimate and proximate mechanisms. Brain Behav. Evol. 70 (4), 218–226. https://doi.org/10.1159/000105485.

Krishnan, V., Han, M.-H., Graham, D.L., Berton, O., Renthal, W., Russo, S.J., LaPlant, Q., Graham, A., Lutter, M., Lagace, D.C., Ghose, S., Reister, R., Tannous, P., Green, T.A., Neve, R.L., Chakravarty, S., Kumar, A., Eisch, A.J., Self, D.W., Lee, F.S., Tamminga, C.A., Cooper, D.C., Gershenfeld, H.K., Nestler, E.J., 2007. Molecular adaptations underlying susceptibility and resistance to social defeat in brain reward regions. Cell 131, 391–404. https://doi.org/10.1016/j.cell.2007.09.018.

Lambert, K.G., Hyer, M.M., Rzucidlo, A.A., Bergeron, T., Landis, T., Bardi, M., 2014. Contingency-based emotional resilience: effort-based reward training and flexible coping lead to adaptive responses to uncertainty in male rats. Front. Behav. Neurosci. 8, 124. https://doi.org/10.3389/fnbeh.2014.00124.

Liu, Q., Ely, B.A., Simkovic, S.J., Tao, A., Wolchok, R., Alonso, C.M., Gabbay, V., 2020a. Correlates of C-reactive protein with neural reward circuitry in adolescents with psychiatric symptoms. Brain Behav. Immun. Health 9, 100153. https://doi.org/10.1016/j.bbih.2020.100153.

Liu, F., Tao, X., Pang, G., Wu, D., Hu, Y., Xue, S., Liu, J., Li, B., Zhou, L., Liu, Q., Zhang, Y.-M., 2020b. Maternal nicotine exposure during gestation and lactation period affects behavior and hippocampal neurogenesis in mouse offspring. Front. Pharmacol. 10, 1569. https://doi.org/10.3389/fphar.2019.01569.

Lo Iacono, L., Catale, C., Martini, A., Valzania, A., Viscomi, M.T., Chiurchiù, V., Guatteo, E., Bussone, S., Perrone, F., Di Sabato, P., Aricò, E., D'Argenio, A., Troisi, A., Mercuri, N.B., Maccarrone, M., Puglisi-Allegra, S., Casella, P., Carola, V., 2018. From traumatic childhood to cocaine abuse: the critical function of the immune system. Biol. Psychiatry 84 (12), 905–916. https://doi.org/10.1016/j.biopsych.2018.05.022.

Macedo, G.C., Morita, G.M., Domingues, L.P., Favoretto, C.A., Suchecki, D., Quadros, I. M.H., 2018. Consequences of continuous social defeat stress on anxiety- and depressive-like behaviors and ethanol reward in mice. Horm. Behav. 97, 154–161. https://doi.org/10.1016/j.yhbeh.2017.10.007.

Malhi, G.S., Das, P., Bell, E., Mattingly, G., Mannie, Z., 2019. Modelling resilience in adolescence and adversity: a novel framework to inform research and practice. Transl. Psychiatry 9, 316. https://doi.org/10.1038/s41398-019-0651-y.

Manzanedo, C., Aguilar, M.A., Rodríguez-Arias, M., Miñarro, J., 2001. Effects of dopamine antagonists with different receptor blockade profiles on morphine-induced place preference in male mice. Behav. Brain Res. 121 (1–2), 189–197. https://doi.org/10.1016/s0166-4328(01)00164-4.

McKay, M.T., Cannon, M., Chambers, D., Conroy, R.M., Coughlan, H., Dodd, P., Healy, C., O'Donnell, L., Clarke, M.C., 2021. Childhood trauma and adult mental disorder: a systematic review and meta-analysis of longitudinal cohort studies. Acta Psychiatr. Scand. 143, 189–205. https://doi.org/10.1111/acps.13268.

Miczek, K.A., Thompson, M.L., Shuster, L., 1982. Opioid-like analgesia in defeated mice. Science 215 (4539), 1520–1522. https://doi.org/10.1126/science.7199758.

Montagud-Romero, S., Reguilon, M.D., Roger-Sanchez, C., Pascual, M., Aguilar, M.A., Guerri, C., Miñarro, J., Rodríguez-Arias, M., 2016. Role of dopamine neurotransmission in the long-term effects of repeated social defeat on the conditioned rewarding effects of cocaine. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 71, 144–154. https://doi.org/10.1016/j.pnpbp.2016.07.008.

Montagud-Romero, S., Nuñez, C., Blanco-Gandia, M.C., Martínez-Laorden, E., Aguilar, M.A., Navarro-Zaragoza, J., Almela, P., Milanés, M.-V., Laorden, M.-L., Miñarro, J., Rodríguez-Arias, M., 2017. Repeated social defeat and the rewarding effects of cocaine in adult and adolescent mice: dopamine transcription factors, proBDNF signaling pathways, and the TrkB receptor in the mesolimbic system. Psychopharmacology 234, 2063–2075. https://doi.org/10.1007/s00213-017-4612-y.

Montagud-Romero, S., Montesinos, J., Pavón, F.J., Blanco-Gandia, M.C., Ballestín, R., Rodríguez de Fonseca, F., Miñarro, J., Guerri, C., Rodríguez-Arias, M., 2020. Social defeat-induced increase in the conditioned rewarding effects of cocaine: role of CX3CL1. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 96, 109753. https://doi.org/10.1016/j.pnpbp.2019.109753.

Montagud-Romero, S., Miñarro, J., Rodríguez-Arias, M., 2021. Unravelling the Neuroinflammatory mechanisms underlying the effects of social defeat stress on use of drugs of abuse. Curr. Top. Behav. Neurosci. https://doi.org/10.1007/7854_2021_260 (Advance online publication).

Navarrete, F., Pérez-Ortiz, J.M., Manzanares, J., 2012. Cannabinoid CB2 receptor-mediated regulation of impulsive-like behaviour in DBA/2 mice. Br. J. Pharmacol. 165, 260–273. https://doi.org/10.1111/j.1476-5381.2011.01542.x.

Navarrete, F., Rubio, G., Manzanares, J., 2014. Effects of naltrexone plus topiramate on ethanol self-administration and tyrosine hydroxylase gene expression changes. Addict. Biol. 19 (5), 862–873. https://doi.org/10.1111/adb.12058.

Nozaki, K., Ito, H., Ohgidani, M., Yamawaki, Y., Sahin, E.H., Kitajima, T., Katsumata, S., Yamawaki, S., Kato, T.A., Aizawa, H., 2020. Antidepressant effect of the translocator protein antagonist ONO-2952 on mouse behaviors under chronic social defeat stress. Neuropharmacology 162, 107835. https://doi.org/10.1016/j.neuropharm.2019.107835.

Platt, B., Kadosh, K.C., Lau, J.Y.F., 2013. The role of peer rejection in adolescent depression. Depress. Anxiety 30, 809–821. https://doi.org/10.1002/da.22120.

Quadros, I.M., Miczek, K.A., 2009. Two modes of intense cocaine bingeing: increased persistence after social defeat stress and increased rate of intake due to extended access conditions in rats. Psychopharmacology 206 (1), 109–120. https://doi.org/10.1007/s00213-009-1584-6.

Reguilón, M.D., Montagud-Romero, S., Ferrer-Pérez, C., Roger-Sánchez, C., Aguilar, M. A., Miñarro, J., Rodríguez-Arias, M., 2017. Dopamine D2 receptors mediate the increase in reinstatement of the conditioned rewarding effects of cocaine induced by acute social defeat. Eur. J. Pharmacol. 799, 48–57. https://doi.org/10.1016/j.ejphar.2017.01.039.

Reguilón, M.D., Ferrer-Pérez, C., Ballestín, R., Miñarro, J., Rodríguez-Arias, M., 2020. Voluntary wheel running protects against the increase in ethanol consumption induced by social stress in mice. Drug Alcohol Depend. 212, 108004 https://doi.org/10.1016/j.drugalcdep.2020.108004.

Reguilón, Marina D., Ferrer-Pérez, C., Manzanedo, C., Miñarro, J., Rodríguez-Arias, M., 2021a. Ethanol intake in male mice exposed to social defeat: environmental enrichment potentiates resilience. Neurobiol. Stress 15, 100413. https://doi.org/10.1016/j.ynstr.2021.100413.

Reguilón, M.D., Ferrer-Pérez, C., Miñarro, J., Rodríguez-Arias, M., 2021b. Oxytocin reverses ethanol consumption and neuroinflammation induced by social defeat in male mice. Horm. Behav. 127, 104875 https://doi.org/10.1016/j.yhbeh.2020.104875.

Roberts, A.J., Gold, L.H., Polis, I., McDonald, J.S., Filliol, D., Kieffer, B.L., Koob, G.F., 2001. Increased ethanol self-administration in δ-opioid receptor knockout mice. Alcohol. Clin. Exp. Res. 25, 1249–1256. https://doi.org/10.1097/00000374-200109000-00002.

Ródenas-González, F., Blanco-Gandía, M., Miñarro López, J., Rodriguez-Arias, M., 2021. Behavioral and neuroimmune characterization of resilience to social stress: Rewarding effects of cocaine. Caracterización conductual y neuroinmune de la resiliencia al estrés social: Efectos reforzantes de la cocaína. Adicciones 33 (4), 319–332. https://doi.org/10.20882/adicciones.1348.

Rodríguez-Arias, M., Miñarro, J., Aguilar, M.A., Pinazo, J., Simon, V.M., 1998. Effects of risperidone and SCH 23390 on isolation-induced aggression in male mice. Eur. Neuropsychopharmacol. 8 (2), 95–103. https://doi.org/10.1016/S0924-977X(97).

Rodríguez-Arias, M., Valverde, O., Daza-Losada, M., Blanco-Gandía, M.C., Aguilar, M.A., Miñarro, J., 2013. Assessment of the abuse potential of MDMA in the conditioned place preference paradigm: role of CB1 receptors. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 47, 77–84. https://doi.org/10.1016/j.pnpbp.2013.07.013.

Rodríguez-Arias, M., Navarrete, F., Blanco-Gandia, M.C., Arenas, M.C., Bartoll-Andrés, A., Aguilar, M.A., Rubio, G., Miñarro, J., Manzanares, J., 2016. Social defeat in adolescent mice increases vulnerability to alcohol consumption: social defeat and ethanol. Addict. Biol. 21, 87–97. https://doi.org/10.1111/adb.12184.

Rodríguez-Arias, M., Montagud-Romero, S., Rubio-Araiz, A., Aguilar, M.A., Martín-García, E., Cabrera, R., Maldonado, R., Porcu, F., Colado, M.I., Miñarro, J., 2017. Effects of repeated social defeat on adolescent mice on cocaine-induced CPP and self-administration in adulthood: integrity of the blood-brain barrier: social defeat, cocaine and BBB. Addict. Biol. 22, 129–141. https://doi.org/10.1111/adb.12301.

Rodríguez-Arias, M., Montagud-Romero, S., Guardia Carrión, A.M., Ferrer-Pérez, C., Pérez-Villalba, A., Marco, E., López Gallardo, M., Viveros, M.P., Miñarro, J., 2018. Social stress during adolescence activates long-term microglia inflammation insult in reward processing nuclei. PLoS One 13, e0206421. https://doi.org/10.1371/journal.pone.0206421.

Russo, S.J., Murrough, J.W., Han, M.H., Charney, D.S., Nestler, E.J., 2012. Neurobiology of resilience. Nat. Neurosci. 15 (11), 1475–1484. https://doi.org/10.1038/nn.3234.

Samson, H.H., 1986. Initiation of ethanol reinforcement using a sucrose-substitution procedure in food-and water-sated rats. Alcohol. Clin. Exp. Res. 10, 436–442. https://doi.org/10.1111/j.1530-0277.1986.tb05120.x.

Sheth, C., McGlade, E., Yurgelun-Todd, D., 2017. Chronic stress in adolescents and its neurobiological and psychopathological consequences: an RDoC perspective. Chronic Stress (Thousand Oaks) 1. https://doi.org/10.1177/2470547017715645, 247054701771564.

Shimizu, T., Ishida, A., Hagiwara, M., Ueda, Y., Hattori, A., Tajiri, N., Hida, H., 2020. Social defeat stress in adolescent mice induces depressive-like behaviors with reduced Oligodendrogenesis. Neuroscience 443, 218–232. https://doi.org/10.1016/j.neuroscience.2020.07.002.

Smith, B.L., 2019. Adaptation as a dynamic construct for studying stress resilience and susceptibility. Brain Behav. Immun. 81, 18–19. https://doi.org/10.1016/j.bbi.2019.07.029.

Solmi, M., Radua, J., Olivola, M., Croce, E., Soardo, L., Salazar de Pablo, G., Il Shin, J., Kirkbride, J.B., Jones, P., Kim, J.H., Kim, J.Y., Carvalho, A.F., Seeman, M.V., Correll, C.U., Fusar-Poli, P., 2021. Age at onset of mental disorders worldwide: large-scale meta-analysis of 192 epidemiological studies. Mol. Psychiatry. https://doi.org/10.1038/s41380-021-01161-7. Advance online publication.

Somerville, L.H., Jones, R.M., Casey, B.J., 2010. A time of change: behavioral and neural correlates of adolescent sensitivity to appetitive and aversive environmental cues. Brain Cogn. 72, 124–133. https://doi.org/10.1016/j.bandc.2009.07.003.

Soria, V., Uribe, J., Salvat-Pujol, N., Palao, D., Menchón, J.M., Labad, J., 2018. Psiconeuroinmunología de los trastornos mentales. Rev. Psiquiatr. Salud Ment. 11, 115–124. https://doi.org/10.1016/j.rpsm.2017.07.006.

Tharp-Taylor, S., Haviland, A., D'Amico, E.J., 2009. Victimization from mental and physical bullying and substance use in early adolescence. Addict. Behav. 34, 561–567. https://doi.org/10.1016/j.addbeh.2009.03.012.

Thompson, S.M., Simmons, A.N., McMurray, M.S., 2020. The effects of multiple early life stressors on adolescent alcohol consumption. Behav. Brain Res. 380, 112449 https://doi.org/10.1016/j.bbr.2019.112449.

Topper, L.R., Castellanos-Ryan, N., Mackie, C., Conrod, P.J., 2011. Adolescent bullying victimisation and alcohol-related problem behaviour mediated by coping drinking motives over a 12month period. Addict. Behav. 36, 6–13. https://doi.org/10.1016/j.addbeh.2010.08.016.

Tornatzky, W., Miczek, K.A., 1993. Long-term impairment of autonomic circadian rhythms after brief intermittent social stress. Physiol. Behav. 53 (5), 983–993. https://doi.org/10.1016/0031-9384(93)90278-n.

Vassilev, P., Pantoja-Urban, A.H., Giroux, M., Nouel, D., Hernandez, G., Orsini, T., Flores, C., 2021. Unique effects of social defeat stress in adolescent male mice on the Netrin-1/DCC pathway, prefrontal cortex dopamine and cognition. eNeuro 8. https://doi.org/10.1523/ENEURO.0045-21.2021. ENEURO.0045-21.2021.

Vidal-Infer, A., Arenas, M.C., Daza-Losada, M., Aguilar, M.A., Miñarro, J., Rodríguez-Arias, M., 2012. High novelty-seeking predicts greater sensitivity to the conditioned rewarding effects of cocaine. Pharmacol. Biochem. Behav. 102 (1), 124–132. https://doi.org/10.1016/j.pbb.2012.03.031.

Wang, W., Liu, W., Duan, D., Bai, H., Wang, Z., Xing, Y., 2021. Chronic social defeat stress mouse model: current view on its behavioral deficits and modifications. Behav. Neurosci. 135, 326–335. https://doi.org/10.1037/bne0000418.

Watt, M.J., Burke, A.R., Renner, K.J., Forster, G.L., 2009. Adolescent male rats exposed to social defeat exhibit altered anxiety behavior and limbic monoamines as adults. Behav. Neurosci. 123 (3), 564–576. https://doi.org/10.1037/a0015752.

Wellman, C.L., Bangasser, D.A., Bollinger, J.L., Coutellier, L., Logrip, M.L., Moench, K. M., Urban, K.R., 2018. Sex differences in risk and resilience: stress effects on the neural substrates of emotion and motivation. J. Neurosci. 38 (44), 9423–9432. https://doi.org/10.1523/JNEUROSCI.1673-18.2018.

Wohleb, E.S., McKim, D.B., Sheridan, J.F., Godbout, J.P., 2015. Monocyte trafficking to the brain with stress and inflammation: a novel axis of immune-to-brain communication that influences mood and behavior. Front. Neurosci. 8 https://doi.org/10.3389/fnins.2014.00447.

Wood, S.K., Bhatnagar, S., 2015. Resilience to the effects of social stress: evidence from clinical and preclinical studies on the role of coping strategies. Neurobiol. Stress 1, 164–173. https://doi.org/10.1016/j.ynstr.2014.11.002.

Wood, S.K., Walker, H.E., Valentino, R.J., Bhatnagar, S., 2010. Individual differences in reactivity to social stress predict susceptibility and resilience to a depressive phenotype: role of corticotropin-releasing factor. Endocrinology 151 (4), 1795–1805. https://doi.org/10.1210/en.2009-1026.

Yates, J.R., Beckmann, J.S., Meyer, A.C., Bardo, M.T., 2013. Concurrent choice for social interaction and amphetamine using conditioned place preference in rats: effects of age and housing condition. Drug Alcohol Depend. 129, 240–246. https://doi.org/10.1016/j.drugalcdep.2013.02.024.

Zhu, Y., Klomparens, E.A., Guo, S., Geng, X., 2019. Neuroinflammation caused by mental stress: the effect of chronic restraint stress and acute repeated social defeat stress in mice. Neurol. Res. 41, 762–769. https://doi.org/10.1080/01616412.2019.1615670.