Neurobiology of Stress 12 (2020) 100221

Contents lists available at ScienceDirect

Neurobiology of Stress

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

Susceptibility and resilience to chronic social defeat stress in adolescent male mice: No correlation between social avoidance and sucrose preference

Leonardo Alves-dos-Santos[a], Letícia de Souza Resende[a], Silvana Chiavegatto[a,b,*]

[a] Department of Pharmacology, Biomedical Sciences Institute (ICB), University of Sao Paulo (USP), Sao Paulo, SP, Brazil
[b] Department of Psychiatry, Institute of Psychiatry (IPq), University of Sao Paulo Medical School (FMUSP), Sao Paulo, SP, Brazil

ARTICLE INFO

Keywords:
Anhedonia
Anxiety
Depression
Mood disorders
Bullying
Animal model
Juvenile

ABSTRACT

Psychosocial stress is the major form of stress faced by children and adolescents and is an important risk factor for the development of mental illnesses. Chronic social defeat stress (CSDS) is a preclinical mouse model that induces an entire spectrum of phenotypes with similar interindividual variability as seen in humans. Following CSDS, adult male mice have been characterized as being either susceptible or resilient to emotional stress on the basis of their social interactions, which was reported to be highly correlated with sucrose preference (SP) when measured after the last defeat episode.

We studied adolescent male C57BL/6 mice (30 days old) for susceptibility and resilience to social avoidance, anhedonia and anxiety-like behaviors, body weight change and basal blood corticosterone concentrations after 10 days of CSDS. Defeated adolescents showed reduced SP, reduced social interaction time (with an unknown adolescent male from their same strain), reduced weight gain and higher basal blood corticosterone concentration when compared to nondefeated mice. Only a small proportion of defeated adolescents were either totally susceptible (20%) or totally resilient (30%) in both the SP and social avoidance tests. The remaining defeated mice had a distinct behavioral impairment - susceptible in one test and resilient in the other. Surprisingly, behaviorally resilient defeated adolescents were the most affected population in terms of both endocrine/physiological outcomes. These findings illustrate that, contrary to prior assumptions in adults, the CSDS responses are more complex and singular in adolescents, and caution should be taken for the correct interpretation of those phenotypes. We propose a better characterization of social defeat stress responses as a critical step to advance our understanding of the mechanisms behind stress resilience that translate to human experience.

1. Introduction

The peak age of onset for many psychiatric disorders is during adolescence, a time of marked physical and behavioral changes (Kessler et al., 2005; Paus et al., 2008). Adolescence is a period when social interaction with peers takes on particular importance and helps shape their adult social behavioral repertoire (Spear, 2000; Burke et al., 2017). During adolescence, frequent exposure to victimization or bullying enhances vulnerability to the development of depression, anxiety, sociophobia, a loss of self-esteem, and suicide attempts (Bjorkqvist, 2001; Brunstein Klomek et al., 2007). Although many adolescents suffer traumatic events such as bullying, only a few develop psychiatric disorders (Dumont and Provost, 1999).

When considering experimental approaches for studying psychosocial stress in adolescents, chronic social defeat in adult rodents appears to be an ethologically based model, showing excellent predictive, discriminative and face validity (Kudryavtseva et al., 1991; Berton et al., 2006; Golden et al., 2011). Furthermore, social defeat models in adult rodents demonstrate similar interindividual variability in response to social stress as seen in humans, thus providing a useful protocol for the study of the biological and molecular basis of susceptibility and resilience to emotional stress (Krishnan et al., 2007; Strekalova et al., 2011, Huang et al., 2013). In particular, adult mice subjected to chronic social defeat have been characterized as being either susceptible or resilient following social defeat on the basis of their social interaction performance (Berton et al., 2006; Krishnan et al., 2007; Russo et al., 2012). Resilience to chronic social defeat stress is defined as the absence of social avoidance, which in male adult

* Corresponding author. Laboratory of Behavioral and Molecular Neurosciences, Department of Pharmacology, Biomedical Sciences Institute, University of Sao Paulo, 1524, Prof. Lineu Prestes Ave – 3rd floor, 05508-000, Sao Paulo, SP, Brazil.
E-mail address: schiaveg@usp.br (S. Chiavegatto).

https://doi.org/10.1016/j.ynstr.2020.100221
Received 18 February 2020; Received in revised form 6 April 2020; Accepted 7 April 2020
Available online 16 April 2020

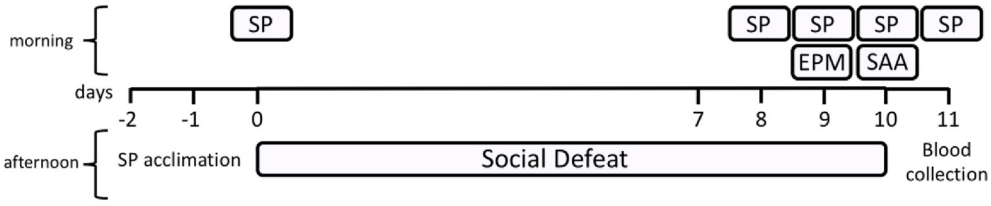

Fig. 1. Experimental design. Timeline showing all the steps of the experimental manipulations. Thirty-day-old C57BL/6 male mice were exposed daily to chronic defeat stress (CSDS) from an aggressive CD-1 mouse during 5 min of physical interaction (occurring in the afternoons), followed by 24 h of protected cohabitation with the aggressor (threat period) over 10 days. Control adolescents were paired in similar cages as the experimental mice (and at the same time) but were placed in different compartments; thus, they were protected from physical interactions. All the pairing compositions were changed every day. All adolescent mice were subjected to an overnight sucrose preference (SP) test before (basal) and on the 7th, 8th, 9th and 10th nights of the CSDS period (with measurements performed the next morning). The elevated plus maze (EPM) test and social approach-avoidance (SAA) test were performed in the morning on the last two days of the CSDS, respectively.

C57BL/6J mice was highly correlated with anhedonia and metabolic syndrome (Russo et al., 2012). It is not known, however, whether the resilience to psychosocial stress in adolescents, when based solely on social interaction, will also reflect their resilience to other behavioral and physiological stress responses. In other words, are adolescent mice resilient to social avoidance also resilient to anhedonia or anxiety-like behaviors after chronic social defeat? This is of importance because the salient value of social interactions in adolescents has been reported to be higher than that in adults (Douglas et al., 2004). Furthermore, exposure to stressors has a greater impact during adolescence than at other ages (see Spear, 2000 and Sheth et al., 2017 for references and reviews). Finally, because resilience results from developmental processes, it can be strengthened over time; thus, the examination of resilience in adolescents is essential (Mahli et al., 2019).

In this study, we aimed to explore male adolescent C57BL/6 mice susceptibility and resilience to social avoidance, anhedonia and anxiety-like behaviors, body weight change and basal blood corticosterone after 10 days of chronic social defeat stress. We tested whether the heterogeneity in their responses in each phenotype was correlated. This study is important because the assessment of social interactions after defeat sessions has been intensively used as a proxy for the evaluation of potential resilience mechanisms in adult animals, whereas the appropriateness of using such assessments among adolescent animals remains unspecified.

2. Methods and materials

2.1. Animals and housing

Male C57BL/6 mice from a breeding colony established in our vivarium (Institute of Tropical Medicine, University of Sao Paulo Medical School, Brazil) were weaned at 21 days of age, maintained in cohorts of three or four in standard polypropylene cages, and then singly housed one day before the first defeat session. Adult male CD-1 mice from our in-house breeding colony were maintained in isolation for at least three weeks. They were tested and selected for high levels of aggressive behavior during social confrontations with nonexperimental C57BL/6 adult males over three days to be used as stimulus aggressors. In the morning of the first defeat session, each CD-1 mouse was transferred into the social defeat cage (polypropylene box with autoclaved wood shavings; 44 x 34 × 16 cm, divided in half by an acrylic perforated partition). All animals had free access to autoclaved rodent chow (Nuvilab CR1/Nuvital, Colombo, PR, Brazil) and tap water. Animals were kept on a 12-h light/dark cycle (lights on at 07:00 h), with temperature and humidity remaining fairly constant in closed and ventilated stands (Alesco, SP, Brazil). Experiments were carried out in accordance with the Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research (ILAR, Washington, D.C., EUA), and the protocol was approved by our local committees (CEUA-ICB nº 173/2013 and CEUA-IMT nº CPE-IMT/172).

2.2. Chronic social defeat stress

The chronic social defeat stress (CSDS) paradigm described for adult male mice (Golden et al., 2011) was used with minor modifications. Thirty-day-old C57BL/6 mice were randomly assigned to control or experimental groups. The experimental mice were individually introduced into the social defeat cage in the compartment of a resident male CD-1 mouse. The aggressor attacked the adolescent intruder for 5 min, and then the experimental mouse was removed and immediately placed in the contiguous empty side for the remaining 24 h. By this procedure, the adolescents were protected from repeated attacks but remained threatened by olfactory, visual, and auditory contact with the aggressor. The CSDS was performed for 10 consecutive days, from 14:00 h to 18:00 h, with the defeated mouse moved into different aggressor cages every day. The pairing between each adolescent and CD-1 mouse was randomized daily to minimize the variability in aggression to which the mice were exposed. Control adolescent mice were paired in identical social defeat cages, but each one was placed in a different compartment; consequently, there was no physical contact between them. This was performed in a distinct experimental room, where control animals were rotated to a new social defeat cage and paired on a daily basis. After the last day of social stress, both defeated and control adolescents were individually housed.

2.3. Determination of body weight gain

Body mass was assessed prior to the first CSDS session (at 30 days old) and again after the last session (at 40 days old) for both experimental and control mice. Body weight gain was determined as the difference between both measurements.

2.4. Behavioral studies

Animals from both groups were tested in an alternating way from 8:00 to 12:00 h according to the experimental design timeline shown in Fig. 1. Sessions were recorded by a video camera. The images were analyzed later by researchers unaware of the experimental groups using ViewPoint® software (Videotrack 3.0.; Viewpoint, Lyon, France).

Sucrose preference (SP) test: Anhedonic behavior was assessed by a standard sucrose preference test (Strekalova and Steinbusch, 2010) with some modifications. Adolescent mice were habituated to the presence of two drinking tubes (50 mL Falcon® with siliconized rubber stops and stainless steel sipper tubes), one containing tap water and the other containing 1% sucrose solution, in their home cages for two consecutive days before the CSDS paradigm was initiated. Sucrose (Merck) in a 1% water solution has been shown in preliminary studies from our group to provide a robust sucrose preference in adolescent C57BL/6 male mice (~80% over tap water). Following this acclimation phase, this procedure was repeated one extra day for the basal measurement recording and again at the four last days of the defeat sessions, when the bottles were given in the social defeat cage (at the compartment of the defeated mouse). Drinking sessions started at 17:00 h and ended at 21:00 h, and the amount consumed by each

mouse was measured by weighing each bottle at the beginning and end of the sessions. Mice had free access to tap water between drinking sessions (9:00 to 17:00 h). To avoid place preference bias, the relative position of bottles (left *vs*. right) was changed every day. No previous food or water deprivation was applied before the test. SP % was calculated as the sucrose solution intake (mL)/total fluid intake (mL) x 100 in each drinking session, and the mean of the last four measurements was regarded as the final SP %.

Elevated plus maze (EPM) test: The test was conducted according to Bibancos et al. (2007). The apparatus was made of black Formica and consisted of two opposing open arms (30 cm long, 5 cm wide) and two closed arms (30 cm long, 5 cm wide, with 15 cm high walls) that extended from a central platform elevated 47 cm above the floor. Each mouse was placed individually on the central area with the head facing an open arm and was allowed to explore the maze for 5 min. This test was performed under standard light conditions (~82 lux), and an increase in the percentage of open-arm entries (open/total) and the percentage of open-arm time (open/open + closed) indicates a decrease in anxiety-like behavior.

Social approach-avoidance (SAA) test: This test was used to assess sociability in mice based on the preference of rodents to spend time with another conspecific rather than remaining alone or to explore nonsocial stimuli (Toth and Neumann, 2013). The apparatus consisted of a transparent plexiglass box (40.5 X 60 × 22 cm) divided into three chambers of equal size (40.5 × 20 cm) by walls with a 7 × 7 cm square opening that could be closed by a slide door (Noldus, Wageningen, Netherlands). We used only two contiguous chambers of the apparatus because, in preliminary studies from our group, this configuration has been shown to provide more reliable social approach time when using adolescent male mice, mainly due to their known elevated drive towards the exploration of unknown environments (Macri et al., 2002). The test mouse (defeated or control) was first placed in the left chamber and allowed to explore for 3 min, with the doorway into the right chamber open. After this 3-min habituation period, the test mouse was then returned to its home cage, and an unfamiliar adolescent C57BL/6 male mouse was placed in the right chamber inside a small cylinder. This cylinder (20 × 10 cm) was made out of 18 transparent plexiglass bars placed 6 mm apart. The test mouse was then placed again in the left chamber for three more minutes and allowed to access the right chamber containing the cylinder with the strange C57BL/6 adolescent mouse. The latency to interact with the strange mouse and the time spent in the perimeter around the cylinder (interaction time) were quantified.

2.5. Blood corticosterone (CORT) concentration

One day after the last episode of CSDS, at approximately 15:00 h, isolated defeated and control adolescents were euthanized by rapid decapitation in a randomized order. Trunk blood samples were collected in microtubes and centrifuged (10,000 ×g for 15 min at 4 °C), and the serum was transferred into clean tubes and stored at −70 °C until the assay. CORT was measured in triplicate by an enzyme immunoassay (Arbor Assays - DetectX® Corticosterone Enzyme Immunoassay Kit, K014–H5, Ann Arbor, MI, USA), which utilizes a microplate reader set at 450 nm. Serum samples were diluted 1:100 in appropriate assay buffers to be within the calibration curve range. The sensitivity of the kit was 18.6 pg/mL with a limit of detection of 16.9 pg/mL.

2.6. Statistical analysis

Data were tested for normal distribution using the Kolmogorov–Smirnov test. Two-tailed Student's *t*-test was used for analyses implicating two-group comparisons except for data that were not normally distributed, in which case a between-subject Mann–Whitney *U* test was performed. For normal data, one-way ANOVA was used to test differences among the control, susceptible and resilient groups. Significant results demonstrated by ANOVA were further analyzed for significance with Tukey's multiple comparison *post hoc* test. The Kruskal-Wallis test was used for comparisons among subgroups when the data did not assume a normal distribution and was followed by Dunn's multiple comparison test. Data are expressed as the mean ± the standard error of the mean (SEM) or the median and range. Differences were considered statistically significant when $p \leq 0.05$. All results were analyzed using GraphPad Prism® (GraphPad Software, San Diego, CA, USA).

3. Results

3.1. Sucrose preference test

To identify the development of anhedonia after chronic social defeat, mice were exposed to the SP test. At the basal measurement, control and defeated adolescents showed similar SP (78.77 ± 6.65%, n = 10 *vs.* 86.25 ± 1.99%, n = 20, respectively; $t = 1.25$, $p > 0.05$) and similar total liquid intake (sucrose + water) (3.73 ± 0.56 mL *vs.* 4.22 ± 0.64, respectively). At the end of CSDS, the mean of the last four measurements of defeated mice showed a significantly lower SP than that of control mice ($t = 2.35$, $p < 0.05$) (Fig. 2B). No differences in total liquid (mL) intake were observed between them ($t = 0.06$, $p > 0.05$) (Fig. 2A). The analysis of sucrose intake revealed remarkable interindividual variability in the defeated group with the existence of two discrete subgroups: one with an SP similar to that of the control animals and another with reduced SP compared to control animals. Based on this observation, mice subjected to chronic social defeat were segregated into susceptible and resilient subpopulations. A decrease in

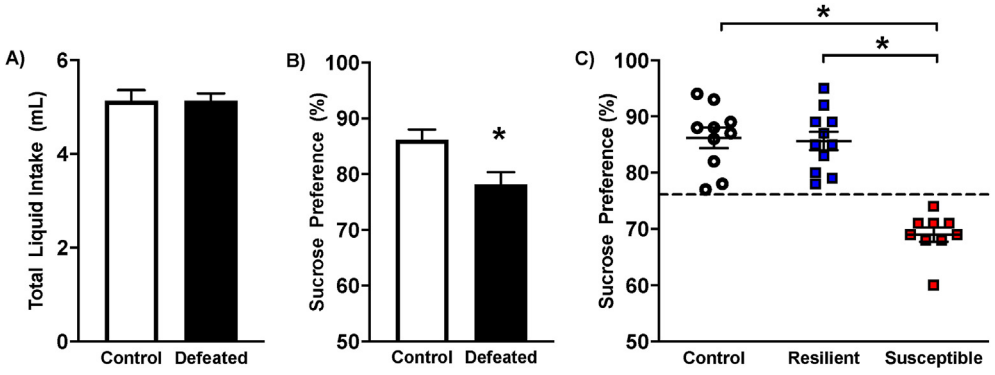

Fig. 2. Sucrose test parameters in male adolescent mice after chronic social defeat stress. The results represent the mean of four days of measurement. A) No difference in total liquid intake (sucrose + water; mL) was observed between the control (n = 10) and defeated groups (n = 20). B) Sucrose preference in the defeated group was significantly lower than that in the control group. C) Vertical scatterplot depicting the distribution of sucrose preference for control, resilient and susceptible mice. The dashed line represents 74% preference for sucrose over water, which corresponds to two standard deviations from the control mean and was taken as the criterion for susceptibility. Only susceptible mice (n = 9) displayed anhedonia, as measured by a reduction in sucrose preference. The mean preference for sucrose shown by resilient (n = 11) mice did not significantly differ from that shown by control mice. Mean ± SEM; *p < 0.05.

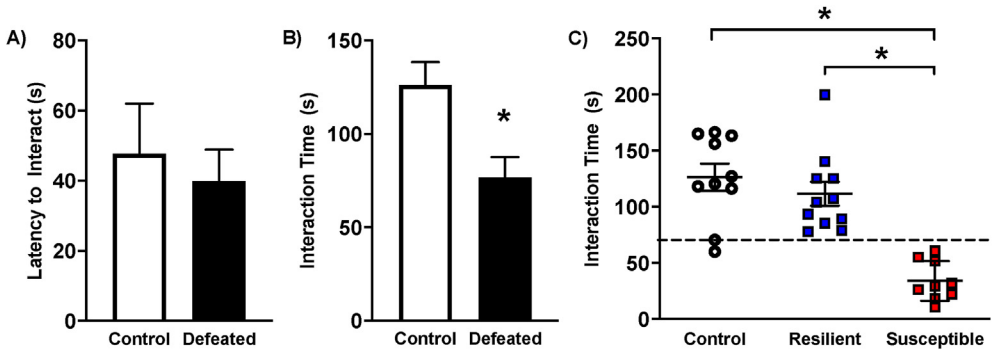

Fig. 3. Social avoidance behavior in male adolescent mice after chronic social defeat stress. A) No differences in the latency to interact with an unfamiliar adolescent C57BL/6 mouse were observed between the control and defeated groups. B) Defeated mice showed social avoidance spending significantly less time interacting with the social target. C) Vertical scatterplot showing the interaction time for the control, resilient and susceptible groups. The dashed line represents two standard deviations from the control mean, which is ~70 s. Susceptible mice (n = 9) spent less time engaged in social interaction than controls. The time spent in social interaction by resilient (n = 11) mice did not significantly differ from controls (n = 10). Mean ± SEM; *p < 0.05.

SP below 74%, which corresponds to two standard deviations from the control mean, was taken as the criterion for susceptibility. Of defeated mice, 45% (n = 9) showed an SP below 74% and were defined as susceptible. In total, 55% (n = 11) of defeated animals demonstrated an SP over 74% and were regarded as resilient (Fig. 2C). We found a significant difference in SP among the control, susceptible and resilient groups ($F_{(2,27)} = 34.15$, $p < 0.001$; Fig. 2C). *Post hoc* comparisons indicated that the susceptible group was different from the control and resilient groups ($p < 0.05$). The mean preference for sucrose shown by resilient mice did not differ from that of control mice ($p > 0.05$) (Fig. 2C).

3.2. Social approach-avoidance test

The effects of social defeat stress during adolescence on social avoidance are shown in Fig. 3. Defeated mice did not differ from controls in the latency to interact with an unfamiliar adolescent male mouse from the same strain ($U = 83.50$, $p > 0.05$) (Fig. 3A). However, the time spent in the interaction zone, another measure of social avoidance, indicated that defeated mice spent less time engaged in social interaction than controls (Fig. 3B) ($t = 3.20$, $p < 0.05$). Mice were also categorized as resilient or susceptible based on the interaction time. An interaction time below 69.82 s was taken as the criterion for susceptibility to social stress, which corresponds to two standard deviations from the control mean. As indicated in Fig. 3C, 11 (55%) out of a total of 20 defeated mice failed to show social avoidance behavior and were considered resilient. A total of 9 mice (45%) showed significant social avoidance behavior in the presence of unfamiliar mice and were considered susceptible. The results demonstrated significant differences in social interaction among susceptible, resilient and control mice, as indicated by a significant change in social interaction time ($F_{(2,27)} = 27.76$, $p < 0.05$). *Post hoc* comparisons demonstrated that susceptible mice spent significantly less time investigating the social target mouse than controls and resilient mice ($p < 0.05$). No significant differences were noted between resilient mice and controls in social interaction ($p > 0.05$) (Fig. 3C).

3.3. Susceptibility and resilience in both sucrose preference and social approach-avoidance tests

To better understand how male adolescent mice respond to chronic social defeat stress, we tested the relationship between their two behavioral quantitative variables: sucrose preference and interaction time values after *z*-score transformation. We found no correlation between SP and SAA values ($p > 0.05$, $f = 0.54$, $r^2 = 0.019$). To visualize the broad distribution of animals through the resilience-susceptibility spectrum, defeated adolescent mice were clustered into four subgroups: resilient in both SP and SAA parameters (n = 6; blue), resilient and susceptible in each of the parameters (n = 5 purple, and n = 5 green), and susceptible in both parameters (n = 4 red) (Fig. 4).

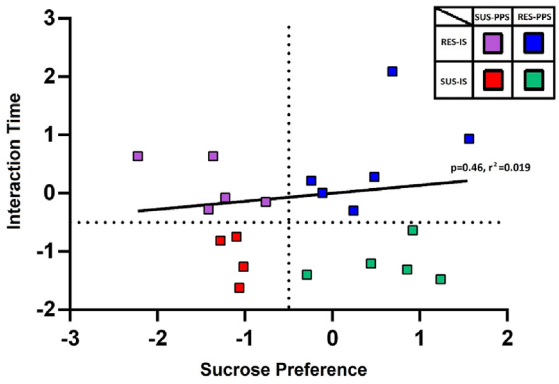

Fig. 4. Relationship between sucrose preference and interaction time parameters of socially defeated adolescent male mice and clustering observation. Sucrose preference and interaction time values of experimental mice (n = 20) were transformed into *z*-scores using control animal data. Defeated adolescents were clustered in subgroups according to resilience and susceptibility to each parameter. No correlation was observed between sucrose preference and interaction time scores ($r^2 = 0.019$; $p > 0.05$). The dashed lines represent the separation criterion value for resilience and susceptibility according to each parameter.

3.4. Elevated plus maze test

The time spent in the open arms ($t = 0.22$, $p > 0.05$) (Fig. 5A), the number of entries in relation to the total entries in closed and open arms ($t = 0.27$, $p > 0.05$) (Fig. 5B), and the distance traveled in the open arms ($U = 95.00$, $p > 0.05$) in the five total minutes the mice spent in the EPM did not differ between the control and experimental groups (Fig. 5C). These results indicate that adolescent defeated mice did not show anxiety-like behaviors. As shown in Fig. 6, defeated mice segregated as susceptible in the SP (Fig. 6A) or susceptible in the SAA (Fig. 6B) tests did not differ from controls or resilient mice in any EPM parameter ($p > 0.05$ for all).

3.5. Body weight gain

Control and experimental adolescent male mice had similar body weights at the beginning of the defeat protocol (12.69 ± 0.65 g, n = 10 *vs.* 13.49 ± 0.48 g, n = 20, respectively; $t = 1.07$, $p > 0.05$). The final body weights of the control and experimental mice were 17.24 ± 0.36 g and 16.63 ± 0.32 g, respectively ($t = 1.15$, $p > 0.05$). Defeated mice, as a group, gained significantly less weight over the 10-day period than controls ($t = 2.91$, $p < 0.05$) (Fig. 7A). When separating animals into resilient and susceptible subgroups

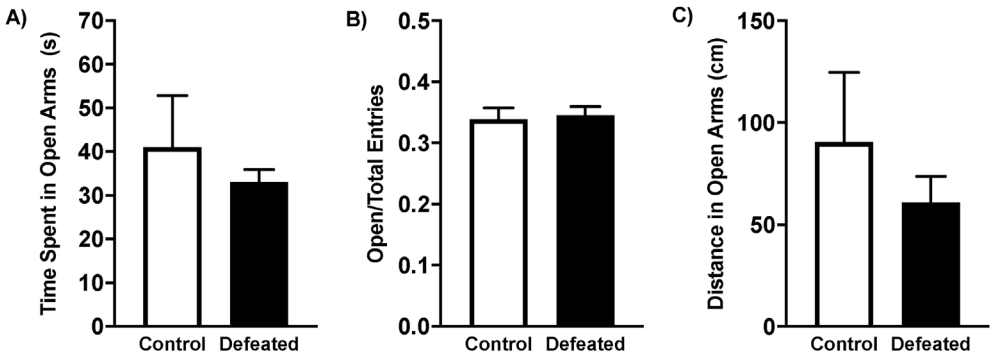

Fig. 5. Anxiety-like behaviors in the EPM of male adolescent mice after chronic social defeat stress. (A) Time spent in the open arms (s), B) the number of entries in the open arms in relation to open + closed arms, and C) the distance traveled in the open arms (cm) in the five total minutes the mice spent in the EPM. There were no differences between the control (n = 10) and the defeated (n = 20) groups (p > 0.05). Mean ± SEM.

according to the SP and SAA test results, body weight gain was different among groups ($F_{(2,27)} = 5.05$, $p < 0.05$; $F_{(2,27)} = 5.61$, $p < 0.05$, respectively). *Post hoc* comparisons showed that only the mice resilient in the SP test or those resilient in the SAA test exhibited decreased body weight gain compared to their respective controls in each test ($p < 0.05$, Fig. 7B). To understand whether defeated mice totally resilient (in both the SP and SAA tests) would be the most affected in relation to body weight gain, we analyzed data from the four clustered subgroups depicted in Fig. 4. The results showed that indeed, only the resilient animals in both the SP and SAA parameters (n = 6; blue) exhibited decreased body weight gain when compared to controls ($H_{(4,25)} = 11.66$ $p < 0.05$), but not the resilient animals exclusively in the SP (n = 5 green, $p > 0.05$) or exclusively in the SAA parameters (n = 5 purple, $p > 0.05$) (Fig. 7C).

3.6. Corticosterone immunoassay

Fig. 8 shows the effect of CSDS on blood serum CORT concentration in adolescent mice. Twenty-four hours after the last episode of social defeat, defeated mice exhibited increased levels of blood CORT compared to control mice ($t = 2.25$, $p < 0.05$) (Fig. 8A). No differences in blood CORT concentration were observed among the control (21.46 ± 5.87 ng/mL), susceptible (43.46 ± 12.01 ng/mL) and resilient groups (61.75 ± 14.78 ng/mL) ($F_{(2,27)} = 3.18$, $p > 0.05$) when susceptibility and resilience were based on SP test results (Fig. 8B). However, when defeated mice were separated into susceptible and resilient groups on the basis of the SAA test results, there was a significant effect of group ($F_{(2,27)} = 3.93$, $p < 0.05$). *Post hoc* comparisons showed that resilient mice had higher CORT levels than controls ($p < 0.05$). Defeated mice were also categorized as high CORT or low CORT based on control concentrations (two standard deviations from the control mean: 56.70 ng/mL; high CORT n = 8 *vs.* low CORT n = 12). We did not find differences in behavior in the SP or SAA tests according the hormone levels of defeated mice (data not shown).

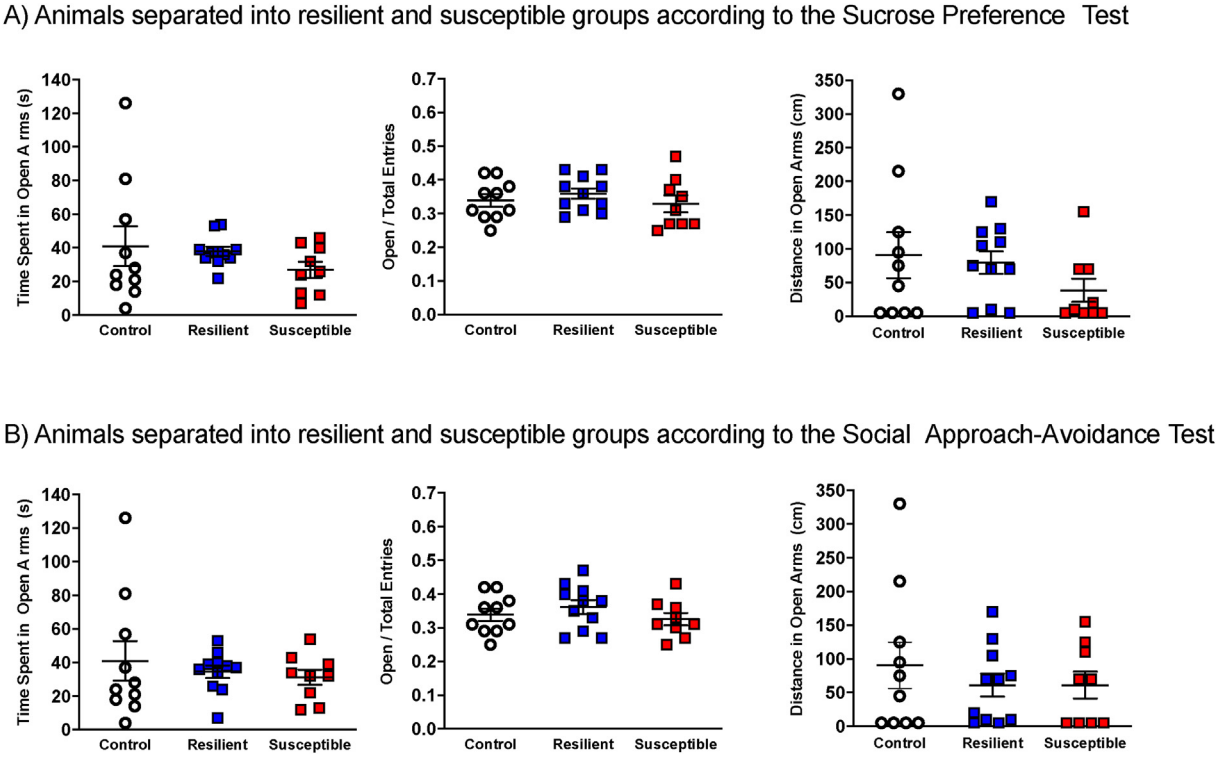

Fig. 6. Anxiety-like behaviors in the EPM of male adolescent mice after chronic social stress according to susceptibility and resilience to the SP and SAA tests. Susceptible animals in the sucrose preference test (n = 9) did not differ from controls (n = 10) and resilient mice (n = 11) in the time spent in the open arms (s), the number of entries in open arms (over total entries), and the distance traveled in the open arms (cm) ($p > 0.05$; Fig. 6A). Susceptible animals in the social approach-avoidance test (n = 9) did not differ from controls (n = 10) and resilient mice (n = 11) in any parameter from the EPM test ($p > 0.05$; Fig. 6B). Mean ± SEM.

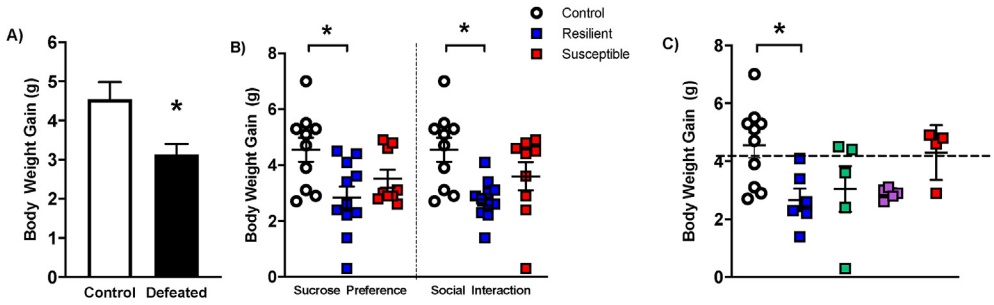

Fig. 7. Body weight gain in male adolescent mice after chronic social defeat stress.
(A) Defeated animals (n = 20) gained significantly less weight over the 10-day period compared to controls (n = 10). (B) Segregation of resilient and susceptible defeated mice according to the SP or SAA tests. Resilient groups in both subsets gained significantly less body weight when compared to controls. (C) Segregation of defeated mice according to both SP and SAA resiliency-susceptibility spectra. In total, resilient mice (to both SP and SAA parameters - blue) had less body weight gain than controls. Resilient animals exclusively in the SP (green) or exclusively in the SAA (purple), and susceptible to both SP and SAA parameters (red). Mean ± SEM; *p < 0.05.

4. Discussion

We explored adolescent behavioral and physiological consequences of chronic psychosocial stress in male C57BL/6 mice (from 30 to 40 days of age) using the CSDS model. After 10 days of daily aggressive social interactions, defeated adolescent mice exhibited reduced SP, reduced social interaction time with an unknown adolescent male from their same strain, reduced weight gain and higher basal blood corticosterone concentration. These phenotypes were observed in the defeated animals as a group, but because there were remarkable individual variabilities, we were able to identify adolescents that were resilient and susceptible to different behavioral outcomes of chronic social defeat stress.

4.1. Defeated male adolescents showed anhedonia-like and social avoidance behaviors

The SP test is a widely used behavioral test that measures stress-induced anhedonia or hedonic deficit in rodents (Katz et al., 1982; Monleon et al., 1995). The 10-day CSDS protocol caused a significant decrease in the SP of defeated adolescents when compared to controls. In our previous study, we also reported decreased SP in adolescent male mice using the 21-day protocol of repeated brief social defeat stress (from 30 to 50 days of age) (Chiavegatto et al., 2013; Resende et al., 2016). Limited data are available for the SP test using juvenile or adolescent mice, and there appear to be inconsistent findings. Whereas Iniguez et al. (2014) found that 10-day-defeated male adolescent mice displayed a reduced preference for the sucrose solution at 45 days of age, Mouri et al. (2018) did not report differences in this test in 31-day-old mice using a modified protocol of social defeat (10 days with 10 min of physical contact only, without sensory threat). It is important to note that in the present study, the SP value represents the mean of measurements conducted on the 4 last days of social defeat, when they had sucrose solution as a free choice during 16 h (including their entire dark phase of the light-dark cycle, from 37 to 40 days old). By doing so, we avoided some problems associated with the experimental context (Bondar et al., 2008) and the technical problems of single-point measurements, such as bottle leakage or clogging, neophobia and side-preference bias (Strekalova and Steinbusch, 2010).

Another behavioral consequence of social defeat stress is social avoidance or reduced social interaction. This behavior is commonly measured after the social defeat paradigm, and, depending on certain circumstances (known or unknown social target, strain, apparatus), it can be interpreted as a fear specific to the context of the defeat, a generalized fear of any social interaction, or a lack of social interest (Toth and Neumann et al., 2013). To avoid the first interpretation (specific fear), we used an unknown conspecific from the same strain (C57BL/6) and age (adolescent) as a social target in the SAA test. We believe this design can better relate to the core symptoms of depression and posttraumatic stress disorders. Accordingly, a recent study showed that not all susceptible adult male mice (classified with respect to the avoidant phenotype in a social interaction test using mice from the same strain as the aggressor, *i.e.*, CD-1) presented social avoidance against an unknown conspecific from a different aggressor strain (Ayash et al., 2020). In our study, defeated adolescent mice, as a group, showed reduced social interaction time with peers compared to controls.

4.2. Identification of susceptible and resilient male adolescents to anhedonia-like and social avoidance behaviors after CSDS

Looking at the individual values of defeated adolescents in the SP and SAA tests, we clearly see a nonhomogeneous distribution, in which some of the subjects maintained the SP and social interaction time similar to controls. In this regard, we used the control group of each behavioral test to establish a cut-off value (mean minus two standard deviations) to segregate the defeated group into two subpopulations: resilient mice (similar to the control group) and susceptible mice (different from the control group). We think this criterion is more adequate since it takes into account the control value distribution in each

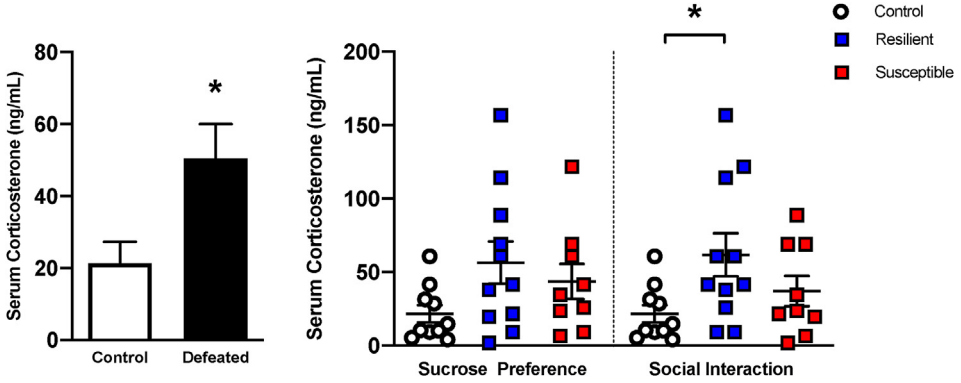

Fig. 8. Serum corticosterone concentrations (ng/mL) in male adolescent mice 24 h after the last episode of CSDS. (A) Defeated mice (n = 20) exhibited increased levels of blood corticosterone compared to controls (n = 10). (B) No difference in blood corticosterone concentration was observed among the control, susceptible (n = 9) and resilient (n = 11) groups when susceptibility and resilience were based on the SP test results, but resilient mice according to the SAA test results showed increased corticosterone when compared to the control group (p < 0.05). Mean ± SEM.

moment of experimental testing instead of using an arbitrary historical lowest control value (Strekalova et al., 2004) or ratio (Krishnan et al., 2007).

4.3. No correlation between anhedonia-like and social avoidance behaviors in socially defeated male adolescents

Interestingly, we found no correlation between these two different behavioral outcomes (Fig. 4), and only a small proportion of defeated adolescents were either totally susceptible (20%) or totally resilient (30%) for both anhedonia and social avoidance induced by the CSDS protocol. The remaining defeated mice had a distinct behavior impairment - susceptible in one test and resilient in the other. This is a novel and important finding, since the social interaction-avoidance test is currently used as a major criterion to investigate the neurobiology and molecular aspects of resilience to CSDS in male adult mice (Berton et al., 2006; Krishnan et al., 2007; Russo et al., 2012), and it is considered a depressive-like phenotype (Krishnan and Nestler, 2008). Our results suggest that, at least in adolescents, we should not assume that resilience to one criterion would automatically align with resistance for others in such heterogeneous and complex phenotypes of stress-induced disorders.

Indeed, the lack of correlation we found between the SP and SAA test results is not surprising, since these behaviors are influenced differently by social stress. Acute episodes of social defeat in male adults are sufficient to induce social fear but not anhedonia (reduced SP), which requires long-lasting stress stimuli (Toth and Neumann, 2013). Additionally, we previously found that social avoidance persisted when tested 20 days after the last defeat episode in male adolescent mice, but not the reduced preference for the sucrose solution (Carrillo et al., 2015), indicating that these behavioral consequences dissociate over time. The social avoidance as an enduring consequence of chronic social defeat was confirmed by other laboratories, where it was observed from 15 days up to 6 weeks after the last defeat episode (Zhang et al., 2016; Hasegawa et al., 2018), differently from anhedonia, which lasted no longer than 18 days (Krishnan et al., 2007; Macedo et al., 2018). Accordingly, a recent study failed to show anhedonia in social-avoidant defeated juvenile mice (Mouri et al., 2018), although we cannot exclude that procedural variables may account for this inconsistency.

4.4. Generalized anxiety is not observed in defeated adolescents from susceptible or resilient subpopulations

Defeated adolescents as a group, or specific to each identified subpopulation (resilient or susceptible mice), did not show anxiety-like behaviors in the EPM test when compared to control animals. The data distribution of each parameter investigated (time, distance, or the number of entries in the open arms) in defeated mice was homogeneous and similar to control mice, preventing any subclassification. Likewise, in our previous study, adolescent defeated mice under brief and repeated social defeat for 21 days did not show anxiety-like behaviors when tested in the EPM or in the open field (Resende et al., 2016). Juvenile male mice after 10 days of social defeat did not show anxiety-like behaviors in several tasks (Mouri et al., 2018), although different findings were also reported (Huang et al., 2013; Iniguez et al., 2014; Kovalenko et al., 2014). Additionally, we have also found that adolescent male mice under chronic social isolation, another type of social stress, did not show anxiety-like behaviors in the EPM, light-dark box, or open-field tests (Bibancos et al., 2007). Collectively, these studies from our laboratory strengthen the notion that chronic social defeat stress in juvenile or adolescent male mice (10- or 21-day protocol) induces the social type, but not the generalized type, of anxiety.

4.5. Behaviorally resilient defeated male adolescents display reduced weight gain and increased basal corticosterone levels after CSDS

Complementary to the behavioral analysis, we quantified body weight gain and basal serum corticosterone concentration one day after the last social defeat episode since animals usually exhibit many physiological responses after chronic stress (Bohus et al., 1987). Our defeated male adolescent mice, as a group, showed reduced body weight gain when compared to controls, similar to what has been previously reported (Iñiguez et al., 2014, 2016; Jianhua et al., 2017; Li et al., 2018). Basal corticosterone levels were higher in defeated mice than in controls, which is also consistent with the literature using the same stress model in adolescents (Iñiguez et al., 2014) or in juveniles immediately after the last defeat episode (Mouri et al., 2018). Although we did not quantify food intake, this phenotype may be due to the elevation in glucocorticoids and the subsequent change in the complex interplay between feeding neuropeptides and peripheral signals (Maniam and Morris, 2012).

Surprisingly, the defeated adolescents resilient to anhedonia, or those resilient to social avoidance, were the most affected mice in terms of both endocrine/physiological outcomes. Although it has already been established that socially defeated animals display fluctuations in body weight gain and corticosterone levels, these fluctuations were thought to be correlated with deleterious effects on behavior. A close inspection of the literature revealed that these paradoxical effects were also reported in adult male mice using the same CSDS model. Recently, a significant decrease in the body weight gain of mice resilient to social avoidance, but not mice susceptible to social avoidance (Gururajan et al., 2019), was reported; these results are similar to those in our defeated adolescents. Regarding basal blood corticosterone levels, Krishnan et al. (2007) reported that defeated adult mice resilient to social avoidance showed a significant increase compared to susceptible mice when studied 28 days after the social defeat protocol, although no differences were found one day after the last defeat. Resilience to CSDS is reported to be associated with other physiological changes (increased relative heart mass, decreased relative thymus weight) and molecular signatures in gene expression and chromatin modifications in specific brain regions that are not seen in susceptible animals (Krishnan et al., 2007; Gururajan et al., 2019; Wilkinson et al., 2009).

The functional relevance of these findings and how they relate to the behavioral resilient phenotype of defeated adolescents is not known. Resilience is an active process of body-brain plasticity in favor of adaptation to stress and can thus involve both passive and active strategies (Russo et al., 2012). Adaptive or maladaptive responses may depend on each particular physiological system, context and timing after stress exposure (Smith, 2019). Future work should address the temporal dynamics of HPA hyperactivity in the resting state and under an acute stress challenge, as well as body weight in resilient adolescents after the cessation of stress and the persistence of behavioral responses.

An important limitation to the present investigation is that we studied only male adolescent mice. Marked sex differences in stress responses have been reported in adult rodents and humans (for reviews, see Wellman et al., 2018 and Hodes and Epperson, 2019), but limited data in female rodents during adolescence are available (Burke et al., 2017). Additional studies should use female adolescents to explore the sex differences in the resilience phenotype after CSDS. Another limitation is related to the social defeat model. Because the social defeat model involves a dyadic interaction (resident-intruder model of aggression), it is not easy to induce similar levels of attacks on the intruders (experimental animals) throughout the 10 days. Although we tried to minimize this variability by a. testing and selecting the aggressors before the experiment (to standardize their attack latency); b. changing the aggressors daily for each defeat session (to maintain the aggressors' interest and the strength of attacks); and c. limiting the physical contact in each session to 5 min (instead of the usual 10 min in most protocols), we cannot ensure total similarity of the aggressive acts,

nor the intruders' perception of the aggression. Indeed, this technical limitation of the social defeat model is also the basis for its translational value. Recently, the nature or quality of social stress of male mice exposed to adult ICR aggressor mice as juvenile (24 days old) and adult (70 days old) for 1, 5, and 10 consecutive days was investigated (Mouri et al., 2018). The duration of defensive behaviors (escape and submissive postures) during the stress between juvenile and adult mice was not significant different suggesting similar perception of the aggression. Because our study is primarily observational, it would be informative to learn how the impact of stress during adolescence is regulated through a distinct mechanism, or even differences in the form of Fos expression, within brain areas across the presumably resilient versus vulnerable populations of adolescent mice.

In summary, we found that male adolescent mice, after being chronic socially defeated, showed reduced SP (anhedonia) and reduced social interaction with peers (generalized social avoidance or social type of anxiety) but did not show generalized anxiety-like behaviors. These two behavioral consequences of social defeat were not correlated. We could distinguish three subpopulations of resilient adolescent mice after CSDS: 1. those resilient to both SP and social avoidance (30%); 2. those resilient only to SP (25%); and 3. those resilient only to social avoidance (25%). Studying the subjects from each subclassification in detail will improve the definition of the resilient phenotype and help advance our understanding of the behavioral spectrum of chronic stress-induced emotional impairments.

Furthermore, we found that male adolescent mice, after being chronically socially defeated, showed reduced weight gain and increased basal blood corticosterone concentration. These endocrine-physiological outcomes were more remarkable in the resilient mice in general. An important next step is to study these results in a time-point manner in each distinct subpopulation of resilient adolescent mice immediately after chronic stress and in adulthood.

5. Conclusion

Social anxiety and depression are common and serious disorders of childhood and adolescence and are associated with more severe and more disabling forms of these illnesses in their future life (Beesdo et al., 2007; Andersen and Teicher, 2008). Psychosocial stress is the major form of stress faced by these young populations and is considered an important risk factor for the development of those disorders. Chronic social defeat stress is an interesting preclinical model that induces a whole spectrum of phenotypes resembling the clinical symptoms in humans. At the opposing ends of the spectrum are individuals resilient and susceptible to defeat stress. Studies in adult mice commonly use a single behavioral readout (social avoidance in a social interaction test after the stress paradigm) to segregate defeated animals in resilient or susceptible populations. Our study in male adolescent mice clearly demonstrates that social avoidance and decreased sucrose preference are independent behavioral responses after CSDS. These findings illustrate that, contrary to prior assumptions in adults, social defeat stress responses are more complex and singular in adolescents, and caution should be taken for the correct interpretation and translation of those phenotypes. Our results also highlight and strengthen the concept of resilience to CSDS as an active process and the importance of attention to individual variability in the subject's responses in different biological systems due to the multidimensional aspects of chronic stress-induced disorders, especially in young ages.

Finally, we propose a better characterization of social defeat stress responses as a critical step to advance our understanding of the mechanisms behind stress resilience that translate to human experience.

Funding

LAS received a master's fellowship from the CNPq-Brazil; LSR received a CAPES/PNPD postdoctoral fellowship from the Department of Pharmacology of the Biomedical Sciences Institute-USP, SP. This study was funded by the São Paulo Research Foundation (FAPESP #2017/06100-8 to SC).

CRediT authorship contribution statement

Leonardo Alves-dos-Santos: Investigation, Methodology, Formal analysis, Validation. **Letícia de Souza Resende:** Formal analysis, Validation. **Silvana Chiavegatto:** Conceptualization, Methodology, Validation, Supervision, Funding acquisition, Writing - review & editing.

References

Andersen, S.L., Teicher, M.H., 2008. Stress, sensitive periods and maturational events in adolescent depression. Trends Neurosci. 31 (4), 183–191. https://doi.org/10.1016/j.tins.2008.01.004.

Ayash, S, Schmitt, U, Müller, MB, 2020. Chronic social defeat-induced social avoidance as a proxy of stress resilience in mice involves conditioned learning. J. Psychiatr. Res. 120, 64–71. https://doi.org/10.1016/j.jpsychires.2019.10.001.

Beesdo, K., Bittner, A., Pine, D.S., et al., 2007. Incidence of social anxiety disorder and the consistent risk for secondary depression in the first three decades of life. Arch. Gen. Psychiatr. 64 (8), 903–912. https://doi.org/10.1001/archpsyc.64.8.903.

Berton, O., McClung, C.A., Dileone, R.J., et al., 2006. Essential role of BDNF in the mesolimbic dopamine pathway in social defeat stress. Science 311 (5762), 864–868. https://doi.org/10.1126/science.1120972.

Bibancos, T., Jardim, D.L., Aneas, I., Chiavegatto, S., 2007. Social isolation and expression of serotonergic neurotransmission-related genes in several brain areas of male mice. Gene Brain Behav. 6 (6), 529–539. https://doi.org/10.1111/j.1601-183X.2006.00280.x.

Björkqvist, K., 2001. Social defeat as a stressor in humans. Physiol. Behav. 73 (3), 435–442. https://doi.org/10.1016/s0031-9384(01)00490-5.

Bohus, B., Benus, R.F., Fokkema, D.S., et al., 1987. Neuroendocrine states and behavioral and physiological stress responses. Prog. Brain Res. 72, 57–70. https://doi.org/10.1016/s0079-6123(08)60196-x.

Bondar, N., Kovalenko, I., Avgustinovich, D., et al., 2008. Anhedonia in the shadow of chronic social defeat stress, or when the experimental context matters. Nat Prec. https://doi.org/10.1038/npre.2008.2682.1.

Brunstein Klomek, A., Marrocco, F., Kleinman, M., Schonfeld, I.S., Gould, M.S., 2007. Bullying, depression, and suicidality in adolescents. J. Am. Acad. Child Adolesc. Psychiatry 46 (1), 40–49. https://doi.org/10.1097/01.chi.0000242237.84925.18.

Burke, A.R., McCormick, C.M., Pellis, S.M., Lukkes, J.L., 2017. Impact of adolescent social experiences on behavior and neural circuits implicated in mental illnesses. Neurosci. Biobehav. Rev. 76 (Pt B), 280–300. https://doi.org/10.1016/j.neubiorev.2017.01.018.

Carrillo, J.F.S., Vasconcelos, P.E.N.S., Araujo, M.R., Honda, F.M., Resende, L.S., Chiavegatto, S., 2015. Behavioral effects of chronic social defeat stress in adolescent male mice and its persistence in adulthood. In: 9th IBRO - World Congress International Brain Research Organization, pp. 736. Pt -577. http://icongresso.itarget.com.br/useradm/anais/?clt=ibr.2&lng=I.

Chiavegatto, S., Amaral, C.E., Soares, R.B., Rodrigues, A.C.D., Reigado, C.N., Resende, L.S., Alves-dos-Santos, L., June 2013. Comparison of two different protocols of chronic social defeat in adolescent C57BL/6 male mice on anxiety and depression-related behaviors. Abstracts Int. Behav. Neurosci. Soc. 22 page 50; abstract 49. https://www.ibnsconnect.org/assets/docs/pastprograms/ibnsprogram2013final.pdf.

Douglas, L.A., Varlinskaya, E.I., Spear, L.P., 2004. Rewarding properties of social interactions in adolescent and adult male and female rats: impact of social versus isolate housing of subjects and partners. Dev. Psychobiol. 45 (3), 153–162. https://doi.org/10.1002/dev.20025.

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 (3), 343–363. https://doi.org/10.1023/A:1021637011732.

Golden, S.A., Covington 3rd, H.E., Berton, O., Russo, S.J., 2011. A standardized protocol for repeated social defeat stress in mice [published correction appears in Nat Protoc. 2015 Apr;10(4):643]. Nat. Protoc. 6 (8), 1183–1191. https://doi.org/10.1038/nprot.2011.361. Published 2011 Jul 21.

Gururajan, A, van de Wouw, M, Boehme, M, et al., 2019. Resilience to chronic stress is associated with specific neurobiological, neuroendocrine and immune responses. Brain Behav. Immun. 80, 583–594. https://doi.org/10.1016/j.bbi.2019.05.004.

Hasegawa, S., Miyake, Y., Yoshimi, A., et al., 2018. Dysfunction of serotonergic and dopaminergic neuronal systems in the antidepressant-resistant Impairment of social behaviors induced by social defeat stress exposure as juveniles. Int. J. Neuropsychopharmacol. 21 (9), 837–846. https://doi.org/10.1093/ijnp/pyy038.

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

Huang, G.B., Zhao, T., Muna, S.S., et al., 2013. Effects of chronic social defeat stress on behaviour, endoplasmic reticulum proteins and choline acetyltransferase in adolescent mice. Int. J. Neuropsychopharmacol. 16 (7), 1635–1647. https://doi.org/10.1017/S1461145713000060.

Iñiguez, S.D., Riggs, L.M., Nieto, S.J., et al., 2014 May. Social defeat stress induces a depression-like phenotype in adolescent male c57BL/6 mice. Stress 17 (3), 247–255. https://doi.org/10.3109/10253890.2014.910650.

Iñiguez, S.D., Aubry, A., Riggs, L.M., et al., 2016. Social defeat stress induces depression-like behavior and alters spine morphology in the hippocampus of adolescent male C57BL/6 mice. Neurobiol Stress 5, 54–64. https://doi.org/10.1016/j.ynstr.2016.07.001. Published 2016 Aug 21.

Jianhua, F, Wei, W, Xiaomei, L, Shao-Hui, W, 2017. Chronic social defeat stress leads to changes of behaviour and memory-associated proteins of young mice. Behav. Brain Res. 316, 136–144. https://doi.org/10.1016/j.bbr.2016.09.011.

Katz, R.J., 1982. Animal model of depression: pharmacological sensitivity of a hedonic deficit. Pharmacol. Biochem. Behav. 16 (6), 965–968. https://doi.org/10.1016/0091-3057(82)90053-3.

Kessler, R.C., Berglund, P., Demler, O., Jin, R., Merikangas, K.R., Walters, E.E., 2005. Lifetime prevalence and age-of-onset distributions of DSM-IV disorders in the national comorbidity survey replication [published correction appears in arch gen psychiatry. 2005 jul;62(7):768. Merikangas, kathleen R [added]. Arch. Gen. Psychiatr. 62 (6), 593–602. https://doi.org/10.1001/archpsyc.62.6.593.

Kovalenko, I.L., Galyamina, A.G., Smagin, D.A., Michurina, T.V., Kudryavtseva, N.N., Enikolopov, G., 2014. Extended effect of chronic social defeat stress in childhood on behaviors in adulthood. PLoS One 9 (3), e91762. https://doi.org/10.1371/journal.pone.0091762. Published 2014 Mar 25.

Krishnan, V., Han, M.H., Graham, D.L., et al., 2007. Molecular adaptations underlying susceptibility and resistance to social defeat in brain reward regions. Cell 131 (2), 391–404. https://doi.org/10.1016/j.cell.2007.09.018.

Krishnan, V., Nestler, E.J., 2008. The molecular neurobiology of depression. Nature 455 (7215), 894–902. https://doi.org/10.1038/nature07455.

Kudryavtseva, N.N., Bakshtanovskaya, I.V., Koryakina, L.A., 1991. Social model of depression in mice of C57BL/6J strain. Pharmacol. Biochem. Behav. 38 (2), 315–320. https://doi.org/10.1016/0091-3057(91)90284-9.

Li, M., Xu, H., Wang, W., 2018. An improved model of physical and emotional social defeat: Different effects on social behavior and body weight of adolescent mice by interaction with social support. Front Psychiatr. 9, 688. https://doi.org/10.3389/fpsyt.2018.00688. Published 2018 Dec 11.

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.

Macri, S., Adriani, W., Chiarotti, F., Laviola, G., 2002. Risk Taking during Exploration of a Plus-Maze Is Greater in Adolescent than in Juvenile or Adult Mice. pp. 541–546. https://doi.org/10.1006/anbe.2002.4004.

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 (1), 316. https://doi.org/10.1038/s41398-019-0651-y. Published 2019 Nov 26.

Maniam, J, Morris, MJ, 2012. The link between stress and feeding behaviour. Neuropharmacology 63 (1), 97–110. https://doi.org/10.1016/j.neuropharm.2012.04.017.

Monleon, S., D'Aquila, P., Parra, A., Simon, V.M., Brain, P.F., Willner, P., 1995. Attenuation of sucrose consumption in mice by chronic mild stress and its restoration by imipramine. Psychopharmacology (Berlin) 117 (4), 453–457. https://doi.org/10.1007/bf02246218.

Mouri, A., Ukai, M., Uchida, M., et al., 2018. Juvenile social defeat stress exposure persistently impairs social behaviors and neurogenesis. Neuropharmacology 133, 23–37. https://doi.org/10.1016/j.neuropharm.2018.01.016.

Paus, T., Keshavan, M., Giedd, J.N., 2008. Why do many psychiatric disorders emerge during adolescence? Nat. Rev. Neurosci. 9 (12), 947–957. https://doi.org/10.1038/nrn2513.

Resende, L.S., Amaral, C.E., Soares, R.B., et al., 2016. Social stress in adolescents induces depression and brain-region-specific modulation of the transcription factor MAX. Transl. Psychiatr. 6 (10), e914. https://doi.org/10.1038/tp.2016.202. Published 2016 Oct 11.

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

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

Smith, BL., 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.

Spear, L.P., 2000. The adolescent brain and age-related behavioral manifestations. Neurosci. Biobehav. Rev. 24 (4), 417–463. https://doi.org/10.1016/s0149-7634(00)00014-2.

Strekalova, T., Couch, Y., Kholod, N., Boyks, M., Malin, D., Leprince, P., Steinbusch, H.M., 2011. Update in the methodology of the chronic stress paradigm: internal control matters. Behav. Brain Funct. : BBF 7, 9. https://doi.org/10.1186/1744-9081-7-9.

Strekalova, T., Spanagel, R., Bartsch, D., Henn, F.A., Gass, P., 2004. Stress-induced anhedonia in mice is associated with deficits in forced swimming and exploration. Neuropsychopharmacology 29 (11), 2007–2017. https://doi.org/10.1038/sj.npp.1300532.

Strekalova, T., Steinbusch, H.W., 2010. Measuring behavior in mice with chronic stress depression paradigm. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 34 (2), 348–361. https://doi.org/10.1016/j.pnpbp.2009.12.014.

Toth, I., Neumann, I.D., 2013. Animal models of social avoidance and social fear. Cell Tissue Res. 354 (1), 107–118. https://doi.org/10.1007/s00441-013-1636-4.

Wellman, C.L., Bangasser, D.A., Bollinger, J.L., et al., 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.

Wilkinson, M.B., Xiao, G., Kumar, A., et al., 2009. Imipramine treatment and resiliency exhibit similar chromatin regulation in the mouse nucleus accumbens in depression models. J. Neurosci. 29 (24), 7820–7832. https://doi.org/10.1523/JNEUROSCI.0932-09.2009.

Zhang, F., Yuan, S., Shao, F., Wang, W., 2016. Adolescent social defeat induced alterations in social behavior and cognitive flexibility in adult mice: effects of developmental stage and social condition. Front. Behav. Neurosci. 10, 149. https://doi.org/10.3389/fnbeh.2016.00149. Published 2016 Jul 20.