

# Individual baseline behavioral traits predict the resilience phenotype after chronic social defeat

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## ABSTRACT

Chronic social defeat (CSD) has been widely used as a psychosocial stress model in mice, with the magnitude of CSD-induced social avoidance as the major behavioral hallmark of the resilient and susceptible groups. Despite significant progress in the study of the neurobiology of resilient and susceptible mice, the nature and ethological relevance of CSD-induced social avoidance and social approach, particularly measured using a CD1 mouse, needs conceptual clarification. Based on the findings of a recent study revealing substantial individuality in genetically homogeneous inbred mice, we investigated whether certain baseline individual characteristics of male C57BL/6J mice predict the resilient outcome after CSD. We focused on two well-studied individual traits that seem to have heritable underpinnings—approach to novelty and avoidance of harm, which are essential for the expression of the exploratory drive. Our results showed that the exploration levels and the approach to novelty and harm were different before and after CSD in resilient and susceptible mice. Before the stress, resilient mice had higher horizontal activity in a novel environment, shorter approach latencies, and higher exploration times for social and non-social targets than susceptible mice. However, susceptible mice performed better in the passive avoidance task than resilient mice as they were more successful in learning to avoid potential adversity by suppressing the spontaneous exploratory drive. Our findings challenge the validity of the current selection criteria for the susceptible and resilient groups and encourage comprehensive assessment of both baseline and stress-induced individual behavioral signatures of mice.

## 1. Introduction

Chronic social defeat (CSD) is a stress paradigm widely used to model psychosocial stress in rodents (Martinez et al., 1998; Toth and Neumann, 2013; Hollis and Kabbaj, 2014). The plethora of neuroendocrine and behavioral changes that defeated rodent display as well as individual variability in response to stress make this paradigm a powerful tool for studying the underlying mechanisms of stress resilience and vulnerability (Bartolomucci et al., 2003; Bhatnagar et al., 2006; Krishnan et al., 2007; Wood et al., 2010; Razzoli et al., 2011). The core feature of the model is the resident-intruder encounter. The development of social avoidance, that is, the loss of preference for social targets over non-social targets in the social interaction test (Krishnan et al., 2007), has been the behavioral hallmark in the context of resilience research in recent years. In a typical setting, the social target is a new, unfamiliar mouse of the same strain as the resident (aggressor) mouse previously

used for the defeat sessions (Golden et al., 2011; Hammels et al., 2015). Most stressed mice develop a decreased drive to approach and explore this particular social target, while resilient mice maintain the preference for the social target at levels comparable to the non-stressed group. Accordingly, the social interaction test has been widely used to stratify the defeated population into susceptible and resilient animals (Golden et al., 2011). Since the hallmark study by Krishnan et al. (2007), CSD stress in combination with the outcome in the social interaction test has inspired numerous studies to investigate the neurobiological mechanisms underlying stress resilience. Deep insights into resilience mechanisms will enable the development of more elaborate and targeted strategies for resilience-promoting interventions and their translation into clinical practice (Krishnan et al., 2007; Friedman et al., 2014, 2016; Henry et al., 2018).

However, to bridge this translational gap, a comprehensive understanding of the ethological relevance of resilient and susceptible groups

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following CSD is of utmost importance (Nestler and Hyman, 2010; Henriques-Alves and Queiroz, 2016). From an evolutionary point of view, social avoidance developed by susceptible mice shortly after CSD can be considered an adaptive coping strategy in the presence of a potentially dangerous stimulus (Blanchard et al., 2001; Meduri et al., 2013; Diaz and Lin, 2020). Meduri et al. (2013) suggested that the social interaction test after CSD is another measure of fear-motivated learning processes. Recently, Ayash et al. (2020) provided clear evidence for the involvement of conditioning in the development of CSD-induced social avoidance. Successful learning of the association between an aversive event and environmental stimuli that predict the event is essential for survival. Based on the latter assumption, we might question whether preserved social preference in the social interaction test reflects the ability to successfully cope with stressful events or it is an indication of the animal's inability to learn threat-associated cues. Furthermore, the question arises whether there are any other underlying individual traits that predispose animals to the development of such behavior in response to CSD.

Based on the finding of a recent study revealing substantial individuality in genetically homogeneous inbred mice (Tuttle et al., 2018), we hypothesized that C57BL/6J mice display certain individual characteristics that enhance their segregation into resilient and susceptible groups based on the social interaction test findings after CSD. To test our hypothesis, we focused on two well-studied individual traits that seem to have heritable underpinnings (Cloninger 1986)—approach to novelty and avoidance of harm. In rodents, novelty seeking indicates an enhanced exploratory behavior toward novel situations, objects, or stimuli, while harm avoidance measures the tendency to either respond intensively to aversive stimuli or learn to avoid punishment and react passively to novelty (Cloninger 1986). These two traits largely influence an animal's exploratory behavior, an innate urge essential for any kind of unconditioned behavioral test. The social interaction test used to characterize the animals after CSD stress combines both the aforementioned aspects—novelty of the social target and the potential harm of the aggressor strain. To this end, we investigated the behavior of naïve male C57BL/6J mice with respect to their approach to a novel social and non-social target and aversive learning under baseline conditions, that is, before the stress, and compared the findings with the social interaction test outcomes after the stress.

From a preclinical perspective, the introduction of basal characterization, an approach adopted from clinical studies (Chmitorz et al., 2020), as a pre-step in the overall assessment of stress-induced readouts in mice would substantially increase the translational validity of the entire mouse model.

## 2. Material and methods

### 2.1. Animals

This study included 8-week-old male C57BL/6J mice weighing 22–25 g purchased from Janvier (France). Animals were habituated to the housing conditions for 7 days before the start of experiments. CD1 mice that were used as aggressors during CSD were retired breeders in the animal facility. Mice were housed individually in an animal care facility, with a 12-h light-dark cycle (lights on 08:00 a.m.), an air temperature of 23 °C, and a humidity of 45%. Water and food were available *ad libitum*. All experimental work was performed during the animals' light phase, between 09:00 a.m. and 01:00 p.m. All experimental procedures were performed in accordance with the European Communities Council Directive regarding care and use of animals for experimental procedures and were approved by the local authorities (Landesuntersuchungsamt Rheinland-Pfalz). All efforts were made to minimize animal suffering and reduce the number of animals used for the study.

### 2.2. Approach and exploration of a novel object before CSD

Experimentally naïve mice (N = 51) were placed in an open-field arena, a rectangular box measuring 40 cm × 40 cm × 40 cm, and behavior was recorded for 5 min. Subsequently, mice were briefly returned to their home cages, and an unfamiliar object (plastic toy bird, 6.5 cm high) was introduced in the center of the open field. Mice were returned to the same arena to explore the object for another 2.5 min. Blood samples for measurement of corticosterone levels were collected by tail cut 20 min after finishing the test. In the first phase of the test, we scored the total distance traveled, distance traveled in the center of the open-field arena (defined as a rectangular zone with borders 5 cm distant from the open-field walls), and number of exploratory rearings against the arena walls (vertical exploration). In the second phase of the test, we scored the total distance traveled, time spent exploring the object, latency to approach the object, and time sitting in the corners. On the next day, mice were divided into control (N = 14) and defeated groups (N = 37) and subjected to handling (controls) or CSD. CSD lasted 10 days and was performed based on the protocol described by Golden et al. (2011), with a slight modification in the duration of the daily defeat sessions. Instead of 5 min, the daily defeat session was limited to 2 min, counting from the first CD1 attack. This modification was introduced to decrease the severity of the defeat and potential physical wounding in the mice. Seven days after the last defeat session, we performed a social interaction test as described by Golden et al. (2011). Briefly, mice were introduced to explore the same rectangular open-field arena as that used for the open-field test; however, for this test, it contained an empty wire mesh (10-cm diameter) placed next to the wall. After 2.5 min, mice were briefly returned to the home cages, the empty mesh was replaced with a wire mesh containing an unfamiliar CD1 mouse, and exploration was recorded for another 2.5 min. The time exploring both the social target and empty mesh, approach latencies, and time sitting in the two corners most distant from the mesh were scored. The time in the corner was considered only when it is > 5 s. The social interaction index was calculated by dividing the time exploring the CD1 mouse with the time exploring the empty mesh. Mice were divided into the resilient and susceptible groups according to their social index (SI) with a cutoff value at SI = 1.0. Data on the exploration times obtained before the stress were organized and analyzed according to this division.

### 2.3. Approach and exploration of an unknown social target before CSD

In this experiment, we used another batch of 49 experimentally naïve male C57BL/6J mice to assess the pattern of social exploration before CSD. The testing was performed as described by Golden et al. (2011), except that the social target was an adult male C57BL/6J mouse of the same age as the experimental mouse. The time exploring the empty mesh and the social target, approach latencies, and time sitting in two corners most distant to the mesh were scored, and the social interaction index was calculated. The next day, mice were divided into control (N = 16) and defeat (N = 31) groups and subjected to handling or social defeat procedures as described above. A social interaction test with CD1 mouse as a social target was performed 7 days after the last defeat session. Twenty minutes after the completion of the social interaction test, blood samples for the serum corticosterone measurements were taken by the tail cut. Mice were divided into resilient and susceptible groups according to the SI, and the data on the exploration times obtained before the stress were analyzed according to this division.

### 2.4. Passive avoidance before CSD

To investigate variability in associative learning and the tendency to avoid harm, we used the passive avoidance test (PA). The chamber for passive avoidance (45 cm × 29 cm) consisted of a light and a dark compartment of equal size, separated by slide doors automatically

operated by the software (TSE Systems GmbH, Germany). For this experiment, we used a batch of 45 experimentally naïve male C57BL/6J mice. On the first day of the experiment, animals were introduced into the lit chamber, and after 60 s of habituation, the door to the dark compartment was opened. The activity of the animals was registered using infrared light beams. Immediately upon entering the dark compartment, the doors were closed, and the animal received a foot shock of 0.7 mA for 2 s via the grid floor. After a 60-s delay, animals were returned to the home cage. On the second day, animals were placed again into the lit chamber, and after 15 s, doors to the dark chamber were opened. The latency to enter the dark compartment (DC) was recorded. The cutoff time was set at 300 s. Mice were given 7 days of pause and were then divided into the control group ( $N = 18$ ) and the defeat group ( $N = 27$ ) and subjected to a CSD procedure as described above. Seven days after the last defeat session, mice were evaluated using the social interaction test, the defeated population was divided into the resilient and susceptible groups, and data from the PA test obtained before CSD were analyzed according to this division.

## 2.5. Video tracking and scoring

The results of the open-field, object exploration, and social interaction tests were recorded using EthoVision software 11.0 (Noldus Information Technology, Wageningen, the Netherlands). Parameters such as total distance traveled and time sitting in the corner were scored automatically by EthoVision, and exploration of the non-social or social target was scored manually by a blind observer using Observer XT 13 (Noldus Information Technology).

## 2.6. Measurement of the serum corticosterone level

Fresh blood samples were centrifuged at 10,000 rpm for 10 min at 4 °C, and the serum was collected and stored at -80 °C until analysis. Corticosterone concentration was measured using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Enzo Life Sciences, Inc.) following the manufacturer's protocol. The absorbance was read at 450 nm using a microplate reader (Multiskan EX, Thermo Scientific, USA).

## 2.7. Statistics

For the preparation of graphs and statistical analysis, we used GraphPad Prism 8.3.0 (GraphPad Software Inc., San Diego, CA, USA) and SPSS (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.). Normal distributed data are presented as mean  $\pm$  standard error (SEM) on the graphs, and skewed data as medians. Depending on the data distribution, comparisons between the control and defeated groups as well as between the resilient and susceptible groups were made using *t*-test or Mann-Whitney *U* test. Differences in the social interaction index after the CSD test were assessed using one-way ANOVA. Comparisons of exploration times during the social interaction test and training and testing times in the passive avoidance task were analyzed using two-way ANOVA, with *post hoc* Tukey test if applicable. A one-way analysis of covariance (ANCOVA) was conducted to compare CD1 exploration times in the control, susceptible, and resilient groups while controlling for the different pre-CSD behavioral scores.

## 3. Results

### 3.1. Approach and exploration of a novel object before CSD

After CSD, the defeated animals had a significantly lower SI than control animals ( $t = 3.402$ ;  $P = 0.0013$ ); one-way ANOVA showed a significant effect of the group ( $F(2,49) = 17.97$ ;  $P < 0.0001$ ) and the *post hoc* test showed that susceptible animals had a significantly lower SI

than control ( $p < 0.0001$ ) and resilient mice ( $p = 0.0001$ ) (Fig. 1a). Two-way ANOVA on exploration times showed a significant effect of the group ( $F(2, 98) = 6.655$ ;  $P = 0.002$ ) and a significant interaction between the group and testing condition ( $F(2, 98) = 13.22$ ;  $P < 0.0001$ ). While the exploration of an empty mesh did not differ among groups, exploration of a CD1 target was significantly lower in the susceptible group than in the control ( $p = 0.0149$ ) and resilient group ( $p < 0.0001$ ). Interestingly, resilient mice had a higher exploration of the CD1 target than control mice ( $p = 0.031$ ) (Fig. 1b).

Analysis of the data obtained before CSD showed that resilient mice tended to have greater activity than susceptible mice, as indicated by the total distance traveled during the initial exploration of an empty open field ( $t = 2.005$ ;  $P = 0.052$ ). The number of vertical explorations did not significantly differ between the two groups (data not shown). During the object exploration session, resilient mice were also more active than susceptible mice. They traveled more distance ( $t = 2.185$ ;  $P = 0.0365$ ), spent more time exploring an unknown object ( $t = 3.209$ ;  $P = 0.0029$ ), and spent less time sitting in the corner than susceptible mice ( $t = 3.644$ ;  $P = 0.002$ ) (Fig. 2a and b). Resilient mice also tend to have lower approach latencies ( $U = 119.5$ ;  $P = 0.07$ ) to the unknown object than susceptible mice (Fig. 2c). The same behavioral pattern was observed during the social interaction test after CSD. Resilient mice spent more time exploring the CD1 target ( $t = 5.789$ ;  $P = 0.0001$ ) (Fig. 2d) and less time sitting in the corner than susceptible mice ( $t = 3.025$ ;  $P = 0.008$ ) (Fig. 2e). Resilient mice tended to approach the CD1 target with shorter latencies than susceptible mice ( $U = 118$ ;  $P = 0.065$ ) (Fig. 2f).

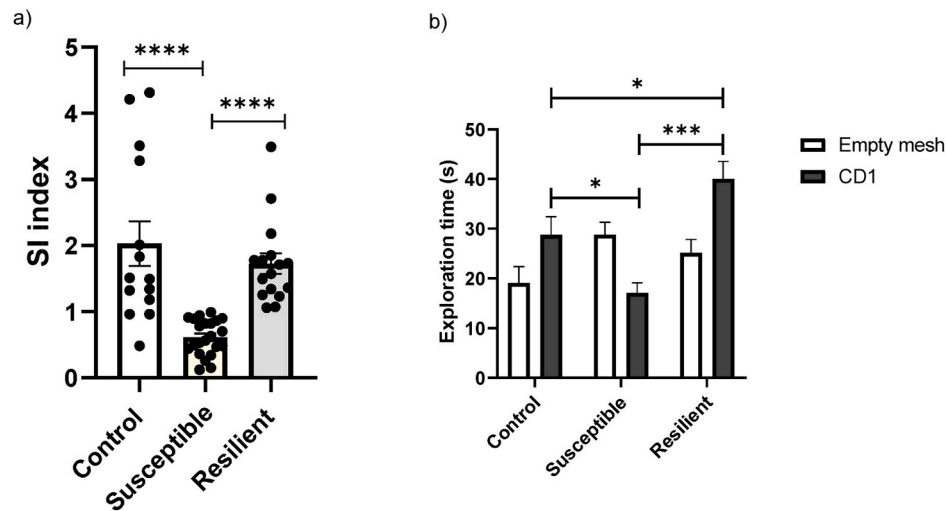
After adjustment by object exploration time as a covariate, the differences in the CD1 exploration times ( $F(2, 47) = 10.175$ ,  $p < 0.001$ ) among the three groups were still significant (one-way ANCOVA), with susceptible mice having a lower CD1 exploration time than resilient mice ( $p < 0.001$ ). However, the differences between control and resilient mice and control and susceptible mice were no longer significant ( $p = 0.098$  and  $p = 0.079$ , respectively).

We were further interested to see if the exploration times obtained before CSD correlated with the exploration time of the CD1 target after CSD. In stressed mice, there was a significant correlation between the exploration of an open field before the stress and exploration of a CD1 mouse after stress ( $N = 37$ ; Pearson  $r = 0.31$ ,  $p = 0.0306$ ) (Fig. 3a) and a significant correlation between exploration of an unknown object before CSD and exploration of a CD1 mouse after CSD ( $N = 37$ ; Pearson  $r = 0.560$ ,  $p = 0.0003$ ) (Fig. 3b). Further, there was a positive correlation between the distance traveled in the center of an open field before CSD and the serum corticosterone levels measured 20 min after the completion of this test (defeated mice,  $N = 37$ ; Pearson  $r = 0.333$ ,  $p = 0.044$ ) (Fig. 3c). None of these correlations was significant in the control group.

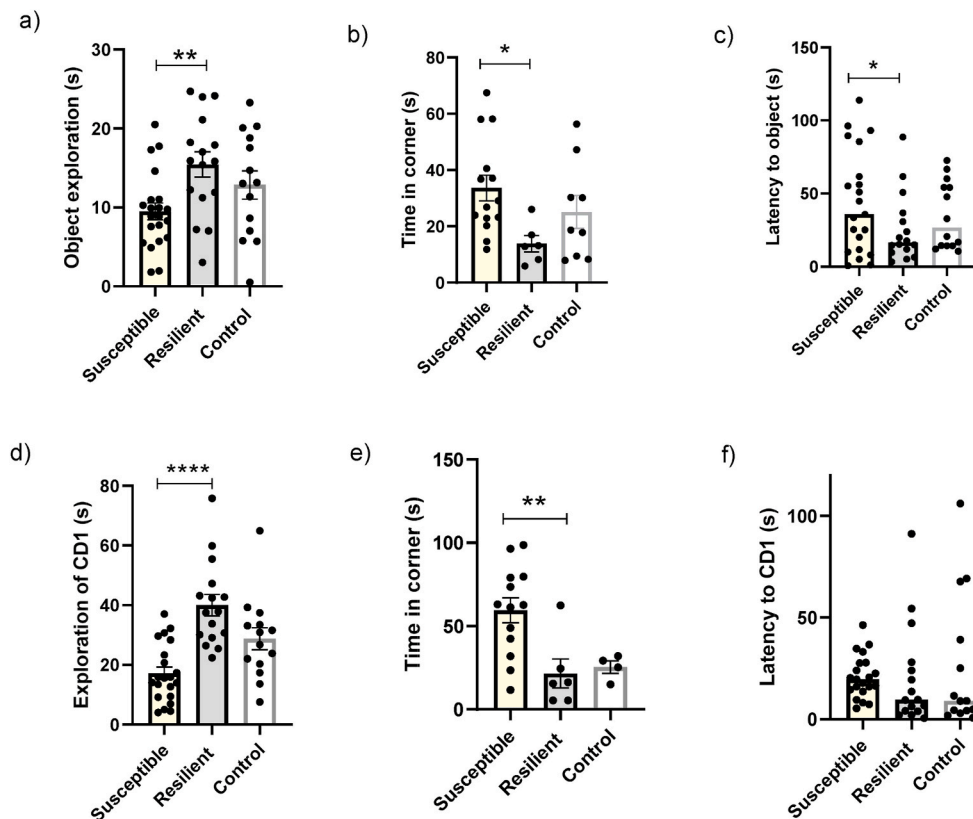
To test how strongly the object exploration test before CSD can predict the segregation of resilient and susceptible groups after CSD, we performed receiver operating characteristic (ROC) curve analysis. The ROC curve is depicted in Fig. 4, and the results are shown in Table 1.

### 3.2. Approach and exploration of the social target before CSD

Fig. 5a shows the differences in SI in control, resilient, and susceptible mice after CSD (one-way ANOVA [ $F(2,44) = 12.38$ ;  $P < 0.0001$ ]). Two-way ANOVA for exploration times showed a significant effect of the group ( $F(2, 44) = 5.604$ ;  $P = 0.0068$ ), significant effect of testing condition ( $F(1, 44) = 6.606$ ;  $P = 0.013$ ), and significant interaction between the group and testing condition ( $F(2, 44) = 17.37$ ;  $P < 0.0001$ ). The exploration times of an empty mesh and the CD1 target for all the groups are presented in Fig. 5b. Multiple comparisons revealed that exploration of an empty mesh did not differ between the groups, but exploration of the CD1 target was significantly lower in the susceptible group than in the resilient group ( $p < 0.0001$ ); the difference between the control and susceptible groups was almost significant ( $p = 0.061$ ). Evaluation of the data obtained before CSD revealed that most animals



**Fig. 1.** Social interaction index (a) and exploration times (b) after CSD in the first experiment (approach and exploration of novel object). \* $p < 0.05$ , \*\*\* $p < 0.001$ , \*\*\*\* $p < 0.0001$ . Control group  $N = 14$ , resilient mice  $N = 16$ , susceptible  $N = 21$ . All data are presented as mean  $\pm$  SEM.

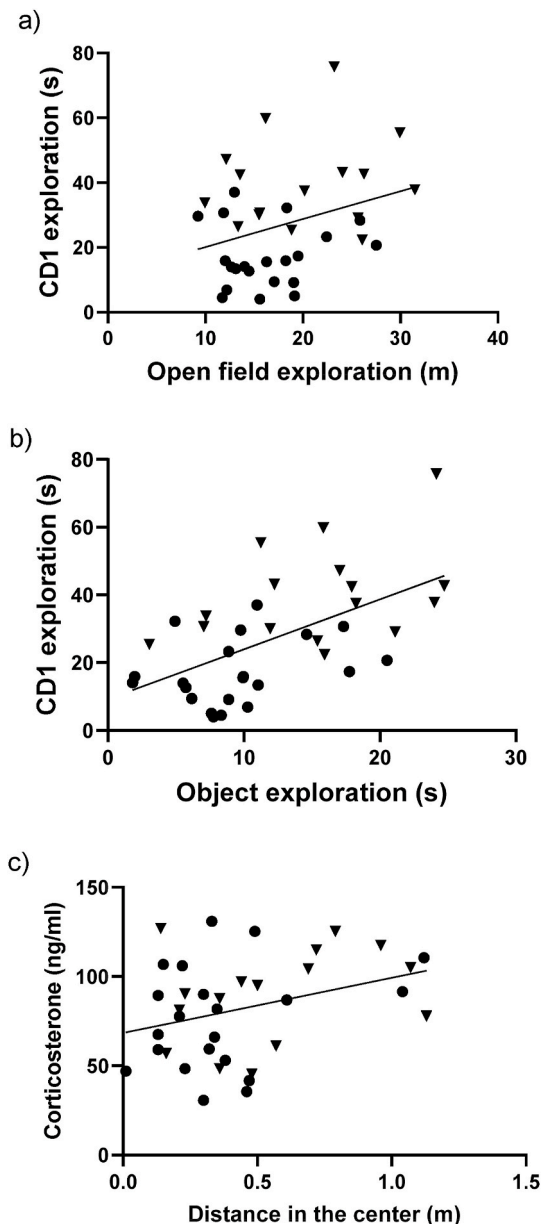


**Fig. 2.** Comparison of the exploration patterns between resilient and susceptible mice before (a–c) and after CSD (d–f) (resilient mice  $N = 16$ , susceptible mice  $N = 21$ , control mice  $N = 14$ ). Of 21 susceptible mice, 14 (66.66%) were sitting in the corner for longer than 5 s during the object exploration, and out of 16 resilient mice, 6 (37.5%) showed this behavior (b). In the social interaction test after CSD, this ratio was 6/16 (37.5%) and 13/21 (61.9%) for resilient and susceptible mice, respectively (e). \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\*\* $p < 0.0001$ . All data are presented as mean  $\pm$  SEM, except latencies that are presented as medians.

had an SI above 1, and there was no statistical difference with regard to the SI between resilient and susceptible mice (Fig. 6a). However, of the nine mice that had a pre-stress SI below 1 in social interaction with C57BL/6J mouse, eight were later categorized as susceptible. Before CSD, resilient mice had a higher exploration time an unknown C57BL/6J mouse compared to susceptible mice ( $t = 2.299$ ;  $P = 0.005$ ) (Fig. 6b). Susceptible mice spent more time sitting in the corner during this exploration session ( $t = 2.827$ ;  $P = 0.018$ ) (Fig. 6c). After CSD, resilient ( $N = 13$ ) and susceptible mice ( $N = 18$ ) had a significantly different SI ( $t = 4.993$ ;  $P < 0.0001$ ) (Fig. 6d) and a significantly different exploration time of the CD1 target ( $t = 5.462$ ;  $P < 0.000$ ) (Fig. 6e). The effect of CSD

on the CD1 exploration time was significant even after controlling for the differences in the pre-stress C57BL/6J exploration time (ANCOVA;  $F(2,43) = 12.690$ ,  $p < 0.001$ ); susceptible mice had a lower CD1 exploration time than resilient mice ( $p < 0.001$ ).

In the stressed mice, there was a significant correlation between exploration of an unfamiliar social target (C57BL/6J) before CSD and exploration of CD1 mice after CSD ( $N = 31$ ; Pearson  $r = 0.351$ ,  $p = 0.034$ ) (Fig. 7a). Further, there was a positive correlation between exploration time of the CD1 target and serum corticosterone levels measured 20 min after the completion of the social interaction test ( $N = 29$ ; Pearson  $r = 0.671$ ,  $p < 0.0001$ ) (Fig. 7b). In control mice, there was a

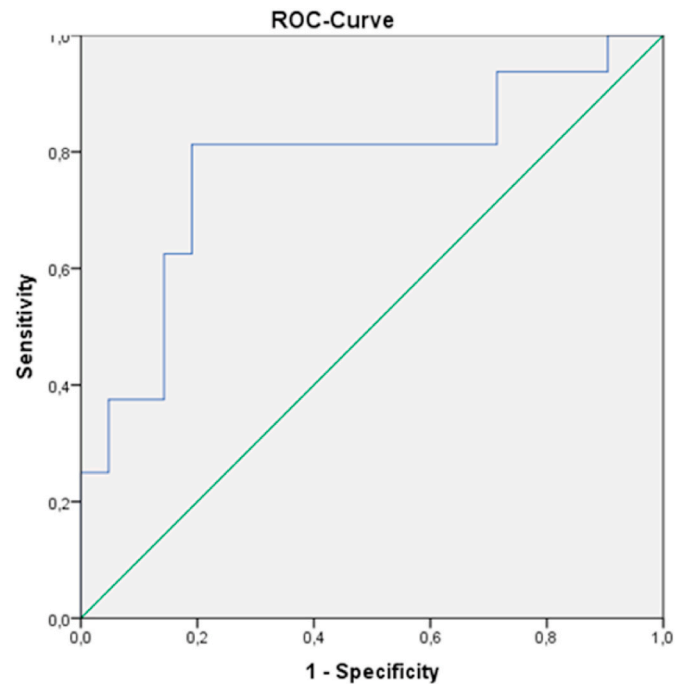


**Fig. 3.** (a) Correlation between exploration of the center of an open field before CSD and exploration of a CD1 mouse after CSD ( $N = 37$ ;  $p = 0.044$ ). (b) Correlation between exploration of an unknown object before CSD and exploration of CD1 mouse after CSD ( $N = 37$ ;  $p = 0.0003$ ). (c) Correlation between the distance in the center of an open field before CSD and corticosterone levels obtained 20 min after the test ( $N = 37$ ;  $p = 0.044$ ).  $\blacktriangledown$  - resilient mice;  $\bullet$  - susceptible mice.

positive and significant correlation between exploration of an empty mesh during social interaction with C57BL/6J mice and exploration of an empty mesh during the CD1 social interaction test (Pearson  $r = 0.501$ ;  $p = 0.047$ ). The correlation between exploration of C57BL/6J mice and exploration of CD1 mice was negative in control mice (Pearson  $r = -0.49$ ;  $p = 0.07$ ).

### 3.3. Passive avoidance before CSD

After CSD, we found significant differences in the SI among the three groups (one-way ANOVA,  $F(2, 41) = 18.39$ ;  $P < 0.0001$ ). Multiple comparison tests are presented in Fig. 8a. For the exploration times, two-way ANOVA showed a significant effect of group ( $F(2, 41) = 3.352$ ;  $P =$



**Fig. 4.** ROC curve for the classification of defeated mice into resilient and susceptible phenotype based on exploration of an unknown object before CSD. The true-positive rate (sensitivity) are plotted against the false-positive rate (1-specificity) for the different possible cutoff values of object exploration times.

**Table 1**

Area under the curve.

Area	Std. Error <sup>a</sup>	Asymptotic Sig. <sup>b</sup>	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
0.78	0.083	0.004	0.614	0.940

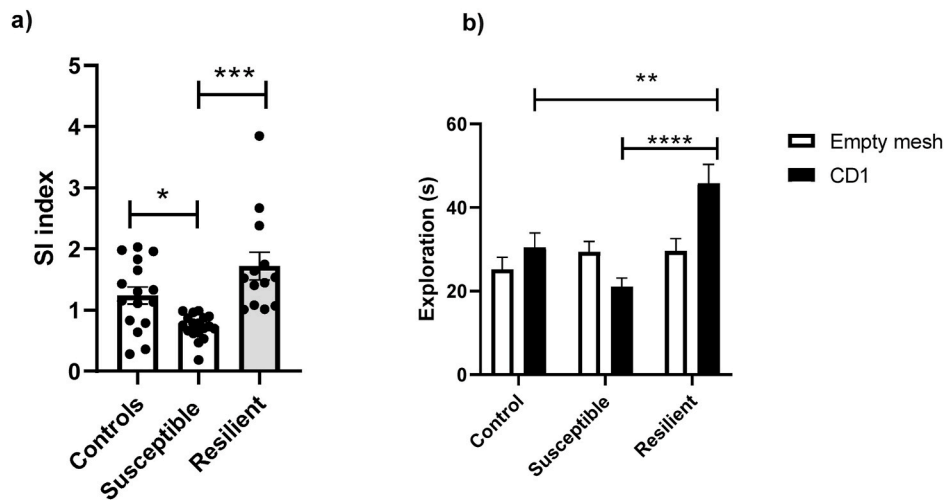
A random classifier has an area of 0.5, while an ideal one has an area of 1.

<sup>a</sup> Under the nonparametric assumption.

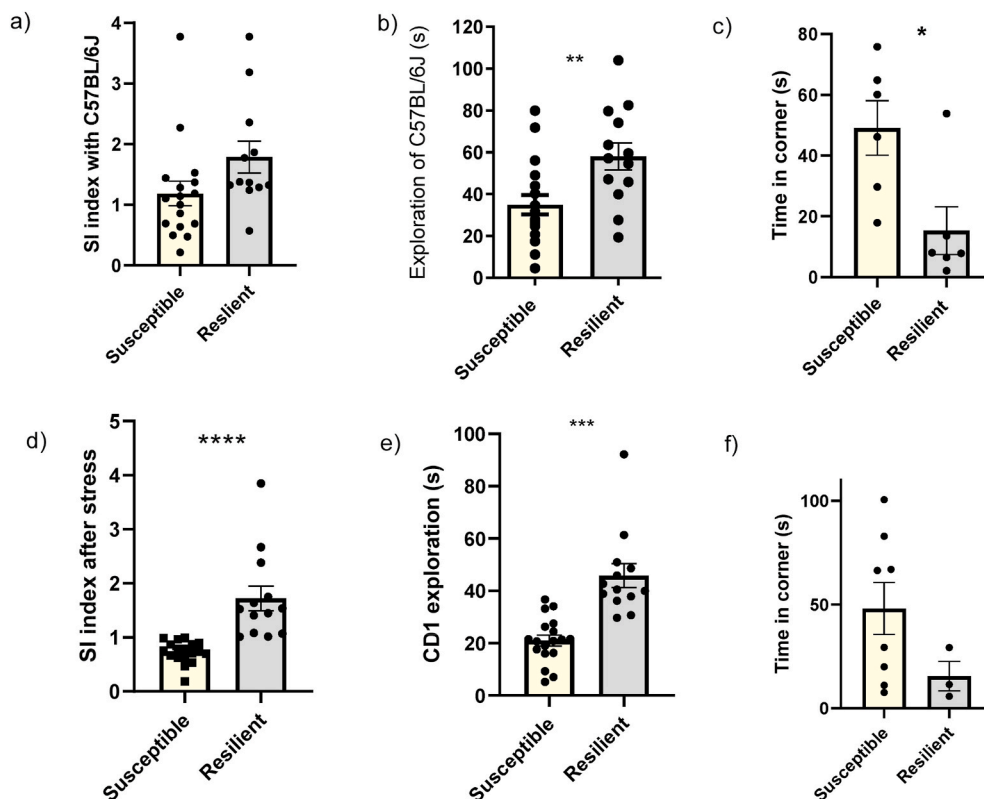
<sup>b</sup> Null hypothesis: true area = 0.5.

0.0044) and a significant interaction between the group and test condition ( $F(2, 41) = 8.667$ ;  $P = 0.007$ ). The three groups had a similar exploration time for an empty mesh, but susceptible mice explored the CD1 target for a lesser time than control mice ( $p = 0.0012$ ) and resilient mice ( $p = 0.0013$ ) (Fig. 8b). In the passive avoidance test prior to CSD, two-way ANOVA showed significant effects for both the testing phase ( $F(1, 25) = 75.09$ ;  $P < 0.0001$ ) and the group ( $F(1, 25) = 11.92$ ;  $P = 0.002$ ) as well as their interaction ( $F(1, 25) = 10.79$ ;  $P = 0.003$ ). *Post hoc* tests revealed that susceptible mice had higher latencies to enter the dark compartment on the testing day than resilient mice ( $p < 0.0001$ ) (Fig. 9a). After CSD, susceptible mice explored the CD1 target for a lesser time than resilient mice ( $t = 4.722$ ;  $P < 0.0001$ ) (Fig. 9b) and had higher latencies to approach the CD1 mouse (Mann-Whitney test,  $P = 0.026$ ) (Fig. 9c). When we performed one-way ANCOVA using a latency to dark compartment in the PA test as a covariate, the differences in the CD1 exploration times ( $F(2, 41) = 2.307$ ;  $p = 0.112$ ) and the differences in the latencies to approach CD1 mouse ( $F(2, 41) = 2.595$ ;  $p = 0.087$ ) among three groups were no longer significant.

Furthermore, there were negative correlations between the latencies to enter the dark compartment and CD1 target exploration (Spearman  $r = -0.557$ ;  $P = 0.002$ ) (Fig. 9d) and the SI (Spearman  $r = -0.506$ ;  $P = 0.007$ ) (Fig. 9e). In control mice, we found a negative and significant correlation (Spearman  $r = -0.57$ ;  $p = 0.013$ ) between the latency to the dark compartment in the PA test and exploration of the CD1 target in the SIT.



**Fig. 5.** Social interaction index (a) and exploration times (b) after CSD in the second experiment (approach and exploration of social target). \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , \*\*\*\* $p < 0.0001$ . Control group  $N = 16$ , resilient mice  $N = 13$ , susceptible  $N = 18$ . All data are presented as mean  $\pm$  SEM.



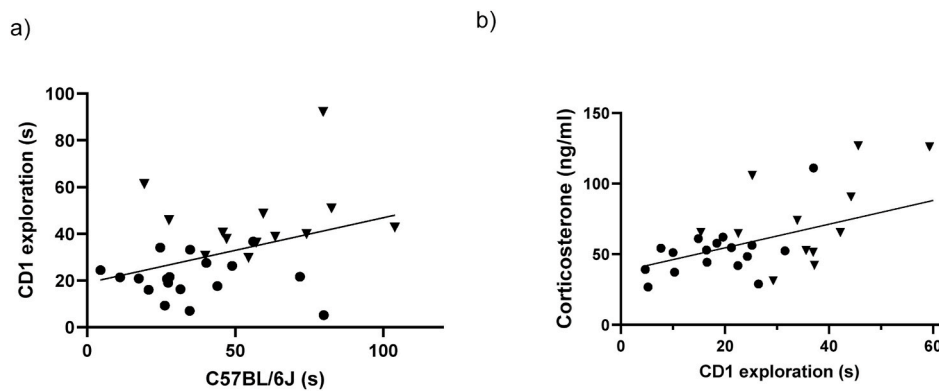
**Fig. 6.** (a) Social preference assessed with C57BL/6J target before CSD. (b) Exploration times of the social target (C57BL/6J) for resilient and susceptible mice before CSD (resilient  $n = 13$ , susceptible  $n = 18$ ). (c) Different time spent in the corner between resilient and susceptible mice ( $N = 6$  in both groups) during the exploration of C57BL/6J target before CSD. (d) Social interaction index with CD1 target after CSD. (e) Exploration times of the social target (CD1) after CSD (resilient  $n = 13$ , susceptible  $n = 18$ ). (f) Time sitting in the corner during CD1 exploration after CSD ( $n = 8$  susceptible,  $n = 3$  resilient). \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , \*\*\*\* $p < 0.0001$ . All data are presented as mean  $\pm$  SEM.

#### 4. Discussion

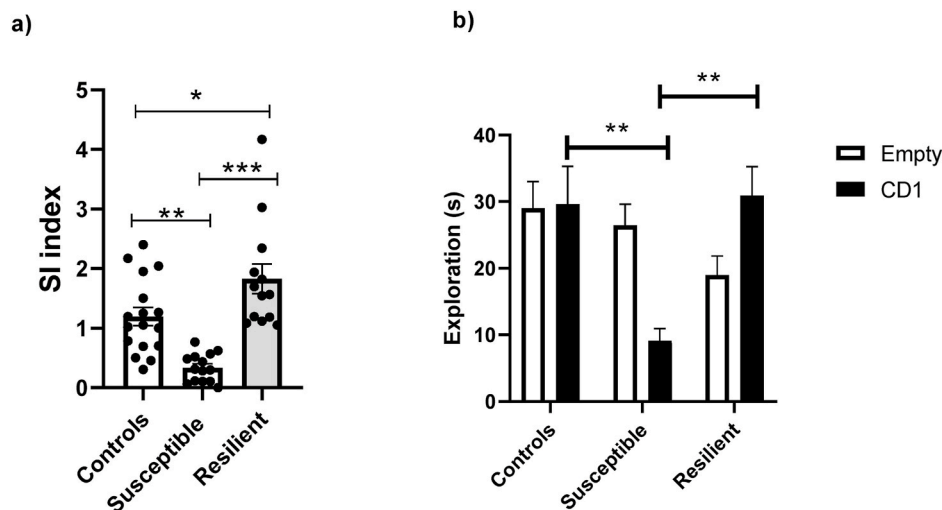
Behavioral heterogeneity within an inbred population is not a new concept (Biggers and Claringbold, 1954; Lathe, 2004; Jensen et al., 2016; Tuttle et al., 2018), and a growing body of evidence has demonstrated that individual differences not only influence the performance in behavioral tests but also shape an animal's response to stress (Jakovcević et al., 2008, 2011; Hager et al., 2014; Torquet et al., 2018). In our study, we provide evidence that behavioral variability observed before stress predicts the segregation of resilient and susceptible groups after chronic stress exposure (CSD).

An organism has two competing innate strategies to deal with

novelty: caution or exploration (Gray and McNaughton, 2000), a propensity that has been consistently followed and challenged in our study design, across different experimental settings. When faced with an unknown object or an unknown social target from the same strain before the stress, the mice that avoid the CD1 exploration (called "susceptible mice") after CSD have significantly higher approach latencies, spend more time sitting in the corner, and explore less than resilient mice. The same behavioral pattern emerged during the social interaction test after CSD when the mice were confronted with a novel mouse from the CD1 aggressor strain, suggesting the existence of a stable individual trait. An overview of the current literature shows that differences in novelty seeking among rodents is an important, behaviorally relevant general



**Fig. 7.** (a). Correlation between exploration of an unfamiliar social target (C57BL/6J) before CSD and exploration of CD1 mouse after CSD ( $N = 31$ ;  $p = 0.034$ ); (b) Correlation between exploration of CD1 mouse during the social interaction test and serum corticosterone levels obtained upon completion of the test ( $N = 29$ ;  $p < 0.0001$ ). Two blood samples from the cohort of 31 mice could not be used for the analysis due to the small amount of blood extracted from the tail vein. ▽ – resilient mice; ● – susceptible mice.



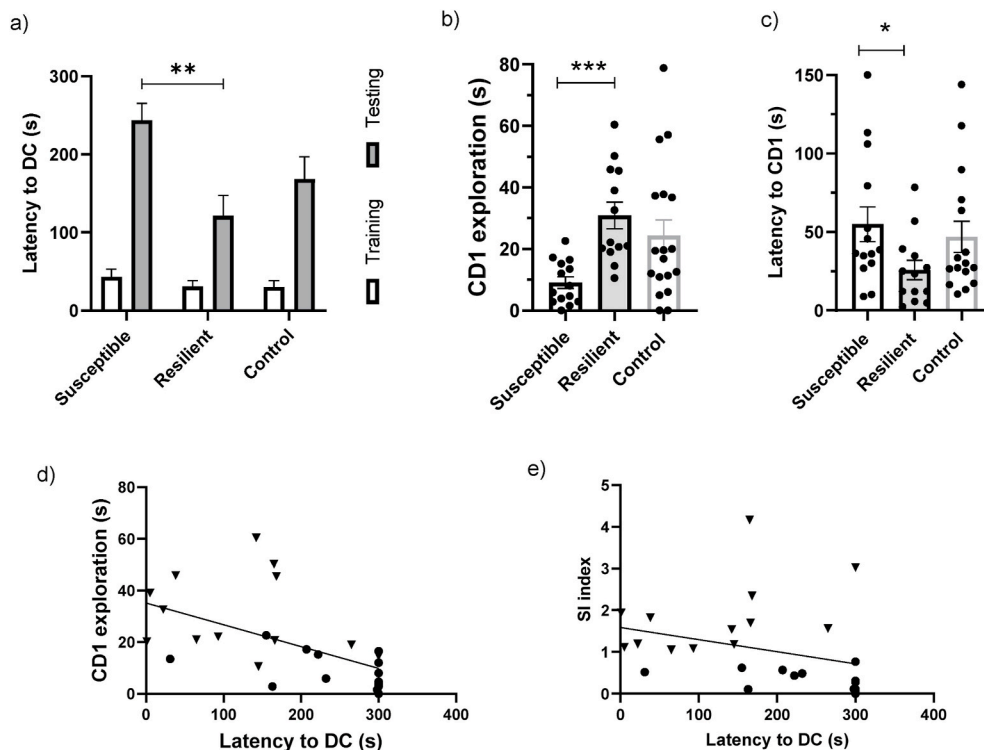
**Fig. 8.** Social interaction index (a) and exploration times (b) after CSD in the third experiment (passive avoidance). \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ . Control group  $N = 18$ , resilient mice  $N = 13$ , susceptible  $N = 14$ . All data are presented as mean  $\pm$  SEM.

trait and could be used to predict vulnerability in different models of psychiatric disorders. In most previous studies, animals with a high exploratory drive appeared to be more active, while animals with a low exploratory drive were more often associated with behavioral inhibition after stress application (Mallo et al., 2007; Walker et al., 2008; Padilla et al., 2010; Stedenfeld et al., 2011).

In our study, resilient mice were characterized by increased horizontal activity when placed in a novel environment with an unknown object before stress. Furthermore, positive correlations between the exploration times obtained before and after the stress indicate that the high and low novelty seekers gravitate to the resilient and susceptible groups, respectively. These findings suggest that the high exploratory drive is a manifestation of a neurobiological asset that promotes resilience and shapes the stress outcome. However, in the present study, we concluded that this phenomenon is a consequence of the selection criterion (i.e., the social interaction test), which largely, but not exclusively, depends on an individual's exploratory drive and, thus, facilitates segregation into the two groups. Interestingly, in the resilient group, exploration times of the CD1 mouse increased not only compared to the susceptible group but also compared to the control group. This finding indicates a tendency of the social interaction index to polarize the cohort of defeated mice with respect to their exploration levels. The relative apparentness of this effect also depends on the composition of the tested cohort of mice and their individual traits. Due to the binary classification of the defeated cohort ( $SI < 1$  or  $> 1$ ), the overlap in the exploration parameters between the two groups, also visible in our data, may be more or less pronounced. According to the ROC curve analysis (AUC),

the amount of exploration of the object before the stress has a remarkable predictive value for the segregation of resilient and susceptible mice in the social interaction test after stress, further supporting our hypothesis that the behavioral performance and outcome in the social interaction test is largely influenced by stable individual traits. It may be possible to find a cutoff value for the prediction of group segregation; however, it is beyond the scope of this paper for several reasons. We used a limited number of samples to generate the curve. Further, there may be variability in the level of exploration due to different experimental and housing conditions in the different laboratories. More importantly, however, is the question of the biological context of the behavior that we aim to predict as the nature of CD1 avoidance and CD1 approach in the SI test after the stress still needs conceptual clarification (Ayash et al., 2020).

In addition to innate behavioral traits, that is, individual exploratory behavior, we showed that increased rates of locomotor activity pre-stress and sustained exploration in a novel environment of resilient mice after the stress were consistently accompanied by higher levels of plasma corticosterone, an association that also exists in high novelty seeking rats (Piazza et al., 1989; Kabbaj et al., 2000). Although we cannot say if this link reflects differences in stress reactivity or in basal corticosterone levels, these data imply the existence of a specific coping style in the face of novelty, which was defined by Koolhaas et al. (1999) as a “coherent set of behavioral and physiological stress responses characteristic for the group of individuals.” In studying the ethological evaluation of the effects of social defeat in mice, Henriques-Alves and Queiroz (2016) found that social avoidance in the defeated group only



**Fig. 9.** (a) Susceptible mice have higher testing latencies than resilient mice after receiving a foot shock in the dark compartment (susceptible mice  $N = 14$ , resilient mice  $N = 13$ , control mice  $N = 18$ ). (b) Exploration of CD1 mouse in the social interaction test after CSD. (c) Latency to approach CD1 mouse during social interaction test after CSD. (d) Mice that had longer latencies to enter the dark compartment in the passive avoidance test before CSD had lower exploration times of CD1 mouse after CSD ( $p = 0.0025$ ). (e) There is a negative correlation between latency to enter the dark compartment in the passive avoidance task before CSD and SI after CSD ( $N = 27$ ;  $p = 0.007$ ). \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ . Data on the graphs (a), (b), and (c) are presented as mean  $\pm$  SEM. ▼ - resilient mice; ● - susceptible mice.

exists during the first 150 s of the social interaction test and then decreases over time. This finding aligns with our repeated observations of susceptible mice in the social interaction test after defeat, showing longer approach latencies and more time spent in the corner in the presence of a CD1 mouse. However, while [Henriques-Alves and Queiroz \(2016\)](#) attributed this feature to novelty-induced anxiety, we show that this pattern of behavior is already present before the stress, probably as a part of an individual's specific coping style.

Nevertheless, basal differences in novelty seeking do not explain the loss of preference for the CD1 mouse in the susceptible group after stress. As analyses of covariance show, even if we control for the different basal exploration levels in resilient and susceptible mice, the effect of stress on the CD1 exploration is still significant. This finding is expected because the animals' behavior in SIT after stress is much more complex than is explained by a one-dimensional approach. To examine what further drives this division, we exploited the fact that the social target in this test was a mouse from the CD1 aggressor strain. Given the previous experience, the difference in the individual exploration times of a CD1 mouse after stress might resemble individual differences in an animal's strategy to approach risk (or avoid harm). In the passive avoidance test, mice learn to inhibit the innate response tendency of entering a dark compartment after receiving a brief electric foot shock. Our data show that susceptible animals performed better than resilient ones in learning this causal link. Furthermore, the latencies to enter the dark compartment negatively correlated with both the SI and the exploration of the CD1 after the stress. As mice are an inherently social species ([Latham and Mason, 2004](#)), our observations of the social interaction test in susceptible mice after CSD is the inhibition of an otherwise normal response. Similarly, short latencies to enter the dark compartment in the PA task and continued social interaction during social interaction in resilient mice, resemble a failure to inhibit inappropriate responses possibly by learning mechanisms. Our findings and interpretation are supported by deficits seen in other forms of inhibitory learning in resilient mice after stress ([Meduri et al., 2013](#); [Dulka et al., 2015](#)). The fact that differences in CD1 exploration times between resilient and susceptible groups are no longer significant when we control for basal

differences in aversive learning, stresses the importance of this particular trait and the magnitude of its influence on later group segregation. Thus, avoidance of the CD1 mouse after CSD has no "negative" value by itself unless one proves, and not only assumes, that it is the expression of an underlying pathological condition. An increasing number of studies have used other strains of mice, that is, C57BL/6J or SV129 mice, to assess social behavior after CSD ([Desbonnet et al., 2012](#); [Ayash et al., 2020](#), [Alves-dos-Santos et al., 2020](#)). Based on these findings, it is apparent that the division into resilient and susceptible mice solely based on interaction with a CD1 mouse becomes irrelevant when the social interaction is performed using another strain ([Ayash et al., 2020](#)). Interestingly, results published thus far do not unequivocally assign any other physiological or behavioral stress-related disturbance to either of these two groups ([Meduri et al., 2013](#); [Krishnan et al., 2007](#); [Chou et al., 2014](#); [Han et al., 2017](#); [Peña et al., 2019](#)), thus challenging its translational relevance with respect to more complex phenotypes of stress-related mental disorders.

The main limitation of our study is that we were not able to address the question of comparability of selected behavioral traits between control and defeated mice before and after CSD. We were able to see some differences in the correlational analysis between the control and defeated groups, but due to our current experimental design, we could not further elaborate.

In conclusion, we showed that resilient and susceptible mice show consistent characteristics in their behavioral response to novelty and harm, and these individual characteristics are stable over time and across different experimental situations. The social interaction test performed with a CD1 mouse after CSD is designed in a way that favors stratification into the two groups based on these two individual traits, but it does not provide any information about the general underlying social behavior of the defeated mice before stress induction. Based on our data, we have identified three weaknesses of this test: 1) exploration is limited to 2.5 min, which encourages the selection of animals with a naturally high exploratory drive and short approach latencies to be classified as resilient; 2) it does not consider basal social behavior of defeated animals to distinguish pre-existing low sociability from stress-

induced sociability; and 3) it uses mice from the aggressor strain to assess social behavior after the stress, thus neglecting the existence and evolutionary benefit of an adaptive coping strategy in the presence of potentially aversive stimuli. We believe that addressing these points is essential for future research and would lead to a constructive reassessment of the field, further enhancing the translational relevance of CSD model.

### CRedit authorship contribution statement

**Marija Milic:** Conceptualization, Methodology, Investigation, Formal analysis, Writing - original draft. **Ulrich Schmitt:** Conceptualization, Methodology, Writing - review & editing. **Beat Lutz:** Conceptualization, Writing - review & editing, Funding acquisition. **Marianne B. Müller:** Conceptualization, Supervision, Writing - review & editing, Funding acquisition.

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