

Research report

Paradoxical enhancement of fear expression and extinction deficits in mice resilient to social defeat



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HIGHLIGHTS

- Mice can be characterized as susceptible or resilient after social defeat.
- Paradoxically, resilient mice display enhanced fear expression and poor extinction.
- These effects are not due to increased anxiety or poor behavioral flexibility.
- Mechanisms of resilience may leave animals vulnerable to maladaptive fear behavior.

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ABSTRACT

The exposure to stress has been associated with increased depressive and anxiety symptoms, yet not all individuals respond negatively to the experience of stress. Recent rodent social defeat models demonstrate similar individual differences in response to social stress. In particular, mice subjected to chronic social defeat have been characterized as being either “susceptible” or “resilient” by the level of social interaction following social defeat. Susceptibility is associated with lasting social avoidance as well as increased anxiety-like behavior, and depressive-like symptoms. Resilient animals, however, do not show social avoidance or increased depressive-like symptoms, but retain increased anxiety-like behavior. Thus, it is unclear what “resilience” as measured by social interaction represents in terms of an overall behavioral and physiological phenotype. Here, we use an acute social defeat procedure, which produces distinct behavioral phenotypes in social interaction with no apparent changes in anxiety-like behavior. Susceptible mice display lasting social avoidance, whereas resilient mice display normal social interaction. Susceptible mice also displayed deficits in fear extinction retention but had normal within-session extinction. Paradoxically, resilience was associated with enhanced fear expression, and severe deficits in fear extinction and extinction retention beyond that observed in susceptible mice. These effects in resilient mice were only apparent after the experience of social stress and were not due to impaired behavioral flexibility. These data suggest that mechanisms controlling resilience to acute social defeat as characterized by social interaction leave animals vulnerable to maladaptive fear behavior.

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1. Introduction

Social stress, primarily in the form of conflict between individuals, is one of the most pervasive forms of stress experienced by many animal species, including humans. Exposure to social stress in humans and non-human animals often produces pronounced changes in physiology and behavior that may lead to the development of stress-related disorders. Although many individuals experience traumatic or stressful events during their lifetime,

only a proportion of these individuals develop stress-related psychopathology. This underscores the importance of understanding the nature of resilience and vulnerability to stress-related psychopathology. For example, only a proportion of individuals who experience trauma develop post-traumatic stress disorder (PTSD), and the risk varies depending on the type of trauma experienced. People exposed to interpersonal violence have a greater propensity for developing PTSD than those exposed to nonpersonal trauma [1]. Rodent models of social defeat are ethologically relevant methods for examining behavioral and physiological responses to stress [2–6] and may have a unique ability to model the symptomatology of stress-related disorders like PTSD and depression [7–9]. Previously, we have demonstrated that behavioral responses to social defeat require amygdala-dependent plasticity, suggesting that social avoidance in response to social defeat may be a

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naturalistic measure of fear-motivated learning [6,9,10]. Associative fear learning and social defeat both produce behavioral effects that persist over a long period of time and we have shown that social avoidance following social defeat may be resistant to extinction [2,5,9–11]. Likewise, current hypotheses of PTSD suggest that the persistence of this disorder involves an inability to appropriately extinguish fear responses [12,13]. Thus, social defeat models may represent a unique way of examining associative fear learning mechanisms as well as the behavioral and physiological consequences of stress exposure, and may be well suited for modeling stress-related psychopathology.

Several recent studies have examined the effects of prior stress on associative and non-associative fear behavior in order to model the complex nature of PTSD symptomology [14–18]. For example, Knox et al. (2012) demonstrated specific fear extinction deficits in rats exposed to a single prolonged stress (SPS) procedure, modeling similar deficits in fear extinction observed in PTSD patients. Additional stressors including immobilization, exposure to shock, and exposure to predator odor produce similar, but varying effects in the acquisition, expression, and extinction of both associative and non-associative fear behavior [8,19–22]. However, less is known about the role of social stressors on associative fear behavior. Recently, studies have demonstrated conflicting findings regarding the effects of chronic social stress on fear learning [23–26]. For example, Yu et al. (2010) provided evidence of potentiated associative fear memory in mice after exposure to chronic social defeat stress. Additional evidence suggests similar effects of chronic social defeat stress including potentiated associative fear memory and impaired recall of fear extinction [25,26]. Inconsistent with the above findings, other reports demonstrated intact associative fear memory in mice after repeated exposure to social defeat [23]. Many of these studies have been used to model the complex nature of PTSD and other stress-related psychopathology. However, there remains a wide range of individual differences in the vulnerability to PTSD in humans and how these individual responses to stress contribute to the development of stress-related psychopathology is poorly understood.

Individual differences in vulnerability to the effects of social stress have been reported in recent chronic stress models [27–33]. Namely, following chronic social defeat stress, mice exhibit two distinct phenotypes that have been characterized as being either susceptible or unsusceptible to the defeat-induced avoidance observed in social interaction with a conspecific [31]. Susceptible mice exhibit a variety of deleterious symptoms following chronic social defeat that include anhedonia-like symptoms, increased anxiety-like behavior, elevated reactivity of the hypothalamic-pituitary-adrenal (HPA) axis, and stress-induced polydipsia [31,34,35]. In contrast, unsusceptible or resilient mice seldom exhibit the depressive-like behaviors of susceptible mice. Thus, this characterization of resilience appears to be well-suited to model resistance to depressive symptoms [31,35–37]. However, resilient mice also show increased anxiety-like behavior and elevated HPA axis reactivity [31]. Therefore, it remains unclear what resilience as measured by social interaction represents in terms of an overall behavioral and physiological phenotype, and how this may relate to resilience to other stress-related psychopathology like PTSD. In the present study, we take advantage of the ability to identify individual differences in stress responsiveness in an acute social defeat model, and also examine whether phenotypic differences in response to social defeat are associated with specific differences in associative fear learning and extinction. This enables us to examine how individual vulnerability to stress is related to alterations in associative fear and extinction, similar to what is observed in PTSD.

2. Material and methods

2.1. Animals

Six- to eight-week-old male C57BL/6J mice bred in our animal facility were used in all experimental procedures. Mice were housed in groups of four per cage until the beginning of each experiment. Mice were then housed individually and maintained on a 12:12 light/dark cycle from 7am to 7pm with *ad libitum* access to food and water. Four- to ten-month-old male CD1 mice bred in our animal facility were used as resident aggressors for social defeat training. Prior to the experiment CD1 mice were screened for their level of aggression and mice that attacked within two minutes were used for the experiment. All animal procedures were carried out in accordance with the National Institutes of Health guidelines and were approved by Kent State University Institutional Animal Care and Use (IACUC) Guidelines.

2.2. Behavioral manipulations

2.2.1. Social defeat stress

Adult male C57BL/6J mice were matched by weight and randomly assigned to defeat or control procedures. Mice assigned to the defeat group were subjected to social defeat stress by CD1 mice on two consecutive days. Non-defeated control mice were allowed sensory contact but no physical contact with a CD1 aggressor for the same duration of time. We used a modified procedure based on our previous acute social defeat studies in hamsters and recent chronic defeat studies in mice [2,31,35]. Briefly, an experimental mouse was placed into the home cage of a larger aggressive CD1 mouse and experienced 5 min of physical contact. A 55 min period of physical separation immediately followed. During separation, a perforated Plexiglas divider was positioned between the mice, dividing the cage in two equal halves, to allow sensory contact but preventing further physical contact. This procedure was repeated four times, with each defeat by a novel CD1 aggressor. Day 2 of the defeat procedure was exactly the same as Day 1 (Fig. 1A). Animals were monitored after every defeat session to ensure no serious wounds were incurred. During the defeats the number of attacks and latency to first submissive posture were videotaped and were scored later by an observer blind to experimental condition. An attack was defined as a lunge followed by a bite. Submissive behaviors that were recorded by intruders included upright defensive posture, side defensive posture, full submissive posture, and fleeing.

2.2.2. Social interaction testing

Social interaction testing followed 24 h after defeat to measure approach and avoidance behavior toward a novel non-threatening mouse (social target mouse). Social target mice were novel non-aggressive male CD1 mice as determined by pre-screening aggression testing. Testing was performed in a dimly lit room with four identical open field arenas (46 cm × 46 cm × 39 cm). A wire-mesh enclosure with Plexiglas frame (20 cm × 12 cm × 12 cm) was positioned against one of the four walls. The social interaction test consisted of two separate trials: Trial 1 (target absent) and Trial 2 (target present). In Trial 1, an experimental mouse was placed in the center of the arena and allotted 150 s to explore the novel environment in the absence of a social target mouse. After 150 s had elapsed, the experimental mouse was momentarily removed from the arena to position a social target mouse within the wire-mesh enclosure. In Trial 2, the experimental mouse was reintroduced into the center of the arena and allotted 150 s to explore in the presence of the social target mouse. A digital camera was positioned above the open field arena and automated tracking software (LimeLight; Coulbourn Instruments) was used to record locomotor activity

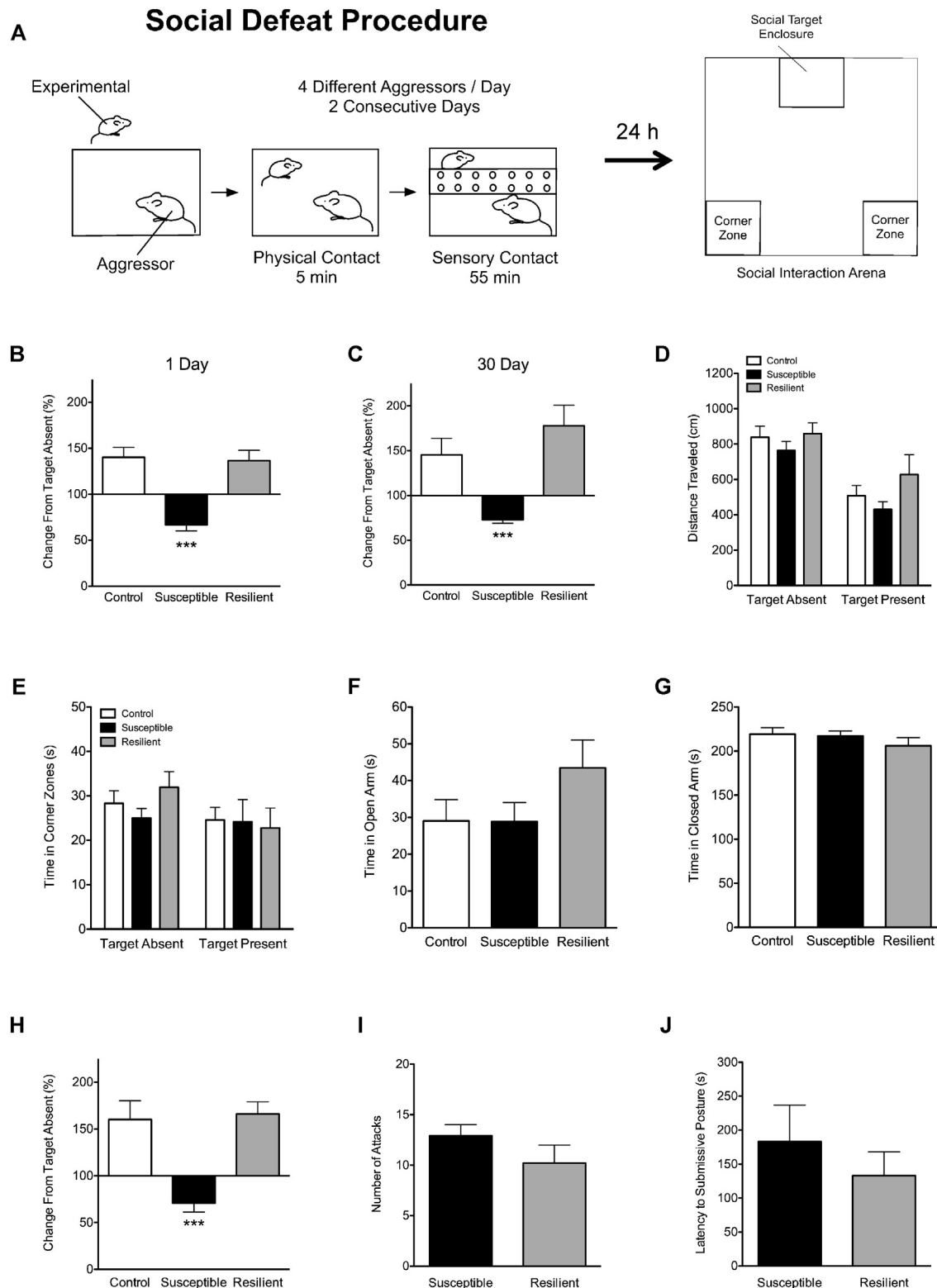


Fig. 1. Social defeat produces prolonged alterations in social behavior. (A) Schematic of social defeat procedure and social interaction arena. (B,C) Percent of change in social interaction time from target absent to target present during the 1 Day and 30 Day social interaction tests. Social defeat decreased interaction ratios in susceptible mice ($n = 12$) as compared with non-defeated controls ($n = 16$) and resilient mice ($n = 9$) that were also defeated. Interaction ratios were comparable between resilient mice and non-defeated controls. (D) There were no differences among groups in locomotor activity as measured by total distance traveled (cm) during social interaction. (E) There were no differences among groups in time spent in the corner zones of the arena during social interaction. (F,G) Time spent in open and closed arms during elevated plus-maze test, respectively. There were no differences in anxiety-like behavior measured during the elevated plus-maze test. (H) Percent of change in time spent in social interaction from target absent to target present in an additional group of mice in which defeat behavior was scored. (I) Number of attacks displayed by resident aggressors during all defeats. There were no differences in the number of attacks on susceptible ($n = 9$) and resilient mice ($n = 11$). (J) Latency to exhibit submissive posture during social defeat. There were no differences observed in the latency to exhibit a submissive posture between susceptible and resilient mice. Data are represented as mean $\pm$ SEM. * $p < .05$; ** $p < .01$; *** $p < .001$.

(distance traveled in cm) and the amount of time spent in the corner zones (seconds). Social interaction and investigation were hand scored by an observer blind to experimental condition. In Trial 1, the amount of time experimental mice spent investigating the wire-mesh enclosure was used as a baseline measure to determine any differences in investigation time, when there was a social target mouse present during Trial 2. Investigation was defined as the experimental mouse orienting towards the enclosure while in very close proximity to the enclosure, as well as the time spent sniffing the enclosure. Social interaction is therefore a measure of the time spent investigating the enclosure versus the time spent investigating the social target mouse within the enclosure during Trial 2. Interaction times were used to extrapolate individual differences among the mice exposed to social defeat. We used an established method for calculating individual differences in response to social defeat stress [31,38]. Briefly, interaction ratios were calculated as percent change in time spent in social interaction in the presence of a social target mouse relative to the absence of a social target mouse by using the equation: $100 \times (\text{interaction time, target present}) / (\text{interaction time, target absent})$. Defeated mice with a score of less than 100 were defined as susceptible and defeated mice with a score equal to or greater than 100 were defined as resilient.

2.2.3. Anxiety testing

Anxiety-like behavior was measured in the elevated plus-maze 24 h after social interaction to investigate general anxiety-like behavior. The plus-maze was elevated 55 cm and consisted of a square platform with four runways (arms), two enclosed (29 cm $\times$ 6 cm $\times$ 15.5 cm) and two exposed (29 cm $\times$ 6 cm), extending from the center (6 cm $\times$ 6 cm). The procedure began by placing an experimental mouse on the center platform facing a closed arm and allotting 5 min to explore the novel maze. A digital camera was positioned above the maze and automated tracking software (LimeLight; Coulbourn Instruments) was used to record the amount of time spent in the open and closed arms of the maze (seconds).

2.2.4. Cued fear conditioning

Fear conditioning and extinction procedures were performed seven days after completion of the social defeat procedure and occurred in two chambers rendered distinct with respect to visual cues, lighting, floor type and odor. Fear conditioning occurred in Context A (30.5 cm $\times$ 8 cm $\times$ 8 cm) that consisted of a grid floor with stainless steel rods, polka-dotted wallpaper covering, dim lighting, exhaust fan, and was cleaned between trials with 70% ethanol. Fear extinction occurred in Context B (30.5 cm $\times$ 8 cm $\times$ 8 cm) that consisted of a flat Plexiglas floor, and lacked lighting, decorative wallpaper, or fan, and was cleaned between trials with 70% Quatricide disinfectant.

The procedure consisted of four main phases: habituation, fear conditioning, extinction, and extinction testing. On Day 1, mice were habituated in Context A for 5 min. On Day 2, mice underwent cued fear conditioning in Context A. Mice were placed into the conditioning chamber and were presented with five CS-US trials consisting of a 30 s, 6 kHz, 75 dB tone that co-terminated with a 1.0 s, 0.6 mA footshock [2 min baseline, intertrial interval (ITI) of 90 s]. On Day 3, mice underwent cued fear extinction in Context B. Mice were placed into the extinction chamber and were presented with 30 tone CS-only trials [2 min baseline, intertrial interval (ITI) of 30 s]. On Day 4, mice underwent an extinction test to examine the consolidation and retention of extinction learning. The extinction test used identical parameters as those used in extinction training on Day 3 and also occurred in Context B. Freezing behavior was defined as the absence of all movement. Percent of time spent freezing during each 30 s tone was recorded

and quantified using automated tracking software (FreezeFrame; Coulbourn Instruments).

2.2.5. Morris water maze

Spatial reference learning and memory was performed in the Morris water maze seven days after completion of the social defeat procedure using the previously described procedure [39]. The water maze consisted of a circular pool (122 cm diameter, 76 cm depth) filled with water ($21 \pm 1^\circ\text{C}$) and made opaque with the addition of white, nontoxic, liquid tempera paint. A circular hidden platform (10 cm diameter) was painted white and submerged 0.5 cm below the surface of the water. The pool was located in a room containing distinguishing visual cues, which provided mice with distal learning cues. A camera was mounted above the pool and trials were recorded and later analyzed using automated tracking software (LimeLight; Coulbourn Instruments). The water maze task consisted of two phases: spatial training and spatial reversal training. Twenty-four hours after the final training trial in each phase, mice were returned to the pool and performed a 60 s probe test to assess spatial memory. During the probe test, mice were released from a novel start position with the hidden platform absent from the pool and the following measures were recorded: latency to reach the area where the platform was located on the previous day (seconds), time spent in each quadrant (seconds), total swimming distance (cm), and path length to platform area (cm).

Spatial training consisted of 24 trials (4 trials per day for 6 days) in which mice were trained to locate and swim to a hidden platform located in the NW quadrant of the pool. For each trial, mice were gently placed into the water facing the pool wall in one of four semi-randomly ordered start positions (NW, NE, SW, SE) and were given 60 s to locate the hidden platform. Mice that located the platform were allowed to remain there for 15 s before being returned to a heated cage filled with shredded paper towel. Mice that failed to locate the platform within 60 s were guided there by the experimenter and allowed to remain on the platform for 15 s. Twenty-four hours after the training probe trial, mice then performed spatial reversal training in which the hidden platform was relocated to the opposite quadrant (SE) of that used for initial spatial training. Spatial reversal training consisted of 16 trials (4 trials per day for 4 days) in which mice were trained to locate and swim to a hidden platform now positioned in the SE quadrant of the pool.

2.2.6. Statistical analysis

Results are expressed as mean $\pm$ standard error of the mean (SEM). Behavioral measures for social defeat, social interaction, elevated plus-maze, and probe trials in Morris water maze were analyzed using Student's t-test or one-way analysis of variance (ANOVA), followed by Tukey's post hoc comparisons, where appropriate. Cued fear acquisition, cued fear extinction, extinction retention, and spatial acquisition in Morris water maze tasks were analyzed using one-way ANOVA with repeated measures, followed by Tukey's post hoc comparisons, where appropriate. Individual analyses were conducted on each day of the spatial reversal task using a Student's t-test. Significance level was set at $p < .05$.

3. Results

3.1. Social defeat produces susceptible and resilient phenotypes during social interaction

Mice were subjected to the two-day social defeat procedure and tested for social approach or avoidance behavior in a social interaction test. After the social interaction test, defeated mice were divided into susceptible ($n = 12$) and resilient ($n = 9$) subgroups based on their interaction ratio (see methods). Results demonstrated significant differences in social interaction

among susceptible, resilient, and non-defeated control mice after introducing a social target mouse (Fig. 1B), as indicated by a significant percent change in social interaction times ($F_{(2,34)} = 17.17$, $p < .001$). Post hoc comparisons demonstrated that susceptible mice spent significantly less time investigating the social target mouse compared with resilient mice and non-defeated controls ($p < .001$ and $p < .001$, respectively). No significant differences were noted between resilient mice and non-defeated controls in social interaction ($p = .966$). To assess whether these effects persist over an extend period of time, we tested for social interaction in the same mice 30 days after social defeat (Fig. 1C). We found a similar pattern of results at 30 days, with significant differences in social interaction times among susceptible, resilient, and non-defeated control mice ($F_{(2,16)} = 8.56$, $p = .003$). Again, post hoc comparisons demonstrated that susceptible mice spent significantly less time investigating the social target mouse compared with resilient mice and non-defeated controls ($p = .003$ and $p = .019$, respectively). No significant differences were noted between resilient mice and non-defeated controls in social interaction ($p = .412$). In a separate set of mice, social defeat behavior was recorded and analyzed for differences in latency to first submissive posture between susceptible and resilient mice and number of attacks exhibited by resident aggressors during the two-day defeat procedure. Results from social interaction demonstrated significant interaction differences among susceptible ($n = 9$), resilient ($n = 11$), and non-defeated control mice ($n = 8$) after introducing a social target mouse (Fig. 1H), as indicated by a significant percent change in social interaction times ($F_{(2,25)} = 13.95$, $p < .001$). Results from social defeats demonstrated no significant differences between susceptible and resilient mice for the mean number of attacks ($t(16.15) = 1.30$, $p = .212$) (Fig. 1I) or in the latency to first submissive posture ($t(18) = 0.81$, $p = .426$) (Fig. 1J). Taken together, our findings demonstrate that resilience to social defeat does not result from differential defeat exposure between susceptible and resilient mice or an inherent difference in initial submissive behavior. Furthermore, these findings demonstrate that social defeat produces drastic and long-term alterations in social investigation that persist for at least 30 days.

3.2. Anxiety and locomotor behavior testing

To further characterize the effects of social defeat anxiety-like behavior and locomotor activity were measured in the social interaction arena during the two-trial interaction test. Results confirmed that differences in social interaction were not due to impaired locomotor activity, as total distance traveled during both target absent and target present was comparable among susceptible, resilient, and non-defeated control mice (all $ps > .188$) (Fig. 1D). No significant differences were noted in the amount of time spent in the corner zones of the interaction arena among the three groups, regardless of whether a social target mouse was absent or present (all $ps > .310$) (Fig. 1E). Additionally, we investigated anxiety-like behavior using the elevated plus-maze. No significant differences were noted in the amount of time spent in the open or closed arms among the three groups (open: $F_{(2,34)} = 1.56$, $p = .226$; closed: $F_{(2,34)} = 0.78$, $p = .466$) (Fig. 1F,G). These findings suggest that differences in social behavior are not due to increased general anxiety but rather specific to an interaction between social defeat stress and the presence of an unfamiliar conspecific.

3.3. Resilience is marked by enhanced fear expression and deficits in fear extinction

Seven days after defeat, mice were fear conditioned with five CS-US trial presentations and were examined further for phenotypic differences in associative fear learning and extinction. Results

demonstrated a significant main effect in the level of within-session learning as all three groups acquired similar levels of freezing at the end of fear conditioning (Trial: $F_{(4,136)} = 163.90$, $p < .001$; Group: $F_{(2,34)} = 1.18$, $p = .318$). Twenty-four hours after conditioning, mice were tested for extinction of conditioned fear with 30 CS-only trial presentations in a different context. Social defeat produced significant main effects in extinction learning among susceptible, resilient, and non-defeated control groups (Group: $F_{(2,34)} = 8.97$, $p = 0.001$; Trial: $F_{(9,306)} = 7.93$, $p < .001$), but no significant interaction effect. Post hoc comparisons demonstrated potentiated fear expression and impaired extinction learning in resilient mice as compared with susceptible mice ($p = .005$) and non-defeated controls ($p = .001$). No significant differences were noted between susceptible mice and non-defeated controls in extinction learning ($p = .844$). Twenty-four hours later, mice were tested for the retention of extinction learning with an additional 30 CS-only trial presentations. As a measure of extinction retention, we compared freezing behavior during the last block of three trials in extinction learning (Extinction) with freezing behavior during the first block of three trials in extinction testing (Extinction Test). A repeated measures ANOVA demonstrated significant differences in the retention of extinction learning among susceptible, resilient, and non-defeated control mice ($F_{(2,34)} = 11.48$, $p < .001$). Post hoc comparisons demonstrated marginal extinction retention deficits in susceptible mice (Extinction: 26.29 ± 17.99 , Extinction Test: 46.03 ± 20.03) as compared to non-defeated controls (Extinction: 21.02 ± 11.71 , Extinction Test: 33.29 ± 15.81 , $p = .093$). Surprisingly, resilient mice (Extinction: 43.30 ± 27.82 , Extinction Test: 65.49 ± 17.90) demonstrated notable extinction retention deficits as compared with susceptible mice ($p = .013$) and non-defeated controls ($p < .001$) (Fig. 2A,B). Furthermore, results from all 30 CS-only trial presentations during the extinction test demonstrated significant main effects in extinction learning among the three groups (Group: $F_{(2,34)} = 7.50$, $p = .002$; Trial: ($F_{(9,306)} = 13.06$, $p < .001$); in particular, these differences were driven by persistent deficits in resilient mice compared to susceptible mice ($p = .010$) and non-defeated controls ($p = .002$) (Fig. 2A).

Taken together, these findings demonstrate that social defeat stress significantly modifies subsequent fear learning and extinction. Resilient mice expressed enhanced conditioned fear and impaired extinction learning. In contrast, susceptible mice exhibited normal within-session extinction learning, yet extinction retention appeared to be compromised as we noted marginal deficits as compared to non-defeated controls.

3.4. No disruption in fear extinction prior to social defeat stress

Individual differences in rates of fear extinction likely exist even within a population of inbred mouse strains. Thus, one possibility is that we artificially selected for mice that extinguish fear poorly based on our measure of resilience, and that these behavioral characteristics were present before experiencing social defeat stress. If specific behavioral characteristics are present before defeat, then conducting our initial experiment in reverse order (fear conditioning then social defeat) should produce similar results. Alternatively, these characteristics may have emerged only after the experience of social defeat, in which case we would observe no differences in fear expression and extinction among the groups. Thus, mice were subjected to social defeat one week after completion of cued fear conditioning and extinction procedures as previously described. Results from social interaction were then analyzed in order to establish susceptible ($n = 10$), resilient ($n = 10$) and non-defeated control ($n = 7$) groups.

We found significant differences among groups in social interaction ($F_{(2,24)} = 10.66$, $p < .001$), again with increased social interaction in resilient and non-defeated control mice as compared to

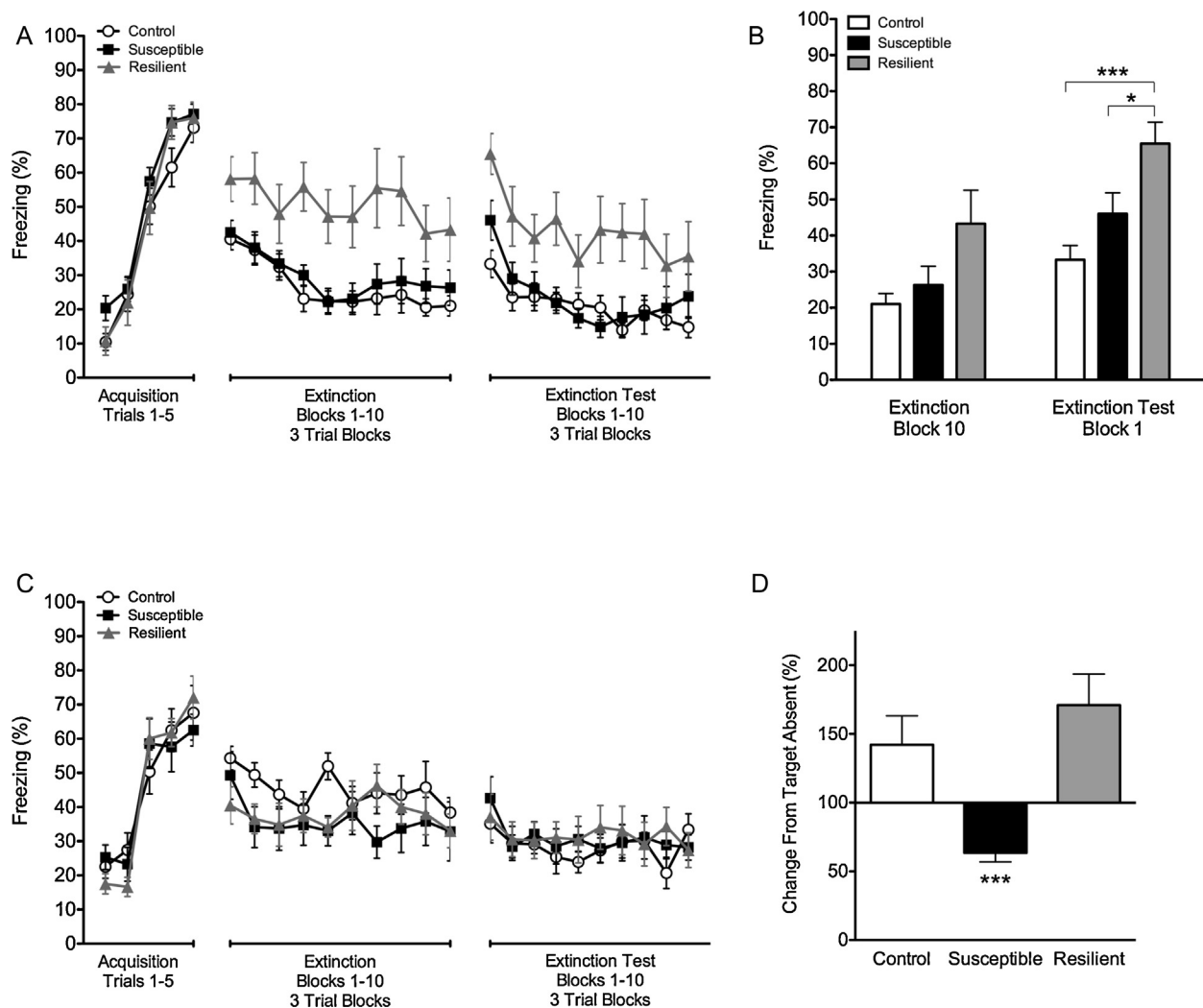


Fig. 2. Enhanced fear expression and deficits in fear extinction after exposure to social defeat. (A) Cued fear conditioning and extinction one week after social defeat. Social defeat disrupted extinction learning in resilient mice ($n=9$) as compared with susceptible mice ($n=12$) and non-defeated controls ($n=16$). Susceptible mice displayed specific deficits in extinction retention. (B) Extinction retention as measured by a repeated measures ANOVA comparing the last block of trials during extinction and the first block of trials during the extinction test. Resilient mice displayed significant extinction retention deficits as compared with non-defeated controls and susceptible mice. Susceptible mice displayed marginal extinction retention deficits compared to non-defeated controls. (C) Cued fear conditioning and extinction procedures one week before exposure to social defeat. No differences were noted in fear conditioning and extinction among susceptible mice ($n=10$), resilient mice ($n=10$), and non-defeated controls ($n=7$). (D) Interaction ratios represent the percent of change in social interaction time from target absent to target present during the social interaction test. Social defeat decreased interaction ratios in susceptible mice as compared with non-defeated controls and resilient mice that were also defeated. Interaction ratios were comparable between resilient mice and non-defeated controls. Data are represented as mean $\pm$ SEM. * $p < .05$; ** $p < .01$; *** $p < .001$.

susceptible mice (Fig. 2D). After establishing the three groups, we examined the fear learning and extinction data for possible group differences already present before experiencing social defeat stress. Similar to our earlier finding, there were no differences in the acquisition of cued fear among groups ($F_{(2,24)} = 0.007$, $p = .993$). Results from fear extinction demonstrated comparable rates of extinction learning among all groups of mice ($F_{(2,24)} = 1.20$, $p = .319$) (Fig. 2C). Our findings demonstrate that resilient mice extinguish fear similar to susceptible and non-defeated control mice when the fear learning precedes exposure to social defeat. Thus, the deficits in fear extinction emerge only after experiencing social defeat stress.

The finding that resilience is associated with deficits in fear extinction is compelling given that resilient individuals generally demonstrate cognitive and emotional flexibility that enables them to recover appropriately from traumatic experiences [40–44]. Perhaps these paradoxical behavioral findings could be explained by impaired cognitive or behavioral flexibility. For example, rather than enhanced behavioral flexibility resilient mice may display a

less flexible emotional response strategy to social defeat, explaining why they do not adjust their social behavior after experiencing defeat, but could also explain their inability to appropriately extinguish fear.

3.5. Resilient mice do not display impaired behavioral flexibility

We tested the behavioral flexibility hypothesis by using spatial reversal learning procedures in the Morris water maze task. Given that fear extinction is considered a form of reversal learning, we predicted that resilient mice would perform poorly in the reversal phase of the Morris water maze. Mice were subjected to social defeat and were characterized into susceptible ($n=9$) and resilient ($n=5$) subpopulations again replicating our initial findings ($F_{(2,19)} = 24.69$, $p < .001$) (Fig. 3A). Seven days after completion of the social defeat procedure, all mice were trained in the Morris water maze task. Mice performed 24 trials of spatial training that required them to locate and swim to a hidden platform in the NW quadrant of the pool. Analysis of latency to reach platform across all training

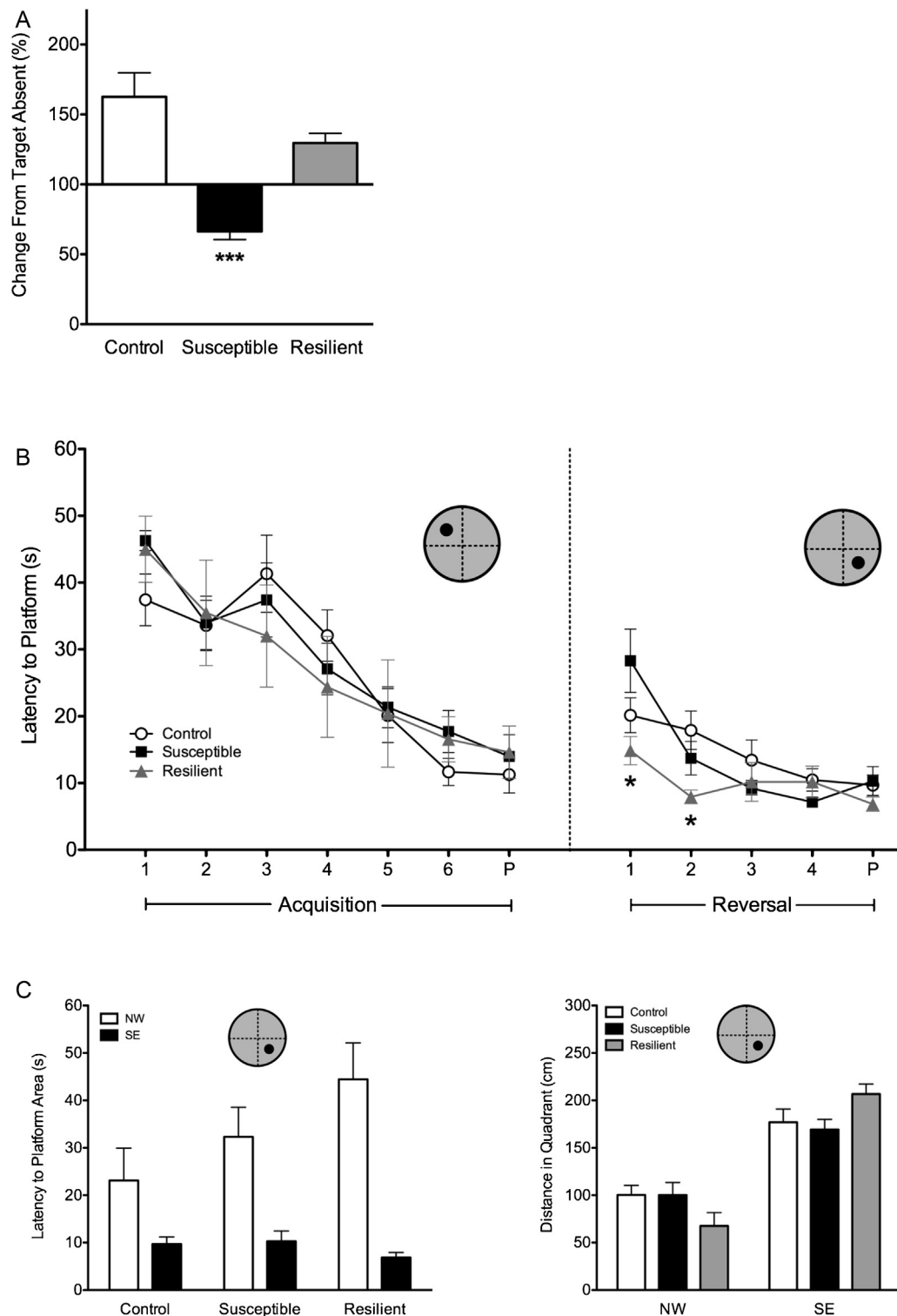


Fig. 3. Resilient mice do not display impaired behavioral flexibility. (A) Interaction ratios represent the percent of change in social interaction time from target absent to target present during the social interaction test. Social defeat decreased interaction ratios in susceptible mice ($n=9$) as compared with non-defeated controls ($n=7$) and resilient mice ($n=5$) that were also defeated. Interaction ratios were comparable between resilient mice and non-defeated controls. (B) Spatial learning and spatial reversal procedures using the Morris water maze. Mice performed four training trials per day to locate and swim to a hidden platform submerged within the pool. Data represent the average latency to reach the hidden platform in blocks of four trials. Probe tests were conducted 24 hours after the spatial phase and again 24 hours after the spatial reversal phase to assess reference memory. (C,D) Latency to the platform area (seconds) and swim distance (cm) in the original quadrant (NW) compared to the reversal quadrant (SE) during the reversal probe test. Data are represented as mean $\pm$ SEM. * $p < .05$; ** $p < .01$; *** $p < .001$.

trials revealed no significant differences in the acquisition and performance among susceptible, resilient, and non-defeated control mice ($F_{(2,18)} = 0.12$, $p = .890$) (Fig. 3B). Furthermore, all groups performed equivalently in the probe trial 24 hours after the last spatial

training session (all $ps > .563$), (Non-defeated control = 11.3 ± 2.7 ; Susceptible = 13.4 ± 2.9 ; Resilient = 14.6 ± 3.9).

After completion of the initial spatial phase, all mice performed 16 trials of spatial reversal training in which the platform was

relocated to the SE quadrant of the pool. This change in platform location provided an index of behavioral flexibility to assess performance in spatial reversal learning. Analysis of latency to reach the platform (new location) across all reversal training trials revealed no significant differences in the acquisition and performance among susceptible, resilient, and non-defeated control mice ($F_{(2,18)} = 1.81, p = .192$) (Fig. 3B). Although our initial analysis revealed no significant differences during spatial reversal training, we performed individual analyses on each day of the reversal phase. Results from the first reversal day demonstrated marginal differences among groups in the latency to reach the platform ($F_{(2,18)} = 2.88, p = .083$). Specifically, we observed shorter latencies to reach the platform in resilient mice as compared to susceptible mice $t(12.01) = 2.31, p = .04$ (Fig. 3B). Results from the second reversal day demonstrated marginal differences among groups in the latency to reach the platform ($F_{(2,18)} = 3.18, p = .066$). Specifically, we observed enhanced reversal learning in resilient mice as compared to non-defeated controls $t(10) = 2.80, p = .019$ (Fig. 3B). No further differences in reversal learning were observed. Results from the reversal probe test revealed comparable levels of spatial memory for the reversal platform location, as no differences were found among groups in latency to reach the platform area, total time in quadrant, and total swimming distance in quadrant (all $ps > .136$) (Fig. 3C,D). These data demonstrate an initial and transient enhancement in spatial reversal learning in mice resilient to social defeat. Thus, poor fear extinction is not likely due to impaired cognitive or behavioral flexibility. More likely, the resilience as characterized by social interaction is perhaps due to slightly enhanced behavioral flexibility in these mice.

4. Discussion and conclusion

The present study used an acute model of social defeat stress to investigate whether phenotypic differences in response to social defeat are associated with specific differences in associative fear learning and extinction. Based on their social interaction with an unfamiliar, non-threatening conspecific, we identified susceptible and resilient phenotypes in socially defeated mice. Susceptible mice responded with lasting reductions in social interaction whereas resilient mice showed no reduction in social interaction following the experience of social defeat stress. These findings are consistent with other reports demonstrating that chronic social defeat stress produces susceptible and resilient (unsusceptible) phenotypes [31,33,36,45]. To our knowledge, no studies have investigated how susceptible and resilient mice respond to cued fear conditioning following social defeat stress. Although a previous report demonstrated deficits in extinction retention following social defeat in 5-HTT^{-/-} mice [26], the relationship between these effects and individual differences in the response to social defeat were not investigated. In the present study, we found that susceptible mice expressed and extinguished fear comparable to non-defeated controls but had specific deficits in extinction retention, whereas resilient mice displayed enhanced fear expression and were more resistant to extinguish conditioned fear across multiple testing days. The present study is the first to demonstrate how susceptible and resilient phenotypes are associated with specific behavioral differences in subsequent cued fear learning and extinction and suggests possible overlapping mechanisms that may underlie stress responsiveness and the ability to extinguish learned fear.

Previous studies have characterized susceptible and resilient phenotypes in response to chronic social defeat stress [27,30,31]. Susceptibility has been associated with maladaptive behavioral changes in a wide range of procedures including social interaction, forced swim, elevated plus-maze, and sucrose preference

[31]. Resilience has been associated with some maladaptive behavioral changes but none of the depressive-like behaviors as seen in susceptible mice. Moreover, resilience has been attributed to alterations in BDNF signaling within the ventral tegmental area-nucleus accumbens circuit [31,46] and epigenetic alterations in the hippocampus and serotonergic neurons [32]. Thus, resilience as characterized by intact social behavior after social defeat, does not extend to all anxiety- and stress-related behaviors [31]. Similar to these previous reports, we found intact social behavior in resilient mice; however, these mice were unable to appropriately extinguish cued fear as compared to non-defeated controls. There are clear differences between the current findings using acute social defeat and those described above using chronic social defeat. For instance, we found no differences in anxiety-like behavior among susceptible, resilient, and non-defeated control mice when tested in the elevated plus-maze. Given the temporal contrast between studies using chronic social defeat and the acute model used here, discrepancies in anxiety-like behavior may be due to differences in the duration of exposure to aggressive mice (both physical and non-physical exposure). In the chronic defeat procedures described above, susceptibility is associated with inadequate coping strategies that lead to the development of maladaptive behavior. In contrast, resilience is associated with adaptive coping strategies in the face of stress and adversity. It is likely that the same holds true for both susceptible and resilient mice in the present study, in part because these effects last for at least 30 days.

Behavioral flexibility has been associated with stress resilience and is critical in permitting successful coping strategies that allow animals to adapt in the face of adversity and stress [42,44]. Therefore, deficient behavioral flexibility may explain the failure to appropriately extinguish fear in resilient mice. As an index of behavioral flexibility, we tested this hypothesis by using a spatial reversal learning procedure in the Morris water maze. Contrary to our hypothesis, we demonstrated that resilient mice exhibited intact behavioral flexibility and displayed transient enhancements in reversal learning. These findings remain consistent with a large literature demonstrating that resilient individuals show behavioral and cognitive flexibility [41–44,47]. However, the fact that resilient mice show deficits in fear extinction seems inconsistent with intact or enhanced behavioral flexibility. Although not truly a reversal task, fear extinction requires an animal to flexibly alter behavior and inhibit a previously learned response. Thus, based on their performance on the reversal learning task, resilient mice should be able to appropriately extinguish a learned fear response, but do not do so efficiently. One possible explanation for this effect may be due to differences in the tasks themselves. Reversal learning in the Morris water maze is an instrumental procedure in which the animal must learn the relationship between their behavior and the consequences of that behavior. Likewise, approach/avoidance behavior during social interaction is a goal-directed task and is also contingent upon the animal's response to the social stimulus animal. Extinction learning, however, is not contingent upon the animal's response, and instead, is a classical conditioning procedure that involves the formation of new learning [48]. Thus, social interaction and reversal learning may share some attributes that enables resilient mice to update behavioral responses to more adaptive strategies based on outcome, whereas conditioned fear extinction does not. Future studies will evaluate the extent to which behavioral flexibility contributes to the observed effects in resilient mice in the present study.

We demonstrated that susceptible mice had deficits in extinction retention, an effect that is similar to what has been observed in previous studies examining the effects of stress on fear learning [15,19–22,49,50]. This behavioral effect is consistent with previous identified neural mechanisms regulating responses to social defeat and those that regulate fear extinction. For example,

exposure to social defeat results in neural activation of a wide variety of brain regions including the cingulate cortex, bed nucleus of the stria terminalis (BNST), the amygdala and several hypothalamic regions [51]. In addition, amygdala-dependent plasticity has been shown to be involved in responses to acute social defeat [2,6]. Furthermore, subordinate hamsters that are susceptible to social defeat show reduced c-Fos activation in the infralimbic cortex (IL) compared to dominant animals that are resistant to defeat [52]. Likewise, susceptible mice show reduced *Arc* mRNA in the medial prefrontal cortex (mPFC) following chronic social defeat [45]. In addition to its involvement in responses to social defeat, the IL is critical for the consolidation and retrieval of extinction. Temporary inactivation of the IL disrupts acquisition of extinction [53], and lesioning the IL results in a specific disruption of extinction retrieval [54]. Moreover, brief uncontrollable stress causes dendritic retraction in the IL and impairs fear extinction [49]. Thus, it seems likely that concurrent activation of the amygdala and reduced activation of the IL may explain the fear extinction retention deficits observed in susceptible mice.

Yet, this explanation seems inadequate to explain the enhanced fear expression and fear extinction deficits of resilient mice. Such a finding is in contrast to what was predicted, and is unexpected based on previously identified mechanisms regulating resilience to social defeat and those regulating fear extinction as described above [45,48,52]. As noted, the IL plays an important role in determining resilience to social defeat. For example, dominant animals have greater activation of the IL during social defeat, which may be necessary in establishing their resilience to the deleterious effects of social stress [52]. In addition, resilient mice show no reductions in *Arc* mRNA in the mPFC following chronic social defeat [45]. Thus, enhanced activation of the IL is associated with resilience to defeat, but is also associated with enhanced cued fear extinction [48,53,55]. Interestingly, resistant and susceptible hamsters show comparable neural activation of the amygdala and BNST [52], suggesting that differences in neural activation of these regions are not likely to explain the behavioral differences between resistant and susceptible animals. Furthermore, stress produces equivalent changes throughout multiple regions of the mPFC. Exposure to chronic stress or glucocorticoids results in decreased volume and dendritic atrophy in the prelimbic cortex (PL), IL, and anterior cingulate cortex (ACC) [56–58]. One intriguing possibility is that individual differences in neurobiological and behavioral responsiveness to stress might underlie the divergent behavior of resilient mice. For instance, differences in morphological alterations of the ACC in response to stress corresponds to the degree of impairment on an attentional-set shifting task [59]. Only those animals with the greatest stress-induced morphological changes showed significant impairment on the behavior task. Moreover, lesions of the ACC have significant effects on the utilization of social information such that ACC lesions cause impairments of memory for social stimuli [60]. Our previous work suggests that social avoidance following the experience of social defeat also requires memory for social stimuli [6]. Thus, it is possible that resilient mice show the greatest stress-induced changes in the ACC, explaining their increased social interaction following social defeat, while reduced IL functioning contributes to their impaired fear extinction, as would be expected. Alternatively, the differences observed between resilient and susceptible mice may be a function of differences in stress-induced alterations of the orbital frontal cortex (OFC). This region is implicated in behavioral flexibility, reward value and emotion regulation [60,61]. Interestingly, the effects of stress on the OFC seem to be opposite to those observed in the IL and ACC [59], but just how this might alter social behavior and fear conditioning is unclear, as its role in these behaviors has not been examined extensively [62]. Whether these neurobiological differences explain why resilient mice also display enhanced fear expression

and deficits in fear extinction is unclear, but should be a focus of future research.

Additional support for individual differences in neurobiological and behavioral responsiveness comes from evidence demonstrating natural variation in fear conditioning and extinction in outbred rats. For example, Bush et al., [63] separated rats based on fear acquisition and extinction and found two distinct phenotypes. One of these phenotypes displayed delayed fear extinction and extinction retention despite equivalent fear acquisition and expression levels. In addition, there were no differences in anxiety-like behavior observed between the two distinct phenotypes [63], similar to the findings of the present study. A further analysis discovered an additional phenotype of rats that failed to extinguish fear in a single extinction session [64], and the extinction rates displayed by resilient mice in the present study appear to match this phenotype. In the present study, however, we did not find significant differences in fear conditioning and extinction unless mice were defeated before fear conditioning. These data suggest an interaction between the environment and variations in stress responsiveness may similarly identify specific fear expression and extinction phenotypes as described above.

The present data suggest that resilience as measured by social interaction does not generalize to other fear-related behaviors and perhaps represents a specific coping strategy in response to social stress. These data suggest that mechanisms controlling resilience to social defeat as characterized by social interaction leave animals vulnerable to maladaptive fear behavior. Perhaps an appropriate way of interpreting the present data is in terms of allostasis and allostatic load/overload [65,66]. Adaptive plasticity of both physiological and neural systems underlies resilience and allostatic responses, but this plasticity may come at a cost when not tightly regulated [67]. Thus, the allostatic adaptations allowing mice to initially be resilient to the effects of social defeat may eventually become deleterious and cause maladaptive fear behavior. Alternatively the observed effects may be due to individual variations in neurobiological and behavioral responsiveness to social stress that reveal differential vulnerabilities in social behavior and fear conditioning. These data may be relevant to PTSD as current theory suggests that an inability to appropriately extinguish learned fear responses underlies PTSD [12,68,69] and several studies show that PTSD patients have enhanced fear expression and impaired fear extinction [70–72]. One might expect differential or competing neural mechanisms underlying such divergent behavioral response patterns in resilient mice, and this will be important for determining why resilient mice are capable of bouncing back in response to social defeat in a social context, but are vulnerable to maladaptive fear-related behavior.

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