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Individual Differences in Novelty-Seeking Behavior in Rats as a Model for Psychosocial Stress-Related Mood Disorders

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Abstract

Most of the neuropsychiatric disorders, including stress-related mood disorders, are complex multi-parametric syndromes. Diagnoses are therefore hard to establish and current therapeutic strategies suffer from important variability in effectiveness, making the understanding of inter-individual variations crucial to unveiling effective new treatments. In rats, such individual differences are observed during exposure to a novel environment, where individuals will present with either high or low locomotor activity and can thus be separated into high (HR) and low (LR) responders, respectively. In rodents, a long-lasting psychosocial stress-induced depressive state can be triggered by exposure to a social defeat procedure. We therefore analyzed the respective vulnerabilities of HR and LR animals to long-lasting, social defeat-induced behavioral alterations relevant to mood disorders. Two weeks after four daily consecutive social defeat exposures, HR animals exhibit higher anxiety levels, reduced body weight gain and sucrose preference, as well as a marked social avoidance. LR animals, however, remain unaffected. Moreover, while repeated social defeat exposure induces long-lasting contextual fear memory in both HR and LR animals, only HR individuals exhibit marked freezing behavior four weeks after a single social defeat. Combined, these findings highlight the critical involvement of inter-individual variations in novelty-seeking behavior in the vulnerability to stress-related mood disorders, and uncover a promising model for posttraumatic stress disorder.

Keywords

Novelty-seeking; individual differences; social defeat; PTSD; depression; fear memory

1. Introduction

Depression is a neuropsychiatric disorder that alters many aspects of the human condition. Therefore, individual vulnerability to a depressive syndrome results from complex interactions between a wide range of personality traits. In support of this critical involvement of individual traits, an significant population of depressive patients exhibits resistance to current antidepressant treatments [1]. However, the molecular basis of this

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resistance to treatment have yet to be elucidated. Moreover, clinical evidence suggests that personality assessment can, in fact, be used to predict further vulnerability to mood disorders [2,3]. It is therefore crucial to obtain animals models mimicking these intra-species resistances in order to better understand the precise role of individual differences in the neurobiology of stress-related mood disorders such as depression and anxiety.

In humans, depression is mainly triggered by prolonged or repeated exposure to a stressor of a psychological nature [4], in combination with genetic and other environmental factors [5]. This repeated exposure to a social stressor can be mimicked in rodents, by using a modified version of the resident-intruder procedure called social defeat, in which a subordinate male rat replaces the female rat in the home cage of an aggressive dominant male. This stressor, unlike many environmental stressors, does not result in habituation upon repeated presentation, and thus generates persistent emotional stress [6]. When flight is barred, the intruder will assume a submissive supine posture and emit loud and frequent ultrasonic distress calls [7]. The intruder will therefore be repeatedly attacked and defeated, resulting in long-term and persistent behavioral and biological changes. In mice, prolonged exposure to social defeat for ten days induces severe deficits in social interactions, measured by an increase in social avoidance behavior, which can be detected even four weeks after the defeat procedure [8]. Interestingly, this social avoidance is not limited to a former aggressor but extended to an unfamiliar social target, suggesting a general social withdrawal [8]. In rats, chronic social defeat has been shown to induce long-lasting changes in circadian rhythms, body weight and decreased locomotor and exploratory activity [9-11]. These alterations are also accompanied by a well-described decrease in the ability to experience pleasure (anhedonia), measured by a reduced preference for a sucrose solution, as well as an increased behavioral despair in the forced-swim test [11-14]. Of note, social defeat-induced behavioral alterations can be reversed by chronic antidepressant treatments [8,12-14]. Taken together, these observations mirror several aspects of the human depressive syndrome and therefore validate social defeat as an acceptable animal model of depression.

In addition, social defeat also provides a powerful tool for discriminating individual differences in response to stress and stress-related depressive behaviors. In subpopulations of rats based on their basal sucrose intake, a daily one hour social defeat exposure for 21 days induces a marked despair behavior in the forced swim test (FST) in animals exhibiting a high sucrose intake, while individuals with a low sucrose preference remain unaffected [15]. Moreover, inherent differences in social defeat reactivity are associated with distinct a corticotrophin-releasing hormone (CRH)-system response and behavioral despair. Individuals with a high reactivity to defeat (short latency to submissive posture) exhibit decreased CRH mRNA levels associated with increased neuronal activation in the paraventricular nucleus (PVN) of the hypothalamus underlying increased immobility time in FST. However, rats with a lower reactivity to social defeat (higher latency to submissive posture) do not exhibit these alterations in the CRH-system or immobility duration in the FST [16]. Several other investigations of individual differences in the behavior displayed before, during and after the social defeat procedure, also reveal significant interactions between coping strategy and long-term effects of social defeat, therefore linking individual differences in response to stress and vulnerability to social defeat exposure [17-19].

A multitude of animal paradigms that model human mood disorders have been developed, based on persistent inter-individual differences in response to stress, in order to study the neurobiology of human affect disorders (for review, see [20]). One of these models relies on the response to the mild stress of a novel environment, where some rats, known as high responders (HR), exhibit high rates of exploratory locomotion while others, known as low responders (LR), exhibit low rates of locomotor activity [21-24].

The locomotor response to a novel environment not only predicts subsequent behavioral responses to drugs such as amphetamine and cocaine [22-27], but also predicts anxiety-related behavior in these animals [28,29]. Indeed, LR rats display higher levels of anxiety in an elevated T-maze, a response that is more strongly enhanced in LR than HR animals following repeated exposure to the test [30]. HR and LR rats also appear to exhibit different behaviors in the forced swim test at basal conditions and following antidepressant treatment, although these points are still unclear. Indeed, Taghzouti and colleagues reported individual differences in the test phase of the FST together with differential effects of subchronic fluoxetine injections [31], whereas a recent study using the same injection protocol revealed no individual differences in the test phase of the FST procedure, an equal effectiveness of fluoxetine in reducing behavioral despair, but an antidepressant effect of desipramine in LR only [32]. Moreover, we recently showed that four exposures to social defeat produce a strong emotional and contextual fear memory that persist over six weeks after the last defeat exposure [33]. Taken together, this evidence strongly suggests that HR and LR animals possess differential vulnerabilities to social defeat that could model the individual differences in vulnerabilities to stress-related mood disorders.

Therefore in this work, we assessed whether HR and LR animals demonstrated differential vulnerabilities to social defeat exposure by measuring several affect parameters related to depression, including the ability to experience pleasure and social avoidance. We also investigated whether these putative individual differences in depression-related behavior could be extended to the emotional and contextual fear memory triggered by social defeat [33] by measuring the persistence of the fear memory up to 4 weeks after the last defeat exposure.

2. Material and methods

2.1. Animals

Eight weeks-old male Sprague–Dawley rats weighing 250–275 g, randomly pair-housed in Plexiglas cages (48.2 × 26.5 × 20.3 cm), were used in this study. As measurement of locomotor activity in response to novelty was carried out five days after reception at the vivarium (see section 2.3.1), and in order to avoid any additional social stress due to further modification of cagemates, the distribution of HR/LR phenotype among cages was random, and both animals of one pair received the same treatment, *i.e.* defeated or not. Additionally, vasectomized male Long–Evans rats weighing 325–350 g were pair-housed with female Long–Evans rats weighing 200–225 g. These Long–Evans males served as the resident attackers during the social defeat procedure and were chosen for consistent aggressive behavior. All animals were ordered from Charles River Laboratories (Wilmington, MA, USA). Rats were maintained on a 12-h light/dark cycle (lights off at 7:00 p.m.) with food and water available *ad libitum* except during testing. All experiments except the sucrose preference study were performed during the light phase of the light/dark cycle and were all conducted in accordance with the guidelines of the Animal Care and Use Committee of Florida State University and National Institutes of Health guidelines.

2.2. Experimental design

After five days of habituation to the animal facility, all animals were first screened for their locomotor activity in order to be assigned to an HR or LR group (see section 2.3.1.), and then either defeated or left undisturbed in their home cage. Four different groups of rats were used for behavioral analyses, in an effort to minimize interferences between the different experimental procedures. The first group of animals thus underwent the open-field procedure only, while a second set was monitored for sucrose preference and body weight gain. The third group was tested for the social approach and avoidance behavior followed by

two contextual fear memory assessments, a first time two days after the social approach and avoidance test, and a second time two weeks later (see section 2.3.6.). The fourth and last group of animals was used for the analysis of short- and long-term contextual fear memory following exposure to acute social defeat.

2.3. Behavioral procedures

2.3.1. Locomotor activity—After five days of habituation to the vivarium and handling on two occasions, locomotor response to novelty was tested in circular activity chambers (Med Associates Inc., St. Albans, Vermont) for one hour to determine HR/LR phenotypes. Four equidistant photo-beam sensors recorded each rat's crossings between adjacent quadrants, allowing an individual locomotor score for later classification into HR and LR groups as previously described [34,35].

2.3.2. Social defeat procedure—The social defeat protocol used in this study was the same as previously described by our group [24,33,36]. Briefly, Sprague-Dawley rats were exposed to one (acute defeat) or four (repeated defeat) consecutive encounters (for 15 minutes each) in the home cage of an aggressive Long-Evans male rat, while non-defeated animals remained undisturbed in their home cage. Rats were first allowed to physically interact for 5 minutes and then, the intruders were transferred to a wire mesh cage placed inside the resident's cage for the remaining ten minutes of the session. This protective cage allowed for full visual, olfactory, and auditory exposure to the resident without unnecessary harm to the intruder. The cage was large enough for the intruders to move freely (10 × 10 × 15 cm). In order to avoid any potential effects between defeated animals and non-defeated cagemates, individuals in the same cage received the same treatments.

2.3.3. Open-field behavior—Two weeks after the last encounter of the repeated defeat procedure, general mobility was examined using a squared arena (90 × 90 cm). The open-field session consisted of placing the rat in the arena and monitoring its movements for 10 minutes using a videocamera. The following parameters were assessed: locomotor activity, time spent in center, immobility duration, speed, as well as rearing and grooming frequencies. Locomotor activity was obtained by virtually dividing the arena into 16 identical squares and counting the number of squares crossed during the 10 minutes period, and the time in center was calculated as the time spent in the four inner squares. Results were analyzed using a two-way ANOVA with HR/LR and defeat as independent factors, followed by a Fisher's PLSD *post-hoc* test when appropriate.

2.3.4. Measure of sucrose preference (anhedonia)—The procedure used to measure the preference for sucrose over water was adapted from Iniguez *et al.* [37] to allow repeated measures over time in order to follow the sucrose preference before, during and after the social defeat procedure. Rats were habituated to drink from two water bottles for five days and then subjected to the first test. Two hours (5:00 *p.m.*) before the test, rats were singly housed in a separate room with free access to food and two bottles of water. At the beginning of the dark phase (7:00 *p.m.*), rats were given access to two preweighed bottles, one containing water and the other one a 0.5% sucrose solution, until 8:00 *a.m.* the next morning. The bottles were weighed every hour from 7:00 *p.m.* to 9:00 *p.m.* and at 8:00 *a.m.* to measure their overnight fluid consumption. The position of the sucrose bottle was changed at every test. The results showed that the contribution of fluid consumption between 9:00 *p.m.* and 8:00 *a.m.* was minor and could be neglected and thus only the fluid consumption during the first two hours of the dark cycle is presented. At the end of the testing period (8:00 *a.m.*), rats were paired-housed again. As we previously reported an inter-individual difference in sucrose preference between HR and LR animals only on the first exposition to sucrose [35], a second test was performed the same day. Then, additional

tests were performed every two days to monitor the sucrose preference before, during and after the social defeat procedure. Results were analyzed using a repeated two-way ANOVA with HR/LR and defeat as independent factors, followed by a Fisher's PLSD *post-hoc* test when appropriate.

2.3.5. Social approach and avoidance test—Four weeks after the last defeat encounter, the social approach and avoidance test, consisting of three 5-min sessions, was performed as described by our group [33]. Briefly, rats were first placed in an empty open field box (90 × 90 cm) for five minutes to both acclimate the animals to the environment and provide a measure of locomotor activity. In the second session ("no target"), each rat was placed into an open field (90 × 90 cm) that contained an empty wire mesh cage (10 × 10 × 15 cm) on one side of the open field, and the time spent by investigating this cage was measured. In the third session ("target"), animals were placed in the open-field containing a caged, unfamiliar Long–Evans male at the same location as in the 'no target' session, and the time spent investigating the caged Long-Evans rat was measured. Results were analyzed using two-way ANOVA with HR/LR and defeat as independent factors, followed by a Fisher's PLSD *post-hoc* test when appropriate.

2.3.6. Contextual fear memory test—The contextual fear memory triggered by the repeated social defeat experience was first assessed as previously described [33]. The animals were tested for freezing behavior in the empty home cage of an unfamiliar Long-Evans resident four weeks after exposure to the last defeat, and then retested two weeks later to identify signs of extinction. During each session of 5 minutes, the time spent freezing was measured. Freezing was defined as the absence of any movement except that necessary for respiration. Thus, the rat was considered to be freezing once all locomotor and exploratory behavior ceased. The number of freezing animals was then calculated by classifying animals as "freezing" only when they demonstrated a total freezing time superior or equal to 20 sec.

In a second set of experiments, animals exposed to acute social defeat were placed in a clean, empty cage either twenty-four hours or 4 weeks later and the time spent freezing was measured for five minutes. This test established a baseline freezing behavior in response to a novel cage. Twenty-four hours after this novel cage exposure, rats were placed inside the empty home cage of a familiar Long–Evans aggressor for five minutes, and their freezing behavior in the context of social defeat was measured. Two parameters of freezing were analyzed: the freezing time, and the number of animals to reach criterion. In the present study, the latter was defined as reached if the difference between the freezing time in the context and novel cage was superior or equal to 20 sec. The percentage of animals to criterion and freezing time therefore respectively provide a quantitative and qualitative measure of the freezing behavior.

The total time spent freezing did not show a normal distribution, and were thus analyzed using the Kruskall-Wallis non-parametric ANOVA, followed by a Mann-Whitney inter-group comparison when appropriate. The percentage of freezing animals and animals to criterion were analyzed using a Chi-squared test.

3. Results

3.1. Long-term sensitivity of HR and LR animals to social defeat-induced anxiety

Several behavioral parameters were investigated using an open-field test, allowing assessment of exploration and general mobility (locomotion, speed, immobility and rearing), stereotyped response (grooming and rearing), and anxiety (time spent in center) in HR and LR, defeated and non-defeated animals. As summarized in Table 1, two-way ANOVA analyses revealed a significant HR/LR effect for the time spent in the center ($F_{(1,23)} = 5.08$,

$p < 0.05$) and the speed ($F_{(1,23)} = 7.97$, $p < 0.01$), with HR animals spending significantly more time in the center of the arena, as compared to LR non-defeated animals. However, we did not find significant effects for HR/LR for any of the other parameters ($F_{(1,23)} = 2.73$, $p > 0.05$ for grid crosses; $F_{(1,23)} < 1$ for immobility and rearing; and $F_{(1,23)} = 1.44$, $p > 0.05$ for grooming). This observation reveals a decreased thigmotaxis, underlying reduced anxiety levels in HR animals, as previously reported by our group and others [28-30].

Two weeks after the last exposure, repeated social defeat stress induced significant alterations in both HR and LR animals with respect to locomotor activity ($F_{(1,23)} = 10.49$, $p < 0.01$), speed ($F_{(1,23)} = 6.90$, $p < 0.05$), and immobility ($F_{(1,23)} = 10.90$, $p < 0.01$), but not grooming behavior ($F_{(1,23)} < 1$). Indeed, social defeat induced a decrease in speed (26% in LR, 16% in HR), a marked increase in immobility duration (303% in LR, 332% in HR), as well as a 32% decrease in locomotion in LR animals, with a non-significant reduction in HR ($p = 0.08$). These observations thus highlight a marked reduction of general mobility and exploratory behavior induced by repeated social defeat stress in both HR and LR animals. While analysis by two-way ANOVA revealed a significant social defeat-induced reduction in both HR and LR animals in the rearing frequency ($F_{(1,23)} = 7.62$, $p < 0.05$) and time spent in the center of the arena ($F_{(1,23)} = 10.02$, $p < 0.01$), *post-hoc* analyses detected a significant reduction in these behaviors in HR animals only. There were no significant interactions for HR/LR × defeat observed among any of the behavioral parameters analyzed. These observations therefore suggest that while repeated social defeat stress induces a significant reduction of general mobility and exploration in both HR and LR animals without affecting their stereotyped responses, only HR animals develop an increased anxiety two weeks following the last exposure to the stressor.

3.2. Long-term sensitivity of HR and LR animals to social defeat-induced alterations in depression-related behavior

Exposure to repeated social defeat is known to induce several depressive-like symptoms in rodents, including anhedonia and decreased body weight gain [9,11,13,14]. We therefore monitored body weight gain and hedonic capacity for a 0.5% sucrose solution in HR and LR rats before, during and after social defeat stress (Fig. 1a).

The body weight was monitored every two days starting two days before the first defeat encounter to six days after, in order to evaluate the body mass gain (Fig. 1b). While all groups significantly increased their body mass (Fr = 73.12, $p < 0.0001$ for LR-ND; Fr = 57.24, $p < 0.0001$ for LR-D; Fr = 142.39, $p < 0.0001$ for HR-ND; and Fr = 55.99, $p < 0.0001$ for HR-D), we observed a significant reduction in body mass gain in HR animals exposed to social defeat compared to their non-defeated counterparts ($F_{(1,60)} = 8.24$, $p < 0.05$). This reduction was evident immediately following the first defeat and again two days after the last encounter (Fig. 1b). Interestingly, this disparity in weight gain was unique to HR animals as no significant difference between LR defeated and non-defeated animals was detected ($F_{(1,76)} < 1$). Moreover, no differences between groups were observed five weeks after the last defeat encounter ($F_{(1,34)} = 1.68$, $p > 0.05$ for HR/LR; and $F_{(1,34)} < 1$ for defeat and interaction; data not shown), suggesting a recovery of the body mass gain reduction in HR animals five weeks after the last defeat exposure.

The sensitivity of HR and LR animals for the rewarding effect of sucrose solution was assessed by monitoring their sucrose preference on several consecutive presentations (Fig. 1a). As shown in figure 1c, HR and LR animals did not exhibit a difference in their sucrose preference ($F_{(1,111)} < 1$, $p > 0.05$) before the beginning of the repeated social defeat procedure. However, a distinct sensitivity appeared following exposure to social stress. Indeed, while the social defeat procedure did not have a significant effect in LR animals ($F_{(1,76)} < 1$, $p > 0.05$), HR rats were vulnerable to this stress ($F_{(1,64)} = 4.87$, $p < 0.05$) and

displayed a significantly lower sucrose preference two days after the last defeat encounter compared to their non-defeated counterparts (Fig. 1d). Neither the HR/LR phenotype nor social defeat had an effect on the total fluid intake ($F_{(1,140)} < 1$ for HR/LR; $F_{(1,140)} = 2.71$, $p > 0.05$ for defeat; and $F_{(1,140)} = 1.29$, $p > 0.05$ for interaction; data not shown). Therefore, these observations suggest that HR rats exhibit a higher vulnerability than LR animals to social defeat-induced alterations of body mass gain and hedonia.

3.3. Long-term sensitivity of HR and LR animals on social defeat-induced social avoidance

We previously reported that four daily social defeat exposures induce a marked reduction of social interactions in rats four weeks after the last defeat encounter, as measured by an increased avoidance of an unfamiliar rat [33]. In order to investigate the individual differences in this particular behavior, we analyzed social approach/avoidance behavior of HR and LR animals, four weeks after the last defeat exposure. All groups exhibited a similar interaction time with an empty cage ($F_{(1,35)} = 1.25$, $p > 0.05$ for HR/LR; $F_{(1,35)} < 1$ for defeat and interaction), suggesting that neither social defeat exposure nor the HR/LR phenotype alter the innate ability of rats to explore and interact with new objects ("no target" session", Fig. 2a). However, when an unfamiliar Long-Evans rat was placed inside the wire mesh cage ("target session", Fig. 2b), HR defeated animals demonstrated a marked reduction in interaction time, compared to their non-defeated counterparts, while both defeated and non-defeated LR animals behaved similarly ($F_{(1,35)} < 1$ for HR/LR; $F_{(1,35)} = 3.71$, $p = 0.06$ for defeat; and $F_{(1,35)} = 6.61$, $p < 0.05$ for the interaction). It should be noted that this effect was not due to decreased exploration of the arena, as no significant difference in locomotion was observed among any of the groups ($F_{(1,35)} = 3.04$, $p > 0.05$ for HR/LR; $F_{(1,35)} = 1.96$, $p > 0.05$ for defeat; and $F_{(1,35)} < 1$ for the interaction; Fig. 2c).

Therefore, while no individual differences are observed in the interaction with an unfamiliar rat in basal conditions, only HR animals develop a social avoidance behavior four weeks following repeated social defeat stress, again suggesting a higher vulnerability in HR animals to the behavioral effects of social defeat.

3.4. Short and long-term sensitivity of HR and LR animals to social defeat fear memory

We previously reported that repeated social defeat triggers a fear memory that persists six weeks after the last social defeat experience [33]. In order to analyze whether individual differences exist in the sensitivity to induction of fear memory by social defeat experience, we assessed the long-term contextual fear memory of HR and LR animals four weeks after the end of a repeated social defeat protocol. We thus observed that while non-defeated animals exhibited almost no freezing behavior at all, both LR- and HR-defeated animals developed a marked freezing response when re-exposed to the social defeat environment four weeks after the last defeat encounter ($H = 36.26$, $p < 0.0001$; Fig. 3a,b).

In order to assess the individual sensitivity to the extinction of this fear-memory engram, the same animals were re-exposed to the contextual fear memory test two weeks later. As expected, a significant decrease in freezing time between the first and second exposure to the test was observed in LR-defeated ($Z = -2.49$, $p < 0.05$) and HR-defeated animals ($Z = -2.98$, $p < 0.01$), which can be interpreted as a sign of extinction of the fear memory after the first exposure to the test (Fig. 3c). Whereas none of the non-defeated animals, HR or LR, showed freezing behavior during the second exposure (Fig. 3d), a significant number of LR animals demonstrated a small, but significant freezing behavior following repeated defeat ($H = 9.46$, $p < 0.05$; Fig. 3c,d). HR-defeated rats did not spend more time freezing than non-defeated animals, however the number of freezing animals approached significance (Fig. 3c,d). These observations demonstrate that HR and LR animals both develop similar long-term fear memory of prior social defeat exposures that can be extinguished by exposure to

the environment without the stressor (social defeat). However, while the contextual fear memory is still detectable in LR animals, HR individuals no longer exhibit a significant fear behavior.

The latter observation, combined with the increased vulnerability to the repeated social defeat-induced anxiety and depressive-like symptoms exhibited by HR animals compared to LR individuals, led us to investigate whether HR rats exhibit higher sensitivity to a single traumatic event. We thus analyzed the fear memory response after a single social defeat episode (acute defeat), both at short- and long-term time points (48 hours and 4 weeks later, respectively). As a lower fear response was expected due to a reduced number of stress exposures (acute defeat, rather than repeated defeat), and as exposure to a novel cage is considered a mild stressor that may interfere, we first measured freezing responses of both defeated and non-defeated rats to an empty, clean cage for five minutes 24 hours prior to the contextual fear exposure. While we did observe freezing behavior in some animals exposed to the novel cage at both 24 hours and four weeks after defeat exposure, overall no significant effect was detected, regardless of HR/LR phenotype ($H = 3.40$, $p > 0.05$ at short-term, Fig. 3e; and $H = 1.57$, $p > 0.05$ at long-term, Fig. 3g). When exposed to the context 48 hours after the social defeat, a significant variation among the groups was observed ($H = 8.26$, $p < 0.05$), but only a small, non-significant trend in freezing behavior can be detected in both HR and LR animals (Fig. 3e). Nevertheless, significantly more HR animals met the freezing criterion following an acute social defeat, while LR individuals did not significantly differ from non-defeated controls (Fig. 3f). This observation suggests that HR animals exhibit a heightened fear response compared to LR individuals, 48 hours after an acute social defeat experience. When animals are exposed to the context four weeks after the acute social defeat experience, no significant variation could be observed among groups, despite a marked trend towards increased freezing time after an acute defeat experience in HR animals ($H = 7.78$, $p = 0.05$; Fig. 3g). Interestingly, analysis of the number of animals that reached criterion revealed that acute defeat exposure induced a freezing response only in HR animals (Fig. 3f). This observation thus suggests that, in HR animals, a single social defeat encounter is sufficient to trigger a fear memory that persists to four weeks after the stress exposure.

4. Discussion

4.1. The HR/LR animals as a model for individual differences in anxiety and depression disorders

Social defeat is already widely validated as a relevant model for psychological stress-related depression, and several studies have demonstrated its ability to analyze individual differences in response to stress and stress-related mood disorders. Based on a different sensitivity to the mild stress of a novel environment, the HR/LR model has been linked to depression through suspected differential responses in FST and antidepressant treatments [31,32]. Moreover, we recently reported differences in social defeat-induced anhedonia between HR and LR animals [35].

We therefore analyzed the vulnerability of HR and LR animals to several aspects of social defeat-induced depressive-like behaviors. First, analysis of global locomotor and exploratory behavior in an open-field confirmed previous reports that non-defeated HR animals exhibit reduced anxiety levels compared to their LR counterparts [28,29]. Exposure to repeated social defeat, however, strongly reduces the time spent by HR animals in the center of the arena to that of the level of LR rats. Moreover, HR and LR animals display similar responses to repeated social defeat in terms of general mobility, exploratory and stereotyped behaviors, demonstrating that the observed reduction of time spent in the center exhibited by HR animals is related to anxiety. Although this suspected higher vulnerability to stress-

induced anxiety remains to be confirmed and analyzed in details using more anxiety-specific behavioral procedures, this point is of particular interest when considering other observations in LR and HR animals. Indeed, while HR animals self-administer higher levels of cocaine than LR individuals under basal conditions, no individual differences can be observed following social defeat exposure [38]. Moreover, while HR animals display a higher preference than LR individuals for a 0.25% sucrose solution during the first presentation, both HR and LR rats exhibit similar sucrose preferences following exposure to social defeat [35]. Finally, the stereotypic decreased immobility duration displayed by HR animals during the first exposure to FST is lost upon repeated FST presentations, as HR and LR animals thereafter present similar immobility behaviors [31]. Together, these observations highlight a pattern of stress response between HR and LR animals, with HR individuals exhibiting higher responses than LR animals in basal conditions, that are then equalized to LR levels by stress exposure.

We recently reported that exposure to repeated social defeat stress induces depressive-like behaviors in male Sprague-Dawley rats [33]. In the second part of this study, we investigated these defeat-induced alterations in the context of individual differences to assess the role that individual responses may play in such depressive-like pathologies. We thus found that while repeated social defeat did not induce alterations in body mass gain, sucrose preference or social withdrawal in LR animals, HR individuals consistently appeared vulnerable to this specific stress experience. It is therefore critical to understand the mechanisms underlying these inter-individuals discrepancies in stress-related mood disorders.

Several animal models of individual differences in stress-related depressive-like states were thus developed, and can be separated in two groups, based on the method of partitioning of subpopulations. The first category of models relies on individual behavioral differences exhibited during the stress. For instance, differences in latency to exhibit classic defeat behavior or in the coping strategy developed during the defeat episode, have both been identified as predictive value of subsequent differential vulnerabilities to social defeat induction of a depressive-like state [16,19]. Interestingly, these behavioral differences are associated with coherent alterations of the hypothalamus-pituitary-adrenal (HPA) axis, affecting corticosterone release, as well as CRH contents and activation of stress-related brain areas in response to stress [16,19]. The second category of models relies on vulnerability to stress-induced depressive-like behaviors. Following exposure to a series of inescapable swim stress, Sprague-Dawley rats can be separated into vulnerable and resilient subpopulations, with vulnerable rats exhibiting higher plasma corticosterone levels immediately after the test [39]. Furthermore, analysis of the learned-helplessness paradigm in rats, used to model several neuropsychiatric disorders such as depression and post-traumatic stress disorder (PTSD), reveals the existence of helpless and non-helpless individuals [40]. Congenitally helpless rats exhibit alterations of the HPA axis characterized by hyperactive PVN, increased plasma ACTH levels and lower release of corticosterone both at baseline and in response to stress [41-44]. Emerging from these observations, a higher stress-induced release of corticosterone appears to be a common parameter among the different models of individual differences in vulnerability to stress-related mood disorders.

Compared to LR counterparts, HR animals possess, at basal conditions, higher levels of CRH mRNA in the hypothalamic PVN but lower levels in the central nucleus of the amygdala, along with lower levels of glucocorticoid receptor (GR) mRNA in hippocampus [29]. These alterations may explain the specific increased and prolonged corticosterone release observed in HR animals in reaction to a mild stressor without baseline modification [29]. Interestingly, these alterations share similarities with other models of individual

differences in stress responsiveness. Although a detailed investigation of the HPA-axis and glucocorticoid signaling response to social defeat in HR and LR animals is still required, this point emerges as a good hypothesis for their higher vulnerability to the repeated social defeat-induced depressive-like behaviors. In addition, the depressive syndrome is a complex multi-parametric disorder and thus, it is important to consider other signaling pathways, such as monoamine systems, where HR and LR animals exhibit marked differences in the levels of monoamines as well as their respective receptors [45-50]. Moreover, the deletion of the serotonin transporter in mice has recently been associated with increased vulnerability to depressive-like state induced by three weeks of chronic social defeat stress [51].

Interestingly, numerous studies investigating long-term effects of social defeat on anxiety- and depression-related behaviors in rats revealed a significant interaction between social environment and several parameters measured in our study. In particular, social housing of animals, as compared to social isolation (*i.e.* animals housed two to five per cage as compared to individual housing), has been demonstrated to exert either partial or full protective effects on social defeat-induced HPA hyperactivity [52], anxiety [52,53], social avoidance [54], as well as decrease in body mass gain and activity during the dark phase of the daily cycle [55]. Moreover, sucrose consumption is differentially affected by defeat in socially or individually housed rats, although isolation or defeat alone do not exhibit significant effect [56]. These data therefore strengthen our observations since we detected marked individual differences in vulnerabilities to social defeat-induced behavioral alterations even under constant pair-housing. Sucrose and body mass gain monitoring, however, have been executed on animals being isolated for several hours every two days and could therefore be considered as receiving isolation stress. Nevertheless, in addition to the fact that this procedure affected all experimental groups evenly, it should be noted that HR and LR animals exhibit a similar increase in sucrose consumption before the first defeat exposure. A specific differential sensitivity to intermittent isolation following social defeat, however, cannot be ruled out.

By unveiling individual differences in vulnerability to repeated social defeat-induced alterations of depression-relevant behaviors, this study adds further evidence for a link between novelty-seeking behavior and depressive-state. Indeed, the fact that individual differences in depressive-like behaviors are detected only after exposure to social defeat suggests that high novelty-seeking behavior is not associated by itself to a depressive phenotype, but rather represents a crucial component in the differential vulnerabilities to stress-related mood disorders. In support of this hypothesis, recent clinical data place the novelty-seeking personality trait as a predictive risk factor in adolescents, for the development of a transitory course depression period [57]. Other studies also reported that suicidal-depressive patients exhibit more novelty-seeking behavior than non-suicidal depressed patients [58], as well as higher comorbidity of major depression with other affective disorders in novelty-seeking patients [59]. Moreover, mood stabilizer treatments reduce novelty-seeking behavior in rodents [60], while novelty-seeking, along with other personality traits, influences the efficacy of antidepressant treatment in humans [61]. Lower scores of novelty-seeking behavior, however, have also been observed in patients with life-time anxiety and depressive disorders [62]. Thus, the direct relationship between novelty-seeking and depressive-state therefore remains unclear. Nevertheless, the observation of differential sensitivities to antidepressant or anxiolytic treatments correlated with differential novelty-seeking behavior [32,63,64] place the HR/LR model as an interesting and promising animal model in individual differences of neuropsychiatric treatments.

4.2. The HR/LR model for individual differences in fear response

In the last portion of this study, we analyzed differences in HR and LR animals vulnerabilities to induction of a fear memory following social defeat exposure [33]. While

no discrepancies between HR and LR animals were observed in the fear response four weeks following repeated social defeat, HR individuals appear to exhibit an increased sensitivity to the extinction of fear memory, as they no longer display significant freezing behavior when re-exposed two weeks after the initial contextual fear memory test. Interestingly, several other recent studies are in line with our results. Indeed, an animal model of individual differences based on differential fear response has recently been developed. Rats were divided into subpopulations based on their freezing duration exhibited during a conditioned fear test, that then correlate to distinct activations of brain structures, GR expression and monoamines levels [65,66]. Interestingly, high-freezing animals present a marked decrease in fear response over two extinction sessions, compared to low-freezing animals [67]. Moreover, a treatment with benzodiazepine accelerated the extinction of the fear response in the high-freezing animals only [68], suggesting that animals exhibiting a higher vulnerability to fear-induction, also demonstrate an increased extinction of fear memory along with higher sensitivity to relevant drugs. This hypothesis is further strengthened by several observations of a positive correlation between the novelty-seeking trait and benzodiazepine treatment efficacy [63,64].

Of particular interest in this study is the induction of a long-lasting fear memory following a single, 15 min social defeat exposure, in HR animals only. This increased sensitivity of HR animals appears to be specific to the fear stimulus and unrelated to a global higher learning ability, as HR and LR animals exhibit similar spatial episodic memory, as assessed in a novel-object recognition procedure (our unpublished data). This observation could be interpreted as either an enhanced HR sensitivity, or a resilience of LR animals to a traumatic event, but nonetheless, our findings pinpoint hyperarousal behaviors in HR animals, compared to their LR counterparts. Interestingly, this particular behavior trait is highly related to another emotional state disorder, PTSD. As defined by the DSM-IV classification, PTSD represents a long-term maladaptive stress response that involves re-experiencing, avoidance of stimuli associated with the traumatic event, and hyperarousal symptoms. In this study, we have observed similar characteristics in our HR defeated animals. First, HR animals demonstrate a long-term avoidance of an unfamiliar Long-Evans rat following repeated social defeat experience, highlighting a global social withdrawal. Second, HR animals develop a fear response to a single acute defeat experience that does not affect LR individuals, denoting a hyperarousal. Finally, while the acute defeat exposure induces a non-significant increase in freezing duration in both HR and LR animals, only HR animals still present a freezing response four weeks after the defeat experience, therefore mimicking the human pattern of response. Indeed, an important individual variation in the vulnerability to PTSD induction is observed, as following exposure to a traumatic event, only a small proportion of the population will experience persistent high stress response, anxiety, and persistent traumatic memories [69]. The HR/LR model therefore meets some of the criteria required for a relevant animal model of PTSD, as previously defined [70,71]: (i) the fear response is triggered by a brief, stressful event; (ii) the fear response persists for several weeks; (ii) symptoms include hyperarousal and social withdrawal; and (iii) an important inter-individual variation is observed.

The concept of HR/LR animals as a putative model of PTSD has the potential to link this emotional disorder with novelty-seeking behavior. Interestingly, an association between PTSD symptoms and novelty-seeking trait has been observed in both clinical studies [72-74] and in an animal model of susceptibility to learned helplessness [40,75]. Further investigations into this link may provide beneficial avenues for individualized PTSD therapeutic strategies.

4.3. Conclusion

During this study, we observed differential vulnerability in HR and LR animals to social defeat-induced alterations of anxiety and depressive-like levels. Indeed, analysis of defeat-induced alterations of several mood disorder-related behaviors, revealed that HR animals possess a higher vulnerability to induction of anxiety, decrease in body weight gain, anhedonia and social withdrawal, whereas LR animals exhibit a lower vulnerability or resilience to all these behavioral symptoms. In addition, HR animals exhibit a long-lasting fear response following a single social defeat exposure mimicking several aspects of human PTSD. HR and LR animals therefore emerge as a promising model for inter-individual variations in stress-related mood disorders vulnerability. Moreover, our present findings also propose HR/LR animals as a very promising tool in the study of inter-individual variations in vulnerability to PTSD.

Research Highlights

- LR animals are resilient to social defeat-induced anxiety and depressive-like state
- Repeated social defeat induces anxiety and depressive-like state in HR animals
- HR animals are more vulnerable than LR to social defeat-induced fear memory

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References

1. Byrne SE, Rothschild AJ. Loss of Antidepressant Efficacy During Maintenance Therapy: Possible Mechanisms and Treatments. Journal of clinical psychiatry. 1998; 59:279–88. [PubMed: 9671339]
2. Cloninger CR, Svrakic DM, Przybeck TR. Can personality assessment predict future depression? A twelve-month follow-up of 631 subjects. Journal of Affective Disorders. 2006; 92:35–44. [PubMed: 16442638]
3. Kotov R, Gamez W, Schmidt F, Watson D. Linking “big” personality traits to anxiety, depressive, and substance use disorders: A meta-analysis. Psychological Bulletin. 2010; 136:768–821. [PubMed: 20804236]
4. Bjorkqvist K. Social defeat as a stressor in humans. Physiol Behav. 2001; 73:435–42. [PubMed: 11438372]
5. Caspi A, Moffitt TE. Gene-environment interactions in psychiatry: joining forces with neuroscience. Nat Rev Neurosci. 2006; 7:583–90. [PubMed: 16791147]
6. Tidey JW, Miczek KA. Acquisition of cocaine self-administration after social stress: role of accumbens dopamine. Psychopharmacology. 1997; 130:203–12. [PubMed: 9151353]
7. Blanchard RJ, Caroline Blanchard D. Aggressive behavior in the rat. Behavioral Biology. 1977; 21:197–224. [PubMed: 562152]
8. Berton O, McClung CA, DiLeone RJ, Krishnan V, Renthal W, Russo SJ, et al. Essential Role of BDNF in the Mesolimbic Dopamine Pathway in Social Defeat Stress. Science. 2006; 311:864–8. [PubMed: 16469931]
9. Meerlo P, Overkamp GJF, Daan S, Hoofdakker RHvd, Koolhaas JM. Changes in Behaviour and Body Weight Following a Single or Double Social Defeat in Rats. Stress: The International Journal on the Biology of Stress. 1996; 1:21–32.

10. Meerlo P, Sgoifo A, Turek FW. The Effects of Social Defeat and Other Stressors on the Expression of Circadian Rhythms. Stress: The International Journal on the Biology of Stress. 2002; 5:15–22.
11. Rygula R, Abumaria N, Flügge G, Fuchs E, Rüther E, Havemann-Reinecke U. Anhedonia and motivational deficits in rats: Impact of chronic social stress. Behavioural Brain Research. 2005; 162:127–34. [PubMed: 15922073]
12. Rygula R, Abumaria N, Havemann-Reinecke U, Rüther E, Hiemke C, Zernig G, et al. Pharmacological validation of a chronic social stress model of depression in rats: effects of reboxetine, haloperidol and diazepam. Behavioural Pharmacology. 2008; 19:183–96. [PubMed: 18469536]
13. Rygula R, Abumaria N, Domenici E, Hiemke C, Fuchs E. Effects of fluoxetine on behavioral deficits evoked by chronic social stress in rats. Behavioural Brain Research. 2006; 174:188–92. [PubMed: 16949682]
14. Rygula R, Abumaria N, Flügge G, Hiemke C, Fuchs E, Rüther E, et al. Citalopram counteracts depressive-like symptoms evoked by chronic social stress in rats. Behavioural Pharmacology. 2006; 17:19–29. [PubMed: 16377960]
15. Kanarik M, Alttoa A, Matrov D, Kõiv K, Sharp T, Panksepp J, et al. Brain responses to chronic social defeat stress: Effects on regional oxidative metabolism as a function of a hedonic trait, and gene expression in susceptible and resilient rats. European Neuropsychopharmacology. 2010 In Press, Corrected Proof.
16. Wood SK, Walker HE, Valentino RJ, Bhatnagar S. Individual Differences in Reactivity to Social Stress Predict Susceptibility and Resilience to a Depressive Phenotype: Role of Corticotropin-Releasing Factor. Endocrinology. 2010; 151:1795–805. [PubMed: 20160137]
17. Stefanski V. Social Stress in Loser Rats: Opposite Immunological Effects in Submissive and Subdominant Males. Physiology & Behaviour. 1998; 63:605–13.
18. Meerlo P, Sgoifo A, De Boer SF, Koolhaas JM. Long-lasting consequences of a social conflict in rats: Behavior during the interaction predicts subsequent changes in daily rhythms of heart rate, temperature, and activity. Behavioral Neuroscience. 1999; 113:1283–90. [PubMed: 10636307]
19. Walker FR, Masters LM, Dielenberg RA, Day TA. Coping with defeat: acute glucocorticoid and forebrain responses to social defeat vary with defeat episode behaviour. Neuroscience. 2009; 162:244–53. [PubMed: 19393295]
20. Harro J. Inter-individual differences in neurobiology as vulnerability factors for affective disorders: Implications for psychopharmacology. Pharmacology & Therapeutics. 2010; 125:402–22. [PubMed: 20005252]
21. Piazza P, Deminiere J, Le Moal M, Simon H. Factors that predict individual vulnerability to amphetamine self-administration. Science. 1989; 245:1511–3. [PubMed: 2781295]
22. Hooks MS, Jones GH, Liem BJ, Justice JB Jr. Sensitization and individual differences to IP amphetamine, cocaine, or caffeine following repeated intracranial amphetamine infusions. Pharmacology Biochemistry and Behavior. 1992; 43:815–23.
23. Pierre P, Vezina P. Predisposition to self-administer amphetamine: the contribution of response to novelty and prior exposure to the drug. Psychopharmacology. 1997; 129:277–84. [PubMed: 9084067]
24. Kabbaj M, Akil H. Individual differences in novelty-seeking behavior in rats: a c-fos study. Neuroscience. 2001; 106:535–45. [PubMed: 11591454]
25. Hooks MS, Jones GH, Smith AD, Neill DB, Justice JB. Response to novelty predicts the locomotor and nucleus accumbens dopamine response to cocaine. Synapse. 1991; 9:121–8. [PubMed: 1821483]
26. Hooks MS, Jones GH, Smith AD, Neill DB, Justice JB Jr. Individual differences in locomotor activity and sensitization. Pharmacology Biochemistry and Behavior. 1991; 38:467–70.
27. Piazza PV, Deroche-Gamonent V, Rouge-Pont F, Le Moal M. Vertical Shifts in Self-Administration Dose-Response Functions Predict a Drug-Vulnerable Phenotype Predisposed to Addiction. J Neurosci. 2000; 20:4226–32. [PubMed: 10818158]
28. Dellu F, Mayo W, Vallée M, Maccari S, Piazza PV, Le Moal M, et al. Behavioral reactivity to novelty during youth as a predictive factor of stress-induced corticosterone secretion in the

elderly--a life-span study in rats. Psychoneuroendocrinology. 1996; 21:441–53. [PubMed: 8888367]

29. Kabbaj M, Devine DP, Savage VR, Akil H. Neurobiological Correlates of Individual Differences in Novelty-Seeking Behavior in the Rat: Differential Expression of Stress-Related Molecules. J Neurosci. 2000; 20:6983–8. [PubMed: 10995843]
30. Verheij M, Veenvliet J, Groot Kormelink T, Steenhof M, Cools A. Individual differences in the sensitivity to serotonergic drugs: a pharmacobehavioural approach using rats selected on the basis of their response to novelty. Psychopharmacology. 2009; 205:441–55. [PubMed: 19434397]
31. Taghzouti K, Lamarque S, Kharouby M, Simon H. Interindividual differences in active and passive behaviors in the forced-swimming test: implications for animal models of psychopathology. Biological Psychiatry. 1999; 45:750–8. [PubMed: 10188005]
32. Jama A, Cecchi M, Calvo N, Watson S, Akil H. Inter-individual differences in novelty-seeking behavior in rats predict differential responses to desipramine in the forced swim test. Psychopharmacology. 2008; 198:333–40. [PubMed: 18438645]
33. Hollis F, Wang H, Dietz D, Gunjan A, Kabbaj M. The effects of repeated social defeat on long-term depressive-like behavior and short-term histone modifications in the hippocampus in male Sprague–Dawley rats. Psychopharmacology. 2010; 211:69–77. [PubMed: 20454892]
34. Dietz DM, Tapocik J, Gaval-Cruz M, Kabbaj M. Dopamine transporter, but not tyrosine hydroxylase, may be implicated in determining individual differences in behavioral sensitization to amphetamine. Physiology & Behavior. 2005; 86:347–55. [PubMed: 16126238]
35. Hollis F, Duclot F, Gunjan A, Kabbaj M. Individual differences in the effect of social defeat on anhedonia and histone acetylation in the rat hippocampus. Hormones and Behavior. 2010 In Press.
36. Kabbaj M, Evans S, Watson SJ, Akil H. The search for the neurobiological basis of vulnerability to drug abuse: using microarrays to investigate the role of stress and individual differences. Neuropharmacology. 2004; 47:111–22. [PubMed: 15464130]
37. Iniguez SD, Vialou V, Warren BL, Cao JL, Alcantara LF, Davis LC, et al. Extracellular Signal-Regulated Kinase-2 within the Ventral Tegmental Area Regulates Responses to Stress. J Neurosci. 2010; 30:7652–63. [PubMed: 20519540]
38. Kabbaj M, Norton CS, Kollack-Walker S, Watson SJ, Robinson TE, Akil H. Social defeat alters the acquisition of cocaine self-administration in rats: role of individual differences in cocaine-taking behavior. Psychopharmacology. 2001; 158:382–7. [PubMed: 11797059]
39. Levay EA, Govic A, Hazi A, Flannery G, Christianson J, Drugan RC, et al. Endocrine and immunological correlates of behaviorally identified swim stress resilient and vulnerable rats. Brain, Behavior, and Immunity. 2006; 20:488–97.
40. Padilla E, Shumake J, Barrett DW, Holmes G, Sheridan EC, Gonzalez-Lima F. Novelty-evoked activity in open field predicts susceptibility to helpless behavior. Physiology & Behavior. 2010 In Press.
41. Edwards E, King JA, Fray J. Hypertension and insulin resistant models have divergent propensities to learned helpless behavior in rodents. Am J Hypertens. 2000; 13:659–65. [PubMed: 10912750]
42. Edwards E, King JA, Fray JCS. Increased basal activity of the HPA axis and renin-angiotensin system in congenital learned helpless rats exposed to stress early in development. International Journal of Developmental Neuroscience. 1999; 17:805–12. [PubMed: 10593616]
43. King JA, Edwards E. Early Stress and Genetic Influences on Hypothalamic-Pituitary-Adrenal Axis Functioning in Adulthood. Hormones and Behavior. 1999; 36:79–85. [PubMed: 10506532]
44. Shumake J, Edwards E, Gonzalez-Lima F. Hypermetabolism of paraventricular hypothalamus in the congenitally helpless rat. Neuroscience Letters. 2001; 311:45–8. [PubMed: 11585564]
45. Piazza PV, Rougé-Pont F, Deminière JM, Kharoubi M, Le Moal M, Simon H. Dopaminergic activity is reduced in the prefrontal cortex and increased in the nucleus accumbens of rats predisposed to develop amphetamine self-administration. Brain Research. 1991; 567:169–74. [PubMed: 1726140]
46. Hooks M, Juncos J, Justice J Jr, Meiergerd S, Povlock S, Schenk J, et al. Individual locomotor response to novelty predicts selective alterations in D1 and D2 receptors and mRNAs. J Neurosci. 1994; 14:6144–52. [PubMed: 7931568]

47. Ballaz SJ, Akil H, Watson SJ. Analysis of 5-HT6 and 5-HT7 receptor gene expression in rats showing differences in novelty-seeking behavior. Neuroscience. 2007; 147:428–38. [PubMed: 17543469]
48. Dietz D, Dietz K, Moore S, Ouimet C, Kabbaj M. Repeated social defeat stress-induced sensitization to the locomotor activating effects of d-amphetamine: role of individual differences. Psychopharmacology. 2008; 198:51–62. [PubMed: 18415082]
49. Blanchard MM, Mendelsohn D, Stamp JA. The HR/LR model: Further evidence as an animal model of sensation seeking. Neuroscience & Biobehavioral Reviews. 2009; 33:1145–54. [PubMed: 19497336]
50. Dracheva S, Lyddon R, Barley K, Marcus SM, Hurd YL, Byne WM. Editing of Serotonin 2C Receptor mRNA in the Prefrontal Cortex Characterizes High-Novelty Locomotor Response Behavioral Trait. Neuropsychopharmacology. 2009; 34:2237–51. [PubMed: 19494808]
51. Bartolomucci A, Carola V, Pascucci T, Puglisi-Allegra S, Cabib S, Lesch KP, et al. Increased vulnerability to psychosocial stress in heterozygous serotonin transporter knockout mice. Disease Models & Mechanisms. 2010; 3:459–70. [PubMed: 20371729]
52. Ruis MAW, te Brake JHA, Buwalda B, De Boer SF, Meerlo P, Korte SM, et al. Housing familiar male wildtype rats together reduces the long-term adverse behavioural and physiological effects of social defeat. Psychoneuroendocrinology. 1999; 24:285–300. [PubMed: 10101734]
53. Nakayasu T, Ishii K. Effects of pair-housing after social defeat experience on elevated plus-maze behavior in rats. Behavioural Processes. 2008; 78:477–80. [PubMed: 18358638]
54. Haller J, Leveleki C, Baranyi J, Mikics É, Bakos N. Stress, social avoidance and anxiolytics: a potential model of stress-induced anxiety. Behavioural Pharmacology. 2003; 14:439–46. [PubMed: 14501256]
55. de Jong JG, van der Vegt BJ, Buwalda B, Koolhaas JM. Social environment determines the long-term effects of social defeat. Physiology & Behavior. 2005; 84:87–95. [PubMed: 15642611]
56. Von Frijtag JC, Reijmers LGJE, Van der Harst JE, Leus IE, Van den Bos R, Spruijt BM. Defeat followed by individual housing results in long-term impaired reward- and cognition-related behaviours in rats. Behavioural Brain Research. 2000; 117:137–46. [PubMed: 11099767]
57. Tijssen MJA, Van Os J, Wittchen HU, Lieb R, Beesdo K, Wichers M. Risk factors predicting onset and persistence of subthreshold expression of bipolar psychopathology among youth from the community. Acta Psychiatrica Scandinavica. 2010; 122:255–66. [PubMed: 20199490]
58. Csorba J, Dinya E, Ferencz E, Steiner P, Bertalan G, Zsadon A. Novelty seeking: Difference between suicidal and non-suicidal Hungarian adolescent outpatients suffering from depression. Journal of Affective Disorders. 2010; 120:217–20. [PubMed: 19386368]
59. Mulder RT, Joyce PR, Cloninger CR. Temperament and early environment influence comorbidity and personality disorders in major depression. Comprehensive Psychiatry. 1994; 35:225–33. [PubMed: 8045113]
60. Herzog CJ, Miot S, Mansuy IM, Giros B, Tzavara ET. Chronic valproate normalizes behavior in mice overexpressing calcineurin. European Journal of Pharmacology. 2008; 580:153–60. [PubMed: 18021766]
61. Joyce PR, Mulder RT, Cloninger CR. Temperament predicts clomipramine and desipramine response in major depression. Journal of Affective Disorders. 1994; 30:35–46. [PubMed: 8151047]
62. Minelli A, Pedrini L, Magni LR, Rotondo A. Personality Traits in an Italian Sample: Relationship with Anxiety and Depression. Clin Pract Epidemiol Ment Health. 2009; 5:26–30. [PubMed: 20498697]
63. Cowley DS, Roy-Byrne PP, Greenblatt DJ, Hommer DW. Personality and benzodiazepine sensitivity in anxious patients and control subjects. Psychiatry Research. 1993; 47:151–62. [PubMed: 8341768]
64. Lane S, Tcheremissine O, Lieving L, Nouvion S, Cherek D. Acute effects of alprazolam on risky decision making in humans. Psychopharmacology. 2005; 181:364–73. [PubMed: 15830221]
65. Lehner M, Taracha E, Skórzewska A, Turzynska D, Sobolewska A, Maciejak P, et al. Expression of c-Fos and CRF in the brains of rats differing in the strength of a fear response. Behavioural Brain Research. 2008; 188:154–67. [PubMed: 18067977]

66. Lehner M, Taracha E, Turzynska D, Sobolewska A, Hamed A, Kolomanska P, et al. The role of the dorsomedial part of the prefrontal cortex serotonergic innervation in rat responses to the aversively conditioned context: Behavioral, biochemical and immunocytochemical studies. Behavioural Brain Research. 2008; 192:203–15. [PubMed: 18499280]

67. Lehner M, Wislowska-Stanek A, Taracha E, Maciejak P, Szyndler J, Skórzewska A, et al. The expression of c-Fos and colocalisation of c-Fos and glucocorticoid receptors in brain structures of low and high anxiety rats subjected to extinction trials and re-learning of a conditioned fear response. Neurobiology of Learning and Memory. 2009; 92:535–43. [PubMed: 19596457]

68. Lehner M, Wislowska-Stanek A, Taracha E, Maciejak P, Szyndler J, Skórzewska A, et al. The effects of midazolam and d-cycloserine on the release of glutamate and GABA in the basolateral amygdala of low and high anxiety rats during extinction trial of a conditioned fear test. Neurobiology of Learning and Memory. 2010 In Press.

69. Davidson JRT, Stein DJ, Shalev AY, Yehuda R. Posttraumatic Stress Disorder: Acquisition, Recognition, Course, and Treatment. J Neuropsychiatry Clin Neurosci. 2004; 16:135–47. [PubMed: 15260364]

70. Yehuda R, Antelman SM. Criteria for rationally evaluating animal models of postraumatic stress disorder. Biological Psychiatry. 1993; 33:479–86. [PubMed: 8513032]

71. Siegmund A, Wotjak CT. Toward an Animal Model of Posttraumatic Stress Disorder. Annals of the New York Academy of Sciences. 2006; 1071:324–34. [PubMed: 16891581]

72. Richman H, Frueh BC. Personality and PTSD II: Personality assessment of PTSD-diagnosed Vietnam veterans using the Cloninger Tridimensional Personality Questionnaire (TPQ). Depression and Anxiety. 1997; 6:70–7. [PubMed: 9451548]

73. Wang S, Mason J, Charney D, Yehuda R, Riney S, Southwick S. Relationships between hormonal profile and novelty seeking in combat-related posttraumatic stress disorder. Biological Psychiatry. 1997; 41:145–51. [PubMed: 9018384]

74. Evren C, Dalbudak E, Cetin R, Durkaya M, Evren B. Relationship of alexithymia and temperament and character dimensions with lifetime post-traumatic stress disorder in male alcohol-dependent inpatients. Psychiatry and Clinical Neurosciences. 2010; 64:111–9. [PubMed: 20132531]

75. Shumake J, Barrett D, Gonzalez-Lima F. Behavioral characteristics of rats predisposed to learned helplessness: Reduced reward sensitivity, increased novelty seeking, and persistent fear memories. Behavioural Brain Research. 2005; 164:222–30. [PubMed: 16095730]

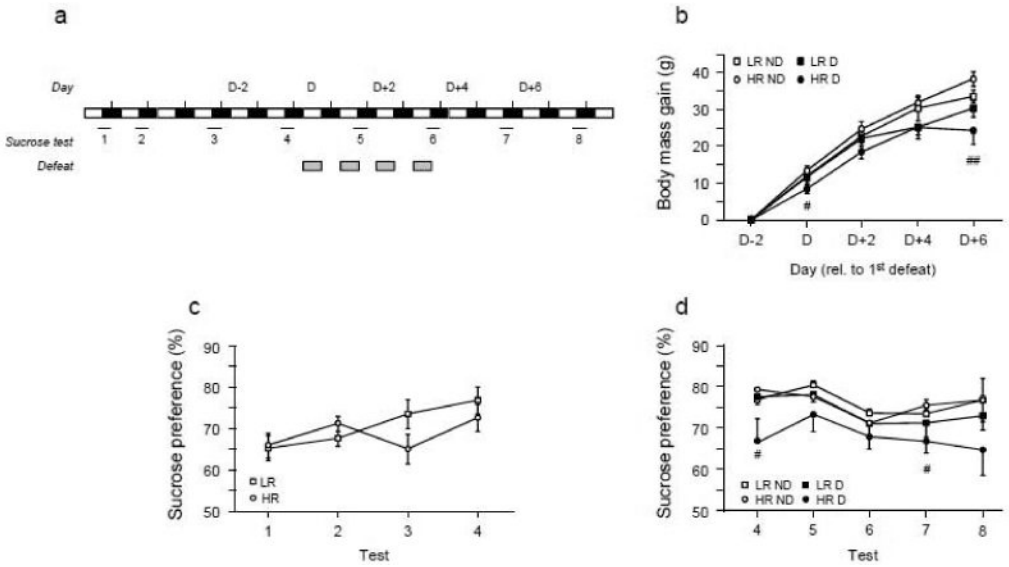

Figure 1.

Sucrose preference and body weight gain alterations in HR animals following repeated social defeat procedure. (a) Schematic representation of the timeline used for experiments, the black and white bars representing the dark and light cycles, respectively. (b) Progression of body weight as compared to two days prior to the first defeat encounter (n = 8-11). (c,d) Monitoring of the sucrose preference during the first two hours of the dark cycle, before (c) and after (d) the first defeat exposure (n = 9-11). $^{\#}$ $p < 0.05$, $^{\#\#}$ $p < 0.01$ vs. the HR ND group at the same time point; Fischer's PLSD *post-hoc* test. ND: non defeat, D: defeat.

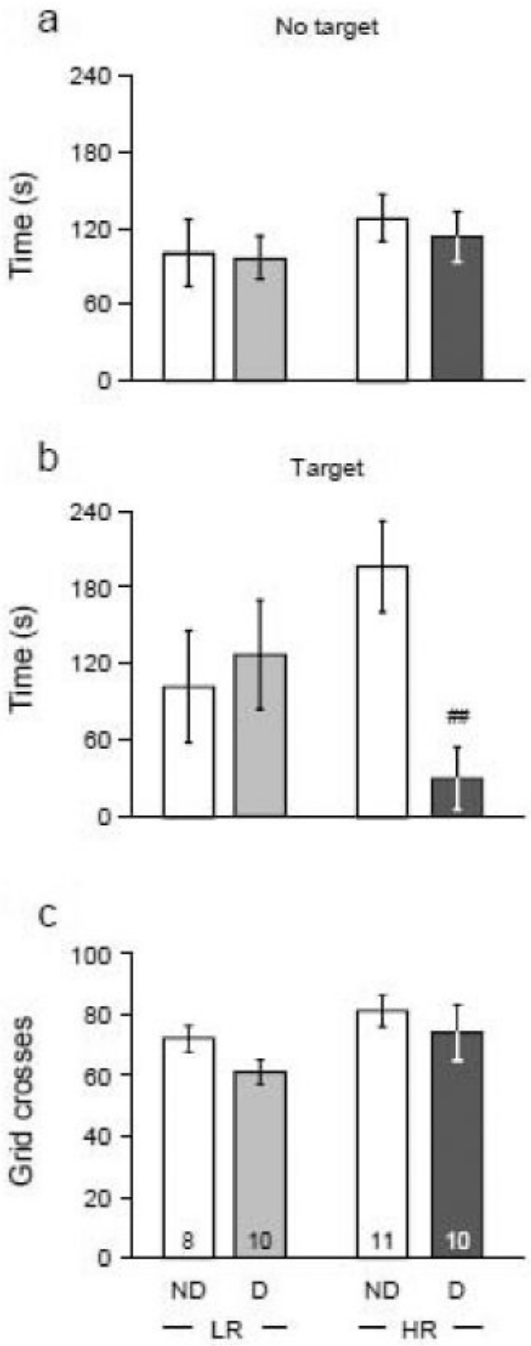

Figure 2.

Social approach and avoidance behavior developed in HR animals four weeks after repeated social defeat. (a,b) Time spent interacting with an empty cage ("no target", a) or a cage containing an unfamiliar Long-Evans rat ("target", b). (c) Locomotion of the animals in the test arena, during the first 5-minutes session of the test, as measured by the number of grid crosses. The number of animals is indicated within columns. $^{\#\#}$ $p < 0.01$ vs. the HR ND group; Fischer's PLSD *post-hoc* test. ND: non defeat, D: defeat.

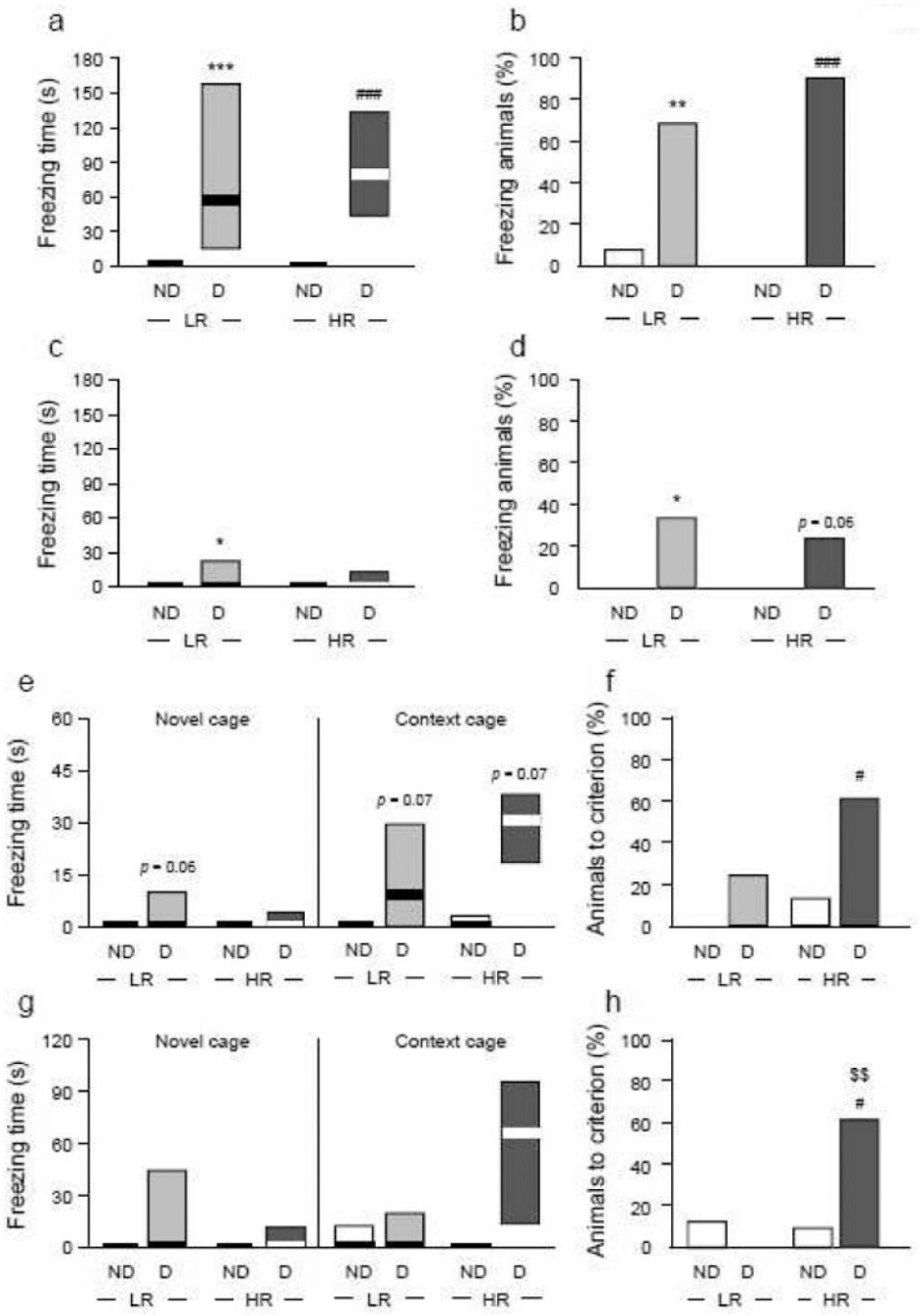

Figure 3.

Contextual fear memory triggered in HR and LR animals, following either repeated or acute social defeat exposure, at a short- and long-term period after the last defeat exposure. (a-d) Following repeated social defeat exposures (n = 12-13), total time spent freezing (a,c) and number of animals developing a freezing response (b,d), measured during a first test four weeks after (a,b) and then again two weeks later (c,d). (e-h) Following an acute defeat exposure, total time spent freezing in the novel and the context cages (e,g), and the number of animals reaching the criterion (f,h), measured either 48 hours (e,f – n = 8), or four weeks (g,h – n = 8-10) after the single social defeat exposure. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. the LR ND group; $^{\#}$ $p < 0.05$, $^{\#\#\#}$ $p < 0.001$ vs. the HR ND group; and $^{\$\$}$ $p < 0.01$

vs. the LR D; Fisher's PLSD *post-hoc* test. *p*-values shown in the graphics are related to ND group of the same phenotype. ND: non defeat, D: defeat.

Table 1

Behavioral parameters of defeated and non-defeated LR and HR rats in the Open-Field Test

	LR		HR	
Parameter	**Non defeat**	**Defeat**	**Non defeat**	**Defeat**
Grid crosses	126.67 ± 11.66	85.43 ± 8.86*	142.17 ± 12.07	108.25 ± 12.58
Time in center (s)	37.60 ± 8.72	23.82 ± 8.51	67.58 ± 8.41*	30.24 ± 6.73#
Speed (m/min)	2.94 ± 0.24	2.18 ± 0.17*	3.35 ± 0 26	2.82 ± 0.19$$
Immobility (s)	18.83 ± 8.51	75.86 ± 21.27*	25.83 ± 14.71	111.75 ± 28.05#
Rearing	41.00 ± 5.54	30.00 ± 4.35	47.17 ± 5.94	30.00 ± 4.64#
Grooming	1.33 ± 0.42	1.86 ± 0.46	2.67 ± 0 92	1.87 ± 0.40
n	6	7	6	8

* $p < 0.05$ vs. LR non-defeat,

# $p < 0.05$ vs. HR non-defeat,

$$ $p < 0.01$ vs. LR defeat group;

Fisher's PLSD *post-hoc* test.