Behavioural Brain Research 216 (2011) 100–108

Contents lists available at ScienceDirect

Behavioural Brain Research

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

Research report

Different susceptibility to social defeat stress of BalbC and C57BL6/J mice

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ARTICLE INFO

Article history:
Received 4 May 2010
Received in revised form 13 July 2010
Accepted 14 July 2010
Available online 21 July 2010

Keywords:
Stress
Social defeat
Metabolism
Vulnerability
Strain
Hormones
Inflammation
Biomarkers

ABSTRACT

Social stress may precipitate psychopathological disorders in susceptible individuals. The present experiments were focused on the biology beyond the differential susceptibility to social stress. Social defeat, an ethologically relevant stressor known to elicit different coping strategies, was used in two mouse strains differing for baseline emotionality, such as C57BL6/J and BalbC. In separate experiments, in both strains a single social defeat decreased home-cage activity without altering social aversion; it diminished body weight only in defeated BalbC mice. In longitudinal experiments, mice experienced repeated social defeats that induced multiple long-term consequences. Defeated C57BL6/J increased their body weight and food intake; defeated BalbC mice diminished their metabolic efficiency. Only defeated BalbC subjects exhibited increased social avoidance levels; no differences from controls were seen on forced swim test response in defeated mice of either strain. No long-term effects of social defeat were detected in peripheral biomarkers of stress, metabolic, and immune responses, although the analysis of selected internal organs revealed decreases in abdominal fat and gonadal organs in all defeated subjects. These results demonstrated a strain-distinctive profile in the susceptibility to social defeat stress, either acutely or chronically, with metabolic consequences more consistently found in C57BL6/J while social aversion induced predominantly in BalbC subjects.

1. Introduction

It is well-established that individuals differ in the perception and in the psychological consequences of adverse life events [1]. In particular, environmental variables, such as stressful experiences, and predisposing genetic factors are thought to interact and to influence resistance/vulnerability to risk for psychiatric disorders, by mediating the adaptive/mal-adaptive stress-coping strategies [1].

The underlying mechanisms of individual variability in stress resilience can be made accessible using animal models of individual differences in response to social stress procedures such as the one represented by social defeat [2]. Social defeat is a meaningful experience particularly in species relying on interactions with conspecifics like the house mice, whose social life organization is mainly determined by aggressive interactions [3]. Social defeat can be accomplished by forcing the experimental subject to intrude into the space occupied by an aggressive and unfamiliar mouse (i.e., the resident animal) leading to subordination of the test mouse [4]. Social defeat stress can have persistent behavioral and neurobiological effects even after a single experience [5–11]. Complementarily, when encountered on an intermittent, unpredictable basis, and experienced over protracted periods, the cost of the biological efforts to adapt may become excessive, thus increasing vulnerability to stress-related pathologies [12].

Multiple approaches can be performed to examine individual differences in response to stress. For example it has been established that, when a considerably large number of C57BL6/J inbred mice is subjected to chronic social defeat, defeated mice can be segregated into susceptible and unsusceptible populations [13]. Considering other experimental stressors, the relationship between stressor experience and stress resiliency/susceptibility has been assessed by studying the stress response of different inbred mouse strains [14,15]. Several behavioral inter-strain differences and the relative contribution of genetic factors to stress/anxiety reactions have been repeatedly demonstrated [14]. However, it must be noted that these differences may depend on the specific behavioral test selected [16,17] and may differ depending on the adopted stressor (i.e. psychogenic/neurogenic versus naturalistic) [18–20]. Therefore the experimental approach based on the comparison of different strains subjected to social defeat, which to our knowledge has not been previously applied, has the potential to identify the variables contributing to depressive-like changes.

We subjected C57BL6/J and BALB/c mice to social defeat and evaluated the expression of different coping strategies on the basis of several behavioral and physiological measures related to stress. We selected these strains because of their frequent use in scientific research and because they diverge in measures of behav-

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doi:10.1016/j.bbr.2010.07.014

ior [14,21–24] and physiology [25–29]. Specifically, several prior studies have demonstrated that C57BL6/J mice are stress-resilient, exhibit a lower level of anxiety, and have less emotionality than BalbC mice that, in turn, are considered more stress-sensitive, anxious, and emotional [14,20,30]. In the BalbC strain, stressors can provoke marked hypothalamic–pituitary–adrenal (HPA) responses and central monoamine variations at hypothalamic and mesolimbic brain regions that could explain the heightened stress reactivity of this strain [20,30–33]. Importantly, the excessive utilization of norepinephrine and serotonin in response to chronic stressors is moderated in C57BL6/J mice (i.e., the initial excessive utilization is tempered), whereas the already high levels of amine utilization becomes further pronounced in BalbC mice [34–36]. These strain-specific neurobiological adaptations may underlie the high responsivity to both acute and chronic stress in BalbC mice, as well as the reduced responsivity to acute stress and a capability to acclimate more readily to chronic stress in C57BL6/J mice.

In the present studies, mice of both strains were subjected, in separate experiments, to acute or chronic social defeat with the aim to highlight strain-dependent coping strategies with this social stress. The immediate consequences of a single social defeat were evaluated on different behavioral parameters, such as home-cage activity, social avoidance, sucrose preference, and body weight that can reflect some of the diagnostic criteria for mood disorders (i.e., psychomotor retardation, social withdrawal, anhedonia and body weight loss/gain) (DSM-IV-TR™, 2000). Furthermore, the long-term consequences of repeated social defeats were evaluated in a longitudinal study that was primarily focused on behavioral measures relevant for anxiety- and depressive-like states (i.e., social avoidance, forced swim test); secondarily, the existence of persistent alterations due to repeated social defeats was evaluated on parameters related to metabolism (i.e., body weight, food intake, leptin, insulin, abdominal fat stores), stress (i.e., ACTH, adrenal glands, gonadal organs), and immune system (i.e., cytokines, thymus, spleen), as a whole associated with psychiatric disturbances.

2. Materials and methods

2.1. Animals

C57BL6/J (n = 55) and BalbC (n = 56) mice (Charles River Labs, Calco, Italy) weighing 18–20 g at the beginning of the experiments served as experimental subjects. Subjects were group housed in 59.5 cm × 38.0 cm × 20.0 cm polycarbonate cages upon arrival under constant temperature (21 ± 2 °C) and a 12/12 h light/dark cycle (dark phase: 1800–0600 h). Food and water were available ad libitum.

All experimental procedures were carried out in accordance with Italian law (Legislative Decree no. 116, 27 January 1992), which acknowledges the European Directive 86/609/EEC, and were fully compliant with GlaxoSmithKline policy on the care and use of laboratory animals and codes of practice.

2.2. General experimental design

5 d before the start of testing mice serving as experimental subjects were housed individually. Two sets of experiments were conducted in separate groups of adult male mice (~2 mo of age at the beginning of testing) (see Fig. 1). In experiments 1 and 2 (single social defeat stress), the immediate effects of a single social defeat stress were evaluated on behavioral and physiological parameters relevant to stress responses in both C57BL6/J (n = 18) and BalbC (n = 18) intruders versus respective control subjects (n = 18/strain). In experiments 3 and 4 (repeated social defeat stress), C57BL6/J (n = 20) and BalbC (n = 20) mice underwent a repeated social defeat procedure (defeated n = 10/strain; control n = 10/strain), followed by a long-term assessment of behavioral, physiological and biochemical responses relevant to stress and depressive-/anxiety-like states.

2.3. Experiment 1—effects of single social defeat stress on C57BL6/J mice

2.3.1. Social defeat stress

CD-1 male mice (Charles River Labs, Calco, Italy), selected on the basis of their attack latencies consistency (shorter than 30 s on 3 consecutive screening tests), were used as aggressive residents. For the social defeat stress, C57BL6/J (n = 18) mice were introduced into the home-cage (42.5 cm × 26.6 cm × 18.5 cm) of an unfamiliar CD-1 resident mouse for a 10 min full interaction. During this exposure all subject mice showed signs of subordination (i.e., sideways or upright submissive postures, withdrawal, fleeing, lying on its back, or freezing). After the 10 min full interaction, the subject mouse (defeated) was separated from the aggressive resident by introducing into the resident home-cage a perforated Plexiglas divider to allow sensory contact. The mice were housed in this way for the next 24 h, with food and water provided ad libitum. Control mice (n = 18) were housed in pairs, separated by the perforated Plexiglas divider.

2.3.2. Body weight

Mice were weighed 3 d before the start of the procedure to allow a balanced distribution between groups. Subsequently, mouse body weight was assessed immediately the start of the social defeat as well as at the end of 24 h cohabitation.

2.3.3. Behavioral assessments

Separate subsets of mice were tested for their home-cage behaviors (n = 16) and the social avoidance test (n = 20) to evaluate the immediate behavioral consequences of the single social defeat stress (Fig. 1(A)).

2.3.4. Home-cage activity

Mouse home-cage activity was evaluated using the LABORAS™ system (Metris b.v., Hoofddorp, The Nederlands). As previously described by Quinn et al. [37], this system consists of a sensing platform that converts the animal's movements into electric signals that can be registered by a computer,

After 24 h cohabitation, defeated (n = 8) and control (n = 8) mice were transferred to the test room within their home-cages and, about 1 h later, were placed individually in clean Macrolon® type II cages with wood chips as bedding, food, and water as in their home-cages. Each Macrolon® cage was placed on a sensing platform for the automated acquisition of home-cage activity that was measured as total duration of locomotion (s) during 24 h.

2.3.5. Social avoidance test

Defeated (n = 10) and control (n = 10) mice were placed individually in a 45 cm × 45 cm arena with an empty wire-mesh cage (10 cm × 4.5 cm) located at one end, and their movement was tracked for 2.5 min ('no aggressor' phase), followed by 2.5 min in the presence of a confined unfamiliar aggressor, represented by one of the resident CD-1 male mice that was introduced into the wire-mesh cage ('aggressor'

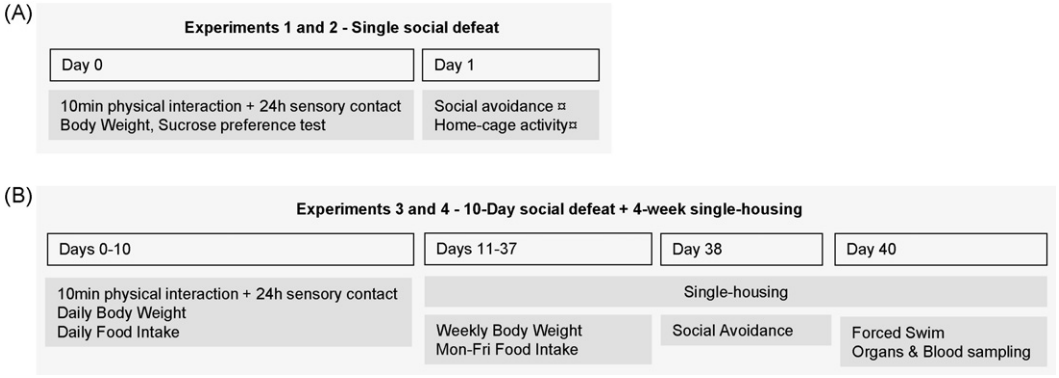

Fig. 1. Experimental procedures: (A) a single social defeat was followed by 24 h cohabitation with the aggressor in experiments 1 and 2 (different groups of defeated and control mice were run through either the social avoidance or the home-cage activity testing); (B) in experiments 3 and 4, 10 daily social defeat experiences were followed by 4 w of single housing.

phase) [38]. Between the two sessions, the subject mouse was removed from the arena and placed back into its home-cage for approximately 1 min. The procedure was performed under red light conditions and video-recordings were performed using a video-camera equipped with infrared filter. The duration of the subject's presence in the "interaction zone" (defined as the 8 cm-wide area surrounding the wire-mesh cage) was obtained using the automated video-tracking system based on the Ethovision XT software (Noldus Information Technology).

2.3.6. Sucrose preference

During 24 h following the social defeat encounter, the preference for 1% sucrose solution was evaluated by giving mice a free choice between two bottles, one with 1% sucrose solution and another with drinking water (Fig. 1(A)).

The consumption of 1% sucrose solution and water was measured by weighing the bottles both at the start and at the conclusion of the 24 h cohabitation. The preference for 1% sucrose solution was calculated as a percentage of the total amount of liquid intake and was used as a measure of mouse sensitivity to reward [39].

2.4. Experiment 2—effects of single social defeat stress on BalbC mice

Thirty-six BalbC mice were tested in the same procedure described in experiment 1 (Fig. 1(A)).

2.5. Experiment 3—repeated social defeat stress on C57BL6/J mice

2.5.1. Social defeat stress

For the repeated social defeat stress, ten C57BL6/J mice were submitted to social defeat stress during 10 consecutive days [40]. The daily social defeat procedure was conducted as in experiment 1; every day the experimental mice were exposed to a new resident. Control mice ($n = 10$) were housed in pairs, separated by the perforated Plexiglas divider, and were handled daily.

2.5.2. Metabolic parameters

Animals were weighed 3 d before the start of the experiment to allow a balanced distribution between groups. Body weight and food intake measures were taken at multiple time-points during the 10 d social defeat stress procedure (see Fig. 1(B)). On experimental days 1–10, mice were weighed immediately before being exposed to the social defeat procedure. Additional body weight measures were taken during the weekly change of the home-cage and at the end of the experimental procedure. Food intake was assessed daily during the social defeat procedure (days 1–10) and daily, Monday to Friday, from experimental day 11 to day 40; chow was removed from the food hopper, weighed, and replaced. To minimize food spill, only food pellets weighing more than 5 g were used for replacing the amount of chow available in the food hopper.

Two feed efficiency indexes were calculated as total body mass gained (g)/cumulative food intake (g) during either the social defeat phase or the single housing phase.

2.5.3. Behavioral assessments

To evaluate the long-term behavioral consequences of the social defeat stress mice were tested in the social avoidance test and in the forced swim test, 28 d and 30 d later respectively (Fig. 1(B)). These behavioral procedures were spaced 2 d apart to minimize possible confounding effects due to the social avoidance testing on the forced swim test response.

2.5.4. Social avoidance test

As in experiment 1.

2.5.5. Forced swim test (FST)

Each experimental subject was placed in an open cylindrical glass container (diameter 10 cm, height 25 cm), containing 10 cm of water at $25 \pm 1\,^{\circ}C$, for 6 min. The water was changed before the introduction of each animal. At the end of the FST, each mouse was returned to its home-cage and placed under a heating lamp to facilitate drying.

Mouse behavior was video-recorded by a video-camera placed in front of the glass cylinders. The duration (s) of floating (minimal activity required for the mouse to keep its head above water level) during the last 4 min of the 6-min test was subsequently scored from videotapes by a trained observer, using The Observer XT 7.0 software (Noldus Information Technology, The Netherlands).

2.5.6. Peripheral biomarker sampling and internal organ weight

Following the completion of the FST, mice were kept in their home-cage for 2 h, a time that previous studies in our laboratory had indicated as sufficient for animal to regain basal levels of immediate stress responses biomarkers. Mice were killed by rapid decapitation for trunk blood collection between 1000 and 1300 h (Fig. 1(B)). At autopsy, internal organs such as testis, seminal vesicles, spleen, adrenal glands, and thymus were dissected and weighed. Organ weight was analyzed as relative weight (i.e. absolute organ weight/body weight).

2.5.7. Blood sampling

Trunk blood was collected in Microtainer BD K_2EDTA tubes (Becton Dickinson Italia, Milano, Italy) with a protease inhibitor cocktail (Sigma–Aldrich) and a DPPIV protease inhibitor (Millipore, Billerica, MA, USA). After 10 min centrifugation at 1800 *g*, 4 °C, plasma was collected, split into aliquots and stored at −80 °C.

2.5.8. Plasma hormone, cytokine and chemokine levels

Analytes were measured with Milliplex kits (Millipore, Billerica, MA, USA) using the Luminex technology in a Bio-Plex instrument (Bio-Rad, Hercules, CA, USA), a technology that simultaneously measures concentrations of multiple analytes. ACTH, insulin and leptin were determined with the Mouse Bone Panel kit (Millipore, Billerica, MA, USA) [Mouse Bone Panel kit inter-assay precision percentage: <11%; Mouse Bone Panel kit intra-assay precision percentage: <4%; insulin assay sensitivity 18.6 pg/mL; leptin assay sensitivity: 3.0 pg/mL; ACTH assay sensitivity: 1.8 pg/mL]. Interleukin (IL)1alpha, IL-1beta, IL-2, IL-6, IL-9, IL-10, IL-12p(40), IL-12p(70), IL13, Il-17, Eotaxin, G-CSF, GM-CSF, Interferon-gamma, KC, MCP-1, MIP-1beta, RANTES and TNF-alpha levels were assessed with the Mouse Cytokine/Chemokine Panel I kit (Millipore, Billerica, MA, USA) [Mouse Cytokine/Chemokine Panel I kit inter-assay precision percentage: 4.2–21.2%; Mouse Cytokine/Chemokine Panel I kit intra-assay precision percentage: 3–22.6%; assay sensitivity: 3.2 pg/mL].

2.6. Experiment 4—effects of repeated social defeat stress on BalbC mice

Twenty BalbC mice ($n = 10$ defeated, $n = 10$ control) were tested in the same procedure described in experiment 3 (Fig. 1(B)).

3. Statistics

Statistical analyses were conducted using Statistica V8 (Statsoft, Inc., Tulsa, OK). Data distribution was checked for satisfying ANOVA's assumptions and, if appropriate, data were log transformed, which resulted to be needed only for internal organ weight data.

Home-cage activity data were analyzed as total values during the 24 h observation time by means of one-way ANOVA with social defeat stress (defeated versus controls) as between-subject variable.

Body weight gain data were analyzed as differences from baseline values.

For data generated after the single social defeat stress experiments, values from two animal subsets (i.e., animals tested in home-cage activity and animals tested in the social avoidance test) were combined into a single dataset. One-way ANOVA with social defeat stress (defeated versus control) as between-subject variable was performed on the body weight gain calculated between the end of the 24 h cohabitation and the basal value.

For experiments 3 and 4, one-way ANOVA with social defeat stress (defeated versus control) as between-subject variable was performed on the body weight gain at the end of the social defeats (1–10 d), as well as on the cumulative amount of food consumed during the 10 d social defeat. ANOVA for repeated measures, with social defeat stress (defeated versus control) as between-subject variable and time as within-subject variable (weeks 1–4), was performed on the differences between each of the weekly body weight measurements (1 w, 2 w, 3 w, 4 w) and the value at the end of the social defeat (day 10). Similarly, ANOVA for repeated measures, with social defeat stress as between-subject variable and time as within-subject variable, was performed on the total weekly food intake values, recorded from weeks 1 to 4 following the end of the social defeat. Significant differences due to main effects were followed by multiple group comparisons using Tukey's HSD test. Feed efficiency (total body weight gain/total food intake) was analyzed by means of one-way ANOVA with social defeat stress (defeated versus control) as between-subject factor, for values recorded both during the social defeat and the single housing phases.

Social avoidance data were analyzed by repeated measures ANOVAs with stress (defeated versus control) as between-subject variable and test phase ('aggressor' versus 'no aggressor') as within-subject variable, followed by Holm corrected planned comparisons.

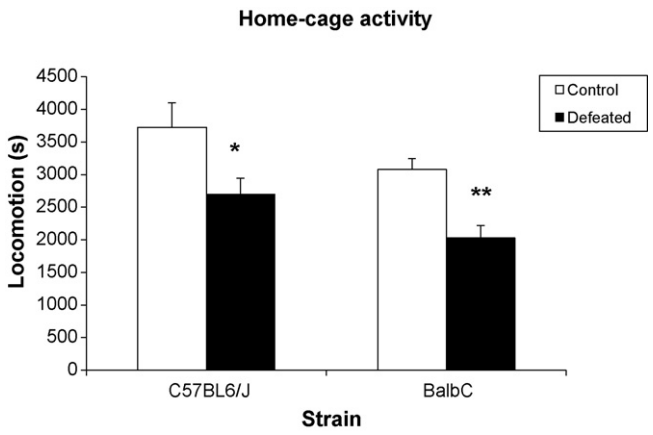

Fig. 2. Home-cage activity measured as duration of locomotion (s) across 24 h. Mouse activity was monitored from the end of 24 h cohabitation with an aggressor which followed a single social defeat episode.

Data from FST, internal organs, peripheral hormones, and inflammation biomarker levels, were analyzed by means of one-way ANOVA, with social defeat stress (defeated versus control) as between-subject variable.

All results are expressed as mean ± standard error of raw data.

For all data levels of statistical significance were set at $p < 0.05$.

4. Results

4.1. Experiment 1—effects of single social defeat on C57BL6/J mice

4.1.1. Body weight

Defeated mice body weight gain did not differ from control values ($F(1,33) = 1.83$; ns) [control: -0.53 ± 0.11 g; defeated: -0.38 ± 0.12 g].

4.1.2. Home-cage activity

The total duration of locomotion was significantly decreased in defeated mice compared to controls (C57BL6/J: $F(1,14) = 5.26$; $p < 0.05$) (Fig. 2).

4.1.3. Social avoidance test

In general the time in the 'interaction zone' was significantly increased in the 'aggressor phase' in C57BL6/J ($F(1,18) = 14.19$; $p < 0.01$) compared to the 'non-social phase' of the test (data not shown). On the other hand, the time spent in the proximity to the confined aggressor was not altered by the single social defeat ($F(1,18) = 0.14$; ns) (Fig. 3(A)).

4.1.4. Sucrose preference

The preference for 1% sucrose solution tended to be decreased in defeated mice compared to control subjects ($F(1,18) = 3.31$; $p = 0.08$; control: 80.90 ± 1.08%; defeated: 70.79 ± 5.45%); the total fluid intake was not altered by social defeat in this strain ($F(1,18) = 0.30$; ns; control: 6.76 ± 0.22 g; defeated: 6.92 ± 0.21 g).

4.2. Experiment 2—effects of single social defeat on BalbC mice

4.2.1. Body weight

BalbC defeated subjects showed a significantly greater decrease in body weight than controls ($F(1,33) = 47.12$; $p < 0.001$) [control: -0.18 ± 0.06 g; defeated: -1.21 ± 0.13 g].

4.2.2. Home-cage activity

The total duration of locomotion was significantly decreased in defeated mice compared to controls ($F(1,14) = 16.74$; $p < 0.01$) (Fig. 2).

4.2.3. Social avoidance test

The time spent in the 'interaction zone' was similar during the 'social' and 'non-social phase' of the test ($F(1,18) = 0.36$; ns) (data not shown), and it was not altered by the single social defeat ($F(1,18) = 0.81$; ns) (Fig. 3(A)).

4.2.4. Sucrose preference

No basal preference for 1% sucrose solution was detected in this strain. No influence of the social stress experience could be seen on this parameter ($F(1,14) = 0.18$; ns; control: 49.36 ± 8.32%; defeated: 44.31 ± 8.35%) or on fluid intake ($F(1,14) = 1.04$; ns; control: 6.73 ± 0.24 g; defeated: 6.27 ± 0.37 g).

4.3. Experiment 3—effects of repeated social stress on C57BL6/J mice

4.3.1. Metabolic parameters

Body weight (Table 1)—at the conclusion of the 10 social defeats, defeated subjects showed a trend to gain more body weight than controls ($F(1,17) = 3.87$; $p = 0.06$).

During the social isolation phase, body weight increased significantly due to the effect of Time ($F(3,51) = 86.80$, $p < 0.0001$). In defeated mice, body weight gain was significantly increased

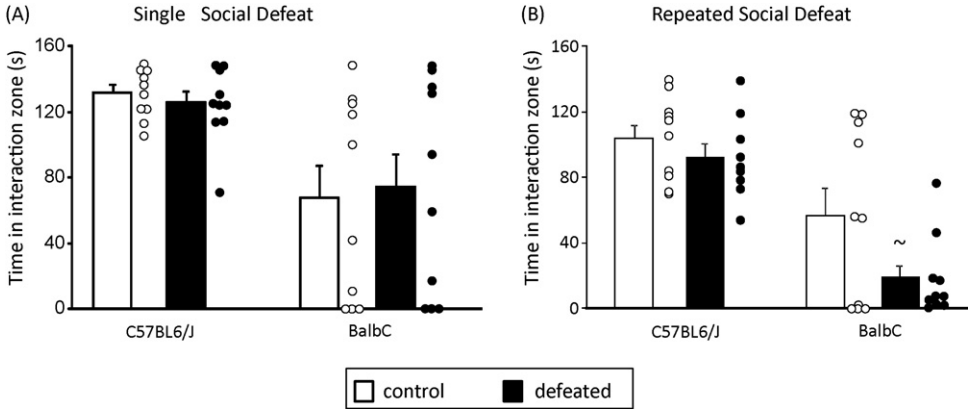

Fig. 3. Social avoidance test conducted 24 h after either a single social defeat (A), or 28 d after the end of 10 d social defeat stress. The social avoidance test comprised 2 phases, either in the absence (2.5 min not shown) and in the presence (2.5 min) of an aggressor mouse confined within a small cage, around which the interaction could take place. The time spent in the interaction zone (s) was measured. Data are represented as group mean ± SEM (histograms) as well as individual values (small circles). ~ represents $p = 0.06$ between defeated and control mice within BalbC strain.

Table 1
Metabolic parameters.

Parameter	Time interval	C57BL6/J		BalbC	
		Control	Defeated	Control	Defeated
Delta body weight (g)	1–10 d	0.41 ± 0.19	0.89 ± 0.13	0.92 ± 0.24	0.02 ± 0.28*
	1 w	1.22 ± 0.16	1.95 ± 0.34	2.39 ± 0.28	1.78 ± 0.31
	2 w	1.86 ± 0.16	2.98 ± 0.25*	3.46 ± 0.28	2.98 ± 0.42
	3 w	2.45 ± 0.19	3.68 ± 0.19*	4.29 ± 0.33	4.48 ± 0.44
	4 w	3.19 ± 0.29	4.62 ± 0.24**	5.16 ± 0.35	6.06 ± 0.46
Food intake (g)	1–10 d	49.31 ± 0.53	51.91 ± 0.96*	49.78 ± 1.40	54.57 ± 2.41
	1 w	17.19 ± 0.75	17.62 ± 0.55	18.48 ± 0.52	21.28 ± 0.38**
	2 w	17.77 ± 0.72	19.05 ± 0.86	17.86 ± 0.57	19.51 ± 0.39
	3 w	18.00 ± 0.93	18.64 ± 0.28	17.65 ± 0.45	19.06 ± 0.38
	4 w	17.43 ± 0.62	18.19 ± 0.43	17.58 ± 0.39	19.23 ± 0.43
Feed efficiency	1–10 d	0.008 ± 0.004	0.02 ± 0.002	0.02 ± 0.004	−0.001 ± 0.005*
	10 d–4 w	0.05 ± 0.005	0.06 ± 0.003*	0.08 ± 0.004	0.08 ± 0.006

Values represent mean ± SEM. Feed efficiency was calculated as body mass gained (g)/cumulative food intake (g), either at the end of the social defeat (1–10 d) or at the end of the single housing phase (10 d–4 w). Delta body weight as well as food intake measures were taken either at the end of the 10d social defeat (1–10 d) or at the end of each of the following 4 w of single housing (1–4 w). n = 9–10/group.
* $p < 0.05$ versus control.
** $p < 0.01$ versus control.

compared to controls ($F(1,17) = 16.89$; $p < 0.001$), at all but first time-points considered (2 w: $p < 0.05$; 3 w: $p < 0.05$; 4 w: $p < 0.01$).

Food intake (Table 1)—during the 10 d social defeat, food intake was significantly increased in C57BL6/J defeated subjects ($F(1,17) = 5.95$; $p < 0.05$). During the 4-w single housing phase, no differences due to either social defeat stress or time or their interaction were found in C57BL6/J subjects ($F(1,17) = 1.10$; ns; $F(3,51) = 1.60$; ns; $F(3,51) = 0.63$; ns, respectively).

Feed efficiency (Table 1)—the feed efficiency index measured at the end of 10 d social defeat tended to be increased compared to controls in C57BL6/J defeated mice ($F(1,17) = 3.40$; $p = 0.08$). At the end of the single housing phase, feed efficiency of defeated subjects was significantly increased compared to control values ($F(1,17) = 7.45$; $p < 0.05$).

4.3.2. Behavioral assessments

Social avoidance test—in general, C57BL6/J subjects spent a significantly longer time in the 'interaction zone' during the 'aggressor' compared to the 'no aggressor' phase of the test ($F(1,17) = 22.67$; $p < 0.001$). On the other hand, no effects were found for either social defeat stress ($F(1,17) = 3.28$; ns) or the interaction social defeat stress × test phase ($F(1,17) = 1.86$; ns) (Fig. 3(B)).

FST—no differences were detected in floating behavior comparing defeated and control subjects (control = 200.1 ± 6.82 s, defeated = 186 ± 7.2 s, $F(1,17) = 2.6$; ns).

4.3.3. Internal organ weight

Social defeat stress induced significant decreases in relative testicle size ($F(1,17) = 4.93$; $p < 0.05$) and relative abdominal fat amount ($F(1,17) = 26.25$; $p < 0.0001$), whereas no effects were found in adrenal gland ($F(1,17) = 1.49$; ns), spleen ($F(1,17) = 0.30$; ns), seminal vesicles ($F(1,17) = 2.67$; ns), and thymus relative weight ($F(1,17) = 0.05$; ns) (Table 2).

4.3.4. Plasma hormones and inflammation biomarkers

None of the parameters differed significantly between defeated and control mice (Table 3).

4.4. Experiment 4—effects of repeated social stress on BalbC mice

4.4.1. Metabolic parameters

Body weight (Table 1)—at the end of the repeated social stress, defeated subjects gained significantly less body weight than controls ($F(1,18) = 6.10$; $p < 0.05$).

During the social isolation phase, body weight increased significantly due to the effect of time ($F(3,54) = 158.78$; $p < 0.0001$). No overall effect of social defeat stress was evident ($F(1,18) = 0.00$; ns), but a significant time × social defeat stress interaction was found ($F(3,54) = 8.36$; $p < 0.001$); defeated mice were gaining significantly less body weight than controls soon after the end of the 10d social defeat (1 w), while the opposite was true at the last time-point examined (4 w).

Food intake (Table 1)—during the 10 d social defeat, food intake did not differ from control animals ($F(1,18) = 2.93$; ns). During the 4-w single housing phase, the repeated social defeat stress induced a subsequent significant increase of food intake ($F(1,18) = 12.46$; $p < 0.01$), particularly during the first week of single housing ($p < 0.01$); time influenced significantly the amount of food intake ($F(3,54) = 12.93$; $p < 0.0001$), independently from its interaction with social defeat stress ($F(3,54) = 2.45$; $p = 0.08$).

Feed efficiency (Table 1)—BalbC defeated subjects showed significantly decreased feed efficiency values compared

Table 2
Internal organs relative weight (g/100 g body weight).

	C57BL6/J		BalbC	
	Control	Defeated	Control	Defeated
Adrenal glands	0.034 ± 0.003	0.029 ± 0.003	0.021 ± 0.002	0.021 ± 0.001
Spleen	0.253 ± 0.008	0.247 ± 0.008	0.400 ± 0.025	0.346 ± 0.012*
Thymus	0.774 ± 0.052	0.659 ± 0.036	0.808 ± 0.029	0.723 ± 0.035
Seminal Vesicles	0.212 ± 0.014	0.206 ± 0.010	0.159 ± 0.006	0.177 ± 0.009
Testicles	0.829 ± 0.014	0.768 ± 0.025*	0.837 ± 0.022	0.818 ± 0.015
Abdominal Fat	1.489 ± 0.032	1.248 ± 0.034***	1.559 ± 0.058	1.101 ± 0.069***

Values represent group mean ± SEM.
* $p < 0.05$.
*** $p < 0.001$ versus control.

Table 3
Peripheral hormones and inflammation biomarkers (pg/mL).

Parameter	C57BL6/J		BalbC	
	Control	Defeated	Control	Defeated
ACTH	11.04 ± 3.6	294.57 ± 275.16	7.34 ± 1.85	6.90 ± 1.96
Insulin	973.81 ± 148.96	943.45 ± 166.54	1840 ± 281.61	1437.09 ± 296.84
Leptin	734.30 ± 101.74	889.69 ± 113.75	1424 ± 180.99	1005.14 ± 190.78
IL-1a	33.01 ± 4.03	37.11 ± 4.51	21.59 ± 1.83	22.08 ± 1.46
IL-1b	139.77 ± 15.41	168.33 ± 17.23	130.13 ± 6.33	133.01 ± 9.90
IL-2	94.72 ± 8.39	93.82 ± 9.38	82.32 ± 5.51	80.62 ± 4.48
IL-6	18.47 ± 2.70	24.41 ± 3.03	8.94 ± 0.96	12.95 ± 2.75
IL-9	160.80 ± 18.09	163.36 ± 20.22	143.54 ± 11.42	203.32 ± 43.47
IL-10	80.24 ± 9.10	93.83 ± 11.32	37.29 ± 3.43	44.50 ± 7.44
IL-12p(40)	287.11 ± 15.61	307.82 ± 17.45	284.96 ± 28.83	225.65 ± 9.53
IL-12p(70)	26.21 ± 3.32	30.02 ± 3.71	12.94 ± 1.01	14.34 ± 1.18
IL-17	90.82 ± 18.17	98.77 ± 20.32	91.68 ± 31.69	121.06 ± 31.07
Eotaxin	579.48 ± 87.54	600.78 ± 97.87	591.62 ± 45.95	560.02 ± 54.77
G-CSF	103.53 ± 9.07	132.15 ± 10.14	51.81 ± 2.98	66.62 ± 9.94
Inf-gamma	18.47 ± 4.54	18.61 ± 5.07	51.09 ± 6.10	50.24 ± 7.05
KC	108.01 ± 5.83	107.40 ± 6.52	107.38 ± 6.15	105.77 ± 7.086
MCP-1	301.71 ± 28.6	292.13 ± 31.98	252.54 ± 12.79	232.93 ± 19.97
MIP-1b	58.29 ± 8.16	70.10 ± 9.13	48.01 ± 3.12	47.14 ± 4.98
RANTES	2.71 ± 0.43	3.55 ± 0.48	1.51 ± 0.31	1.64 ± 0.14
TNF-alpha	2.83 ± 0.32	3.69 ± 0.51	1555.05 ± 111.99	1517.87 ± 127.81

Data represent plasma levels (mean ± SEM) of peripheral biomarkers measured at the end of 4 w of single housing that followed 10 d social defeat. n = 9–10/group. Inf: interferon.

to controls at the end of 10 d social defeat ($F(1,18) = 7.68$; $p < 0.05$).

At the end of the single housing phase, social defeat stress lacked of any effect ($F(1,18) = 0.43$; ns).

4.4.2. Behavioral assessments

Social avoidance test—social defeat stress and test phase had no effect if considered separately ($F(1,18) = 1.41$; ns and $F(1,18) = 0.07$; ns, respectively). A close to significant interaction was found for the two main factors ($F(1,18) = 3.55$; $p = 0.07$), due to a significantly shorter time spent by BalbC defeated mice in the 'interaction zone' compared to their respective controls ($p < 0.05$) (Fig. 3(B)).

FST—no differences were detected in floating behavior between defeated and control subjects (control = 204.9 ± 8.26 s, defeated = 215.0 ± 6.51 s, $F(1,18) = 0.75$, ns).

4.4.3. Internal organ weight

Defeated subjects showed a significant decrease in relative spleen weight ($F(1,18) = 4.69$; $p < 0.05$), relative abdominal fat amount ($F(1,18) = 22.31$; $p < 0.0001$), and a close to significant decrease in seminal vesicle relative weight ($F(1,18) = 3.59$; $p = 0.07$); the remaining organ weights measured in defeated subjects were not different from the control group (adrenal glands: ($F(1,18) = 0.05$; ns); thymus: ($F(1,18) = 2.17$; ns); testicles: ($F(1,18) = 0.43$; ns) (Table 2).

4.4.4. Plasma hormones and inflammation biomarkers

None of the parameters differed significantly between defeated and control mice (Table 3).

5. Discussion

5.1. Experiments 1 and 2—effects of single social defeat on C57BL6/J and BalbC mice

In the present experimental conditions, a single social defeat triggered strain- and parameter-dependent consequences.

Acute social defeat triggers a prompt stress response based upon increases in markers of HPA axis activation, suggestive of a fast activation of the sympatho-adrenal system [41]. Home-cage activity was reliably decreased in both strains of defeated subjects, in accordance with data of stress-induced immobility obtained across different acute stress procedures and mouse strains [42].

In the present conditions, a loss of body weight was induced exclusively in the BalbC strain, in agreement with previous data showing body weight loss in this strain starting as early as 24 h after one social defeat [8]. Interestingly, in the BalbC but not in the C57BL6/J strain, body weight had been shown to be a socially sensitive response, being dependent upon social housing conditions [43].

The preference for the sucrose solution was altered following the single social defeat in the C57BL6/J strain. On the other hand, in BalbC mice neither defeated nor control subjects exhibited a preference for the sucrose solution, such that it was not possible to further assess a potential stress-dependent modulation of this parameter. In basal conditions, clear mouse strain differences exist in multiple sucrose intake measures [44]. C57BL6/J mice have been previously demonstrated to consume the highest amount of sucrose compared to several inbred strains, including the BalbC. The influence of stress on this parameter has been mostly demonstrated in chronic stress procedures, although increases, decreases, or lack of changes in sucrose preferences and intake have been reported and could be related to the phase of the stress but also to individual vulnerability to anhedonia [45,46]. Therefore, in consideration of the lack of robust results in the present study and of the overall inconsistencies of published data about chronic stress effects on sucrose preference [47], we decided not to include this parameter in the further experiments.

Albeit proving its efficacy in home-cage activity and body weight, the single defeat experience did not induce social avoidance for a confined aggressor in either mouse strain. The majority of the relevant publications are in support of the induction of social aversion, either short- or long-term, following chronic stress exposure [38,40,48]. The experience of stress over time appears to be crucial for the development of social avoidance. A repeated exposure to social defeat was indeed demonstrated to induce changes in gene expression, such as *Bdnf* gene, and chromatin remodeling in a variety of brain regions relevant to the stress as well as to the avoidant response [38,40] that could not develop only 24 h following a single social defeat experience.

5.2. Experiments 3 and 4—effects of repeated social defeat stress on C57BL6/J and BalbC mice

Based on multiple parameters, it was possible to highlight a strain-specific metabolic reactivity to repeated social defeats. Body weight was increased in defeated C57BL6/J subjects, while it was diminished in defeated BalbC mice that were able to recover to control levels only at the final stage of the experimental procedure. Food intake was increased only in C57BL6/J subjects during the 10 social defeats but not during the single housing phase, when it was highly increased exclusively in BalbC mice explaining the late recovery in body weight gain seen in the defeated subjects. Although rodents exposed to repeated social stress situations have generally been found to lose body weight [4,49,50], a growing number of reports is supportive of the opposite [48,50–53]. In C57BL6/J subjects body weight and food intake were increased up to 40 d following 10 social defeat experiences [48], whereas, at least to our knowledge, there are no similar published reports in BalbC mice.

The feed efficiency parameter, calculated to reflect the relationship between energy intake and energy storage, tended to be increased in C57BL6/J defeated mice. On the other hand, this index was decreased in BalbC defeated subjects, thereby further supporting the emergency of opposite energy expenditure adaptations induced by the repeated social defeat in the two strains. It has in fact been shown that metabolic differences are present across inbred mouse strains at baseline and can translate into different degrees of responsiveness to changing dietary environments [25].

When experimental animals were subjected to behavioral tests relevant to mood disturbances, C57BL6/J mice were found to be resilient, while BalbC defeated mice exhibited a persistent social aversion. As mentioned earlier, this test has proven to be a reliable procedure to confirm the persistently aversive nature of social stimuli after experiences of aggression, mostly in the C57BL6/J strain [13,38,40]. Therefore, the observed lack of social withdrawal in C57BL6/J is quite unexpected, although in a recent publication a similarly modest social avoidance was shown in this strain, particularly in animals fed on normal chow versus high fat diet [48]. Only C57BL6/J defeated mice on a high fat diet demonstrated a worsening of social avoidance, indicating that the access to calorically rich food can interfere with the recovery from stress-induced behavioral deficits [48]. On the other hand, to our knowledge, this is the first report of social avoidance induced by social defeat in BalbC mice, and it can be considered a further evidence of the overall enhanced emotionality of this strain [54]. Differently from the social avoidance test, FST behavior did not vary as a long-term consequence of 10 d social defeat stress in either mouse strain. Social defeat stress effects on mouse FST response are not consistent, since increases in immobility as well as lack of effects have been reported following a single as well as a series of aggressive encounters [55–57]. In the present procedure, the occurrence of spontaneous recovery of the defeated animals cannot be excluded, and possible consequences of social defeat stress on FST behavior should be further assessed in longitudinal studies.

Stressful conditions have well-established consequences on rodent physiology. Effects such as enlarged adrenal glands, diminished thymus and spleen, and decreased reproductive organ weight have been repeatedly reported [58–60]. In the present conditions, social defeat stress decreased testicle size and abdominal fat amount of C57BL6/J subjects; in BalbC defeated mice spleen, abdominal fat, and seminal vesicle weight was decreased. Nonetheless, none of the considered peripheral hormones and inflammation biomarkers differed significantly between defeated and control mice in either strain, despite the fact that social stressors may influence the functionality of the endocrine/immune system, acting upon multiple levels, depending upon the individual coping abilities [61–63]. The majority of the mouse social defeat studies are consistent with altered immune/endocrine responses that are normally evident promptly after the end of the stress experience or upon challenge [62,64–66]. Defeated mice are indeed capable to return to control levels if allowed to fully recover [41], but, given the observation of multiple physiological alterations 4 w after the final social defeat, the observed lack of differences in biomarkers parameters could be alternatively explained by the static, single time-point analyses used in the present experiments. As such, a differential response following a more dynamic assessment and/or a differential reactivity to a challenge of these systems cannot be ruled out. Nevertheless, the present data suggest that the repeated social defeat did not induce a tonic pathological state of either endocrine or immune nature that would be likely to require longer stress experiences (i.e. between 2 and 3 w) [41].

6. General conclusions

Individual variability is of pivotal importance when assessing the negative consequences of stress experiences. In the present studies, the use of the ethologically relevant social defeat stress in two mouse lines demonstrated strain-specific coping strategies, following either its single or repeated experience. One social defeat episode decreased the home-cage activity in both strains, and it elicited body weight loss in BalbC but not in C57BL6/J defeated mice. As for the repeated exposure to social defeat stress and its long-term consequences, defeated C57BL6/J and BalbC mice showed opposite consequences on metabolic efficiency. A dichotomic response was found also for social behavior, since a long-term social withdrawal could only be observed in defeated BalbC subjects. Overall, the two strains can therefore be considered representative of different adaptive strategies to the consequences of social defeat stress. These data can offer a valid case to devise translational strategy leading to a better understanding of the biological nature of the proposed link [67,68] between stress vulnerability and risk of disease.

Acknowledgements

The authors wish to thank Claudia Granato, Alessia Guidi, Valentina Pavoni, Alessandro Poffe and Claudio Righetti for their excellent technical assistance in the conducting of experiments and Dr. Francesca Michielin for her valuable discussion with respect to the statistical analyses.

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