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Strain-specific outcomes of repeated social defeat and chronic fluoxetine treatment in the mouse

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ABSTRACT

Social stress is a risk factor for affective disorders in vulnerable individuals. Although the biological nature of stress susceptibility/resilience remains to be elucidated, genetic variation is considered amongst the principal contributors to brain disorders. Furthermore, genetic predisposition may be determinant for the therapeutic outcome, as proposed for antidepressant treatments. In the present studies we compared the inherently diverse genetic backgrounds of 2 mouse strains by assessing the efficacy of a chronic antidepressant treatment in a repeated social stress procedure. C57BL/6J and BalbC mice underwent 10-day social defeats followed by 28-day fluoxetine treatment (10 mg/kg/mL, p.o.). In C57BL/6J, most of the social defeat-induced changes were of metabolic nature including persistently altered feed efficiency and decreased abdominal fat stores that were ameliorated by fluoxetine. BalbC mouse behavior was persistently affected by social defeat both in the social avoidance and the forced swim tests, and in either procedure it was restored by chronic fluoxetine, whereas their endocrine parameters were mostly unaffected. The highlighted strain-specific responsivity to the metabolic and behavioral consequences of social defeat and to the chronic antidepressant treatment offers a promising research tool to further explore the underlying neural mechanisms and genetic basis of stress susceptibility and treatment response.

1. Introduction

The most recent translational biomedical research has been focused on the neurobiology of the individual perception of adverse life events and on its role in the development of psychiatric conditions in vulnerable subjects (Krishnan et al., 2007; Jarrell et al., 2008; Miller et al., 2008; Balu et al., 2009; Gillespie et al., 2009; Lagace et al., 2010; Wood et al., 2010). The complexity of this matter is further increased having to factor in the individual variability in the response to psychotropic drugs, a phenomenon potentially related to different levels of stress vulnerability (Rush et al., 2006; Richardson-Jones et al., 2010).

Multiple experimental approaches can be applied to better understand the biological nature of the proposed link between stress vulnerability and variability in the response to psychotropic drugs (Rutter, 2006; Richardson-Jones et al., 2010). In particular, social stress such as social defeat in rodents is a significant and ethologically relevant experience in that, even when faced once, it can have persistent behavioral and neurobiological effects (Miczek et al., 1999; Marini et al., 2006). In mice the chronic exposure to social defeat stress has the potential to segregate defeated subjects into susceptible and unsusceptible populations, on the basis of a considerable individual variance to social defeat behavioral outcomes (Krishnan et al., 2007). Alternatively, the comparison of stress effects across different mouse strains may enable the identification of the stress-induced neurochemical alterations promoting depressive-like phenotypes; several behavioral inter-strain differences and the relative contribution of genetic factors to stress/anxiety reactions have been repeatedly demonstrated (reviewed in Crawley et al., 1997).

We have recently highlighted strain-specific social defeat coping styles by subjecting mice of two inbred lines to a repeated social defeat procedure (Razzoli et al., 2011). The C57BL/6J and BalbC strains were chosen for their differential stress reactivity, as the former is reported to be stress-resilient, exhibiting a lower level of anxiety and emotionality compared to the latter (Shanks et al., 1994; Crawley et al., 1997; Belzung et al., 2001), and we were able to highlight a greater metabolic susceptibility to chronic social defeat stress in C57BL/6J and a greater behavioral susceptibility in BalbC defeated subjects.

Thus we sought to expand the translational potential of these results by assessing the efficacy of a standard serotonergic antidepressant treatment in this social defeat model based on several assumptions. Firstly, variations in serotonin (5-HT) neurotransmission are included among the genetic factors that modulate individual differences in the stress response as well as the potential adverse health consequences associated with chronic stress exposure (Barr et al., 2003; Graeff et al., 1996; Price and Lucki, 2001; Jarrell et al., 2008; Jansen et al., 2010). Secondly, a deficient serotonergic system in the brain has been related to increased risk of developing psychiatric pathologies (Owens and

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Nemeroff, 1994; Melke et al., 2001; Lesch et al., 1996; Gonda et al., 2007; Holmes, 2008). Thirdly, the therapeutic potential of agents increasing the synaptic concentration of 5-HT or enhancing the serotonergic neurotransmission is observed in the treatment of mood and eating disorders (reviewed in Butler and Meegan, 2008; Capasso et al., 2009). These agents include selective serotonin reuptake inhibitors (SSRIs), such as fluoxetine, that act by blocking the 5-HT transporters, thus prolonging the elevation of extracellular 5-HT in response to activation of serotonergic neurons (Nutt et al., 1999). Fluoxetine represents the prototypical SSRI whose therapeutic applications, owing to the manifold effects of 5-HT in the brain, expand beyond depression to the treatment of anorexia and bulimia nervosa, obsessive–compulsive disorder, premenstrual dysphoria and generalized anxiety disorder (Stokes and Holtz, 1997; Wong et al., 2005).

Nonetheless, the human response to antidepressant treatment is highly variable. Complete remission of depression occurs in only 50–60% of patients and considerable individual variability is assessed in the clinical efficacy of any given treatment (Nestler et al., 2002; Trivedi et al., 2006), both of which could be accounted for by, amongst others, polymorphisms in genes involved in monoaminergic signaling (Serretti et al., 2005; Binder and Holsboer, 2006).

It has been proposed that the variation in the response of different mouse strains to fluoxetine resembles the observed variation in human response to SSRIs treatment (Rush et al., 2006; Miller et al., 2008). Hence, to gain insight into the neurobiological factors conferring vulnerability or resiliency to stress, with its implications in psychiatric diseases and treatment response, we evaluated the long-term consequences of social defeat stress and of a chronic fluoxetine treatment in C57BL/6J and BalbC mice in a variety of behavioral, physiological, endocrine, and immune parameters relevant to stress responses and depressive-/anxiety-like states.

2. Materials and methods

2.1. Animals

Forty C57BL/6J and 40 BalbC male mice (Charles River Labs, Calco, Italy) weighing 18–20 g at the beginning of the experiments served as experimental subjects. Mice were housed under constant temperature (21 ± 2 °C) and a 12 h/12 h light/dark cycle (lights on 06.00–18.00). 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 the Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC) and GlaxoSmithKline policy on the care and use of laboratory animals and codes of practice.

2.2. General experimental design

In 2 separate experiments, adult mice (~2 months of age at the beginning of testing) of the C57BL/6J and BalbC strains underwent a repeated social defeat procedure, followed by 4-week individual housing, during which they were administered with either 10 mg/kg fluoxetine p.o. or vehicle in their drinking bottle (see Fig. 1). The efficacy of the chronic antidepressant treatment was evaluated on behavioral, physiological and biochemical responses, thought to be relevant to stress and depressive-/anxiety-like states.

2.3. Social defeat stress

The social defeat stress was performed for 10 days, using a similar method to that described by Berton et al. (2006). CD-1 male mice (Charles River Labs, Calco, Italy), selected on the basis of their attack latency consistency (shorter than 30 s on 3 consecutive screening tests), were used as aggressive residents. Experimental subjects of either strain (n = 20 C57BL/6J, n = 20 BalbC) were exposed to a different unfamiliar CD-1 resident mouse each day 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 unrestricted interaction, subject mice were separated from the aggressive resident by introducing into the resident home-cage a Plexiglas divider perforated with small holes 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 = 20 C57BL/6J, n = 20 BalbC) were housed in pairs, separated by the perforated Plexiglas divider, and handled daily.

2.4. Drug treatment

Following the end of the 10-day social defeat (Fig. 1), defeated and control animals of either mouse strain received either fluoxetine or vehicle for 30 days, thus originating 4 experimental groups within each strain: Control–Vehicle (CV, n = 10), Control–Fluoxetine (CF, n = 10), Defeated–Vehicle (DV, n = 10), Defeated–Fluoxetine (DF, n = 10).

Fluoxetine hydrochloride (Trifarma SpA, Milan, Italy) (10 mg/kg/mL) was dissolved in the drinking water with 0.5% wt/vol sucrose (Sigma-Aldrich) to increase palatability. Solutions were no more than 3 days old and stored at 4 °C. Vehicle treated mice received sucrose 0.5% in the drinking water.

Fluoxetine plasma levels were determined by liquid chromatography-tandem mass spectrometry assay [Phenomenex Luna C18(2) for chromatographic separation followed by detection with Perkin-Elmer API3000 mass spectrometer].

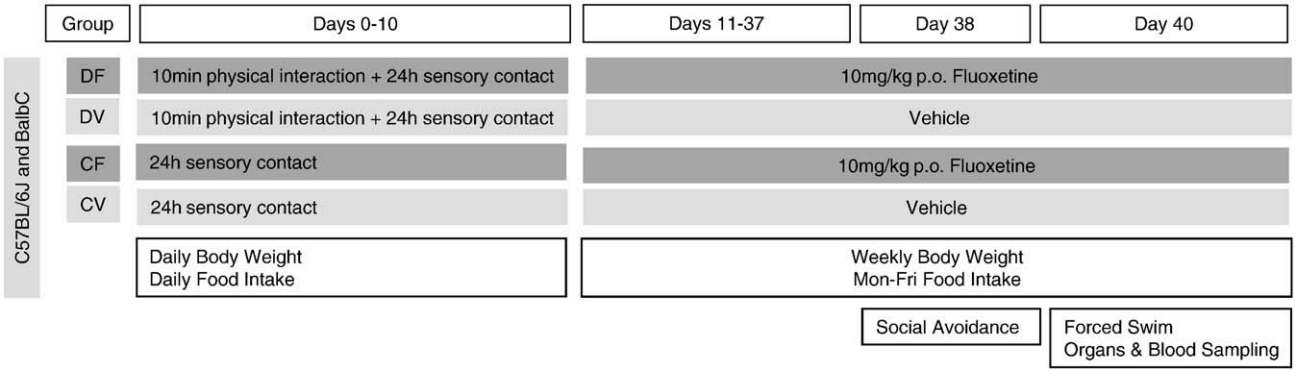

Fig. 1. Experimental procedures: 10 daily social defeat experiences were followed by 4 weeks of single housing during which animals were administered with either 10 mg/kg p.o. fluoxetine or vehicle. D = socially defeated; C = control; F = fluoxetine; V = vehicle.

2.5. Metabolic parameters

Body weight and Food Intake measures were taken at multiple time points during the 10-day social defeat stress procedure (see Fig. 1; Figs. S1–S2). Animals were weighed 3 days before the start of the experiment to allow a balanced distribution between groups. On experimental days 1 to 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. Finally, animals were weighed 30 days following the last social defeat exposure.

Body weight gain data were analyzed as differences from baseline values at the end of the social defeat stress procedure (d10-d1) as well as at the end of the fluoxetine treatment (d40-d10).

Food intake was assessed daily during the social defeat procedure (days 1 to 10) and daily, Monday to Friday, from experimental days 11 to 40, during the fluoxetine treatment; 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 (Fig. 1).

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

2.6. Behavioral assessments

Mice were tested in the social avoidance test and in the forced swim test, 28 and 30 days after the last social defeat stress respectively (Fig. 1). These behavioral procedures were spaced 2 days apart to minimize possible confounding effects due to the Social Avoidance testing on the forced swim test response.

2.6.1. Social avoidance test

As previously described by Berton et al. (2006), a video-tracking system was used to score approach–avoidance toward an unfamiliar social target. All subjects were individually placed in a 45×45 cm arena with an empty wire-mesh cage (10×4.5 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 CD-1 male mouse residents that was introduced into the wire-mesh cage ("aggressor" phase). Between the two phases, 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 (s) of the subject's presence in the "interaction zone" (defined as the 8 cm-wide area surrounding the wire-mesh cage) and in the "avoidance zone" (defined as the corners opposite to the aggressor cage) as well as the total distance moved (cm) were obtained using Ethovision XT software (Noldus Information Technology, The Netherlands).

2.6.2. Forced swim test (FST)

Mice were individually forced to swim in an open cylindrical glass container (diameter 10 cm, height 25 cm), containing 10 cm of water at 25 ± 1 °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. A trained observer scored videotapes recorded using The Observer XT 7.0 software (Noldus Information Technology, The Netherlands). The duration (s) of floating (minimal activity required for the mouse to keep its head above water level) and climbing (upward directed movement of the forepaws along the walls of the cylinder) was scored from videotapes during the last 4 min of the 6 min test.

2.7. 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 animals to normalize biomarkers of immediate stress response after the FST. Mice were killed by rapid decapitation to allow trunk blood collection, between 10.00 and 13.00. At autopsy, internal organs such as abdominal fat (represented by epididymal white adipose depot located around both testes and intra-abdominal mesenteric adipose depot), testis, seminal vesicles, spleen, adrenal glands, and thymus were dissected and weighed (relative organs weight was calculated from the ratio between absolute organ weight and body weight).

2.7.1. 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.

2.7.2. 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. Adrenocorticotropic hormone (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%; 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), IL-13, IL-17, eotaxin, granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), Interferon-gamma, keratinocyte chemoattractant (KC), monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory protein-1beta (MIP-1beta), RANTES and tumor necrosis factor (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].

2.8. Statistics

Statistical analyses were conducted using Statistica V8 (Statsoft, Inc. Tulsa, OK). When appropriate, data were log transformed to satisfy ANOVA's assumptions.

Body weight gain, food intake and feed efficiency values during the social defeat phase were analyzed with ANOVA with Stress (Defeated versus Control) as between-subject variable; two-way ANOVA with Stress (Defeated versus Control) and Drug (Fluoxetine versus Vehicle) as between-subject variables was performed during the chronic treatment phase. Significant differences due to main effects were followed by Holm corrected planned comparisons.

Social avoidance data were analyzed by ANOVA for repeated measures, with "test phase" ("no aggressor" and "aggressor" phases) as within-subject variable, followed by Holm corrected planned comparisons.

Data from FST, internal organs, peripheral hormones and inflammation biomarkers levels were analyzed by means of 2-way ANOVA, with Stress (Defeated versus Control) and Drug (Fluoxetine versus Vehicle) as between-subject variables, followed by Holm corrected planned comparisons.

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

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

3. Results

3.1. Serum fluoxetine levels

Serum fluoxetine levels, assessed in a subset of C57BL/6J mice at the end of the chronic drug treatment, corresponded to 343.50 ± 74.93 ng/mL. It is important to notice that the serum levels of fluoxetine achieved in our conditions were consistent with previous reports (Dulawa et al., 2004); values for the 10 mg/kg/day dose were within the range found in patients taking 20–80 mg/day Prozac (100–700 ng/mL), at a time when the steady state has long been achieved (Koran et al., 1996; Santarelli et al., 2003).

3.2. Metabolic parameters

3.2.1. Body weight

At the end of the 10 social defeats, the body weight gain of C57BL/6J defeated subjects did not differ from control (Fig. 2A); in the BalbC strain, the defeated subjects gained significantly less weight compared to controls ($F(1,38) = 8.51$, $p < 0.01$) (Fig. 3A).

During the fluoxetine treatment, defeated C57BL/6J mice gained more body weight than controls ($F(1,35) = 3.83$, $p = 0.058$), although no significant effects were found for either Drug or its interaction with Stress (Fig. 2D). No significant differences were observed in BalbC mice, due to Stress, Drug, or the Stress × Drug interaction (Fig. 3D).

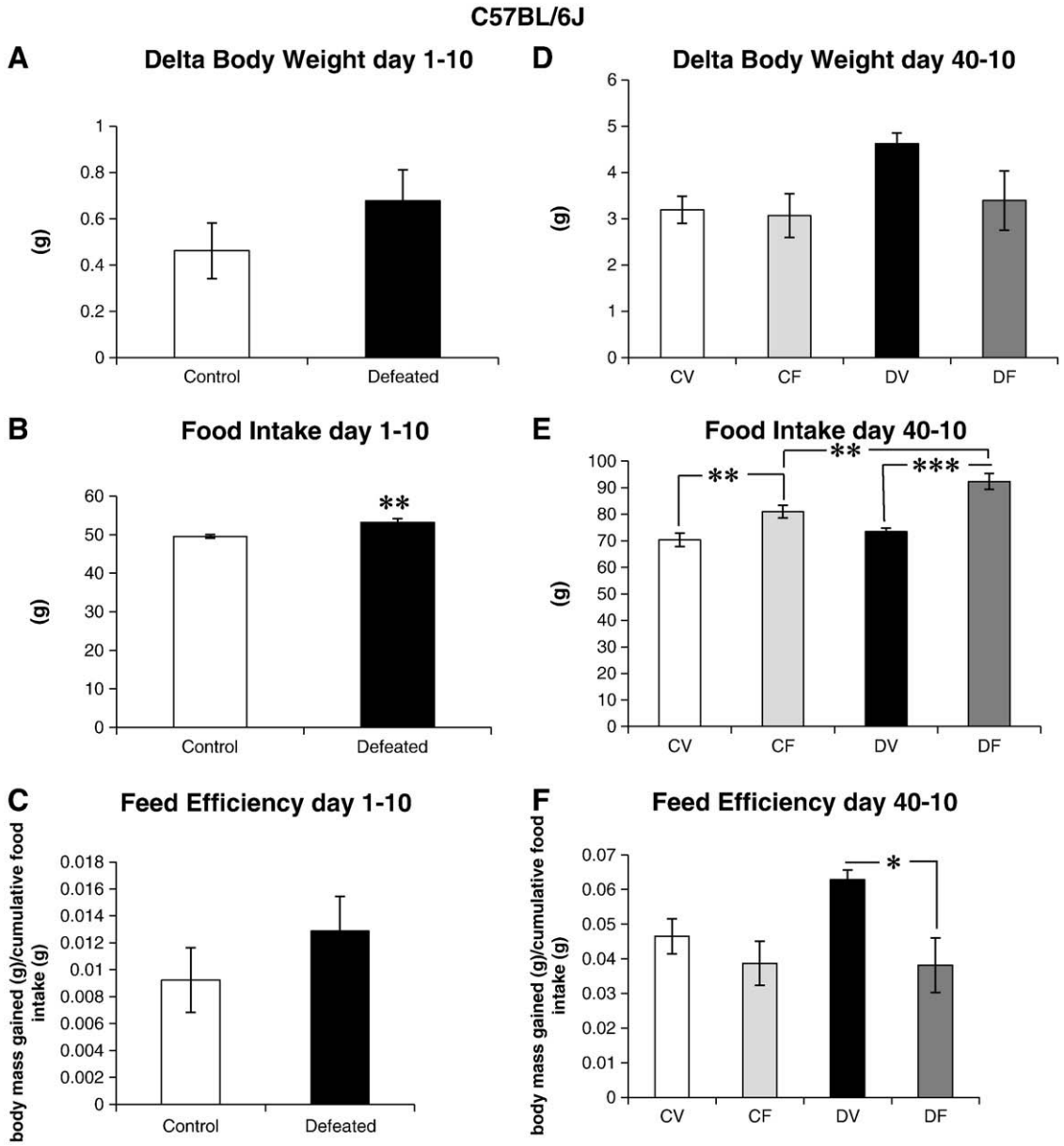

Fig. 2. Metabolic parameters in C57BL/6J during the social defeat phase (A through C) and the single housing phase (D through F). A) The delta body weight (g) was calculated as the difference between the first and the last (tenth) social defeat. B) Food intake (g) was calculated as the total amount of food consumed during the 10 days of social defeat. C) Feed efficiency was calculated as the ratio between the body mass gained (g) and the amount of food ingested (g) over the 10 days of social defeat. D) The delta body weight (g) was calculated as the difference between the last day of the single housing phase and the end of social defeat. B) Food intake (g) was calculated as the total amount of food consumed during the 30 days social housing phase following social defeat. C) Feed efficiency was calculated as the ratio between the body mass gained (g) and the amount of food ingested (g) over the 30 days of single housing following social defeat. The data are represented as the mean ± SEM. D = socially defeated; C = control; F = fluoxetine; V = vehicle. The asterisks indicate significant group differences (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$).

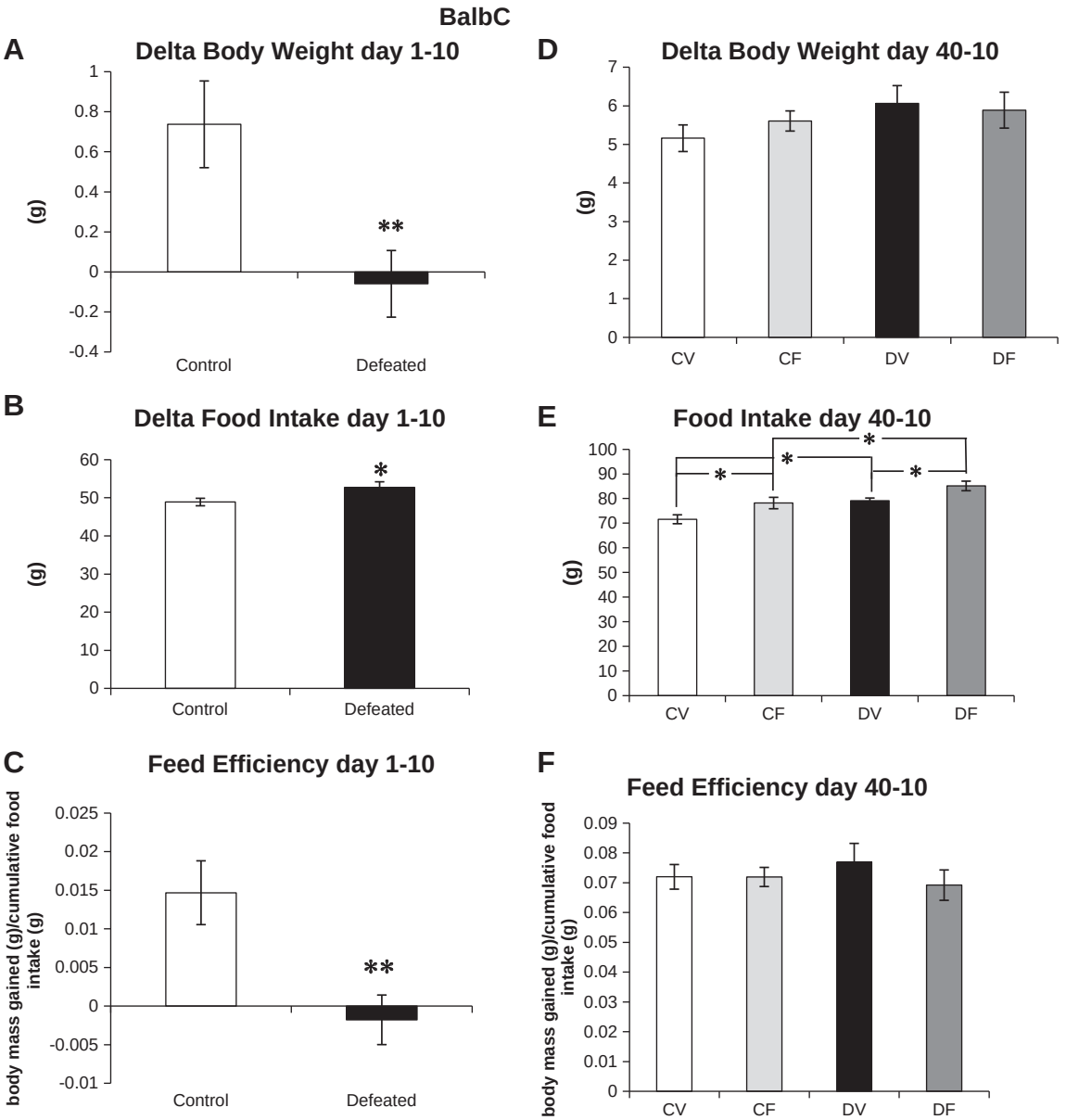

Fig. 3. Metabolic parameters in BalbC during the social defeat phase (A through C) and the single housing phase (D through F). A) The delta body weight (g) was calculated as the difference between the first and the last (tenth) social defeat. B) Food intake (g) was calculated as the total amount of food consumed during the 10 days of social defeat. C) Feed efficiency was calculated as the ratio between the body mass gained (g) and the amount of food ingested (g) over the 10 days of social defeat. D) The delta body weight (g) was calculated as the difference between the last day of the single housing phase and the end of social defeat. B) Food intake (g) was calculated as the total amount of food consumed during the 30 days of social housing phase following social defeat. C) Feed efficiency was calculated as the ratio between the body mass gained (g) and the amount of food ingested (g) over the 30 days of single housing following social defeat. The data are represented as the mean ± SEM. D = socially defeated; C = control; F = fluoxetine; V = vehicle. The asterisks indicate significant group differences (*$p<0.05$; **$p<0.01$).

3.2.2. Food intake

During the social defeat, food intake was significantly increased in defeated subjects of both C57BL/6J ($F(1,37)=11.23, p<0.01$) (Fig. 2B) and BalbC strain ($F(1,38)=4.99$, $p<0.05$) (Fig. 3B). During the fluoxetine treatment, a significant increase of food intake due to Stress ($F(1,35)=8.83$, $p<0.01$) and Drug ($F(1,35)=36.71$, $p<0.0001$) was found in C57BL/6J subjects, with their interaction reaching close to significance levels ($F(1,35)=2.91$, $p=0.096$) (Fig. 2E). Specifically, within animals receiving fluoxetine, defeated subjects consumed significantly more food than controls (CF versus DF, $p<0.01$). Similarly for BalbC mice, a significant increase of food intake was found for both Stress ($F(1,36)=15.29$, $p<0.001$) and Drug ($F(1,36)=11.64$, $p<0.01$), whereas no significant role was highlighted for their interaction (Fig. 3E).

3.2.3. Feed efficiency

At the end of 10-day social stress, the feed efficiency of C57BL/6J defeated mice did not differ from controls (Fig. 2C); BalbC defeated subjects showed a significant decrease in this parameter ($F(1,38)=9.11$, $p<0.01$) (Fig. 3C). After the conclusion of the treatment with fluoxetine, no differences due to Stress or to its interaction with Drug were found in the C57BL/6J strain; Drug per se significantly altered the feed efficiency ($F(1,35)=7.66$, $p<0.01$), mostly because of significantly lower values

within C57BL/6J defeated subjects treated with fluoxetine (DF versus DV, $p<0.05$) (Fig. 2F). In BalbC mice, no differences among groups were found (Fig. 3F).

3.3. Behavioral assessments

3.3.1. Social avoidance

The behavioral parameters originated during both the non social phase (no aggressor present — not shown) and the social phase (aggressor present) of the test (Fig. 4). In C57BL/6J, the time in the interaction zone increased significantly during the social phase compared to the non social phase of the test ($F(3,17)=51.94$, $p<0.001$); a close to significant interaction was detected between Stress and Test Phase ($F(3,35)=2.63$, $p=0.065$), with no main effect of Stress alone (Fig. 4B). No differences were found either in time in avoidance zone or in total distance moved (Fig. 4A, C). In BalbC, the time in the interaction zone tended to be increased in the social phase ($F(1,35)=4.03$, $p=0.052$), it was not significantly influenced by Stress, whereas it was significantly altered by the Stress × Drug interaction ($F(3,35)=4.21$, $p<0.05$) (Fig. 4E), due to the longer time in the interaction zone spent by defeated subjects that had received fluoxetine compared to the defeated subjects administered with vehicle (DF versus DV: $p=0.07$). No differences were found either in time in avoidance zone or in total distance moved (Fig. 4D, F).

3.3.2. FST

In C57BL/6J mice, Stress reduced significantly the duration of floating ($F(1,35)=5.51$, $p<0.05$), independently of Drug or Stress × Drug interaction (Fig. 5A). Coherently, climbing duration was significantly enhanced in defeated C57BL/6J compared to controls ($F(1,34)=5.68$, $p<0.05$), with no effect of either Drug or the Stress × Drug interaction.

In BalbC subjects, nor Stress or Drug had a significant effect on floating duration, although their interaction did ($F(1,34)=6.178$, $p<0.05$) (Fig. 5A). In particular, a significant decrease in this parameter was found in defeated subjects receiving fluoxetine treatment compared with drug-treated controls (DF versus CF, $p<0.05$). At the same time, while Stress and Drug did not influence climbing behavior, their interaction had a significant effect ($F(1,33)=6.372$, $p<0.05$). Mice that received chronic fluoxetine treatment following repeated social defeats did climb for significantly longer durations than controls (DF versus CF, $p<0.05$).

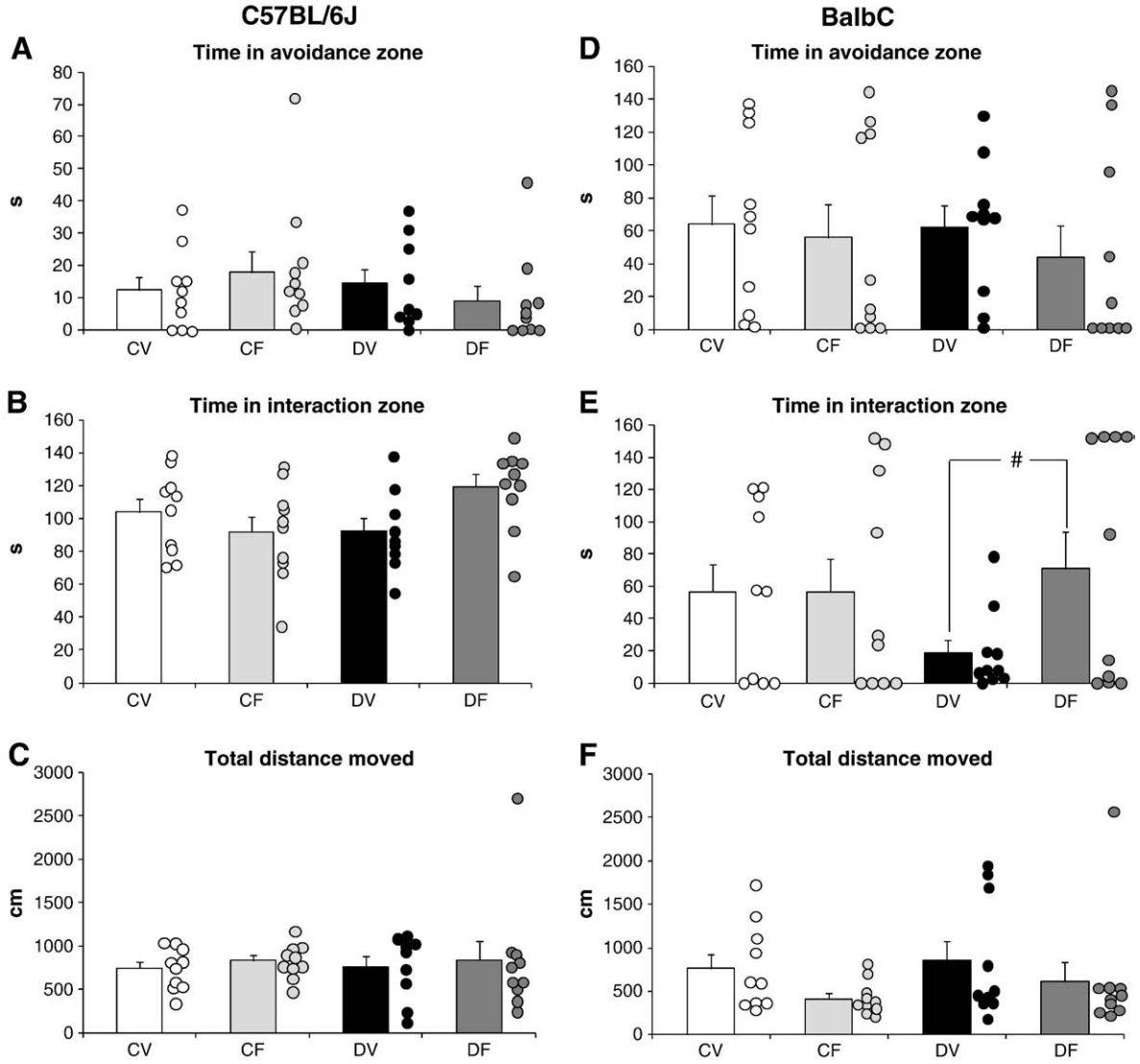

Fig. 4. Social avoidance test conducted 28 days after the end of the social defeat stress in C57BL/6J (A) and BalbC (B) subjects. The test comprised two phases, in the absence (2.5 min) and in the presence (2.5 min) of an aggressor CD1 mouse confined within a small cage, around which the interaction could take place. The time spent in proximity to the aggressor (s) was measured. The values represent group mean ± SEM. D = socially defeated; C = control; F = fluoxetine; V = vehicle. # represents $p=0.07$ between DV and DF within BalbC strain.

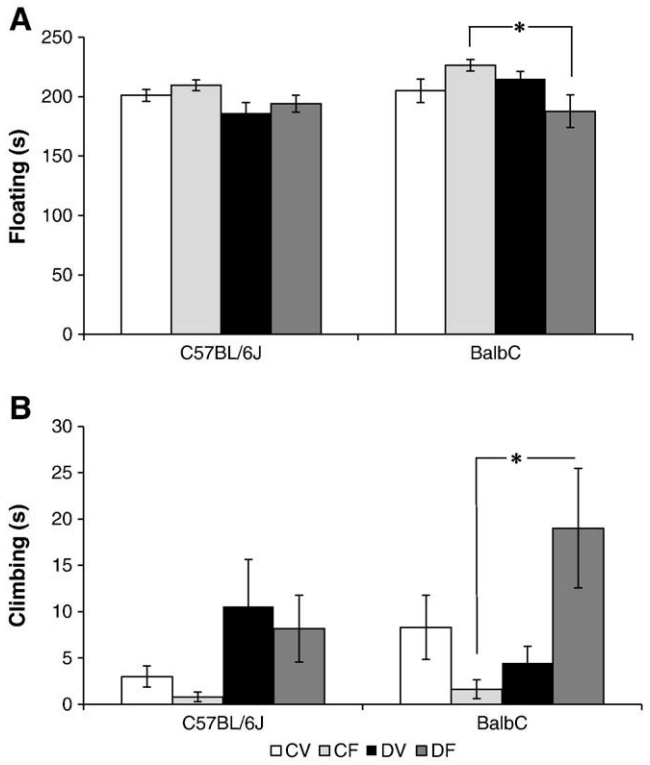

Fig. 5. Forced swim test behaviors: floating (A) and climbing (B) durations (s). The FST was conducted 30 days after the end of the social defeat stress in C57BL/6J and BalbC subjects. Values represent group mean ± SEM. D = socially defeated; C = control; F = fluoxetine; V = vehicle. * represents $p<0.05$ between CF and DF within BalbC strain.

3.4. Internal organs weight and peripheral biomarkers

3.4.1. Internal organ weight

In C57BL/6J no effects of any considered factor were detected for organs such as adrenal glands, spleen, and thymus. On the other hand, gonadal organ weight was significantly reduced by Stress [seminal vesicle: $F(1,35)=6.44$, $p<0.05$; testicle: $F(1,35)=4.697$, $p<0.05$], although no effect was detected for either Drug or the Stress × Drug interaction. The amount of abdominal fat was significantly decreased by Stress ($F(1,35)=26.13$, $p<0.0001$) and increased by Drug ($F(1,35)=42.39$, $p<0.0001$), but it was not influenced by their interaction (Table 1).

Of all measured parameters (Table 1), in BalbC only thymus and testicle weight did not vary. Adrenal glands weight was not affected by Stress or by the Stress × Drug interaction, but it was significantly reduced by fluoxetine treatment ($F(1,36)=4.978$, $p<0.05$), mostly due to a decrease observed, within the defeated, in the subjects receiving the drug (DF versus DV, $p<0.05$). Spleen weight was significantly decreased both by Stress ($F(1,36)=9.531$, $p<0.01$) and Drug ($F(1,36)=12.057$, $p<0.01$) with no effect of their interaction. Within drug-free animals, defeated subjects' spleen weighed significantly less than controls (DV versus CV, $p<0.05$), whilst Drug effect was found to be mostly dependent on a significant reduction within control group (CV versus CF, $p<0.05$). Seminal vesicle weight was significantly decreased by Stress ($F(1,36)=5.672$, $p<0.05$); Drug and the interaction Stress × Drug had no effect. Finally, abdominal fat was significantly decreased by Stress ($F(1,36)=20.829$, $p<0.001$) and, although Drug per se was without influence, it resulted significantly altered depending upon the interaction Stress × Drug ($F(1,36)=4.286$, $p<0.05$). A significant decrease in this parameter was found comparing, within vehicle treated animals, defeated to control subjects (DV versus CV, $p<0.0001$).

3.4.2. Peripheral biomarkers

In C57BL/6J (Table 2), leptin plasma levels did not differ due to Stress, while they were significantly increased in the fluoxetine-treated animals as a whole ($F(1,34)=11.59$, $p<0.01$). A close to significant interaction between Stress and Drug was also detected ($F(1,34)=3.27$, $p=0.079$). Chronic fluoxetine significantly increased leptin plasma levels only within control subjects (CV versus CF, $p<0.01$). Both IL-6 and G-CSF were significantly increased in defeated subjects as a whole ($F(1,32)=5.072$, $p<0.05$ and $F(1,32)=4.621$, $p<0.05$, respectively), whereas nor Drug or the Stress × Drug interaction had any effect.

In BalbC (Table 2), various inflammation biomarkers were significantly altered. Though IL-6 and IL-10 were not influenced by Stress, a tendency to a significant effect for Drug (respectively: $F(1,35)=3.65$, $p=0.064$; $F(1,35)=3.975$, $p=0.054$) and a significant interaction (respectively: $F(1,35)=8.19$, $p<0.01$; $F(1,35)=4.808$, $p<0.05$) were highlighted. In particular, for both IL-6 and IL-10, fluoxetine-treated defeated subjects were found with significantly lower levels than both drug-free defeated mice (IL-6: DF versus DV, $p<0.01$; IL-10: DF versus DV, $p<0.05$) and fluoxetine-treated controls (IL-6 and IL-10: DF versus CF, $p<0.05$). IL-12p40 and MCP-1 were significantly decreased by Stress (respectively: $F(1,35)=8.75$, $p<0.01$; $F(1,35)=4.866$, $p<0.05$), but not by Drug or by the Stress × Drug interaction. On the other hand, eotaxin was not affected by Stress nor by the Stress × Drug interaction, but it was significantly decreased by Drug ($F(1,35)=7.607$, $p<0.01$).

4. Discussion

The present experiments demonstrated strain-specific responses to social defeat and to the associated chronic fluoxetine treatment. In general, considering each strain response to the experimental procedure, while C57BL/6J showed alterations in metabolic parameters supportive of a predominantly metabolic susceptibility, mice belonging to the BalbC strain demonstrated to be mostly behaviorally sensitive, given their social avoidance and FST responses. A similar diverging reactivity to the behavioral and physiological consequences

Table 1
Internal organs absolute weight (mg).

	C57BL/6J				BalbC			
	CV	CF	DV	DF	CV	CF	DV	DF
Adrenals	7.85 ± 0.61	8.41 ± 1.19	6.95 ± 0.63	8.74 ± 00.94	5.05 ± 0.30	4.79 ± 0.30	5.17 ± 0.19	4.41 ± 0.21°
Spleen	59.02 ± 2.52	53.84 ± 3.16	59.03 ± 1.77	58.89 ± 3.34	98.79 ± 6.03	85.24 ± 2.15°	86.63 ± 2.82*	78.53 ± 2.12
Thymus	49.62 ± 3.57	51.45 ± 5.06	49.33 ± 2.17	44.24 ± 4.07	39.27 ± 1.72	42.21 ± 2.13	44.56 ± 2.76	40.77 ± 2.68
Seminal vesicles	180.58 ± 12.67	174.22 ± 11.32	157.84 ± 8.60	149.15 ± 14.10	199.84 ± 8.87	206.79 ± 9.36	108.49 ± 7.74	192.10 ± 11.01
Testicles	193.03 ± 2.93	188.41 ± 4.02	184.04 ± 6.42	186.93 ± 5.48	206.18 ± 5.18	201.02 ± 4.76	204.75 ± 3.68	185.01 ± 17.67
Abdominal fat	346.72 ± 8.59	433.59 ± 24.65	299.07 ± 8.42	365.24 ± 12.41	386.58 ± 21.05	367.93 ± 26.36	277.29 ± 19.72***	327.99 ± 24.98

The values represent group mean ± SEM. These data were analyzed as relative values over 100 g body weight. CV = Control–Vehicle; CF = Control–Fluoxetine; DV = Defeated–Vehicle; DF = Defeated–Fluoxetine. * $p<0.05$, *** $p<0.001$: 'stress' effect at level of Holm corrected planned comparisons; ° $p<0.05$: 'drug' effect at level of Holm corrected planned comparisons.

Table 2
Peripheral hormones and inflammation biomarkers (pg/mL).

Parameter	C57BL/6J				BalbC			
	CV	CF	DV	DF	CV	CF	DV	DF
ACTH	8.87 ± 3.19	5.34 ± 1.10	11.60 ± 5.34	5.82 ± 1.31	7.36 ± 1.30	8.47 ± 2.69	6.90 ± 2.46	5.71 ± 1.34
Insulin	973.82 ± 159.46	1248.95 ± 135.08	943.46 ± 150.11	1213.70 ± 201.45	1840.86 ± 310.14	2106.93 ± 521.64	1437.10 ± 258.86	1456.94 ± 157.67
Leptin	734.31 ± 63.30	1413.72 ± 111.46°°	889.70 ± 152.10	1086.88 ± 144.48	1424.80 ± 198.37	1280.78 ± 159.84	1005.14 ± 180.33	1256.57 ± 188.63
IL-1a	32.16 ± 3.52	34.63 ± 3.36	39.38 ± 4.26	35.61 ± 4.58	21.60 ± 1.84	24.18 ± 1.44	22.08 ± 1.50	18.85 ± 2.68
IL-1b	146.73 ± 15.22	159.91 ± 16.38	171.71 ± 17.80	400.69 ± 219.71	130.13 ± 6.33	132.82 ± 10.12	133.01 ± 9.90	109.89 ± 14.45
IL-2	95.71 ± 7.42	87.05 ± 7.87	98.71 ± 10.69	93.54 ± 6.81	82.32 ± 5.51	76.47 ± 3.89	80.62 ± 4.49	66.29 ± 6.20
IL-6	19.53 ± 2.81	18.61 ± 2.58	25.67 ± 1.92	78.94 ± 48.68	8.94 ± 0.96	10.36 ± 1.35	12.95 ± 2.75	6.45 ± 0.97*,°°
IL-9	172.10 ± 18.78	176.87 ± 17.58	170.13 ± 18.82	181.49 ± 29.60	143.54 ± 11.43	188.9 ± 50.49	203.33 ± 43.47	164.77 ± 31.05
IL-10	80.24 ± 9.10	69.81 ± 10.44	86.33 ± 13.85	80.31 ± 8.09	37.30 ± 3.43	39.55 ± 5.51	44.50 ± 7.44	25.28 ± 4.00*,°
IL-12p(40)	287.43 ± 18.05	234.67 ± 26.03	313.21 ± 15.48	362.60 ± 77.04	284.96 ± 28.83	258.74 ± 20.85	225.65 ± 9.53	205.61 ± 8.86
IL-12p(70)	26.42 ± 3.18	29.31 ± 3.60	31.46 ± 3.31	28.79 ± 4.25	12.94 ± 1.01	14.30 ± 1.56	14.34 ± 1.18	11.19 ± 1.82
IL-13	1476.67 ± 144.71	1447.69 ± 126.74	1534.50 ± 119.82	1739.95 ± 348.86	1161.99 ± 73.66	1170.11 ± 84.79	1191.19 ± 95.76	960.86 ± 106.97
IL-17	90.85 ± 16.86	111.23 ± 22.98	110.46 ± 21.63	147.77 ± 57.11	91.98 ± 31.70	80.92 ± 19.22	121.06 ± 31.07	72.89 ± 11.13
Eotaxin	645.58 ± 74.39	636.55 ± 102.59	641.78 ± 71.66	722.47 ± 71.32	532.66 ± 71.87	325.53 ± 85.32	504.22 ± 74.29	260.66 ± 93.56
G-CSF	101.70 ± 7.69	105.47 ± 9.93	136.92 ± 8.96	279.92 ± 87.24	51.81 ± 2.98	51.69 ± 3.44	66.62 ± 45.72	54.77 ± 4.55
G-MCSF	59.09 ± 8.31	58.88 ± 7.87	64.81 ± 9.04	75.54 ± 14.39	41.87 ± 7.81	44.07 ± 6.38	45.72 ± 7.76	26.17 ± 7.45
Inf-gamma	18.15 ± 4.54	16.69 ± 3.55	20.85 ± 4.75	31.72 ± 17.12	2.86 ± 0.90	4.34 ± 1.40	4.88 ± 1.40	3.99 ± 1.70
KC	106.67 ± 6.24	103.78 ± 4.68	108.72 ± 5.88	143.29 ± 28.89	107.38 ± 6.15	110.17 ± 6.79	105.77 ± 7.86	96.11 ± 4.26
MCP-1	313.79 ± 23.55	290.15 ± 26.92	313.17 ± 34.64	436.84 ± 145.30	250.54 ± 12.79	257.79 ± 13.92	232.93 ± 19.97	194.76 ± 24.70
MIP-1b	59.71 ± 7.30	64.85 ± 8.46	73.19 ± 10.80	172.20 ± 105.38	48.00 ± 3.12	48.27 ± 3.21	47.14 ± 4.98	35.59 ± 4.91
RANTES	2.83 ± 0.32	3.12 ± 0.65	3.69 ± 0.51	3.63 ± 1.02	1.51 ± 0.31	1.64 ± 0.23	1.65 ± 0.14	1.05 ± 0.24
TNF-alpha	1330.74 ± 105.21	1223.57 ± 103.52	1298.56 ± 151.18	1709.10 ± 323.00	1555.05 ± 112.00	1373.52 ± 92.02	1517.87 ± 127.80	1289.03 ± 82.84

The data represent plasma levels (mean ± SEM) of peripheral biomarkers measured at the end of 4 weeks of single housing, in the presence/absence of fluoxetine treatment that followed 10-day social defeat. CV = Control–Vehicle; CF = Control–Fluoxetine; DV = Defeated–Vehicle; DF = Defeated–Fluoxetine. Inf: Interferon. * $p<0.05$: 'stress' effect at level of Holm corrected planned comparisons; ° $p<0.05$ °°, $p<0.01$: 'drug' effect at level of Holm corrected planned comparisons.

of repeated social defeat experiences had been previously reported by our laboratory in these mouse strains (Razzoli et al., 2011). Thus the present data confirm this profile and extend it to strain-specific responsiveness to a chronic SSRI treatment in the social defeat model.

The most recent studies in mice demonstrate metabolic alterations induced by social stress in agreement with the increases in body weight and food intake reported in the present experiments (Pardon et al., 2004; Moles et al., 2006; Bartolomucci et al., 2009; Chuang et al., 2010a,b). In particular, notwithstanding the strain-specific metabolic reactivity to the social defeat stress, body weight and food intake were persistently increased in C57BL/6J and in BalbC following social defeat. On the other hand, different metabolic profiles are known to exist across different inbred mouse strains (Kirk et al., 1995) and to be maintained following the experience of social defeat (Razzoli et al., 2011). In the present studies fluoxetine induced an increase in food intake in both strains and contributed to normalize the feed efficiency index that was altered by social defeat, particularly in C57BL/6J mice. The observed fluoxetine-induced hyperphagia is difficult to explain since this drug exerts anorectic effects both in humans and rodents (Yen and Fuller, 1987; McGuirk et al., 1992; Tao et al., 2002; Halford et al., 2005), even though most of the studies in rodents were done in basal conditions (Currie et al., 2004; Gobshtis et al., 2007), and different procedures, such as species, sex, and/or composition of test diets could explain the discrepancy with the present results. Nevertheless, the serotonergic stimulation of the hypothalamic paraventricular nucleus has been shown to enhance energy metabolism (Sakaguchi and Bray, 1989), and in humans fluoxetine has been found to increase energy expenditure (Bross and Hoffer, 1995), both evidences endorsing the observed findings. The metabolic implications of this experimental procedure are further supported by the results on abdominal fat that was diminished in defeated subjects consistently with previous studies and similarly in both strains (Bartolomucci et al., 2004; Chuang et al., 2010a,b). The decrease in adipose tissue of defeated mice has been shown to be accompanied by stress-induced alterations in the utilization of nonesterified fatty acids (Chuang et al., 2010b). The stress experience would indeed trigger an autonomic nervous system hyperactivity (Deshaies et al., 1993; Sgoifo et al., 1999; Keeney et al., 2001; Davies et al., 2009), leading to redistribute store excess calories, such as lipids, into alternate peripheral organs than adipose tissue such as the liver (Chuang et al., 2010a,b).

The reported alterations in abdominal fat stores were not paralleled by significant changes in leptin levels, even though a trend for a positive relationship between their values was observed. Leptin plasma levels were increased by fluoxetine within C57BL/6J subjects, where controls had the largest amount of adipose tissue. Leptin is mostly secreted by adipocytes and participates in the regulation of body weight acting upon the control of food intake (Inui, 1999; Loftus, 1999; Prolo and Licinio, 1998), providing critical information about the size of the fat stores (Loftus, 1999; Sandoval and Davis, 2003). Furthermore, multiple evidences support the interaction between leptin and the serotonergic systems (Collin et al., 2000; Finn et al., 2001; Hastings et al., 2002). Further studies are warranted to shed more light into the mechanisms involved in the regulation of the metabolic adaptations to social stress and their functional implications.

The effects of fluoxetine on the long-term consequences of the social stress were also evaluated in behavioral procedures relevant for mood disturbances. The social avoidance test is a procedure whereby to assess the aversive nature of social stimuli after repeated experiences of aggression, sensitive to chronic but not acute antidepressant treatment (Berton et al., 2006; Tsankova et al., 2006; Krishnan et al., 2007). The FST is a reliable assay with predictive validity for antidepressant compounds following acute (Porsolt et al., 1977; Lucki et al., 2001) and chronic drug treatments (Dulawa et al., 2004; Holick et al., 2008).

In the social avoidance test, the C57BL/6J subjects demonstrated, as a whole, a higher social propensity than BalbC (70% time in interaction zone by C57BL/6J versus ~30% by BalbC), as expected on the basis of the distinctively lower sociability level of BalbC compared to other strains (reviewed in Brodkin, 2007). When the effects of stress were taken into account, defeated C57BL/6J mice did not avoid the interaction zone; nonetheless, an increase in this parameter was induced by fluoxetine. BalbC mice displayed greater behavioral vulnerability to social defeat and responsivity to fluoxetine compared to C57BL/6J the interaction time of BalbC defeated subjects was diminished compared to control mice, but it was normalized by fluoxetine. In addition, defeated BalbC mice appeared to segregate in two subpopulations concerning avoidance and interaction time. Interestingly, different levels of approach/

avoidance could be observed also in control mice. Data link different degrees of sociability in this strain to a highly variable development of the corpus callosum, that in ~30–40% BalbC mice is under- or not developed at all (Wahlsten, 1974; Brodkin, 2007; Fairless et al., 2008) and that, therefore, could be related to the observed variability in the social parameters, although further studies should be performed to verify this hypothesis.

On the other hand, although fluoxetine appeared to increase the interaction time in defeated C57BL/6J, the lack of social avoidance in the drug-free defeated subjects is quite unexpected, since most of the published studies show social defeat-induced social avoidance in C57BL/6J mice (Berton et al., 2006; Tsankova et al., 2006; Krishnan et al., 2007; Chuang et al., 2010a,b). This could be explained by the intrinsic variability of this parameter, since following defeat stress social avoidance is induced in approximately half the C57BL/6J subjects, whereas the other half exhibits stress resiliency, i.e. same social interaction levels as undefeated controls (Krishnan et al., 2007; Lagace et al., 2010). The present result reproduces what previously observed in our lab (Razzoli et al., 2011) and is coherent with a similarly modest induction of social avoidance reported by others, particularly when subjects were fed on normal chow versus high fat diet (Chuang et al., 2010a). If procedural differences/parameter intrinsic variability could account for the conflicting findings in C57BL/6J at the same time the social avoidance observed in defeated BalbC mice further supports the overall enhanced emotionality of this strain (Cohen et al., 2008), and highlights their responsivity to chronic fluoxetine.

A comparable behavioral resistance of C57BL/6J subjects was seen in the FST; defeated mice appeared to display fewer 'passive' and more 'active' behaviors that were not modulated by the drug treatment. As for BalbC subjects, the stress-induced increase in "passive" behaviors was counteracted by fluoxetine that was particularly effective in enhancing the active behaviors of defeated subjects (i.e. climbing). These results are in agreement with FST studies in baseline animals showing that C57BL/6J failed to respond to chronic fluoxetine treatment, whereas BalbC mice offered a reliable model for detecting the temporal specificity of fluoxetine behavioral effects (Lucki et al., 2001; Dulawa et al., 2004; Holick et al., 2008). In general a high stress resiliency should be factored in when trying to explain the difficulty in developing chronic depressive-like models using the C57BL/6J strain, since the use of the highly anxious BalbC demonstrated the efficacy of chronic SSRI in subjects stressed with the same procedure, consistently with similar findings in basal conditions (Dulawa et al., 2004; Holick et al., 2008). Importantly, the strain dependent efficacy of the chronic SSRI treatment can be considered consistent with human findings, showing how antidepressant drugs exert their effect on mood in psychiatric patients but not in healthy individuals (Barr et al., 1997; Gelfin et al., 1998; Geyer and Markou, 2002). The existence of marked strain differences in immobility baselines, in the pharmacological responses to antidepressants in the FST, as well as in the social avoidance tests suggests the existence of significant genetic factors contributing to the behavioral performances of rodent in these procedures. Since strain-specific behavioral responses are elicited by SSRIs, it can be inferred that endogenous differences in the response of 5-HT transmission to stressors and to 5-HT receptor activation may provide a basis for the divergent behavioral sensitivity seen between the two strains. Interestingly, owing to their genotype carrier of reduced 5-HT synthesis information (1437G), BalbC mice show ~50% reductions in brain 5-HT synthesis compared to C57BL/6J (reviewed in Brodkin, 2007). In agreement with these data, a dependence of basal anxiety-like behavior and coping ability with negative social experience in adult life was recently demonstrated to be dependent on the modulation of 5-HT release into the synaptic cleft in both heterozygous and knock out mouse for the 5-HT transporter (Jansen et al., 2010).

The deleterious effects of social stress on mouse physiology were mostly consistent with previous reports of decreases in organs weight related to the gonadal as well as the immune function (Selye, 1950; Raab et al., 1985; Bartolomucci et al., 2001; van Kampen et al., 2002). The observed reduction in androgen-dependent target organs could depend upon decreased testosterone levels that can be induced in male mice by defeat and stress of submission during fighting (Parmigiani et al., 1989). On the other hand, in spite of the established detrimental side effects of fluoxetine on reproductive function in both humans and animals (reviewed in de Jong et al., 2006; Serretti and Chiesa, 2009), in neither strain gonadal organ alterations were affected by this drug as, conversely, it can only be expected at toxic chronic dose ranges (Bataineh and Daradka, 2007). In addition, defeated BalbC subjects showed decreased spleen size that was not responsive to chronic fluoxetine treatment. In turn, fluoxetine induced a decrease in spleen weight in control animals. It must be considered that fluoxetine elevates the synaptic concentrations of 5-HT not only in the central nervous system but also in the periphery, and the enhanced accumulation of 5-HT may facilitate the occurrence of apoptotic processes in the spleen, thus explaining the observed findings (Josefsson et al., 1996).

In rodents different stress schedules may have suppressive effects on cell-mediated immune responses that can be at least partially ameliorated by chronic fluoxetine (Freire-Garabal et al., 1993, 1997, 2002; Nunez et al., 2006). Furthermore, murine genetic background had been shown to influence immune responses. BalbC mice present a greater susceptibility to bacterial infection and response to antibiotic therapy compared to C57BL/6J (Pellegrini et al., 2007), due to, amongst other factors, a distinct activity of IL-10 (Roque et al., 2007). In the present study defeated mice showed strain-specific alterations in inflammation biomarkers (IL-6 and G-CSF in C57BL6/J, and IL-6, IL-10, IL-12p40, MCP-1 in BalbC). This result further confirms previous data showing the existence of differences in immune functioning depending on the strain and the stressor employed as well as the immune parameters considered (Lu et al., 1998). Nonetheless, the changes detected in the levels of peripheral biomarkers reported herein are small, that could be due to the sampling of the subjects at baseline. The static, single time-point biochemical analysis poses a further constraint; the existence of a differential response cannot be ruled out unless a more dynamic assessment or a challenge of these systems will be carried out.

In conclusion, present data demonstrated how strain differences in coping with social stress can be translated into differential strain responsivity to SSRI treatment, as it was evidenced by C57BL/6J metabolic adaptations and by BalbC behavioral response pattern. Future studies should be performed to help clarify the biological nature of the highlighted diverging profile, to help strengthening the link with human data on differential degrees of stress vulnerability, insurgence of stress-related psychopathology, and treatment response.

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Appendix A. Supplementary data

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