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What is stressful for females? Differential effects of unpredictable environmental or social stress in CD1 female mice

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ABSTRACT

Stressful life events are a major factor in the etiology of several diseases, such as cardiovascular, inflammatory and psychiatric disorders (i.e., depression and anxiety), with the two sexes greatly differing in vulnerability. In humans and other animals, physiological and behavioral responses to stress are strongly dependent on gender, and conditions that are stressful for males are not necessarily stressful for females. Hence the need of an animal model of social chronic stress specifically designed for females. In the present study we aimed to compare the effects of two different chronic stress procedures in female mice, by investigating the impact of 4 weeks of nonsocial unpredictable, physical stress by the Chronic Mild Stress paradigm (CMS; Exp.1) or of Social Instability Stress (SIS; Exp.2) on physiological, endocrine and behavioral parameters in adult female mice. CMS had a pronounced effect on females' response to novelty (i.e., either novel environment or novel social stimulus), body weight growth and hormonal profile. Conversely, 4 weeks of social instability did not alter females' response to novelty nor hormonal levels but induced anhedonia. Our findings thus showed that female mice were more sensitive to nonsocial stress due to unpredictable physical environment than to social instability stressors. Neither of these stress paradigms, however, induced a consistent behavioral and physiological stress response in female mice comparable to that induced by chronic stress procedures in male mice, thus confirming the difficulties of developing a robust and validated model of chronic psychosocial stress in female mice.

1. Introduction

The impact of stressful life events in the etiology of several diseases is widely recognized in clinical and preclinical research (Schmidt et al., 2008). Chronic stress has been described to increase the risk for developing metabolic syndrome (Chandola et al., 2006), cardiovascular and inflammatory diseases (Holmes et al., 2006) and psychopathologies, such as Alzheimer's disease (Wilson et al., 2003), depression and anxiety disorders (Belmaker and Agam, 2008; Cryan and Slattery, 2007; Kendler et al., 1999; Kessler, 1997; Kessler et al., 2005; Lupien et al., 2009; Nestler et al., 2002; Wang, 2005). Many of these stress-related disorders exhibit gender bias in frequency, severity, or response to treatment (Wittchen et al., 2011; Kessler, 2007; Gobinath et al., 2017). Major depression, which is the most prevalent mental disorder worldwide according the World Health Organization statistics (WHO,), can be caused or enhanced by chronic stress exposure (Belmaker and Agam, 2008; Kendler et al., 1999), presents high comorbidity with anxiety-related disorders (Hirschfeld, 2001; Kessler et al., 2005; Kennedy, 2008) and is twice as common in women than in men (Gorman, 2006; Kendler et al., 1995, 2002; Kessler, 2007; Tafet and Bernardini, 2003; Wang, 2005; Wittchen et al., 2011).

Animal models involving chronic stress are particularly appropriate for emulating several neuropsychiatric disorders, to determine their underlying mechanisms and to find pharmacological treatment (Chaouloff, 2013; Davis and Pfaff, 2014; Goel and Bale, 2009; Pryce and Fuchs, 2016; Schmidt et al., 2008). However, human and animal data clearly indicate that individuals' perception of the stressfulness of a situation as well as the physiological and behavioral responses to stress are strongly dependent on gender; conditions that are stressful for males are not necessarily stressful for females, and the reverse (Bangasser and Wicks, 2017; Kokras and Dalla, 2014; Kudielka and Kirschbaum, 2005; Palanza, 2001; Palanza and Parmigiani, 2017). The

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development of appropriate animal models to investigate the biological basis of sex-biased differences in vulnerability to chronic stress is a major challenge of modern bio-medical research.

Unpredictability, novelty, lack of control, threat to self-esteem are key factors making people perceive a situation as stressful and eliciting a physiological and psychological stress response (Gruenewald et al., 2004; Hammen, 2005; Havranek et al., 2016; Wood et al., 2015). As Koolhaas et al. (2011) pointed out in their critical re-evaluation of the stress concept, "the term 'stress' should be restricted to conditions where an environmental demand exceeds the natural regulatory capacity of an organism, in particular situations that include unpredictability and uncontrollability". Accordingly, animal models of stress-induced disorders generally use chronic, unpredictable stressors involving either the physical or the social environment.

The chronic mild stress (CMS) procedure is a well-established rodent model for inducing behavioral changes commonly associated with clinical depression (Willner et al., 1992; Willner, 2017a). In this procedure mice or rats are exposed chronically to unpredictable environmental mild stressors such as cold, footshock, restraint, bright light, or forced swimming, resulting in behavioral changes, such as decreased response to rewards (considered a behavioral correlate of the core symptom of depression, anhedonia), decreased locomotor and explorative behavior, impairment of feeding, drinking and sexual behavior (Willner et al., 1987, 1992; Willner, 2017b). The CMS procedure induces several physiological changes (e.g., hypercortisolemia, hypertension) that are clinically associated with depression and many effects of CMS can indeed be reversed by antidepressant agents (Willner et al., 1987, 1996). Thus the CMS procedure appears to have face and predictive validity, at least when involving male mice or rats. A few studies have examined the effects of CMS on female rodents and they produced conflicting data; CMS can have an opposite effect to the expected depressed-like profile and the result was reported as an "anomalous" response to chronic stress (Willner, 2005). On the other hand, Dalla et al. (2005) reported that female rats were more vulnerable to chronic mild stress than males. Following CMS exposure, females showed decreased sucrose intake and open field activity, increased corticosterone levels, alteration in estrous cycle and decreased serotonergic activity in hippocampus and hypothalamus. In males CMS procedure induced only behavioral changes, such as decreased sucrose intake and open field activity (Dalla et al., 2005).

Human studies indicate that social rather than physical stress is associated to depression (Blazer and Hybels, 2005; Kessler, 1997). Thus animal models using unpredictable social stressors are currently considered the best available models of human psychopathological disorders (Blanchard et al., 1995; van Kampen et al., 2002). Chronic stress induced by social defeat and subordination has been a valuable tool to induce depressive-like behavior (Becker et al., 2008; Björkqvist, 2001; Fuchs and Flügge, 2002; Rygula et al., 2005). In male mice, defeat in aggressive encounters and chronic subordination due to unescapable exposure to the dominant animal, induce psychopathological changes and depressive-like behavior in male mice, accompanied by consistent alterations of hormonal, physiological, behavioral, immune and metabolic responses (Bartolomucci et al., 2004, 2005, 2009). Because of its congruence with the human condition, the defeat-induced loss of status in mice and rats has been proposed as a model of loss of self-esteem and depression in humans that parallels human psychiatric disorders related to negative emotions provoked by loss of social role, resources, and adverse social environment (Blanchard et al., 1995; Blanchard et al., 2001; Marrow and Brain, 1998; Willner et al., 1995). These models of defeat-related, psychosocial stress used for male mice (or rats) are, however, not appropriate for females, because female mice do not show high levels of competitive, territorial aggression or a strong dominance relationship when they are not in a reproductive state (Berry and Bronson, 1992; Palanza et al., 2005; Palanza and Parmigiani, 2017).

A number of models of social stress in female rodents have been proposed in order to elicit consistent behavioral, hormonal and metabolic changes. For instance, social deprivation by different lengths of social isolation have been widely used to induce a depressive-like profile in females (Brain, 1975; Jesberger and Richardson, 1985; Koike et al., 2009; Martin and Brown, 2010; Palanza et al., 2001; Hong et al., 2012), as well as the crowding procedure (Finger et al., 2012; Lin et al., 2015; Reiss et al., 2007). Moreover, social instability stress, consisting either of alternation of crowding and social isolation or housing in an unpredictable social environment, was reported to induce some stress-related changes in female rats and mice (Haller et al., 1999; Jarcho et al., 2016; Schmidt et al., 2010). Results from such studies, however, are often conflicting and the problem is finding a consistent behavioral and physiological profile indicative of stress-related alterations in female mice (Jarcho et al., 2016; Schmidt et al., 2010). Virtually none of the proposed model of physical or social stress is, indeed, recognized to produce a consistent and coherent set of behavioral and physiological responses indicative of depression- and anxiety-like profile in adult female mice. In addition, not all depression and anxiety disorders in women, but also in men, are alike (Goldberg, 2011; Insel et al., 2010; Nandi et al., 2009). Therefore, different rodent models are likely to apply to different expressions of depressive and/or anxiety disorder (Brand et al., 2015; O'Leary and Cryan, 2013). Although women are more vulnerable to several stress-related disorders, such as major depression and anxiety, an apparent paradox is that rodent models are still mostly based on males (Palanza and Parmigiani, 2017; Zucker and Beery, 2010).

The aim of the present study was to compare the effects of two different chronic stress procedures, based on unpredictability of physical or social environment, upon several behavioral and physiological responses of female mice, in order to validate a behavioral procedure that may serve as a female animal model for stress-related depressive or anxiety disorders. Since large variability among different mouse strains is reported for stress responses, anxiety and depression-like behaviors (Belzung and Griebel, 2001; Ibarguen-Vargas et al., 2008; Milner and Crabbe, 2008; Mineur et al., 2006), we examined CD1 outbred mice, which reflect the genetic diversity in natural populations and can be used in behavioral studies in a translational perspective (Vogt et al., 2017; Palanza and Parmigiani, 2017). The ethogram of CD1 mice, among many mouse strains, is similar to that of wild mice, in particular for their social and emotional behavior (Chalfin et al., 2014; Holmes et al., 2000; Parmigiani et al., 1998, 1999). We carried out two experiments investigating the consequences of 4 weeks of either unpredictable CMS (Exp.1) or social instability (Exp.2) on behavioral, metabolic and endocrine parameters in adult female mice. At variance with Haller et al.'s (1999) procedure in rats, which also employed social isolation and crowding, social instability was achieved here by daily switching the cagemates that the experimental female was housed with. Isolation and crowding per se may indeed be stressful conditions for female mice (Beery and Kaufer, 2015; Martin and Brown, 2010; Reiss et al., 2007), while we aimed to evaluate the impact of instability of the social network in group-housed female mice.

We examined the experimental mice in different behavioral test for assessing anxiety, anhedonia, social exploration and novelty responses and evaluated the metabolic consequences of the stress procedures on females' body weight growth, food intake and glucose plasma level. At the end of the study, we measured plasma Corticosterone (C) and Adrenocorticotropic hormone (ACTH) and organs' weigh to determine stress-related physiological alterations.

2. General methods and materials

2.1. Experimental subjects

Experimental subjects were outbred CD1 female mice (*Mus musculus*) derived from Charles River Italia (Calco, Italy), born and reared in our *vivarium* at the Laboratory of Behavioral Biology at the University of Parma. Animal room temperature was set at 22 ± 2 °C with a 12 h light-dark cycle (lights on 07:00). Food (4rf21 standard diet, Mucedola, Italy) and water were available ad libitum. After weaning (25 days) mice were housed in same-sex groups of siblings (5 ± 1 per cage) in Plexiglas cages (45 cm × 25 cm × 20 cm) with wooden dust-free bedding (Mucedola, Italy) changed weekly. When group-housed, females remained in cages measuring 45 cm × 25 cm × 20 cm, whereas when individually housed females remained in Plexiglas cages measuring 20 × 25 × 15 cm. Housing and experiments were conducted in accordance with the animal experimentation European Communities Council Directive of 24 November 1986 (86/EEC) and approved by the Italian Institute of Health. When 70 days old, adult female mice underwent experimental procedures and were randomly assigned to one of the following 2 experimental procedures of 4-weeks exposure to chronic stress:

1) Unpredictable Chronic Mild Stress Procedure (UCMS, Experiment 1) and
2) Social Instability Stress Procedure (SIS, Experiment 2), described below.

Throughout the experiments we monitored body weight and food intake and performed behavioral tests as described below. Following each behavioral test, females were checked for the estrous phase by vaginal smears. No significant differences were observed with respect to the stage of the estrous cycle. The majority of females was found to be in the diestrous phase of the cycle; the small number of females for each phase prevented a statistical analysis.

2.2. Body weight and food intake

At the beginning of each experiment, mice were weight-ranked to ensure similar body weights between groups. On day 2, day 14 and day 28 we monitored with a digital balance accurate to 0.01 g. (Sartorius, Guxhagen, Germany) body weight and food intake as an indicator of eating disorders as reported in several stress-related disorders (Block et al., 2009; Razzoli and Bartolomucci, 2016). During CMS we did not measure the daily food intake because the amount of food available to the animal varied different times in a week, making the food intake measurement not feasible.

2.3. Behavioral tests

At different time-points of Experiment 1 and Experiment 2 stress procedures (Fig. 1), experimental females underwent the following behavioral tests to evaluate anxiety and/or depression-like responses induced by either environmental or social stress:

- ***Sucrose preference test*** (day 2, 14 and 28). Sucrose preference is the most widely used measure of anhedonia (as decreased response to rewards), which is a symptom of depression (e.g., Willner et al., 1992; Konkle et al., 2003). Briefly, 2 bottles were provided to each experimental animal: 1 with a 1% sucrose solution and the other only water. The bottles were weighed before the test and immediately after its completion 24 h later. Sucrose solutions were prepared by weight from commercial-grade sugar and tap water.
- ***Free exploratory open field (free OF) test*** (day 16). The testing procedure used in this study was based on the method described by Palanza et al. (2001). The apparatus consisted of 2 sections: a familiar area, the home cage (30 × 20 × 20 cm) in which animals were individually housed for 24 h before testing and an unfamiliar area, an open field, OF (73 × 110 × 50 cm). The floor of the home cage was covered by sawdust and food and water were freely available. The top of the chamber was made of transparent Plexiglas to allow observation and videotape recording. The home-cage was connected to the OF by means of a small opening (5-cm diameter) which was closed with a removable barrier until testing. In the OF, a light positioned behind and above the OF wall, so as to cast a shadow along the length of the OF, produced a bright and a dark area. 24 h before testing, subjects were gently handled and moved into the experimental home-cages. Tests were conducted between 14:00 and 18:30 in a room with a dim white light. 5 min before testing, the home cage was placed at one end of the OF. Once the barrier was removed, a cutoff of 10 min was used for animals that did not enter the OF. Risk assessment behavior and time spent to explore each zone were recorded. A maximum time of OF exploration of 5 min was given to animals after the first entry into the unfamiliar arena. In the OF there were the following 8 zones: "near dark", "near bright", "far dark", "far bright", "wall dark", "wall bright", "center bright" and "center bright" zones. To reduce any olfactory cues, the maze was cleaned with water and a 2% ethanol solution between trials. All behavioral tests were recorded by means of a camera above the apparatus and carried out starting 16:30 to 18:30.
- ***Elevated Plus Maze (EPM) test*** (day 20). The EPM apparatus consisted of a black wooden platform covered with transparent plastic, with 2 open arms (30 × 5 cm) and 2 closed arms (30x5x40 cm). The arms extended from a central platform (5 × 5 cm) and the maze was elevated 50 cm above the floor. Mice were individually placed in a central platform facing an open arm and allowed to explore the maze freely for 5 min. The frequency of entries and time spent in the both the open and closed arms were recorded and analyzed by a trained observer using a specific software (The Observer, Noldus, The Netherlands).
- ***Novelty induced suppression of feeding (NISF) test*** (day 24). NISF test is a novel, minimally invasive test, which measures the latency of an animal to approach and eat a familiar food in a novel environment and assesses the animal anxiety-like state (Dadomo et al., 2011). Starting from day 24, animals underwent the NISF test in their home cages. Mice were presented in the home cage with a novel but highly palatable food, i.e. half a peanut, on a petri dish once a day for 4 consecutive days (days 12–15) starting at 9:00. On day 4, when the latency to eat the palatable food was expected to be decreased because of habituation to novelty and hedonic response to the palatable food, the peanuts were presented in a normal housing

Fig. 1. Timeline of behavioral tests and physiological data collection during CMS and SIS procedure.

cage in which bedding was changed (following the rationale previously established for the novelty induced suppression of feeding test for anxiety (Merali et al., 2003)). Latency to eat the peanut was recorded with a cut-off time of 600 s. If mice did not eat the peanut after 600 s the peanut was left in the cage. NISF test was scheduled daily at 9:00 to avoid a direct effect of aggression received and far away from circadian maximum of food intake which occurs before lights off in mice. Only CMS female were subjected to NISF test.

- ***-Social Open Field (social OF) test*** (day 26). The mouse was introduced into an open field (54 cm × 54 cm) for 2 consecutive trials of 2.5 min each (Bartolomucci et al., 2009). During the first trial (T1, "no target'), the open field contained an empty wire–mesh cage (10 cm diameter, 30 cm high). During the second trial (T2, 'Target'), an unfamiliar female mouse of the same age and strain of the experimental subject was introduced into the wire–mesh cage and served as unfamiliar social stimulus. The "social area" was an 8 cm-wide area around the wire–mesh cage in which the unfamiliar social stimulus was located before the beginning of T2. Between trials, the focal mouse was placed back into its home-cage. Locomotion, total distance moved (cm), total time spent in social area and corners of open field (8-cm square areas in the corners at the opposite end to the "social area"), and latency to reach the social area were recorded and analyzed by a trained observer using a specific software (Ethovision, Noldus, The Netherlands). Walking, risk assessment, stretching, rearing, wall rearing and self-grooming behaviors and immobility were scored by means of The Observer software (Noldus, The Netherlands).

2.3.1. Glucose level

On day 29 of the stress procedure, following an overnight fasting, Glucose blood concentration was determined in mice tail blood using a glucometer (Accucheck Aviva; Roche Diagnostics, Indianapolis, IN, USA).

2.3.2. Blood and organ collection

Mice were sacrificed 2 h after lights were on (lights on at 08:00). In order to determine plasma concentrations of corticosterone and ACTH, trunk blood was collected in heparinized tubes, centrifuged and frozen at − 20 °C for later analysis. Adrenals, spleen, thymus, ovaries, uterus, white (perigonadal, visceral) and brown (intra scapular) adipose tissues were dissected and weighed.

2.3.3. Corticosterone and ACTH concentrations

Circulating levels of corticosterone and ACTH were directly measured in plasma. Hormonal concentrations were determined by enzyme-linked immuno-absorbent assay, respectively for ACTH (Mouse ACTH – adrenocorticotropic hormone ELISA E-EL-M0079, Elabscience Biotechnology Co., Ltd. Whuan, Hubei, China) and Corticosterone (Mouse CORT – corticosterone hormone ELISA E-EL-M0349, Elabscience Biotechnology Co., Ltd. Whuan, Hubei, China). Both ACTH and CORT samples were treated in duplicate and in the single assay in randomized order. The intra and inter assay variation coefficient were 4.4 and 6.5% for ACTH; 6.5 and 9.3% for CORT. The sensitivities were 9.38 pg/ml for ACTH and 0.19 ng/ml for CORT. The intra- assay coefficient of variation was < 10% for both the ACTH and CORT measures.

2.4. Statistical analysis

For each experiment, 1-way ANOVA was run to analyze data from EPM and free OF tests, glucose level and organ weight. A 2-way ANOVA for repeated measures was used to analyze data of social OF, NISF, sucrose preference, body weight, and food intake. When necessary, post hoc comparisons were assessed by Tuckey test. To calculate effect sizes, eta squared (η2) or Cohen's d were calculated, where appropriate.

Data were analyzed by Statistica 8.0 Software (Stat-Soft, Tulsa, OK, USA).

3. Experiment 1: effects of unpredictable chronic mild stress in female mice

CMS has been largely used to study affective disorders induced by unpredictable environmental stressors, such as cold, footshock, restraint, or forced swimming, water/food deprivation, continuous light/noise. We followed a modified CMS procedure as described by Willner et al. (1987) and Grønli et al. (2004).

3.1. Procedure

Twelve female mice were individually housed and exposed to the CMS procedure; 11 control females were individually housed and left undisturbed throughout the experiment. Following 1 week of isolation, the CMS procedure lasted four weeks. Each week consisted of the following periods:

1. 6 h of food deprivation during daylight followed by 15 h of restricted access to food (one pellet);
2. 5 h of water deprivation followed by 1-h exposure to an empty bottle, twice a week;
3. 15 h in a wet cage (200 ml of water in 100 g of sawdust bedding), twice a week;
4. 15 h in an empty cage, twice a week;
5. 24 h of continuous light;
6. 4 h of white noise, twice a week;
7. 30 min of restraint stress in a 50-ml falcon, twice a week; and
8. each animal was daily injected with saline.

Since unpredictable stressors have a greater negative impact than predictable stressors due to their uncertainty (Bondi et al., 2008), stressors were presented in an unpredictable way as described in Table 1. Throughout the experiment, we monitored body weight and performed behavioral tests as described above in the general method section and in the testing timetable in Fig. 1.

3.2. Results

3.2.1. Free Open Field

When challenged in the free OF test (Fig. 2A, B, C, D and E), CMS mice did not differ from control females for the latency to enter the OF, time spent exploring the arena, time in the center area and locomotor activity. However, CMS females spent significantly less time in the bright area of the OF compared to controls (CMS: $F_{1,22} = 4.66$, $p < 0.05$, $\eta^2 = 0.17$, Fig. 2D).

3.2.2. EPM

CMS mice showed a no anxious-like behavioral profile, since they spent significantly more time in the open arm ($F_{1,21} = 6$, $p < 0.05$, $\eta^2 = 0.22$, Fig. 2F), less time in the center of EPM ($F_{1,21} = 6.05$, $p < 0.05$; $\eta^2 = 0.22$, Fig. 2G), and they showed higher locomotor activity as measured by the frequency of transitions ($F_{1,21} = 4.35$, $p < 0.05$, $\eta^2 = 0.17$, Fig. 2H) than controls.

3.2.3. NISF test

During the habituation phase of NISF test (Fig. 2I), females exposed to CMS showed increased latency to feed ($F_{1,22} = 5.55$; $p < 0.05$, $\eta^2 = 0.19$), specifically on day 1 ($p < 0.05$, d = 0.97; CMS vs con-

Table 1
Time and length of stressors used in 1 week of the chronic mild stress procedure.

Day	Restrain test	Empty cage	Wet cage	Water deprivation	Empty bottle	White noise	Continous light	Food deprivation	Food Restricted	Saline
Monday	1000 ? 1030	1830 ? 0000								0930
Tuesday		0000 ? 0930	1830 ? 0000	1000 ? 1700	1700 ? 1800					0930
Wednesday			0000 ? 0930			1200 ? 1600	1900 ? 0000			0930
Thursday	1500 ? 1530	1830 ? 0000					0000 ? 0700			0930
Friday		0000 ? 0930		1000 ? 1700	1700 ? 1800					0930
Saturday			1830 ? 0000					1230 ? 1830	1830 ? 0000	0930
Sunday			0000 ? 0930			1200 ? 1600			0000 ? 0930	0930

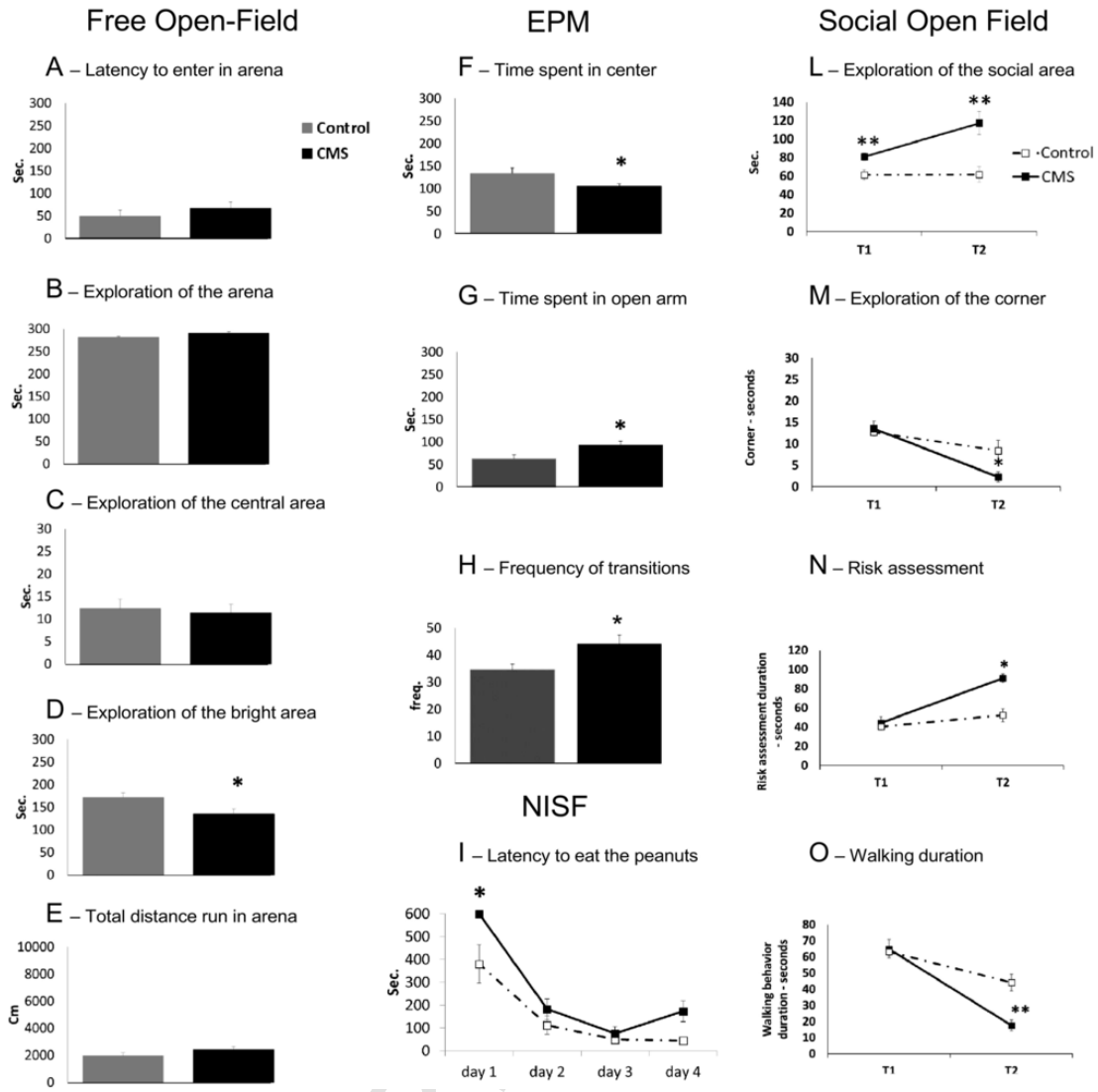

Fig. 2. Behavioral responses of CMS and Control female mice. (A to E) Behavior of CMS (black bar) and control (grey bar) females in the free exploratory paradigm; (F to H) behavioral responses in the EPM test; (I) Latency to consume a highly palatable food over four consecutive days in the NISF procedure by CMS (black line) and control (dashed line) mice; (L to O) activity in the social open field test by CMS (black line) or control mice (dashed line), T1 = no target condition; T2 = target condition. Data are expressed as mean ± SEM. $^{*}p < 0.05$, $^{**}p < 0.01$ vs control.

trols). As expected, all mice habituated to the novel food over time (Trials: $F_{3,66} = 44.65$; $p < 0.0001$, $\eta^2 = 0.20$).

3.2.4. Social Open-field

During the social OF, when presented to an unfamiliar conspecific (T2), CMS mice spent significantly more time in the "social area" ($p < 0.0001$, d = 1.60; CMS × trial: $F_{1,14} = 10.55$; $p < 0.01$, $\eta^2 = 0.26$, Fig. 2L) and in risk assessment behavior ($p < 0.05$; d = 1.08; CMS × trial: $F_{1,17} = 7.7961$, $p < 0.05$; $\eta^2 = 0.23$; Fig. 2N), and less time in the corners ($p < 0.05$; d = 1; Fig. 2M) and walking ($p < 0.005$, d = 1,53; CMS × trial: $F_{1,14} = 14.24$; $p < 0.005$; $\eta^2 = 0.29$; Fig. 2O) as compared to controls. During both trials (T1 and T2), CMS females spent significantly more time in the social area than controls ($F_{1,21} = 15.30$; $p < 0.001$, $\eta^2 = 0.45$; Fig. 2L).

3.2.5. Body weight

As shown in Fig. 3A, CMS significantly reduced body weight gain ($F_{2,42} = 8.56$, $p < 0.005$, $\eta^2 = 0.31$).

3.2.6. Anhedonia

As shown in Fig. 3B, CMS did not affect females' preference for a sucrose solution.

3.2.6.1. Physiological measures Following an overnight fasting, baseline glucose blood levels were significantly higher in CMS mice than controls ($p < 0.05$; d = 1.5 ; Fig. 3C). CMS significantly increased circulating corticosterone levels ($p < 0.01$; d = 1.10; $F_{3,66} = 5.82$, $p < 0.0001$; $\eta^2 = 0.18$; Fig. 3D), without affecting ACTH levels (Fig. 3E) nor adrenal weight (Fig. 3F) or other organs' weight (data not shown).

4. Experiment 2: effects of social instability stress in female mice

In female rodents, chronic social stress involving social instability generally results in higher anxiety-related behaviors and physiological alterations (in rats: Haller et al., 1999; Herzog et al., 2009; in mice: Jarcho et al., 2016; Schmidt et al., 2010). Under present conditions we exposed female mice to unpredictable daily changes of their social companions. We employed a procedure for inducing social instability

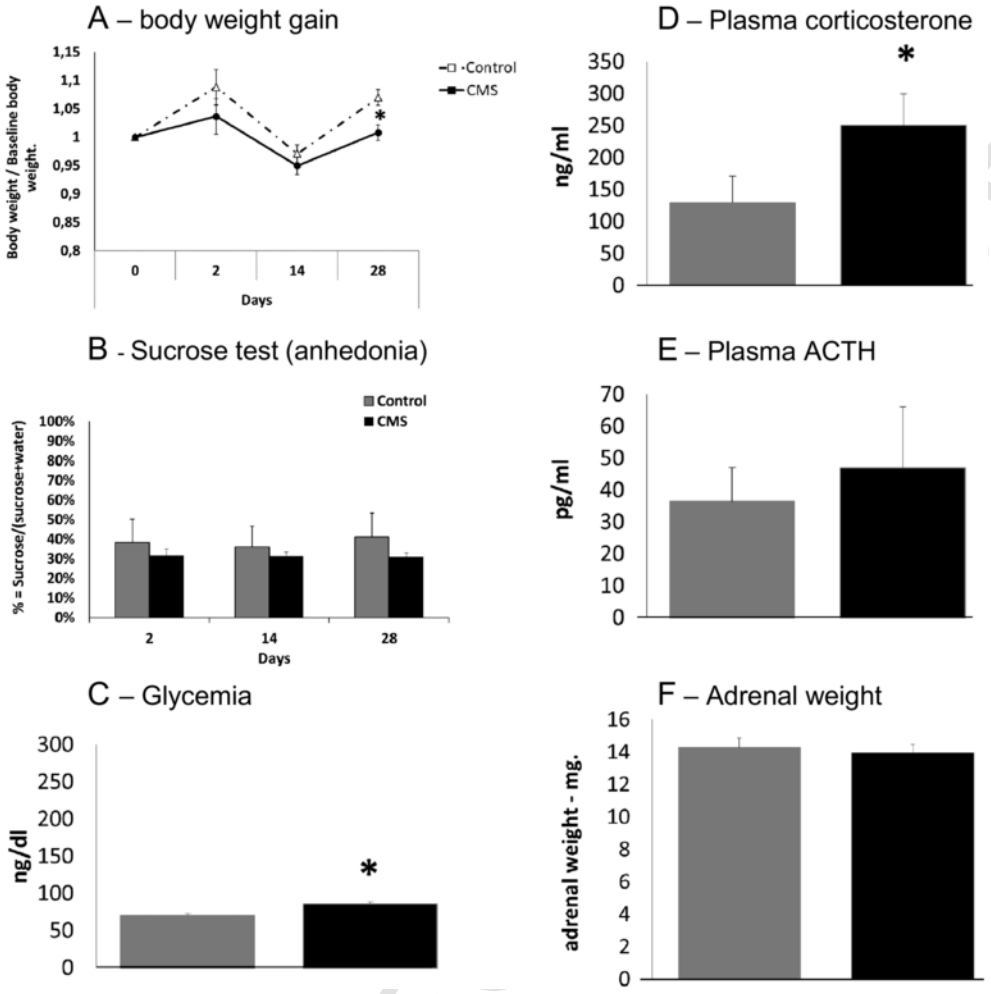

Fig. 3. Physiological responses and anhedonia in CMS and Control female mice. (A) Body weight gain at day 2 (T0), 14 (T1) and 28 (T2) in CMS (black line) and control mice (dashed line). (B) Sucrose test for anhedonia: percentage of preference for sugar consumption at day 2 (T0), day 14 (T1) and day 28 (T2). C) Plasma glucose level at the end of the stress procedure in CMS (black bar) and control (grey bar) mice. (D) Plasma corticosterone levels; (E) plasma ACTH; (F) adrenal glands weight. Data are expressed as mean ± SEM. *$p < 0.05$, vs control animals.

differing from that used in Haller et al.'s (1999) and Jarcho et al.'s (2016) studies, in which isolation and crowding were used in addition to changing the experimental subject's cagemates, and from that reported in s study (in mice,), in which females were exposed to social instable groups since adolescence.

4.1. Procedure

Female mice ($n = 22$) were randomly assigned to a social stress ($n = 11$) or to a control ($n = 11$) group. The social stress group received 4 weeks of daily social instability stress. Every day the experimental resident female was group-housed with 2 unfamiliar female CD1 mice ("Unfamiliar-Group" females). The group composition was changed every day at a different random time by removing from the experimental subject's cage the two female partners and introducing two new, unfamiliar females. The rotation schedule was accurate to minimize the likelihood of a repeated encounter with the same mice throughout the experiment. A "Sister Group", in which the experimental female was group-housed with two sibling females since weaning, served as control group. In the control "sister group", the experimental female's cagemates were gently handled at the same time and in a similar fashion as in the Unfamiliar-Group condition.

Throughout the experiment, we monitored body weight and food intake and performed behavioral tests as described above in the general method section and in the time-schedule pictured in Fig. 1.

4.2. Results

4.2.1. Behavioral tests

Social Instability stress did not significantly alter mouse behavioral profile in the free OF, in the EPM, and in the social OF tests (data not shown).

4.2.2. Anhedonia

SIS induced an anhedonic response, with SIS females drinking significantly less sugar solution than controls during all the trials ($F_{1,17} = 8.00$, $p < 0.05$, $\eta^2 = 0.26$, Fig. 4C). In both experimental groups, however, sucrose preference significantly increased throughout the experiment ($F_{2,24} = 7.05$, $p < 0.005$, $\eta^2 = 0.24$; Fig. 4C).

4.2.3. Body weight and food intake

SIS significantly reduced food intake ($F_{1.20} = 12.42$, $p < 0.005$; $\eta^2 = 0.35$; Fig. 4B) and body weight gain at all-time points ($F_{1.20} = 20.39$, $p < 0.0005$; $\eta^2 = 0.47$; Fig. 4A; SIS females vs controls

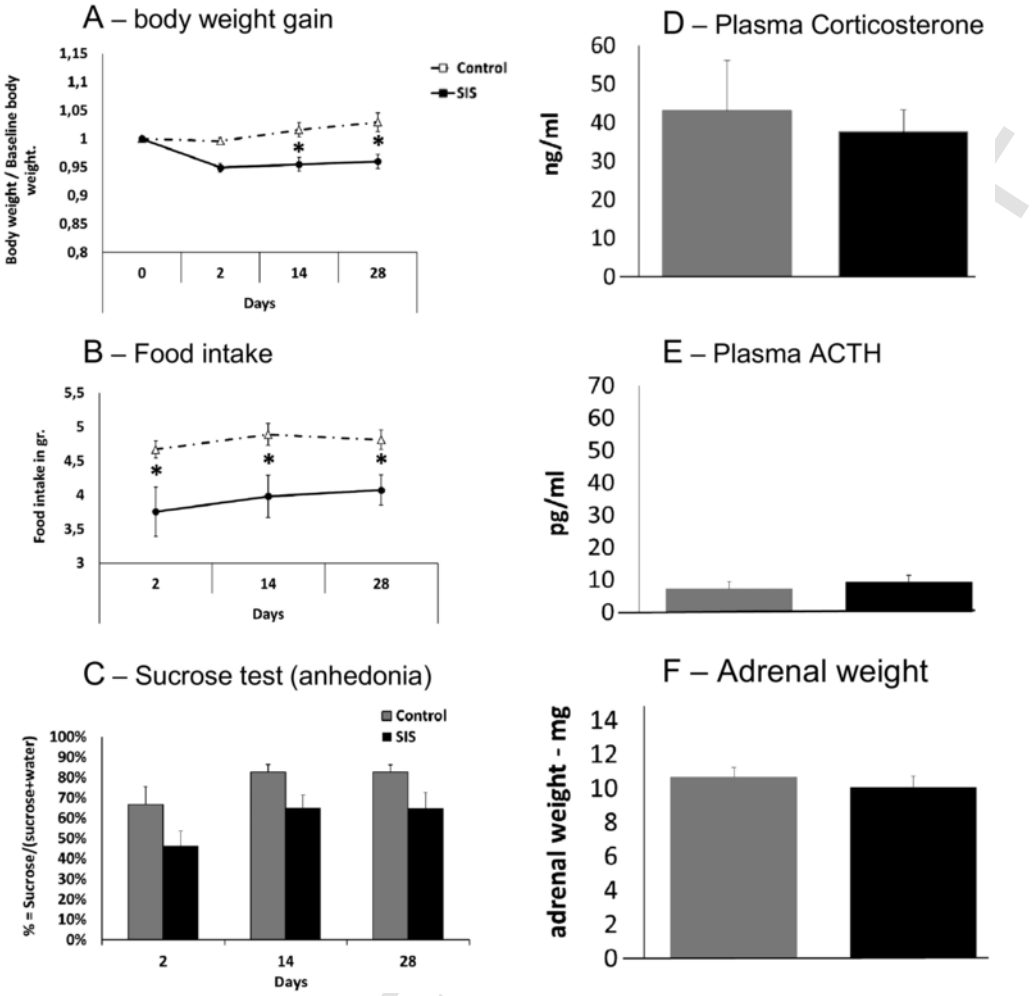

Fig. 4. Physiological responses and anhedonia in SIS and Control female mice. A) Body weight gain on day 2 (T0), day 14 (T1) and day 28 (T2) in SIS (black line) and control (dashed line) mice; B) food intake at day 2 (T0), day 14 (T1) and day 28 (T2); C) sucrose test for anhedonia: percentage of preference for sugar consumption at day 2 (T0), day 14 (T1) and day 28 (T2). D) Plasma corticosterone levels; E) plasma ACTH; F) adrenal glands weight. Data are expressed as mean ± SEM. $^{*}p < 0.05$, vs control mice.

on day 2, 14 and 28, $p = 0.06$, d = 3.7; $p < 0.01$ d = 2.12 and $p < 0.005$ d = 3.4 vs controls, respectively).

SIS did not affect circulating levels of CORT (Fig. 4D) and ACTH (Fig. 4E) nor adrenal weight (Fig. 4F) or other organs' weight (data not shown).

Discussion

We evaluated the effects of chronic stress due to unpredictable environment of either physical or social nature on behavioral and hormonal profiles of female mice. We employed two different chronic stress protocols, the unpredictable chronic mild stress-CMS (experiment 1) or a social instability stress paradigm (SIS, experiment 2), which differentially affected physiology and behavior of female mice. CMS had a pronounced effect on females' behavioral response to novelty (i.e., either novel environment or novel social stimulus), hormonal profile and body weight. Conversely, SIS only marginally affected females' behavioral and hormonal profile, whereas it increased anhedonic response and decreased body weight growth and food intake.

Following CMS exposure, female mice were less anxious (more time on the open arms than controls) and more active on the EPM (greater number of arm transitions). In the free open field, however, females showed a conflicting profile. They displayed similar levels of exploration and locomotor activity in the arena as controls, but they spent lower time in the bright area than controls. Present findings on EPM and free open-field tests contrast with our expectations and literature. Indeed, previous studies reported a clear increase in anxiety following CMS exposure as indicated by increased anxious-like behaviors displayed by female mice in both the EPM (Guilloux et al., 2011; Mineur et al., 2006; Zhu et al., 2014) and the open field (Mineur et al., 2006; Zhu et al., 2014; Filho et al., 2015). Discrepancies between previous studies and present data could be ascribed to strain differences or to different methodological procedures. To the best of our knowledge, there is virtually no study assessing the effects of CMS procedure on CD1 outbred female mice, and previous experimental findings only refer to specific inbred strains. As remarked by Kokras and Dalla (2014) in their comprehensive review, it can be difficult to test female rodents in traditional paradigms measuring anxiety-like behaviors (especially the EPM test; Imhof et al., 1993), since they might not be well validated to discriminate between anxiety-like behaviors and arousal (Fernandes et al., 1999) or to reflect the same emotional states in females and in males. The reported inconsistencies in the outcome measures for anxiety and depression-like responses might indicate that distinct processes or emotional states are involved (Shansky, 2015).

When subjected to the social open field test, CMS females explored more a novel mouse but showed higher risk assessment behavior than control females. Again, CMS females seem to show a conflicting profile as increased social exploration should indicate lower anxiety towards

novel conspecifics (Koolhaas et al., 1999, 2007), whereas risk-assessment behavior is traditionally considered as an anxiety-like marker (Blanchard et al., 2011; Dadomo et al., 2013). Risk assessment is, however, an "active coping" defensive strategy, as opposed to freezing behavior, which is considered a "passive coping" defensive strategy (Lu et al., 2008; Yen et al., 2013). The fact that CMS stressed females risk assessed more the environment when exposed to an unfamiliar mouse should therefore not be interpreted as an index of social anxiety or social avoidance but rather indicative of higher arousal, since they remained more time in the social area compared to controls. Accordingly, in the novelty-induced suppression of feeding test, during the training period CMS females showed more initial food neophobia compared to controls but they did not show suppression of feeding induced by a novel environment, which is a traditionally accepted measure of animal anxiety-like response (Joeyen-Waldorf et al., 2009; Guilloux et al., 2011; Zhu et al., 2014).

The measure most commonly used to track CMS effects is a decrease in consumption of a palatable sweet solution as a model of anhedonia (decreased response to rewards), which is considered a key index of depressive-like behavior (Willner, 2005). In our experiment, the sucrose preference test showed no CMS effects on females' response to rewarding stimuli (i.e., anhedonic-like response), as opposed to other studies reporting anhedonia in female mice exposed to CMS procedures (Zhu et al., 2014; Filho et al., 2015; Karisetty et al., 2017). Again, strain and methodological differences might explain this discrepancy. For instance in our study animals were not water deprived before testing and were in their home cage (e.g., Zhu et al., 2014; Filho et al., 2015). Basic strain differences are reported for sucrose preference and reward sensitivity (Pothion et al., 2004; Ducottet and Belzung, 2005; Mineur et al., 2006) as well as for stress-related HPA activation (Anisman et al., 1998; Novais et al., 2017) and behavioral alterations (Belzung and Griebel, 2001; Bartolomucci et al., 2005; Sterlemann et al., 2008).

Present findings overall indicate that CMS did not induce depression-like nor high anxiety behaviors in female mice but rather it seemed to increase females' responsiveness to novelty, indicative of increased arousal. The physiological profile of female mice subjected to CMS supports this hypothesis, since 4 weeks of CMS affected both metabolic and hormonal profiles in females. More specifically, CMS reduced body weight gain and increased blood glucose and corticosterone levels. Generally, body weight reduction is regarded as a marker of stress severity in female (Bruder-Nascimento et al., 2013; Sachs et al., 2014) and male rodents (Kurhe et al., 2014a, 2014b). Previous studies reported no CMS effect on body weight in female BALB/c (Stanley et al., 2014) or an opposite effect with increased weight gain in BALB/c males (Isingrini et al., 2012). Again, different mouse strains, different diets, as well as the different stressors and test-timing employed may account for discrepancies among different studies. In addition, high corticosterone and glucose levels are considered chronic stress biomarkers (McEwen and Sapolsky, 1995; Ibarguen-Vargas et al., 2008; Rubinstein et al., 2010; Silverman and Sternberg, 2012). As stress response activates the HPA axis, the subsequent corticosteroid hypersecretion helps maintain glucose homeostasis by means of gluconeogenesis, lipogenesis and cellular glucose uptake (Chrousos, 2009). Specifically, cortisol influences mobilization of stored fat and carbohydrate reserves resulting in increased glucose blood levels (Ibarguen-Vargas et al., 2008). Our data are consistent with previous CMS studies in indicating circulating glucocorticoid increase in female (Stanley et al., 2014; Doron et al., 2014) and male (Filho et al., 2015) mice. Present results thus provide further evidence for CMS paradigm inducing stress-related disorders and HPA dysregulation in female mice. Moreover, present results are consistent with the observed behavioral effects and our hypothesis of increased reactivity to novelty of female mice exposed to unpredictable changes of the physical environment.

Present results on CMS-induced changes in females are marginally similar to what reported in previous studies in male Swiss albino mice (Sharma and Thakur, 2015; Willner et al., 1996), kunming mice (Tang et al., 2005; Jin et al., 2015), C57BL/6j mice (Liu Y. et al., 2015; Yang et al., 2015), Tph2KI mice (Sachs et al., 2014), or different mixed strain (Pothion et al., 2004). Male mice exposed to CMS generally showed a consistent increase of anxiety both in the EPM and in the OF test, associated to decreased consumption of palatable sucrose solution (i.e., increased anhedonia), decreased body weight growth and increased corticosterone level (Swiss albino or CD1: Kormos et al., 2016; Sharma and Thakur, 2015; Willner et al., 1996; C57BL/6J: Zhu et al., 2014; Yang et al., 2015; ICR: Liu B et al., 2015; Deng et al., 2015; Jin et al., 2015; Tang et al., 2005). Sex differences in physiological and behavioral effects of CMS procedures are reported, though only a limited number of studies have been conducted on both sexes and they only involved inbred strains (Autry et al., 2009; Hill et al., 2012; Franceschelli et al., 2014).

Instability of the social environment (4 weeks of social instability SIS) did not affect females' behavioral responses to a novel environment or to a novel social stimulus but affected females' anhedonic response. Female mice exposed every day to unpredictable social environment showed a reduced consumption of a palatable sweet solution, an anhedonic-like response. Consistently, SIS decreased both food intake and body weight of females. Concerning the hormonal profile, in the present study 4 weeks of SIS did not alter CORT and ACTH plasma levels.

Haller et al. (1999) were first to suggest that social instability induced by alternation of isolation and crowding, was a powerful social stressor for female rats, whereas social defeat was stressful for male rats. Schmidt et al. (2010) applied an instable social setting paradigm to mice by changing twice a week the social group, and concluded that housing in an unpredictable social environment from weaning throughout adulthood resulted in chronic stress in female CD1 mice. In Schmidt's study, female mice exposed to social instability showed physiological responses indicative of stress (e.g., higher corticosterone and adrenal gland weigh, lower thymus weigh, stress-system-related genes expression), but they did not display a consistent behavioral profile indicative of anxiety and/or depression-like responses. Indeed, only one (NISF) out of three (NISF, EPM and OF) anxiety tests showed a significant alteration of an outcome measure (Schmidt et al., 2010). Remarkably, in male mice the same social instability procedure induced a more consistent set of stress-related effects (Sterlemann et al., 2008). At variance with Schmidt's study, under present conditions social instability (i.e., switching cagemates daily) did not alter corticosterone levels, adrenal weight and anxiety-like responses, but it decreased body weight and food intake, and induced anhedonia. These contrasting results may be due to different procedures, as we exposed females to 4 weeks of SIS as adults, while Schmidt's procedure lasted 7 weeks and included the post-weaning, "adolescent" period (puberty), which is a sensitive developmental period (Holder and Blaustein, 2014). In a recent study, Jarcho et al. (2016) reported that CD1 female mice exposed to an alternation of social isolation and crowding for 5 weeks showed none or marginal behavioral differences on the elevated plus maze and in the open field tests, reduced body weight and higher hair corticosterone when compared to control females. Despite methodological discrepancies, all these studies, including the present one, indicate that social instability does not induce a coherent set of physiological and behavioral changes in female mice, comparable to those induced by social stress procedures in male mice (Bartolomucci et al., 2004, 2005; Sterlemann et al., 2008).

Present data point out that the nature of the stress is relevant and it differently matters in males and females. Based on current literature, male CD1 mice subjected to CMS (Willner et al., 1996; Willner, 2005; Kormos et al., 2016) or to social instability (Schmidt et al., 2007;

Sterlemann et al., 2010; Boleij et al., 2014) showed a similar, consistent set of behavioral and physiological alterations, though SIS procedure did not affect body weight in males (Schmidt et al., 2007). Here we demonstrated that both stress paradigms appear to induce some kind of "stress" response in female mice. However, we must distinguish between the chronic stress elicited by interactions with the non -social environment (i.e. cold, heat, water and food supply, predators – CMS) and the chronic stress elicited by the social interactions with conspecifics (i.e. social instability). Despite social instability appeared to be stressful for females, as depicted by anhedonia and reduction of body weight growth and food intake, female mice adjusted to it by maintaining (or restoring) homeostasis as they showed neither hormonal nor behavioral alterations, i.e. they seemed resilient to social instability stress. One hypothesis is that the length of the stress procedure could have affected females' adaptation to stress. An alternative hypothesis is that instability of the social context is a "disturbance" for females, but it does not represent a threat for their fitness to induce persistent behavioral and physiological stress-responses.

As a whole, CMS did not induce depression-like responses in female mice but it rather induced higher arousal and reactivity to novelty, partially associated to anxiety. Detailed behavioral analysis suggested that female mice subjected to CMS showed an active rather than a passive coping response when challenged with novelty. This seems a highly adaptive response to cope with adverse environmental conditions. From an evolutionary perspective, considering the social and reproductive biology of the House mouse, we should recognize a major role of environmental constraints for female reproductive success as compared to social constraints, because for mammalian females, reproductive success tends to be mostly limited by access to resources (Krebs and Davies, 1997). Male reproductive success is mainly limited by access to females and therefore, social role is a major constraint for male mouse reproductive success, as only dominant males can reproduce (Bronson, 1985; Vom Saal et al., 1995; Palanza et al., 1996, 2005). Indeed, based on potential threat to reproductive success and on elicited HPA response, social defeat is reported to be a major stress for male rodents (i.e., Koolhaas et al., 2011), while our study indicates that unpredictable negative environment is a major stress for female mice.

It should be considered, however, that both procedures (CMS and SIS) decreased females' food-intake and body weight growth. For female mice body mass and food consumption are generally positively correlated with reproductive success (Bronson, 1985; Ruff et al., 2017; Sadowska et al., 2013). Thus it is possible that reduced body weight and food intake might impact females' reproduction and fitness over time. Next challenge in female stress research would be assessing potential long-term costs of environmental or social instability stress on female mouse reproduction.

Overall our findings showed that female mice were more sensitive to unpredictable physical stressors than to social instability stressors. Furthermore, our findings, together with previous literature, confirm the difficulties of inducing a consistent behavioral and physiological stress response in female mice and point out that we do not have a robust and validated model of chronic psychosocial stress in female mice, yet. This represents a scientific flaw in animal modelling of human stress-related disorders. Since human data clearly indicate that women are more susceptible to several mental disorders, it is necessary that translational neuroscience incorporate sex as a biological variable in order to understand the complex aspects of individual susceptibility to stress and stress-related disorders (Joel and McCarthy, 2017). Further studies are needed to deepen our knowledge on the compensation mechanisms females use to cope with chronic stress.

Conflict of interest statement

None.

Authors' contributions

HD planned and executed experiment 1, performed data analysis and graphics and collaborated writing the paper; LG planned and executed experiment 2, performed data analysis and graphics and collaborated writing the paper; JC collaborated in the execution of experiments and data analysis; GC performed and discussed hormonal analysis; SP contributed to data interpretation, revision and discussion of the final draft of the manuscript; PP designed and coordinated the study, integrated data analysis and interpretation, and wrote the final version of the paper.

Uncited references

Kessler, 2003
Willner, 1997

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References

Anisman, H., Lacosta, S., Kent, P., McIntyre, D.C., Merali, Z., 1998. Stressor-induced corticotropin-releasing hormone, bombesin, ACTH and corticosterone variations in strains of mice differentially responsive to stressors. Stress 2 (3), 209–220.

Autry, A.E., Adachi, M., Cheng, P., Monteggia, L.M., 2009. Gender-specific impact of brain-derived neurotrophic factor signaling on stress-induced depression-like behavior. Biol. Psychiatry 2009 Jul 1;66 (1), 84–90.

Bangasser, D.A., Wicks, B., 2017. Sex-specific mechanisms for responding to stress. J. Neurosci. Res. 95 (1–2), 75–82.

Bartolomucci, A., Pederzani, T., Sacerdote, P., Panerai, A.E., Parmigiani, S., Palanza, P., 2004. Behavioral and physiological characterization of male mice under chronic psychosocial stress. Psychoneuroendocrinology 29 (7), 899–910.

Bartolomucci, A., Palanza, P., Sacerdote, P., Panerai, A.E., Sgoifo, A., Dantzer, R., Parmigiani, S., 2005. Social factors and individual vulnerability to chronic stress exposure. Neurosci. Biobehav. Rev. 29, 67–81.

Bartolomucci, A., Cabassi, A., Govoni, P., Ceresini, G., Cero, C., Berra, D., Dadomo, H., Franceschini, P., Dell'Omo, G., Parmigiani, S., Palanza, P., 2009. Metabolic consequences and vulnerability to diet-induced obesity in male mice under chronic social stress. PLoS One 4 (1), e4331.

Becker, C., Zeau, B., Rivat, C., Blugeot, A., Hamon, M., Benoliel, J.J., 2008. Repeated social defeat-induced depression-like behavioral and biological alterations in rats: involvement of cholecystokinin. Mol. Psychiatry 13, 1079–1092.

Beery, A.K., Kaufer, K., 2015. Stress, social behavior, and resilience: insights from rodents. Neurobiol. Stress 1, 116–127.

Belmaker, R.H., Agam, G., 2008. Major depressive disorder. N. Engl. J. Med. 358 (1), 55–68.

Belzung, C., Griebel, G., 2001. Measuring normal and pathological anxiety-like behaviour in mice: a review. Behav. Brain Res. 125 (1), 141–149.

Berry, R.J., Bronson, F.H., 1992. Life history and bioeconomy of the house mouse. Biol. Rev. Camb. Philos. Soc. 67 (4), 519–550.

Björkqvist, K., 2001. Social defeat as a stressor in humans. Physiol. Behav. 73 (3), 435–442.

Blanchard, D.C., Spencer, R.L., Weiss, S.M., Blanchard, R.J., McEwen, B., Sakai, R.R., 1995. Visible burrow system as a model of chronic social stress: behavioral and neuroendocrine correlates. Psychoneuroendocrinology 20, 117–134.

Blanchard, D.C., Griebel, G., Blanchard, R.J., 2001. Mouse defensive behaviors: pharmacological and behavioral assays for anxiety and panic. Neurosci. Biobehav. Rev. 25 (3), 205–218.

Blanchard, D.C., Griebel, G., Pobbe, R., Blanchard, R.J., 2011. Risk assessment as an evolved threat detection and analysis process. Neurosci. Biobehav. Rev. 35 (4), 991–998.

Blazer, D.G., Hybels, C.F., 2005. Origins of depression in later life. Psychol. Med. 35 (9), 1241–1252.

Block, J.P., et al., 2009. Psychosocial stress and change in weight among US adults. Am. J. Epidemiol. 170, 181–192.

Boleij, H., Willems, J., Leijten, M., van't Klooster, J., Lesscher, H., Kirchhoff, S., Lavrijsen, M., Arndt, S.S., Ohl, F., 2014. Chronic social stress does not affect behavioural habituation in male CD1 mice. Behav. Brain Res. 273, 34–44.

Bondi, C.O., Rodriguez, G., Gould, G.G., Frazer, A., Morilak, D.A., 2008. Chronic unpredictable stress induces a cognitive deficit and anxiety-like behavior in rats that is prevented by chronic antidepressant drug treatment. Neuropsychopharmacology 33 (2), 320–331.
Brain, P., 1975. What does individual housing mean to a mouse?. Life Sci. 15;16 (2), 187–200.
Brand, J.S., Moller, M., Harvey, H.B., 2015. A review of biomarkers in mood and psychotic disorders: a dissection of clinical vs. preclinical correlates. Curr. Neuropharmacol. 2015;13 (3), 324–368.
Bronson, F.H., 1985. Mammalian reproduction: an ecological perspective. Biol. Reprod. 32 (1), 1–26.
Bruder-Nascimento, T., Campos, D.H.S., Alves, C., Thomaz, S., Cicogna, A.C., Cordellini, S., 2013. Effect of chronic stress and high fat diet on metabolic and nutritional parameters in Wistar rats. Arq. Bras. Endocrinol. Metabol. 57, 642–649.
Chalfin, L., Dayan, M., Levy, D.R., Austad, S.N., Miller, R.A., Iraqi, F.A., Dulac, C., Kimchi, T., 2014. Mapping ecologically relevant social behaviours by gene knockout in wild mice. Nat. Commun. 5, 4569.
Chandola, T., Brunner, E., Marmot, M., 2006. Chronic stress at work and the metabolic syndrome: prospective study. BMJ 4;332 (7540), 521–525.
Chaouloff, F., 2013. Social stress models in depression research: what do they tell us?. Cell Tissue Res. 354 (1), 179–190.
Chrousos, G.P., 2009. Stress and disorders of the stress system. Nat. Rev. Endocrinol. 5 (7), 374–381.
Cryan, J.F., Slattery, D.A., 2007. Animal models of mood disorders: recent developments. Curr. Opin. Psychiatry 20 (2007), 1–7.
Dadomo, H., Sanghez, V., Di Cristo, L., Lori, A., Ceresini, G., Malinge, I., Parmigiani, S., Palanza, P., Sheardown, M., Bartolomucci, A., 2011. Vulnerability to chronic subordination stress-induced depression-like disorders in adult 129SvEv male mice. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 35 (6), 1461–1471.
Dadomo, H., Parmigiani, S., Nicolini, Y., Freschini, S., Gioiosa, L., Patrelli, T.S., Palanza, P., Volpi, R., 2013. Repeated and chronic administration of Vardenafil or Sildenafil differentially affects emotional and socio-sexual behavior in mice. Behav. Brain Res. 253, 103–112.
Dalla, C., Antoniou, K., Drossopoulou, G., Xagoraris, M., Kokras, N., Sfikakis, A., Papadopoulou-Daifoti, Z., 2005. Chronic mild stress impact: are females more vulnerable?. Neuroscience 135 (3), 703–714.
Davis, E.P., Pfaff, D., 2014. Sexually dimorphic responses to early adversity: implications for affective problems and autism spectrum disorder. Psychoneuroendocrinology 49, 11–25.
Deng, X.Y., Xue, J.S., Li, H.Y., Ma, Z.Q., Fu, Q., Qu, R., Ma, S.P., 2015. Geraniol produces antidepressant-like effects in a chronic unpredictable mild stress mice model. Physiol. Behav. 152 (Pt A), 264–271.
Doron, R., Lotan, D., Versano, Z., Benatav, L., Franko, M., Armoza, S., Rehavi, M., 2014. Escitalopram or novel herbal mixture treatments during or following exposure to stress reduce anxiety-like behavior through corticosterone and BDNF modifications. PLoS One 9 (4), e91455.
Ducottet, C., Belzung, C., 2005. Correlations between behaviours in the elevated plus-maze and sensitivity to unpredictable subchronic mild stress: evidence from inbred strains of mice. Behav. Brain Res. 156, 153–162.
Fernandes, C., González, M.I., Wilson, C.A., File, S.E., 1999. Factor analysis shows that female rat behaviour is characterized primarily by activity, male rats are driven by sex and anxiety. Pharmacol. Biochem. Behav. 64 (4), 731–738.
Filho, C.B., Jesse, C.R., Donato, F., Giacomelli, R., Del Fabbro, L., da Silva, Antunes M., de Gomes, M.G., Goes, A.T., Boeira, S.P., Prigol, M., Souza, L.C., 2015. Chronic unpredictable mild stress decreases BDNF and NGF levels and Na +, K +-ATPase activity in the hippocampus and prefrontal cortex of mice: antidepressant effect of chrysin. Neuroscience 289, 367–380.
Finger, B.C., Dinan, T.G., Cryan, J.F., 2012. The temporal impact of chronic intermittent psychosocial stress on high-fat diet-induced alterations in body weight. Psychoneuroendocrinology 37 (6), 729–741.
Franceschelli, A., Herchick, S., Thelen, C., Papadopoulou-Daifoti, Z., Pitychoutis, P.M., 2014. Sex differences in the chronic mild stress model of depression. Behav. Pharmacol. 25 (5–6), 372–383.
Fuchs, E., Flügge, G., 2002. Social stress in tree shrews: effects on physiology, brain function, and behavior of subordinate individuals. Pharmacol. Biochem. Behav. 73, 247–258.
Gobinath, A.R., Choleris, E., Galea, L.A., 2017. Sex, hormones, and genotype interact to influence psychiatric disease, treatment, and behavioral research. J. Neurosci. Res. 2;95 (1–2), 50–64.
Goel, N., Bale, T.L., 2009. Examining the intersection of sex and stress in modelling neuropsychiatric disorders. J. Neuroendocrinol. 21 (4), 415–420.
Goldberg, D., 2011. The heterogeneity of "major depression". World Psychiatry. 10 (3), 226–228.
Gorman, J.M., 2006. Gender differences in depression and response to psychotropic medication. Gend. Med. 3 (2), 93–109.
Grønli, J., Murison, R., Bjorvatn, B., Sørensen, E., Portas, C.M., Ursin, R., 2004. Chronic mild stress affects sucrose intake and sleep in rats. Behav. Brain Res. 150 (1–2), 139–147.
Gruenewald, T.L., Kemeny, M.E., Aziz, N., Fahey, J.L., 2004. Acute threat to the social self: shame, social self-esteem, and cortisol activity. Psychosom. Med. 66 (6), 915–924.
Guilloux, J.P., Seney, M., Edgar, N., Sibille, E., 2011. Integrated behavioral z-scoring increases the sensitivity and reliability of behavioral phenotyping in mice: relevance to emotionality and sex. J. Neurosci. Methods 197 (1), 21–31.
Haller, J., Fuchs, E., Halasz, J., Makara, G.B., 1999. Defeat is a major stressor in males while social instability is stressful mainly in females: towards the development of a social stress model in female rats. Brain Res. Bull. 50, 33–39.
Hammen, C., 2005. Stress and depression. Annu. Rev. Clin. Psychol. 1, 293–319.
Havranek, M.M., Bolliger, B., Roos, S., Pryce, C.R., Quednow, B.B., Seifritz, E., 2016. Uncontrollable and unpredictable stress interacts with subclinical depression and anxiety scores in determining anxiety response. Stress 19 (1), 53–62.
Herzog, C.J., Czéh, B., Corbach, S., Wuttke, W., Schulte-Herbrüggen, O., Hellweg, R., Flügge, G., Fuchs, E., 2009. Chronic social instability stress in female rats: a potential animal model for female depression. Neuroscience 159 (3), 982–992.
Hill, R.A., YW, Wu, Kwek, P., van den Buuse, M., 2012. Modulatory effects of sex steroid hormones on brain-derived neurotrophic factor-tyrosine kinase B expression during adolescent development in C57Bl/6 mice. J. Neuroendocrinol. 24 (5), 774–788.
Hirschfeld, R.M., 2001. The comorbidity of major depression and anxiety disorders: recognition and management in primary care. Prim Care Companion J. Clin. Psychiatry 3 (6), 244–254.
Holder, M.K., Blaustein, J.D., 2014. Puberty and adolescence as a time of vulnerability to stressors that alter neurobehavioral processes. Front. Neuroendocrinol. 35 (1), 89–110.
Holmes, A., Parmigiani, S., Ferrari, P.F., Palanza, P., Rodgers, R.J., 2000. Behavioral profile of wild mice in the elevated plus-maze test for anxiety. Physiol. Behav. 71 (5), 509–516.
Holmes, S.D., Krants, D.S., Rogers, H., Gottdiener, J., Contrada, R.J., 2006. Mental stress and coronary disease: a multidisciplinary guide. Prog. Cardiovasc. Dis. 49 (2), 106–122.
Hong, S., Flashner, B., Chiu, M., ver Hoeve, E., Luz, S., Bhatnagar, S., 2012. Social isolation in adolescence alters behaviors in the forced swim and sucrose preference tests in female but not in male rats. Physiol. Behav. 105 (2), 269–275.
Ibarguen-Vargas, Y., Surget, A., Touma, C., Palme, R., Belzung, C., 2008. Multifaceted strain-specific effects in a mouse model of depression and of antidepressant reversal. Psychoneuroendocrinology 33 (10), 1357–1368.
Imhof, J.T., Coelho, Z.M., Schmitt, M.L., Morato, G.S., Carobrez, A.P., 1993. Influence of gender and age on performance of rats in the elevated plus maze apparatus. Behav. Brain Res. 56 (2), 177–180.
Insel, T., Cuthbert, B., Garvey, M., et al., 2010. Research domain criteria (RDoC): toward a new classification framework for research on mental disorders. Am. J. Psychiatry 167 (7), 748–751.
Isingrini, E., Belzung, C., Freslon, J.L., Machet, M.C., Camus, V., 2012. Fluoxetine effect on aortic nitric oxide-dependent vasorelaxation in the unpredictable chronic mild stress model of depression in mice. Psychosom. Med. 74 (1), 63–72.
Jarcho, M.R., Massner, K.J., Eggert, A.R., Wichelt, E.L., 2016. Behavioral and physiological response to onset and termination of social instability in female mice. Horm. Behav. 78, 135–140.
Jesberger, J.A., Richardson, J.S., 1985. Animal models of depression: parallels and correlates to severe depression in humans. Biol. Psychiatry 20 (7), 764–784.
Jin, P., Yu, H.L., Tian-Lan, Zhang F., Quan, Z.S., 2015. Antidepressant-like effects of oleoylethanolamide in a mouse model of chronic unpredictable mild stress. Pharmacol. Biochem. Behav. 133, 146–154.
Joel, D., McCarthy, M.M., 2017. Incorporating sex as a biological variable in neuropsychiatric research: where are we now and where should we be?. Neuropsychopharmacology 2017 Jan;42 (2), 379–385.
Joeyen-Waldorf, J., Edgar, N., Sibille, E., 2009. The roles of sex and serotonin transporter levels in age- and stress-related emotionality in mice. Brain Res. 1286, 84–93.
van Kampen, M., Kramer, M., Hiemke, C., Flügge, G., Fuchs, E., 2002. The chronic psychosocial stress paradigm in male tree shrews: evaluation of a novel animal model for depressive disorders. Stress 5 (1), 37–46.
Karisetty, B.C., Maitra, S., Wahul, A.B., Musalamadugu, A., Khandelwal, N., Guntupalli, S., Garikapati, R., Jhansyrani, T., Kumar, A., Chakravarty, S., 2017. Differential effect of chronic stress on mouse hippocampal memory and affective behavior: role of major ovarian hormones. Behav. Brain Res. 318, 36–44.
Kendler, K.S., Kessler, R.C., Walters, E.E., MacLean, C., Neale, M.C., Heath, A.C., Eaves, L.J., 1995. Stressful life events, genetic liability, and onset of an episode of major depression in women. Am. J. Psychiatry 152 (6), 833–842.
Kendler, K.S., Karkowski, L.M., Prescott, C.A., 1999. Causal relationship between stressful life events and the onset of major depression. Am. J. Psychiatry 156 (6), 837–841.
Kendler, K.S., Gardner, C.O., Prescott, C.A., 2002. Toward a comprehensive developmental model for major depression in women. Am. J. Psychiatry 159 (7), 1133–1145.
Kennedy, S.H., 2008. Core symptoms of major depressive disorder: relevance to diagnosis and treatment. Dialogues Clin. Neurosci. 10 (3), 271–277.
Kessler, R.C., 1997. The effects of stressful life events on depression. Annu. Rev. Psychol. 48, 191–214.
R.C.. . J. Affect. Disord. 74 (1), 5–13 (2003).
Kessler, R.C., 2007. The global burden of anxiety and mood disorders: putting the European Study of the Epidemiology of Mental Disorders (ESEMeD) findings into perspective. J. Clin. Psychiatry 68 (Suppl. 2), 10–19.
Kessler, R.C., Berglund, P., Demler, O., Jin, R., Merikangas, K.R., Walters, E.E., 2005. Lifetime prevalence and age-of-onset distributions of DSM-IV disorders in the National Comorbidity Survey Replication. Arch. Gen. Psychiatry 62, 593–602.
Koike, H., Ibi, D., Mizoguchi, H., Nagai, T., Nitta, A., Takuma, K., Nabeshima, T., Yoneda, Y., Yamada, K., 2009. Behavioral abnormality and pharmacologic response in social isolation-reared mice. Behav. Brain Res. 202 (1), 114–121.

Kokras, N., Dalla, C., 2014. Sex differences in animal models of psychiatric disorders. Br. J. Pharmacol. 171 (20), 4595–4619.
Konkle, A.T., Baker, S.L., Kentner, A.C., Barbagallo, L.S., Merali, Z., Bielajew, C., 2003. Evaluation of the effects of chronic mild stressors on hedonic and physiological responses: sex and strain compared. Brain Res. 992 (2), 227–238.
Koolhaas, J.M., Korte, S.M., De Boer, S.F., Van Der Vegt, B.J., Van Reenen, C.G., Hopster, H., … Blokhuis, H.J., 1999. Coping styles in animals: current status in behavior and stress-physiology. Neurosci. Biobehav. Rev. 23 (7), 925–935.
Koolhaas, J.M., De Boer, S.F., Buwalda, B., Van Reenen, K., 2007. Individual variation in coping with stress: a multidimensional approach of ultimate and proximate mechanisms. Brain Behav. Evol. 70 (4), 218–226.
Koolhaas, J.M., Bartolomucci, A., Buwalda, B., de Boer, S.F., Flügge, G., Korte, S.M., Meerlo, P., Murison, R., Olivier, B., Palanza, P., Richter-Levin, G., Sgoifo, A., Steimer, T., Stiedl, O., van Dijk, G., Wöhr, M., Fuchs, E., 2011. Stress revisited: a critical evaluation of the stress concept. Neurosci. Biobehav. Rev. 35 (5), 1291–1301.
Kormos, V., Gáspár, L., Kovács, , Farkas, J., Gaszner, T., Csernus, V., Balogh, A., Hashimoto, H., Reglődi, D., Helyes, Z., Gaszner, B., 2016. Reduced response to chronic mild stress in PACAP mutant mice is associated with blunted FosB expression in limbic forebrain and brainstem centers. Neuroscience 330, 335–358.
Krebs, J.R., Davies, N.B., 1997. An Introduction to Behavioural Ecology. Blackwell Science, Oxford.
Kudielka, B.M., Kirschbaum, C., 2005. Sex differences in HPA axis responses to stress: a review. Biol. Psychol. 69, 113–132.
Kurhe, Y., Radhakrishnan, M., Gupta, D., 2014. Ondansetron attenuates depression co-morbid with obesity in obese mice subjected to chronic unpredictable mild stress; an approach using behavioral battery tests. Metab. Brain Dis. 29 (3), 701–710.
Kurhe, Y., Mahesh, R., Devadoss, T., Gupta, D., 2014. Antidepressant-like effect of a novel 5-HT3 receptor antagonist N-(benzo [d] thiazol-2-yl)-3-ethoxyquinoxalin-2-carboxamide 6k using rodents behavioral battery tests. J. Pharmacol. Pharmacother. 5 (3), 197.
Lin, E.J., Sun, M., Choi, E.Y., Magee, D., Stets, C.W., During, M.J., 2015. Social overcrowding as a chronic stress model that increases adiposity in mice. Psychoneuroendocrinology 51, 318–330.
Liu, B.B., Luo, L., Liu, X.L., Geng, D., Liu, Q., Yi, L.T., 2015. 7-Chlorokynurenic acid (7-CTKA) produces rapid antidepressant-like effects: through regulating hippocampal microRNA expressions involved in TrkB-ERK/Akt signaling pathways in mice exposed to chronic unpredictable mild stress. Psychopharmacology 232 (3), 541–550.
Liu, Y., Lan, N., Ren, J., Wu, Y., Wang, S.T., Huang, X.F., Yu, Y., 2015. Orientin improves depression-like behavior and BDNF in chronic stressed mice. Mol. Nutr. Food Res. 59 (6), 1130–1142.
Lu, A., Steiner, M.A., Whittle, N., Vogl, A.M., Walser, S.M., Ableitner, M., Refojo, D., Ekker, M., Rubenstein, J.L., Stalla, G.K., Singewald, N., Holsboer, F., Wotjak, C.T., Wurst, W., Deussing, J.M., 2008. Conditional mouse mutants highlight mechanisms of corticotropin-releasing hormone effects on stress-coping behavior. Mol. Psychiatry 13 (11), 1028.
Lupien, S.J., McEwen, B.S., Gunnar, M.R., Heim, C., 2009. Effects of stress throughout the lifespan on the brain, behaviour and cognition. Nat. Rev. Neurosci. 10 (6), 434–445.
Marrow, L.P., Brain, P.F., 1998. An evaluation of the use of defeat-induced loss of status in the rat as a model of loss of self-esteem and depression in humans. Aggress. Behav. 24 (4), 297–305.
Martin, A.L., Brown, R.E., 2010. The lonely mouse: verification of a separation-induced model of depression in female mice. Behav. Brain Res. 207 (1), 196–207.
McEwen, B.S., Sapolsky, R.M., 1995. Stress and cognitive function. Curr. Opin. Neurobiol. 5, 205–216.
Merali, Z., Levac, C., Anisman, H., 2003. Validation of a simple, ethologically relevant paradigm for assessing anxiety in mice. Biol. Psychiatry 54 (5), 552–565.
Milner, L.C., Crabbe, J.C., 2008. Three murine anxiety models: results from multiple inbred strain comparisons. Genes Brain Behav.
Mineur, Y.S., Belzung, C., Crusio, W.E., 2006. Effects of unpredictable chronic mild stress on anxiety and depression-like behavior in mice. Behav. Brain Res. 175 (1), 43–50.
Nandi, A., Beard, J.R., Galea, S., 2009. Epidemiologic heterogeneity of common mood and anxiety disorders over the lifecourse in the general population: a systematic review. BMC Psychiatry 9, 31.
Nestler, E.J., Gould, E., Manji, H., Buncan, M., Duman, R.S., Greshenfeld, H.K., Hen, R., Koester, S., Lederhendler, I., Meaney, M., Robbins, T., Winsky, L., Zalcman, S., 2002. Preclinical models: status of basic research in depression. Biol. Psychiatry 15;52 (6), 503–528.
Novais, A., Monteiro, Susana, Roque, Susana, Correia-Neves, Margarida, Sousa, Nuno, 2017. How age, sex and genotype shape the stress response. Neurobiol. Stress 6, 44–56.
O'Leary, O.F., Cryan, J.F., 2013. Towards translational rodent models of depression. Cell Tissue Res. 354 (1), 141–153.
Palanza, P., 2001. Animal models of anxiety and depression: how are females different?. Neurosci. Biobehav. Rev. 25, 219–233.
Palanza, P., Parmigiani, S., 2017. How does sex matter? Behavior, stress and animal models of neurobehavioral disorders. Neurosci. Biobehav. Rev. 76 (Pt A), 134–143.
Palanza, P., Re, L., Mainardi, D., Brain, P.F., Parmigiani, S., 1996. Male and female competitive strategies of wild house mice pairs (Mus musculus domesticus) confronted with intruders of different sex and age in artificial territories. Behaviour 133, 11–21.
Palanza P., Rodgers, R.J., Ferrari, P.F., Parmigiani, S., 1996. Effects of chlordiazepoxide on maternal aggression in mice depend on experience of resident and sex of intruder. Pharmacol. Biochem. Behav. 54 (1), 175–182.
Palanza, P., Gioiosa, L., Parmigiani, S., 2001. Social stress in mice: gender differences and effects of estrous cycle and social dominance. Physiol. Behav. 73, 411–420.
Palanza, P., Della Seta, D., Ferrari, P.F., Parmigiani, S., 2005. Female competition in wild house mice depends upon timing of female/male settlement and kinship between females. Anim. Behav. 69 (6), 1259–1271.
Parmigiani, S., Ferrari, P.F., Palanza, P., 1998. An evolutionary approach to behavioral pharmacology: using drugs to understand proximate and ultimate mechanisms of different forms of aggression in mice. Neurosci. Biobehav. Rev. 23 (1998), 143–153.
Parmigiani, S., Palanza, P., Rogers, J., Ferrari, P.F., 1999. Selection, evolution of behavior and animal models in behavioral neuroscience. Neurosci. Biobehav. Rev. 23 (7), 957–969.
Pothion, S., Bizot, J.C., Trovero, F., Belzung, C., 2004. Strain differences in sucrose preference and in the consequences of unpredictable chronic mild stress. Behav. Brain Res. 155 (1), 135–146.
Pryce, C.R., Fuchs, E., 2016. Chronic psychosocial stressors in adulthood: studies in mice, rats and tree shrews. Neurobiol. Stress 6, 94–103.
Razzoli, M., Bartolomucci, A., 2016. The dichotomous effect of chronic stress on obesity. Trends Endocrinol. Metab. 27 (7), 504–515.
Reiss, D., Wolter-Sutter, A., Krezel, W., Ouagazzal, A.M., 2007. Effects of social crowding on emotionality and expression of hippocampal nociceptin/orphanin FQ system transcripts in mice. Behav. Brain Res. 184 (2), 167–173.
Rubinstein, M.R., Cremaschi, G.A., Oliveri, L.M., Gerez, E.N., Wald, M.R., Genaro, A.M., 2010. Possible involvement of stress hormones and hyperglycaemia in chronic mild stress-induced impairment of immune functions in diabetic mice. Stress 13 (5), 384–391.
Ruff, J.S., Cornwall, D.H., Morrison, L.C., Cauceglia, J.W., Nelson, A.C., Gaukler, S.M., Meagher, S., Carroll, L.S., Potts, W.K., 2017. Sexual selection constrains the body mass of male but not female mice. Ecol. Evol. 7, 1271–1275.
Rygula, R., Abumaria, N., Flugge, G., Fuchs, E., Ruther, E., Havemann- Reinecke, U., 2005. Anhedonia and motivational deficits in rats: impact of chronic social stress. Behav. Brain Res. 162, 127–134.
Sachs, B.D., Ni, J.R., Caron, M.G., 2014. Sex differences in response to chronic mild stress and congenital serotonin deficiency. Psychoneuroendocrinology 40, 123–129.
Sadowska, J., Gębczyński, A.K., Konarzewski, M., 2013. Basal metabolic rate is positively correlated with parental investment in laboratory mice. Proc. Biol. Sci. 280 (1753), 20122576.
Schmidt, M.V., Sterlemann, V., Ganea, K., Liebl, C., Alam, S., Harbich, D., Greetfeld, M., Uhr, M., Holsboer, F., Müller, M.B., 2007. Persistent neuroendocrine and behavioral effects of a novel, etiologically relevant mouse paradigm for chronic social stress during adolescence. Psychoneuroendocrinology 32 (5), 417–429.
Schmidt, M.V., Sterlemann, V., Müller, M.B., 2008. Chronic stress and individual vulnerability. Ann. N. Y. Acad. Sci. 1148, 174–183.
Schmidt, M.V., Scharf, S.H., Liebl, C., Harbich, D., Mayer, B., Holsboer, F., Müller, M.B., 2010. A novel chronic social stress paradigm in female mice. Horm. Behav. 57 (4–5), 415–420.
Shansky, R.M., 2015. Sex differences in PTSD resilience and susceptibility: challenges for animal models of fear learning. Neurobiol. Stress 1, 60–65.
Sharma, H.R., Thakur, M.K., 2015. Correlation of ERα/ERβ expression with dendritic and behavioural changes in CUMS mice. Physiol. Behav. 145, 71–83.
Silverman, M.N., Sternberg, E.M., 2012. Glucocorticoid regulation of inflammation and its functional correlates: from HPA axis to glucocorticoid receptor dysfunction. Ann. N. Y. Acad. Sci. 1261 (1), 55–63.
Stanley, S.C., Brooks, S.D., Butcher, J.T., d'Audiffret, A.C., Frisbee, S.J., Frisbee, J.C., 2014. Protective effect of sex on chronic stress-and depressive behavior-induced vascular dysfunction in BALB/cJ mice. J. Appl. Physiol. 117 (9), 959–970.
Sterlemann, V., Ganea, K., Liebl, C., Harbich, D., Alam, S., Holsboer, F., Müller, M.B., Schmidt, M.V., 2008. Long-term behavioral and neuroendocrine alterations following chronic social stress in mice: implications for stress-related disorders. Horm. Behav. 53 (2), 386–394.
Sterlemann, V., Rammes, G., Wolf, M., Liebl, C., Ganea, K., Müller, M.B., Schmidt, M.V., 2010. Chronic social stress during adolescence induces cognitive impairment in aged mice. Hippocampus 20 (4), 540–549.
Tafet, G.E., Bernardini, R., 2003. Psychoneuroendocrinological links between chronic stress and depression. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 27 (6), 893–903.
Tang, X., Xiao, J., Parris, B.S., Fang, J., Sanford, L.D., 2005. Differential effects of two types of environmental novelty on activity and sleep in BALB/cJ and C57BL/6J mice. Physiol. Behav. 85, 419–429.
Vogt, M.A., Pfeiffer, N., Le Guisquet, A.M., Brandwein, C., Brizard, B., Gass, P., Belzung, C., Chourbaji, S., 2017. May the use of different background strains 'strain' the stress-related phenotype of GR +/− mice?. Behav. Brain Res. 335, 71–79.
Vom Saal, F.S., Franks, P., Boechler, M., Palanza, P., Parmigiani, S., 1995. Nest defense and survival of offspring in highly aggressive wild Canadian female house mice. Physiol. Behav. 58 (4), 669–678.
Wang, J., 2005. Work stress as a risk factor for major depressive episode(s). Psychol. Med. 35 (6), 865–871.
, P.. . 134, –3 (1997).
Willner, P., 2005. Chronic mild stress (CMS) revisited: consistency and behavioural-neurobiological concordance in the effects of CMS. Neuropsychobiology 52 (2), 90–110.
Willner, P., 2017. Reliability of the chronic mild stress model of depression: a user survey. Neurobiol. Stress. 6, 68–77.
Willner, P., 2017. The chronic mild stress (CMS) model of depression: history, evaluation and usage. Neurobiol. Stress 24 (6), 78–93.

Willner, P., Towell, A., Sampson, D., Sophocleous, S., Muscat, R., 1987. Reduction of sucrose preference by chronic unpredictable mild stress, and its restoration by a tricyclic antidepressant. Psychopharmacology 93, 358–364.
Willner, P., Muscat, R., Papp, M., 1992. Chronic mild stress-induced anhedonia: a realistic animal model of depression. Neurosci. Biobehav. Rev. 16, 525–534.
Willner, P., D'Aquila, P.S., Coventry, T., Brain, P., 1995. Loss of social status:preliminary evaluation of a novel animal model of depression. J. Psychopharmacol. 9, 207–213.
Willner, P., Moreau, J.L., Nielsen, C.K., Papp, M., Sluzewska, A., 1996. Decreased hedonic responsiveness following chronic mild stress is not secondary to loss of body weight. Physiol. Behav. 60, 129–134.
Wilson, R.S., Evans, D.A., Bienias, J.L., Mendes de Leon, C.F., Schneider, J.A., Bennett, D.A., 2003. Proneness to psychological distress is associated with risk of Alzheimer's disease. Neurology 61, 1479–1485.
Wittchen, H.U., Jacobi, F., Rehm, J., Gustavsson, A., Svensson, M., Jönsson, B., Olesen, J., Allgulander, C., Alonso, J., Faravelli, C., Fratiglioni, L., Jennum, P., Lieb, R., Maercker, A., van Os, J., Preisig, M., Salvador-Carulla, L., Simon, R., Steinhausen, H.C., 2011. The size and burden of mental disorders and other disorders of the brain in Europe 2010. Eur. Neuropsychopharmacol. 21 (9), 655–679.
Wood, K.H., Wheelock, M.D., Shumen, J.R., Bowen, K.H., Ver Hoef, L., Knight, D.C., 2015. Controllability modulates the neural response to predictable but not unpredictable threat in humans. NeuroImage 119, 371–381.
World Health Organization, 2009. Global Health Risks: Mortality and Burden of Disease Attributable to Selected Major Risks. World Health Organization.
Yang, C.R., Bai, Y.Y., Ruan, C.S., Zhou, H.F., Liu, D., Wang, X.F., Shen, L.J., Zheng, H.Y., Zhou, X.F., 2015. Enhanced aggressive behaviour in a mouse model of depression. Neurotox. Res. 27 (2), 129–142.
Yen, Y.C., Anderzhanova, E., Bunck, M., Schuller, J., Landgraf, R., Wotjak, C.T., 2013. Co-segregation of hyperactivity, active coping styles, and cognitive dysfunction in mice selectively bred for low levels of anxiety. Front. Behav. Neurosci. 7.
Zhu, S., Shi, R., Wang, J., Wang, J.F., Li, X.M., 2014. Unpredictable chronic mild stress not chronic restraint stress induces depressive behaviours in mice. Neuroreport 25 (14), 1151–1155.
Zucker, I., Beery, A.K., 2010. Males still dominate animal studies. Nature 465, 690.