Neuroscience 192 (2011) 524–536

INCREASED SENSITIVITY TO THE EFFECTS OF CHRONIC SOCIAL DEFEAT STRESS IN AN INNATELY ANXIOUS MOUSE STRAIN

H. M. SAVIGNAC,[a,b] B. C. FINGER,[b] R. C. PIZZO,[b] O. F. O'LEARY,[b] T. G. DINAN[a,c] AND J. F. CRYAN[a,b,d]*

[a]*Laboratory of Neurogastroenterology, Alimentary Pharmabiotic Centre, University College Cork, Cork, Ireland*

[b]*Neuropharmacology Research Group, School of Pharmacy, University College Cork, Cork, Ireland*

[c]*Department of Psychiatry, University College Cork, Cork, Ireland*

[d]*Department of Pharmacology and Therapeutics, University College Cork, Cork, Ireland*

Abstract—Stress and genetic predisposition are two of the major risk factors for a variety of psychiatric illnesses. Inbred mouse strains are considered useful tools in dissecting the genetic basis of complex disorders. Indeed, mice of the C57BL/6 and BALB/c strains, differing markedly in anxiety behaviours, are among the most widely used in psychopharmacological research. However, there is a paucity of studies investigating the impact of social stress in these two strains. Moreover, whether these two mouse strains exhibit different sensitivities to chronic social defeat stress remains poorly studied. Thus in this study we compared the impact of repeated (10 days) social defeat stress on a variety of behavioural and endocrine parameters including social interaction, locomotor activity, plasma corticosterone, body weight and stress-related physiological parameters in both mouse strains. Given that the duration of stress exposure may differentially affect such responses we also compared stressors of short (Social Defeat-Short; SD-S) and of long (Social Defeat-Long; SD-L) duration. Our results show that although mice from both strains were defeated in both social defeat paradigms, only BALB/c mice displayed social interaction impairments following SD-S, whereas both strains were behaviourally sensitive to SD-L. Moreover, both strains also differed in some of the physiological alterations induced by social defeat stress. Specifically, SD-S did not induce any change in corticosterone levels in either of the two strains, whereas SD-L was able to induce significant changes in C57BL/6 mice only. SD-S induced differential effects on bodyweight gain in both strains, increasing it in C57BL/6 and decreasing it in BALB/c mice, whereas SD-L had no effect. On the other hand, exposure to SD-S resulted in cardiac hypertrophy in C57BL/6 mice and SD-L induced spleen hypertrophy and thymus atrophy in BALB/c mice in addition to decreasing faecal output. Overall, the innately anxious BALB/c mice were more sensitive to social stress than C57BL/6, with differential behavioural and physiological alterations emerging as a function of stress severity. These data suggest different coping strategies to social interaction stress between the two mouse strains. The genetic basis of this stress-resilience/susceptibility warrants further investigation.

Key words: genetic background, social defeat stress severity, mouse model, susceptibility, resistance, corticosterone.

Chronic stress causes a variety of behavioural and physiological modifications including altered endocrine function, social impairment and increased risk for a large variety of psychiatric and somatic disorders (Leonard and Song, 1996; Connor and Leonard, 1998; Koob, 1999; McEwen, 2000; de Kloet et al., 2005). However, sensitivity to the effects of chronic stress varies in the population with certain individuals being more susceptible to its negative effects, while others remain resistant to these effects (Feder et al., 2009). Accordingly, genetic factors must play a role in the manifestation of various stress-induced psychiatric illnesses including anxiety and depression or irritable bowel syndrome (Mayer et al., 2001; Murphy et al., 2004; de Kloet et al., 2005; Wurtman, 2005; Cryan and Slattery, 2007; Krishnan and Nestler, 2008).

Animal models of stress-related disorders are powerful tools to feature and investigate human psychopathologies, giving a direct insight into the pathophysiology, underlying mechanisms and behavioural consequences of a given disorder (Cryan and Holmes, 2005; Koolhaas et al., 2007; Anisman et al., 2008; Miczek and de Wit, 2008). Notably, over the past decade, chronic social stress-based models have increasingly been used, as models of depression (Kudryavtseva et al., 1991; Sheridan et al., 2000; Vialou et al., 2010) or in the discovery of novel antidepressant and anxiolytic agents (Cryan and Slattery, 2007; Krishnan and Nestler, 2010), largely because social stressors are among the most potent sources of stress in humans, inducing strong neuroendocrine and long-lasting behavioural impairments (Sheridan et al., 2000; Bartolomucci et al., 2005; Beitia et al., 2005; Miczek et al., 2008).

Notably, one social stress model that is widely used is the chronic social defeat paradigm (Fano et al., 2001; Avgustinovich et al., 2005; Berton et al., 2006), which involves a short (5–10 min) physical interaction with an unfamiliar aggressive animal, combined with sensory contact throughout. This stress results in physiological changes ranging from elevations in corticosterone, alterations in immune organs and bodyweight changes (Bartolomucci et al., 2001; Gryazeva et al., 2001; Krishnan et al., 2007), as well as behavioural modifications such as increased anxiety, depression-like behaviour and social im-

*Correspondence to: J. F. Cryan, Cavanagh Pharmacy Building, University College Cork, College Road, Cork, Ireland. Tel: +353-21-490-1676; fax: +353-21-490-1656.
E-mail address: j.cryan@ucc.ie (J. F. Cryan).

Abbreviations: IBS, irritable bowel syndrome; SD, social defeat; SD-L, social defeat-long; SD-S, social defeat-short; SI, social interaction; TPH, tryptophan hydroxylase; 5-HT, serotonin.

0306-4522/11 $ - see front matter © 2011 IBRO. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.neuroscience.2011.04.054

pairment (Avgustinovich et al., 1997; Bartolomucci et al., 2001; Beitia et al., 2005; Krishnan et al., 2007).

Genetically identical inbred mouse strains are very useful tools to analyze the complex genetic basis of stress-related disorders (Jacobson and Cryan, 2007, 2010). For social stress studies, mice of the C57BL/6 strain have been used extensively (Gryazeva et al., 2001; Berton et al., 2006). C57BL/6 also constitutes one of the background strains, on which most transgenic mice are bred (Crawley, 2008). This strain is also widely used in anxiety research where they display normal anxiety responses across a variety of paradigms compared with other mouse strains (Jacobson and Cryan, 2007). Moreover, C57BL/6 mice have often been compared with a range of other strains in basal anxiety or in stress studies in order to assess gene-environment interaction (Jacobson and Cryan, 2007; Millstein and Holmes, 2007). Notably, C57BL/6 markedly differ in their anxiety behaviour from BALB/c mice (Anisman et al., 1998; Griebel et al., 2000; Millstein and Holmes, 2007; O'Mahony et al., 2010), another inbred mouse strain being proposed to be a model of pathological anxiety due to its reported higher anxiety and depression-related behaviour than other strains (Belzung and Griebel, 2001; Cryan and Holmes, 2005; Millstein and Holmes, 2007). We have also shown in our laboratory that C57BL/6 differ in stress-induced brain activation patterns with BALB/c mice (O'Mahony et al., 2010). However, in another set of experiments, both strains remained resistant to the deleterious effects of two different early-life stress in adulthood (Savignac et al., 2011b), suggesting that stress susceptibility is dependent on both the context and the nature of stress itself. BALB/c mice have also been used in social stress studies, but in a much more limited number, also displaying both behavioural and physiological impairments (Merlot et al., 2004; Savignac et al., 2011a). Moreover, there is a paucity of studies investigating the impact of social stress on both behaviour and physiology of these two strains. Therefore, the comparison of these two mouse strains in stress studies should give rise to useful information on which to build future investigations focused on understanding the genetic basis of stress sensitivity and resilience in the context of predisposition to developing psychiatric illnesses.

Accordingly, in the present study, we sought to compare social stress susceptibility in C57BL/6 and BALB/c mice using a chronic social defeat model, for 10 consecutive days. Given that the duration of stress exposure may differentially affect such responses we also sought to assess whether daily stressors of short (Social Defeat-Short; SD-S) or of long (Social Defeat-Long; SD-L) duration would impact differentially on responses in either strain. We firstly adapted a protocol from previous studies (Fano et al., 2001; Gryazeva et al., 2001; Beitia et al., 2005; Krishnan et al., 2007) that had been validated to induce stress in C57BL/6 mice (Krishnan et al., 2007), allowing 10-min physical interaction before 24-h sensory contact (Social Defeat-Long, SD-L). Then, we developed a second protocol allowing a single physical interaction with separation at the first sign of defeat from the stressed mouse (Bartolomucci et al., 2001; Keeney et al., 2006), followed by 24-h sensorial contact (Social Defeat-Short, SD-S). In both protocols, stress-induced behavioural alterations were assessed using the social interaction test at the end of the 10-day stress course, as previously described (Berton et al., 2006); we assumed that social defeat-sensitive mice would become more socially avoidant than control or less sensitive animals. We hypothesized that SD-S would constitute a milder social stress than SD-L, in both strains, as the physical interaction allowed is shorter; this protocol difference would also allow to assess the impact of the length of interaction between mice and the impact of a social stress mainly psychological, such as SD-S. Due to their higher basal anxiety, we also hypothesized that BALB/c mice may be more susceptible to the stress-induced behavioural and physiological impairment following the milder SD-S and that they would become more sensitive than C57BL/6 to SD-L. Finally, we expected these differences in behaviour to be paralleled by alterations in commonly reported stress-sensitive physiological parameters, such as changes in plasma levels of the stress hormone (corticosterone), organs weight modifications including heart and spleen hypertrophy, thymus atrophy, as well as reduced colon length and body weight changes (Bartolomucci et al., 2005; Krishnan et al., 2007; Reber et al., 2007; Savignac et al., 2011a).

EXPERIMENTAL PROCEDURES

Animals

A total of 44 male BALB/cOlaHsd (BALB/c) and 48 male C57BL/6JOlaHsd (C57BL/6) mice; both aged 8–9 weeks old and a cohort of CD1 male mice, (9–10 weeks old; aggressive resident mice) were obtained from Harlan Laboratories, UK and housed under standard controlled laboratory conditions (temperature 21±1 °C, 55–60% humidity) on a 12 h light/dark cycle (lights on 7:30 AM) and were provided with standard laboratory diet and water *ad libitum*. Animals were allowed 10 days habituation to laboratory conditions, remaining group-housed in Plexiglas cages (33×15×13 cm^3, l×w×h) in groups of four per cage as widely described in literature (Berton et al., 2006). During the social defeat procedure, stressed mice of either strain BALB/c or C57BL/6, were housed two per cage with an aggressive CD1 mouse, separated by a transparent perforated Plexiglas wall. Control mice also remained housed two per cage but with a non-aggressive mouse of the same strain. All experiments were conducted in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC), the Recommendation 2007/526/65/EC and approved by the Animal Experimentation Ethics Committee of University College Cork. All efforts were made to minimize the number of animals used and their suffering.

Selection of aggressive residents

Prior to the social defeat stress, CD1 mice were screened in a preliminary study for aggressive behaviour towards a separate cohort of BALB/c and C57BL/6 mice to ensure the defeat of the intruder experimental mice, as previously described (Berton et al., 2006; Savignac et al., 2011a). Latency of first attack was monitored and dominance status of mice was visually determined by observing key behaviours as previously described (Savignac et al., 2011a). Briefly, mice were deemed dominant if they displayed aggressive behaviour toward their opponent such as tail rattling, chasing, biting and fight-attacks. Mice were submissive if they

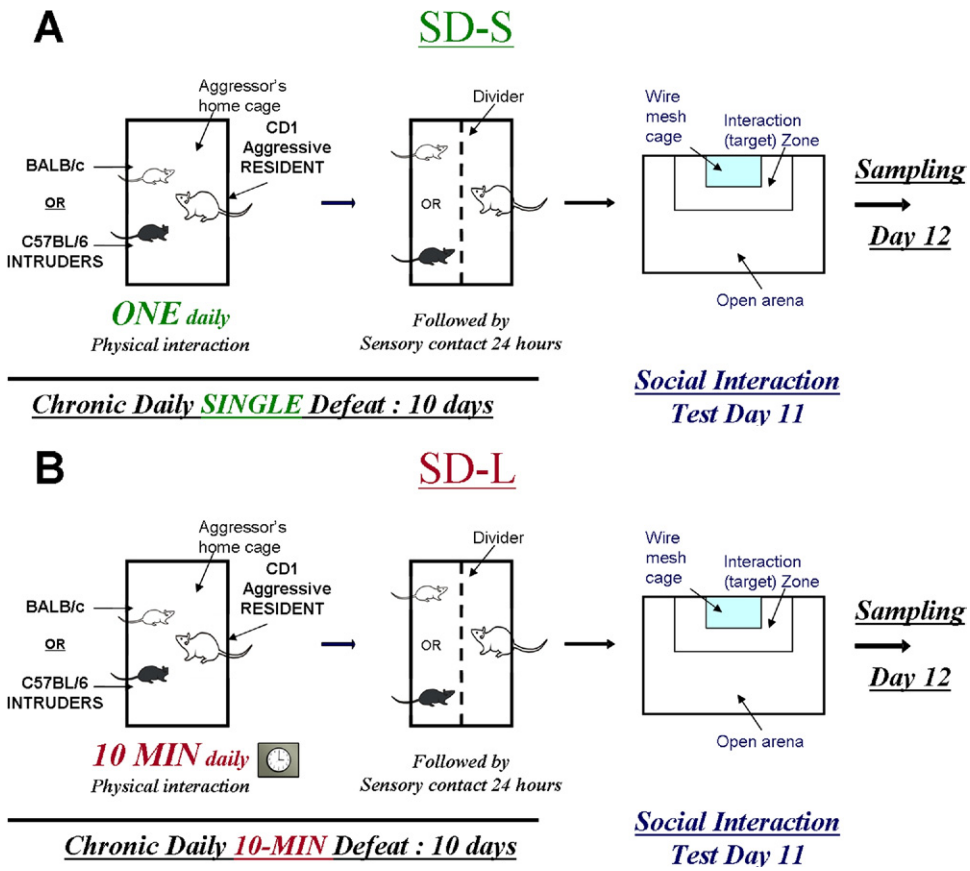

Fig. 1. Social defeat procedures. Two separate sets of experiments were conducted. For both social defeat procedures, innately anxious BALB/c or C57BL/6 mice were introduced in the home cage of an aggressive resident mouse (CD1) for 10 days, either for a single (Social Defeat-Short, (A)) or a 10-min (Social Defeat-Long, (B)) social exposure with physical interaction allowed. Thereafter, mice remained in sensory contact throughout. Mice of the stress group were housed daily with a new aggressor. On day 11, all mice (control and stress groups) were tested in the social interaction test to assess stress-induced social avoidance and related behavioural parameters. The following morning, animals were sacrificed and samples harvested for further physiological analysis. For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.

displayed defending and avoidance behaviour such as escaping, defensive response, upright posture and defensive immobility. Mice with latencies to attack of >30 s were not selected. The selected mice were those that were the most aggressive, most dominant, the heaviest and those which had the lowest latency to attack, for three consecutive days.

Social stress procedures

Two separate sets of experimental protocols were utilized, differing in the length of social defeat interaction, in BALB/c and C57BL/6 mice as shown in Fig. 1.

Experiment 1. (Social Defeat-Short, (SD-S), Fig. 1A) was a 10-day social defeat stress adapted from previous studies (Bartolomucci et al., 2001; Keeney et al., 2006) that consisted of placing, daily, a test mouse in the home cage of a new aggressive resident mouse until the first aggressive attack from the aggressor resulting in defeat from the intruder mouse. A 3-min cut-off for latency of attack was observed in order to maintain the interaction between mice short. If a test mouse (C57BL/6 or BALB/c) was not attacked by an aggressor within 3 min, the aggressor was encouraged to move and attack. Thereafter, mice were physically separated by a perforated transparent wall and remained in sensory contact for 24 h until the next defeat by a different aggressor. Control mice were left undisturbed housed in pairs in their own home cage with another control mouse of the same strain, in the same sensory conditions (behind a transparent plastic separator as well) as stressed mice but without any interaction allowed.

Experiment 2. (Social Defeat-Long, (SD-L), Fig. 1B) was a more severe and modified version of SD-S, while still lasting 10 days, but allowing 10-min physical interaction before physical separation and 24-h sensory contact, similarly to previous descriptions (Kudryavtseva et al., 1991; Beitia et al., 2005; Krishnan et al., 2007). If a test mouse (C57BL/6 or BALB/c) was not attacked by an aggressor within 10 min, the aggressor was forced to move and attack. The frequency and severity of attacks from the aggressors were controlled, with a prolonged, or too severe, single fight event inducing the mice to be separated. Control mice were left in pairs with another control mouse of the same strain, in the same sensory conditions as stressed mice but without any interaction allowed. Mice were handled daily and housing partners rotated to better control for the social defeat exposure undergone by defeated mice.

In both protocols, social status (dominant/submissive) was visually assessed as described for selection of aggressive resident (Savignac et al., 2011a). Precautions were taken to ensure that the intruder was indeed defeated, such as daily rotation of the aggressor to avoid habituation from the defeated mice. Wound and fur score were visually assessed by a trained experimenter to

ensure no major wounds occurred and that animals subjected to social defeat presented with the expected deteriorated fur score. The severity of the interaction was controlled with mice to be separated in case of hazardous attacks from the aggressors. SD-S was hypothesized to reduce the possible risk of minor physical contact-induced wounds occurring, which is often observed in physical interaction-based stress models and may interfere with the social nature of the stress (Merlot et al., 2004; Kinsey et al., 2007; Savignac et al., 2011a). Experiments occurred between 4.00 and 6.30 PM. All mice were weighed before each physical interaction, starting day 1. Following the last social defeat, all mice were singly-housed prior to the social interaction test the following morning as previously described (Berton et al., 2006; Krishnan et al., 2007).

Social interaction test

Social avoidance behaviour has been widely used as a key indicator of the effects of social stress with defeated animals spending less time exploring a social target containing an aggressive mouse than a non social target (Berton et al., 2006). Thus, we employed the social interaction test (SI) on the morning following the last defeat (day 11), as previously described (Berton et al., 2006). Mice were individually tested, one after each other. Briefly, mice were placed in a white-painted open arena (40×30×25 cm^3, l×w×h) comprising an empty wire-mesh cage of 10×6 cm^2, on one side of the box for two 2.5 min sessions as shown in Fig. 1. During the first trial ("No Target"), mice were placed facing the wall at the opposite side of the wire-mesh cage and allowed to explore the empty arena for 2.5 min. Mice were then placed back in their home cage for 1 min. Meanwhile, an aggressive mouse that had been used during the social defeat stress, was placed inside the wire-mesh cage of the open arena. The aggressor (CD1 mouse) was different for every test mouse (C57BL/6 or BALB/c, stress or control group), test mice were then placed back into the arena and allowed for a second trial of a 2.5-min social exploration ("Target") with the aggressor. At the end of the second session, mice were returned to their home cage. Social interaction boxes were cleaned between each mouse with 70% ethanol to avoid odour cues. Experiments occurred under red-light conditions, between 10 AM and 2 PM and were videotaped with infra-red camera. Behaviour was measured and analyzed post-test with Ethovision tracking system (Noldus). Social avoidance behaviour was assessed by measuring the time spent in the zone of interaction, the target zone (Fig. 1), with an empty cage (no target, non social conditions) or in the presence of an aggressor (target, social conditions). An interaction ratio was also calculated, as control animals spent the same amount of time in the interaction (target) zone, under social and non-social conditions. The ratio was obtained by dividing the interaction time when the target was present, by the interaction time without target, ×100. Other behavioural measure included locomotor activity as assessed by the distance moved. Finally, to assess stress-induced defaecation, the number of faecal pellets was counted (Barone et al., 2008; Julio-Pieper et al., 2010).

Sample collection

The day following social interaction test, between 10 AM and 2 PM, mice were sacrificed and trunk blood was collected in EDTA (ethylene-diamine tetra-acetic acid) tubes, centrifuged (15 min, 5000 rpm) and the plasma was collected and stored at −80 °C until corticosterone analysis. The colon was removed, mechanically cleaned and its length was measured, to 0.1 cm precision, as an index of colonic inflammation, colon length reductions have been associated with the occurrence of an inflammatory process (Reber et al., 2006). Body weight, thymus, heart and spleen weight changes were also investigated as chronic social stress is often associated with body weight change, thymus hypotrophy, heart hypertrophy and splenomegaly due to the effects of stress on the immune system activation, immune cells survival, as well as interactions with the autonomic nervous system and metabolic pathways (Bartolomucci et al., 2005; Engler et al., 2005; Krishnan et al., 2007; Reber et al., 2007).

Corticosterone assay

To measure the effect of the social defeat procedure on hypothalamic-pituitary-adrenal axis (HPA-axis) activation, plasma corticosterone levels were measured from both social defeat experiments.

Plasma corticosterone levels were determined using an Enzyme Immunoassay Kit (Assay Designs, Inc., MI, USA) according to the manufacturer's instructions. 20 μl plasma per sample was used for the assay. Samples were analyzed in duplicate in a single assay; the threshold detection was less than 32 pg/ml; coefficient of variation limit=20%; the concentrations are expressed in pg/ml. The number of samples per group in SD-S was for both strains, $n=12$ for control animals and $n=11$ for stressed mice; and in SD-L, C57BL/6 control $n=10$, stress $n=12$; BALB/c control $n=6$, stress $n=10$.

Statistical analysis

Data distribution for normality was assessed by a Shapiro–Wilk test and data were normalized where necessary, and assessed using SPSS software (version 17, outliers' exclusion). For the body weight evolution, two-way ANOVA repeated measures was used to assess stress and strain effect over days; for within-day comparison between groups, a one-way ANOVA was further conducted followed by Fisher LSD post hoc test. For other data, a two-way ANOVA was used for stress and strain effect, which was followed, where appropriate by Fisher LSD post hoc test for multiple comparisons. Statistical significance was set at $P<0.05$. Data are expressed as mean±SEM.

RESULTS

Chronic social defeat stress—defeat behaviour

Assessment of submissive behaviours revealed that all mice demonstrated a defeated phenotype in response to both SD paradigms, displaying the typical defensive upright or immobile submissive positions (Beitia et al., 2005; Savignac et al., 2011a). The nature and length of physical contact allowed between test and aggressive resident mice before being separated, was controlled in both SD, with a cut-off latency of attack of 3 min for SD-S, and a total of 10 min interaction allowed for SD-L. Aggressors were attacking test mice within usually a few seconds in both SD. For SD-S, mice were separated at the first attack from the aggressor whereas for SD-L, repeated moderate attacks were allowed, until the end of the 10-min allocated time. No fight-induced severe wounding occurred. Some minor wounds appeared at the start of the procedures that remained superficial, and disappeared after a few days, as animals became more submissive and fight-avoiding. As a result, the time of interaction between test mice and aggressors was at least three times longer in SD-L than SD-S.

Social interaction test—social behaviours

SD-S. On examining the effects of SD-S on social avoidance behaviour and the time spent in the target zone

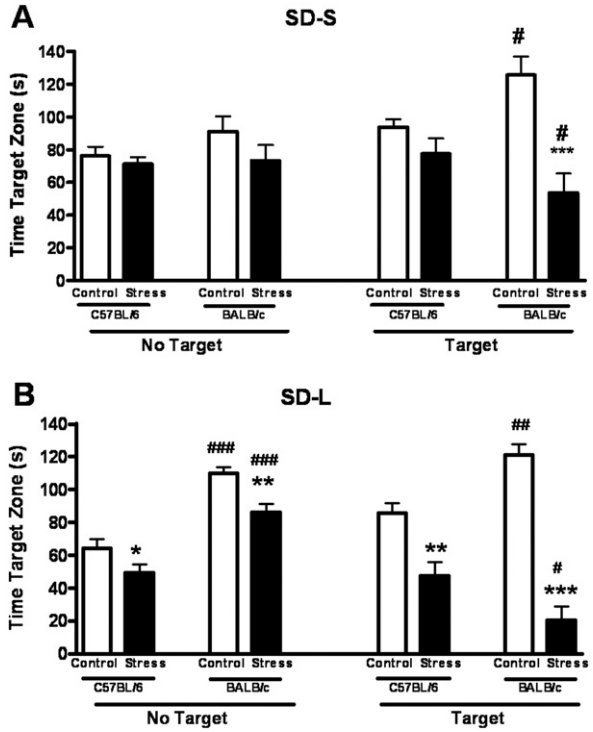

Fig. 2. Effects of the two chronic social defeat paradigms on social avoidance in the social interaction test. Stress-induced social avoidance was assessed in the social interaction test by measuring the time mice spent in the target zone, containing either an empty cage (non social conditions, no target) or an aggressive CD1 mouse placed inside the cage (social conditions, target). Following SD-S (A), only BALB/c mice displayed social avoidance compared with control animals (target conditions). Also, under social conditions, control and stressed C57BL/6 mice spent less and more time in the target zone, respectively, than control and stressed BALB/c mice. Following SD-L, (B), defeated mice of both strains spent less time in the target zone than control animals, in both conditions. However, although control C57BL/6 mice spent less time in the target zone than control BALB/c animals, defeated C57BL/6 mice became less socially avoidant (target conditions) than defeated BALB/c animals. Data are expressed as mean±SEM. Two-way ANOVA followed by Fisher LSD post hoc test, * $P<0.05$; ** $P<0.01$; *** $P<0.001$ control vs. stress difference; # $P<0.05$; ## $P<0.01$; ### $P<0.001$ strain difference.

(Fig. 2A), under non-social conditions (no aggressor in the target zone, “no target”), there was no effect of stress ($F(1,40)=2.28$, $P=0.139$) or strain ($F(1,40)=1.29$, $P=0.264$) and no stress×strain interaction ($F(1,40)=0.75$, $P=0.393$).

However, under social conditions (aggressor present in target zone, “target,” Fig. 2A), there was a general stress effect ($F(1,40)=20.50$, $P<0.0001$), with a stress×strain interaction ($F(1,40)=8.10$, $P<0.01$), but no overall strain effect. Post hoc analysis revealed that stress had a specific impact on BALB/c mice, in that they spent significantly less time in the target zone than control mice ($P<0.0001$), whereas there was no stress effect in C57BL/6 mice. Also, post hoc tests showed control C57BL/6 mice spent significantly less time in the target zone than control BALB/c did ($P<0.005$), whereas there was a trend of the contrary for stressed mice, with C57BL/6 tending to spend more time in the target zone than stressed BALB/c mice ($P=0.092$).

The calculation of the interaction ratio (target/no target) confirms these data, with two-way ANOVA showing an overall stress effect ($F(1,34)=7.63$, $P<0.01$), but no strain effect or stress×strain interaction. Post hoc analysis confirmed that stressed BALB/c mice had a significantly lower interaction ratio than control animals ($P<0.01$), whereas there was no difference in C57BL/6 mice (data not shown). Together these data indicated that SD-S affected BALB/c mice only and only in a social context whereas C57BL/6 seemed to be resistant to the effects of SD-S.

SD-L. Regarding the effects of SD-L on social avoidance behaviour (Fig. 2B), under non-social conditions (no target, Fig. 2B), there was a general effect of stress ($F(1,40)=14.54$, $P<0.001$) and strain ($F(1,40)=63.34$, $P<0.0001$), but no stress×strain interaction. Post hoc analysis revealed that stressed mice of both strains spent a modest but significantly lower amount of time in the target zone than control animals (C57BL/6 $P<0.05$, BALB/c $P<0.01$) showing that stress can induce avoidance behaviour even in non-social conditions. Post hoc analysis also showed that both control and stressed C57BL/6 mice spent significantly less time in the target zone than their respective BALB/c mice ($P<0.0001$ both groups).

Under social conditions (target, Fig. 2B), there was also a general stress effect ($F(1,38)=77.45$, $P<0.0001$), and a stress×strain interaction ($F(1,38)=15.86$, $P<0.0001$), but no overall strain effect. Post hoc analysis showed stressed mice of both strains investigated significantly less the target area than control animals (C57BL/6 $P<0.05$, BALB/c $P<0.0001$). Regarding strain differences, post hoc analysis revealed that control C57BL/6 mice displayed a significant lower social investigation than control BALB/c mice ($P<0.01$), whereas stressed C57BL/6 mice became then less avoidant than stressed BALB/c animals ($P<0.05$). Moreover, the magnitude of stress-induced avoidance was not very different in C57BL/6 mice under social and non-social conditions, whereas it was substantially greater in the social context in BALB/c mice. The calculation of the interaction ratio (target/no target) confirms these data with two-way ANOVA showing there was an overall stress effect ($F(1,36)=49.79$, $P<0.0001$), and an overall strain effect ($F(1,36)=13.40$, $P<0.001$) but no stress×strain interaction ($F(1,36)=2.98$, $P=0.093$). Post hoc analysis confirmed that stressed mice of both strains had a significantly lower interaction ratio than control animals (C57BL/6, $P<0.001$; BALB/c, $P<0.0001$). There was also a significant difference between stressed mice, with C57BL/6 presenting with a higher interaction ratio than BALB/c mice ($P<0.0001$) (data not shown). Overall, these data suggest that this social defeat procedure was able to induce stress and behavioural impairments in both strains, but more significantly so in BALB/c mice.

Social interaction test—distance moved

SD-S. Under non-social conditions (non target, Fig. 3A), there was a general strain effect in the distance moved ($F(1,40)=13.13$, $P<0.001$) but no stress effect or

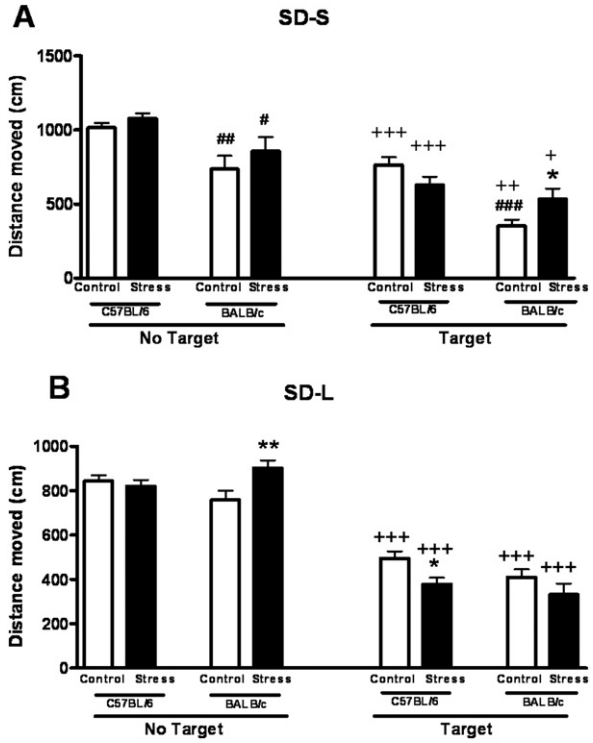

Fig. 3. Effects of the two chronic social defeat paradigms on locomotor activity in the social interaction test. SD-S (A) did not induce any difference between groups under non-social conditions in locomotor activity (distance moved), however, C57BL/6 mice of both control and stress groups travelled more distance than their respective BALB/c. Under social conditions, social defeat exposure reduced the distance moved in C57BL/6 mice whereas it increased it in BALB/c compared with control animals. The presence of a social target also induced all mice to travel less distance than in a non social context. Also, control C57BL/6 travelled more distance than control BALB/c mice. SD-L (B) increased locomotor activity in BALB/c mice compared with control group under non-social conditions whereas activity was decreased in C57BL/6 under social conditions. Overall, a social context also induced lower activity in all mice compared with a non-social context. Data are expressed as mean±SEM. Two-way ANOVA followed by Fisher LSD post hoc test, * $P<0.05$, ** $P<0.01$; control vs. stress difference; # $P<0.05$, ## $P<0.01$, ### $P<0.01$ strain difference; + $P<0.05$, ++ $P<0.01$, +++ $P<0.001$ target conditions difference.

stress×strain interaction. Post hoc analysis further revealed that C57BL/6 mice of both the control and stress groups travelled significantly more distance than their respective BALB/c mice (control, $P<0.01$; stress $P<0.05$).

Under social conditions (target, Fig. 3A), social defeat stress induced differential effects between strains as there was a general effect of strain ($F(1,40)=20.35$, $P<0.0001$) and a stress×strain interaction ($F(1,40)=8.12$, $P<0.01$), although there was no overall stress effect. Indeed, post hoc analysis revealed that stressed C57BL/6 displayed a trend of lower locomotor activity than control animals ($P=0.096$), whereas stressed BALB/c mice displayed significantly higher locomotor activity compared with control mice ($P<0.05$). As a result, C57BL/6 control mice displayed a significant higher locomotor activity than control BALB/c mice (distance moved, $P<0.0001$). Overall, the presence of an aggressive encounter induced a decrease in motor activity in both control and stressed animals of both strains (C57BL/6, $P<0.001$ for both control and stressed mice, BALB/c, $P<0.01$ for control and $P<0.05$ for stressed mice).

SD-L. Under non-social conditions (no target, Fig. 3B), there was a stress×strain interaction in the distance moved ($F(1,37)=6.68$, $P<0.05$), although no overall stress or strain effect emerged. Post hoc analysis further showed stressed BALB/c mice travelled significantly more distance than control mice ($P<0.01$), whereas there was no difference between groups for C57BL/6 mice.

Under social conditions (target, Fig. 3B), there was an overall stress effect ($F(1,40)=6.39$, $P<0.05$) but no strain effect or stress×strain interaction. Post hoc analysis showed stressed C57BL/6 mice travelled significantly less distance than control ones ($P<0.05$). However, there was no difference between groups in BALB/c mice. Finally, overall, both control and stress groups of both strains travelled significantly less distance in social than non-social conditions (C57BL/6, $P<0.0001$, BALB/c, $P<0.0001$).

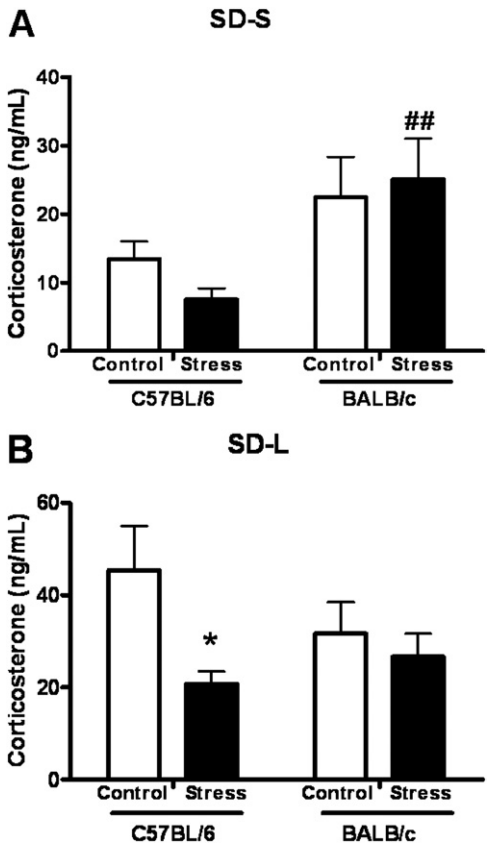

Fig. 4. Effects of the two chronic social defeat paradigms on plasma corticosterone levels. SD-S (A) did not induce any change in corticosterone levels in none of the two strains. However, there was an overall strain effect with post hoc analysis revealing stressed BALB/c mice displayed higher corticosterone levels than stressed C57BL/6 animals. On the contrary, SD-L (B) induced a significant decrease in corticosterone levels in C57BL/6, but not BALB/c mice. Data are expressed as mean±SEM. Two-way ANOVA followed by Fisher LSD post hoc test, * $P<0.05$, control vs. stress difference; ## $P<0.01$ strain difference.

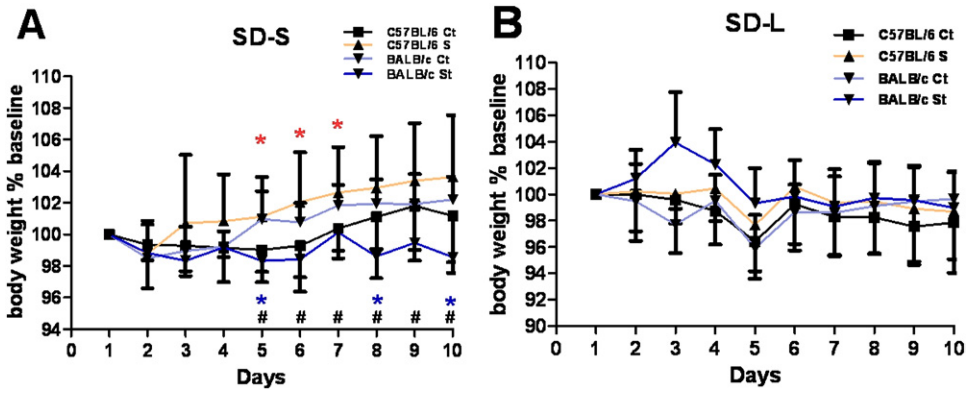

Fig. 5. Effects of the two chronic social defeat paradigms on body weight gain over days. SD-S (A) induced a significant increase in body weight gain (expressed as % baseline) in C57BL/6 (days 5, 6 and 7) and decrease in BALB/c mice (days 5, 8 and 10) compared with their respective control group. Control C57BL/6 also presented with a lower body-weight gain than control BALB/c mice on day 6, whereas stressed C57BL/6 displayed a higher body-weight gain than stressed BALB/c mice from day 5 to 10. On the contrary, SD-L (B) did not induce any change, regardless of stress condition or strain. Data are expressed as mean±SEM. Two-way ANOVA repeated measures for stress and strain effects over days, one-way ANOVA for within day comparison, followed by Fisher LSD post hoc test, * $P<0.05$, control vs. stress difference; # $P<0.05$ strain difference. SD-S full statistics: stressed BALB/c, day 5 $P<0.01$, 8 $P<0.05$, 10 $P<0.01$; stressed C57BL/6, days 5, 6 and 7 $P<0.05$; strain differences: day 5 $P<0.01$, 6 $P<0.01$, 7 $P<0.05$, 8 $P<0.01$, 9 $P<0.01$, 10 $P<0.0001$. For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.

Corticosterone levels

SD-S. There was an overall strain effect (Fig. 4A, $F(1,40)=8.72$, $P<0.01$), but no overall stress effect or stress×strain interaction. Post hoc analysis further revealed that although there was no statistical difference in basal levels between control C57BL/6 and BALB/c mice, there was a strain difference between mice as stressed C57BL/6 mice displayed significantly lower corticosterone levels than BALB/c animals ($P<0.01$).

SD-L. There was an overall stress effect (Fig. 4B, $F(1,32)=5.32$, $P<0.05$) but no strain effect or stress×strain interaction. Post hoc analysis revealed stressed C57BL/6 mice displayed significantly lower corticosterone levels than control animals ($P<0.05$).

Body and tissue weight

Body weight was measured daily and the % change over days from baseline (day 1, 100%) is represented in Fig. 5. Data for body weight gain, thymus, heart and spleen weight changes are presented in Table 1.

SD-S. Regarding the daily evolution of body weight (% baseline on day 1, Fig. 5A), there was a significant effect of time (days) ($F(9,396)=12.12$, $P<0.0001$), a

Table 1. Effects of the two chronic social defeat paradigms on body and tissue weight and colon physiology

SD-S	Factor	C57BL/6		BALB/c	
		Control ($n=12$)	Stress ($n=12$)	Control ($n=12$)	Stress ($n=12$)
Body weight	BWG (g)	0.52±0.14	1.43±0.33*	0.96±0.11	0.3±0.26$^{\#}$
Relative tissue weight	Thymus	0.183±0.007	0.141±0.008**	0.150±0.011$^{\#}$	0.145±0.013
	Heart	0.549±0.02	0.622±0.02*	0.552±0.02	0.549±0.02
	Spleen	0.260±0.008	0.263±0.009*	0.425±0.011$^{\#\#\#}$	0.457±0.016$^{\#\#\#}$
Colon physiology	Colon length	7.6±0.21	7.4±0.19	9.5±0.25$^{\#\#\#}$	9.03±0.22$^{\#\#\#}$
	Stress-induced defaecation	0.6±0.28	1.1±0.45	3.4±0.67$^{\#}$	3.1±0.7$^{\#}$
		Control ($n=11$–12)	Stress ($n=10$–12)	Control ($n=8$–9)	Stress ($n=10$–12)
Body weight	BWG (g)	−0.1±0.25	0.008±0.27	0.11±0.14	0.48±0.55
Relative tissue weight	Thymus	0.152±0.013	0.137±0.007	0.139±0.007	0.08±0.006**,$^{\#\#\#}$
	Heart	0.516±0.008	0.589±0.020**	0.483±0.015	0.513±0.019$^{\#\#}$
	Spleen	0.167±0.008	0.181±0.011	0.271±0.011$^{\#\#}$	0.460±0.052***,$^{\#\#\#}$
Colon physiology	Colon length	7.7±0.3	8.2±0.4	11.1±0.4$^{\#\#\#}$	10.4±0.5$^{\#\#\#}$
	Stress-induced defaecation	1.3±0.5	0.4±0.19	7.1±1$^{\#\#\#}$	1.6±0.2***

BWG, total body weight gain expressed in (g), tissue weight expressed in percentage body weight. SD–S induced in C57BL/6 an increased total BWG and heart weight and a decrease in thymus weight, but no change in BALB/c mice. SD-L induced in C57BL/6 an increased heart weight and in BALB/c mice a decreased thymus weight and increased spleen weight and decreased faecal output in the social interaction (SI) test. Data are expressed as mean±SEM. Two-way ANOVA followed by Fisher LSD post hoc test, * $P<0.05$; ** $P<0.01$; *** $P<0.001$ control vs. stress difference; # $P<0.05$; ## $P<0.01$; ### $P<0.001$ strain difference.

stress×strain interaction ($F(1,44)=8.97$, $P<0.01$) and a time×stress×strain interaction ($F(9,396)=4.49$, $P<0.0001$). However, there was no overall effect of stress or strain ($P=0.061$) and no time×stress or time×strain interaction. Post hoc analysis on individual days revealed stress induced a significant decrease in weight gain in BALB/c mice on days 5 ($P<0.01$), 8 ($P<0.05$) and 10 ($P<0.01$) and a significant increase in C57BL/6 on days 5 ($P<0.05$), 6 ($P<0.05$) and 7 ($P<0.05$). There was also a strain difference in control mice on day 5 ($P<0.05$), with BALB/c mice presenting with a higher weight gain than C57BL/6 and in stressed mice as well, on days 5 ($P<0.01$), 6 ($P<0.01$), 7 ($P<0.05$), 8 ($P<0.01$), 9 ($P<0.01$) and 10 ($P<0.0001$), with stressed C57BL/6 mice gaining more body weight than BALB/c stressed animals.

Regarding the total body weight change, there was a stress×strain interaction ($F(1,44)=11.77$, $P<0.01$), but no overall stress or strain effect. Post hoc analysis showed that stress induced a significantly higher body weight gain in C57BL/6, but not BALB/c mice compared with control animals ($P<0.05$).

For thymus weight, there was an overall effect of stress ($F(1,43)=5.29$, $P<0.05$), and a trend of stress×strain interaction ($F(1,43)=3.18$, $P=0.082$), but no overall strain effect. Post hoc analysis further revealed that stress specifically had an effect in C57BL/6 mice, which presented with a lighter thymus than control animals ($P<0.01$), whereas stress had no effect in BALB/c mice. There were also strain differences under baseline conditions, between control animals as C57BL/6 mice significantly displayed a heavier thymus than BALB/c mice. As stress decreased thymus weight in C57BL/6 mice, as a result, there was no difference in thymus weight between stressed animals.

Regarding heart weight, there was no effect of stress or strain and no stress×strain interaction; however, small trends emerged for a stress effect, ($F(1,44)=2.99$, $P=0.091$), strain effect ($F(1,44)=3.04$, $P=0.088$) and stress×strain interaction ($F(1,44)=3.47$, $P=0.069$). Therefore, an a priori two-group comparison was conducted and Student *t*-test revealed stress increased heart weight in C57BL/6 mice compared with control animals ($P<0.05$).

Finally, regarding spleen weight, there was an overall strain effect ($F(1,42)=232.69$, $P<0.0001$) but no stress effect or stress×strain interaction. Post hoc analysis revealed that although overall stress did not have an effect on spleen weight in any strain, there was a trend for a heavier spleen in stressed BALB/c mice compared with control group ($P=0.057$). Also, C57BL/6 mice of both control and stress groups displayed significantly lighter spleens than their respective BALB/c mice (control, $P<0.0001$; stress, $P<0.0001$).

SD-L. Regarding the daily evolution of body weight (% baseline on day 1, Fig. 5B), there was only a significant effect of time ($F(9,369)=4.95$, $P<0.0001$) but no overall effect of stress or strain and no stress×strain, time×stress, time×strain or time×stress×strain interaction. Further, post hoc analysis did not reveal any statistical difference between groups on single days.

There was no stress or strain effect and no stress×strain interaction in the total body weight-gain.

Regarding thymus weight, there was an overall stress effect ($F(1,37)=14.99$, $P<0.001$), strain effect ($F(1,37)=13.68$, $P<0.001$), and a stress×strain interaction ($F(1,37)=5.03$, $P<0.05$). Post hoc analysis revealed that stress induced significantly lower thymus weight in BALB/c mice, compared with control animals ($P<0.01$), whereas there was no difference in C57BL/6 mice. Further, post hoc test showed there was no basal difference in thymus weight between control C57BL/6 and BALB/c animals, but as stress induced lighter thymus in BALB/c mice, stressed C57BL/6 animals then displayed a significantly higher thymus weight than stressed BALB/c mice ($P<0.0001$).

For the heart weight, there was a stress effect ($F(1,38)=8.44$, $P<0.01$) and a strain effect ($F(1,38)=9.52$, $P<0.01$), but no stress×strain interaction. Post hoc analysis further showed that although there was no basal difference in heart weight between control C57BL/6 and BALB/c mice, stress increased heart weight in C57BL/6, but not BALB/c, mice ($P<0.01$). As a result, stressed C57BL/6 mice had a heavier heart than stressed BALB/c animals ($P<0.01$).

For the spleen weight, there was a stress effect ($F(1,34)=5.04$, $P<0.05$) and a strain effect ($F(1,34)=24.39$, $P<0.0001$), but only a trend of stress×strain interaction ($F(1,34)=3.28$, $P=0.079$). Post hoc analysis revealed control C57BL/6 mice presented with a significantly lighter spleen than control BALB/c mice ($P<0.01$). Further, post hoc analysis showed stress induced an increase in spleen weight in BALB/c, but not C57BL/6 mice, compared with control group ($P<0.0001$). As a result, stress increased strain differences as stressed C57BL/6 mice displayed lower spleen weight than stressed BALB/c ($P<0.001$).

Colon physiology

SD-S. Data for colon length colon and colon motility (number of faecal pellets produced during the social interaction test) are presented in Table 1.

There was an overall strain effect in colon length (Table 1, $F(1,42)=68.94$, $P<0.0001$), but no stress effect or stress×strain interaction. Post hoc analysis showed C57BL/6 mice of both control and stress groups displayed significantly shorter colon than their respective BALB/c mice ($P<0.0001$).

Regarding colon motility, there was also a significant effect of strain ($F(1,42)=18.09$, $P<0.0001$), but no stress effect or stress×strain interaction. Post hoc test showed C57BL/6 mice of both control and stress groups produced significantly less outputs than their respective BALB/c mice ($P<0.05$).

SD-L. For the colon length, there was a strain effect ($F(1,40)=43.79$, $P<0.0001$), but no stress effect and no stress×strain interaction. Post hoc analysis showed C57BL/6 mice of both groups displayed significantly shorter colon than BALB/c mice ($P<0.001$).

Regarding the number of faecal pellets, there was a significant effect of stress ($F(1,40)=37.87$, $P<0.0001$), strain ($F(1,40)=45.40$, $P<0.0001$) and a stress×strain in-

teraction ($F(1,40)=19.26$, $P<0.0001$). Post hoc test revealed basal strain differences, with control C57BL/6 mice defaecating significantly less than BALB/c ones ($P<0.0001$). Further, stress induced significantly less defaecation in BALB/c mice than controls ($P<0.0001$) whereas it had no significant effect in C57BL/6 mice. Therefore, this suggests that BALB/c mouse strain may present a higher colonic motility than C57BL/6 mouse strain and this difference may be abolished by a repeated social defeat.

DISCUSSION

Social stress is one of the most potent stressful stimuli in mammals of all species (Sheridan et al., 2000; Blanchard et al., 2001). In this study, we investigated the effects of two different 10-day social defeat paradigms, relative to their severity, in two mouse strains that differ in their anxiety-related behaviour (O'Mahony et al., 2010). Our data show that BALB/c mice, which display elevated anxiety behaviour (Belzung and Griebel, 2001; Kalueff and Tuohimaa, 2005; Millstein and Holmes, 2007; O'Mahony et al., 2010) and which also differ in their baseline behaviour in the SI test (present data) are also more sensitive to social stress. Specifically, BALB/c mice demonstrated reduced social interaction following both short (SD-S) and long (SD-L) stress protocols. On the other-hand, C57BL/6 mice only showed such deficits following the more severe stressor (SD-L). Taken together, this suggests that BALB/c mice are useful background study to investigate the sensitivity to repeated social stressors.

With regard to the shorter stress protocol, BALB/c, but not C57BL/6 mice, were sensitive to the chronic social exposure and presented behavioural alterations. In both strains, prior social defeat did not affect the time spent in the target zone in the arena devoid of the aggressor (non-social conditions). However, when the target (aggressor) was present in the arena, BALB/c mice demonstrated reduced exploration of the target area, whereas C57BL/6 mice had no overt behavioural impairment. However, under the more severe SD-L paradigm, both strains demonstrated behavioural alterations in both a non social and social context, although clearly more pronounced effects were observed under social conditions and these modifications were more pronounced in BALB/c than C57BL/6 mice. Noteworthy, although in SD-L, BALB/c mice clearly displayed a lower time in the interaction zone under social than non-social conditions, C57BL/6 displayed the same reduction for both conditions compared with control mice thus confirming that BALB/c mice were more sensitive to SD-L than C57BL/6. It is also possible that the C57BL/6 mice may have simply displayed a general anxiety state rather than a specific avoidance of social targets. Whilst the relationship between stress-sensitivity and baseline anxiety is important, it is clear that performance in social-stress interactions is also dependent on other behavioural traits. It has been shown in rats that sociability and aggression behaviours are better predictors for social stress sensitivity in anxious rat strains than anxiety behaviour displayed in non-social anxiety tasks (Berton et al., 1997). Interestingly, BALB/c mice have also been studied for their high level of aggression (Dow et al., 2011) and their low level of sociability compared with C57BL/6 mice (Fairless et al., 2008) which may contribute to their overall phenotype.

In parallel to the behavioural assessments, we also conducted various physiological analyses determining the effects of social defeat paradigm on parameters that are commonly altered by stress (Bartolomucci et al., 2005; Krishnan et al., 2007; Reber et al., 2007; Savignac et al., 2011a). The levels of corticosterone, the main stress hormone, were measured in the plasma as an indicator of HPA-axis activation. Interestingly, SD-S did not induce any change in corticosterone levels in either of the two strains whereas SD-L was able to induce decreased levels in C57BL/6 mice. Of note, it was interesting to observe that although there was no difference in basal levels (control animals) between strains, SD-S induced opposite levels between C57BL/6 and BALB/c mice, as the latter displayed higher levels following stress. Nonetheless, higher corticosterone levels would have been expected following a chronic social stress as we have previously shown in BALB/c mice (Savignac et al., 2011a). However, in the latter study, samples were collected 2 h post last social stress (6-day repeated social stress exposure) whereas in the present study, plasma was taken one day following SI, and may reflect adaptive responses of the HPA axis to chronic stress. Another important point is that in SD-L, control mice, and in particular those of the C57BL/6 strain, displayed relatively high basal corticosterone levels, that may have masked any stress effect on corticosterone in defeated mice. In comparison, corticosterone levels of control SD-S mice were lower. One reason for these differences observed between SD-L and SD-S control mice was their housing conditions, which were adapted to each social defeat protocol; as a result, housing conditions of SD-L mice may have been a mild stressor, as suggested by their corticosterone levels. Nevertheless, there is much discrepancy in the literature regarding the impact of chronic stress on corticosterone levels in these two mouse strains, with many studies reporting either an increase, decrease or an absence of effect in stressed compared with control mice (Bartolomucci et al., 2001; Fano et al., 2001; Engler et al., 2005; Keeney et al., 2006; Krishnan et al., 2007; Michaud et al., 2008; Savignac et al., 2011a). Reasons for this may depend on, amongst other factors, the social status of the animals, the number of social defeat episodes the animals underwent and the time of sampling following stress termination. Moreover, it has been reported that the effects of chronic social stress on corticosterone levels in C57BL/6 mice may only be unmasked when animals are sacrificed immediately before the dark phase, and not in the morning (Reber et al., 2007), as in the current and other social stress models (Krishnan et al., 2007; Reber et al., 2007). Also chronic social-stress-induced alterations in the HPA axis may only become apparent following an acute stress challenge. Interestingly, our data are in agreement with a recent study employing a 10-day social defeat protocol similar to ours, that C57BL/6 mice did not display any change in their basal corticoste-

rone levels when sacrificed one day following the last defeat on day 11 (Krishnan et al., 2007).

Chronic stress is a risk factor for irritable bowel syndrome (IBS) and alterations in colon structure and function have been reported in stress-related disorders including IBS (Mayer et al., 2001; Reber et al., 2007; O'Mahony et al., 2009; Savignac et al., 2011a). Therefore, we have also assessed some parameters related to colonic function and hypothesized that BALB/c mice would present with more pronounced colonic changes. Stress-induced defaecation was also monitored during the SI test, as exposure to a novel environment increases faecal output (Kalueff and Tuohimaa, 2005; Barone et al., 2008; Wang et al., 2010). In the present studies, SD-S had no effect on faecal excretion in either BALB/c or C57BL/6 mice, however, unexpectedly, SD-L induced lower defaecation in stressed BALB/c mice compared with controls. Although somewhat surprising, we have obtained similar results in mice that underwent early-life stress (Savignac et al., 2011b). Moreover, the data may suggest decreases in colonic motility which is relevant to the constipation phenotype observed in some IBS patients, which has not been reproduced to date in IBS mouse models (Bercik et al., 2004; Kimball et al., 2005; Coates et al., 2006). Future studies should focus on analyzing the secretomotor function of the gastrointestinal tract from socially stressed animals. Finally, neither of the two social defeat procedures in either mouse strain used induced changes in colon length, which is a marker of colonic tissue inflammation (Reber et al., 2007). Together these findings suggest the magnitude of these two stress exposures was not sufficient enough to induce any significant colonic inflammation, which is consistent with the literature reporting that histological impairments following a similar type of social stress do not occur within the 15 first days of stress exposure but occur later (Reber et al., 2007).

Chronic stress has been shown to alter body weight (Bartolomucci et al., 2009; Savignac et al., 2011a). SD-S resulted in an increased total body weight gain in C57BL/6 mice, as well as during the course of the 10-days stress, whereas it induced a decrease in body weight gain across the 10 days in BALB/c mice. On the contrary, there was no change in any group following SD-L. The body weight findings at first glance may seem surprising, as chronic social stress has often been reported to decrease body weight gain regardless of strain investigated (Reber et al., 2006; Krishnan et al., 2007; Savignac et al., 2011a). However, no alterations in body weight, or increases, have also been described (Avitsur et al., 2001; Bartolomucci et al., 2005, 2009). Together, these data suggest that stress-induced body weight alterations are under complex regulatory processes specific to the nature of the stress employed.

Previous studies have shown that chronic stress results in alterations in spleen, thymus and heart weight (Leonard, 2000; Engler et al., 2005; Krishnan et al., 2007; Reber et al., 2007). SD-L induced spleen hypertrophy and thymus atrophy in BALB/c mice and cardiac hypertrophy in C57BL/6 animals. Our data are in agreement with previous findings showing that changes in thymus and spleen weight are commonly reported in social-stress studies (Avitsur et al., 2001; Gryazeva et al., 2001; Engler et al., 2005; Reber et al., 2006). Splenomegaly has been associated with increased immune cells recruitment to fight infection or combat the effects of stress, whereas thymus atrophy results in a decrease in the number of immature thymocytes, which are sensitive to stress-induced activation of the HPA-axis and sympathetic adrenomedullary system (Reber et al., 2007). Heart weight increase has also been described following 10-day social defeat in C57BL/6 mice (Krishnan et al., 2007), whereas mice regularly defeated have a blunted cardiac adaptation to stressor (Sgoifo et al., 2005). In contrast to SD-L, SD-S only induced minor physiological changes and in C57BL/6 mice only, with heart weight increase. Interestingly, while SD-S induced behavioural changes in BALB/c mice, these occurred independently from changes in spleen and thymus weight that required a more severe stressor to manifest. Other important parameters that would have been important to measure in future studies is the size of the adrenal glands weight, as adrenals hypertrophy is a good indicator of stress state (Reber et al., 2007).

Further studies are also needed to examine the neurobiological and genetic basis of this stress-sensitivity in BALB/c mice. One logical explanation for these behavioural differences lies in the innate variation in the gene encoding central tryptophan hydroxylase (TPH) between BALB/c and C57BL/6 mice that may be responsible for the lower serotonin (5-HT) rates in BALB/c mice (Zhang et al., 2004; Cervo et al., 2005; Jacobson and Cryan, 2007). It is widely accepted that lower serotonin rates predispose to the pathophysiology of a broad range of psychiatric disorders including depression; with numerous studies regularly showing deficiencies in serotonergic system is linked to dysfunctions in social behaviour and aggression (Holmes et al., 2002; Young and Leyton, 2002; Popova, 2008). Therefore, the reduced amount of serotonin in BALB/c mice may induce a heightened tendency to explore, and interact with other mice. Moreover, we have recently shown stress-induced alterations in serotonergic system between both strains (Browne et al., 2011). Indeed, 5-HT turnover was significantly increased in the majority of the brain regions assessed following acute stress in C57BL/6, whereas BALB/c mice exhibited significant increases in 5-HT turnover in the striatum and hippocampus only following repeated stress. On the other hand, TPH activity was significantly decreased in the brainstem and cortical regions of C57BL/6 but not BALB/c mice following both acute and chronic stress (Browne et al., 2011). Whilst variation in genes relevant to the 5-HT system are perhaps the most parsimonious explanation for the differential responses to stress observed in these two inbred mouse strains, it must be acknowledged that these strains also differ in multiple other genetic polymorphisms which are likely to significantly contribute phenotypically to animals' behavior under basal and stressful conditions.

Another explanation for the ability of BALB/c mice to be more socially avoidant than C57BL/6, is the potential of

stress to amplify memory for social hierarchy via protein synthesis changes (Cordero and Sandi, 2007). Therefore, as BALB/c mice are more anxious, the stress induced by daily social defeat may be stronger than that induced in C57BL/6, resulting in BALB/c mice remembering better the negative social interaction and as a result are more avoidant of any further social interaction (i.e. the social target), via modification in gene expression and protein synthesis in key brain areas involved in fear learning. This hypothesis is in line with previous findings from our laboratory showing BALB/c mice display alterations in stress-induced c-Fos activation in cortical and hippocampal brain areas (O'Mahony et al., 2010).

The molecular basis of stress resilience has been under intense scrutiny recently. Indeed, it has been shown that even within the C57BL/6 strain diverging phenotypes can emerge at the extremes of stress-sensitivity with some mice being extremely stress-susceptible whereas others are stress-resilient (Krishnan et al., 2007; Renthal et al., 2007; Vialou et al., 2010). These studies showed that the neurotrophin brain derived neurotrophic factor (BDNF) in reward pathways plays a key role and also point to the importance of epigenetic mechanisms. Future studies should focus on the role of these mechanisms in defining inter-strain stress susceptibility factors. Indeed, it has been very recently shown that epigenetic regulation via histone modifications and DNA methylation of the promoter for the gene encoding another neurotophin glial cell-derived neurotrophic factor (GDNF) within the ventral striatum, plays a crucial role in the control of behavioral responses to chronic stress in BALB/c mice (Uchida et al., 2011). Interestingly, it has also been shown that these BALB/c and C57BL/6 differ in the level of maternal care mothers give to their pups which can lead to differences in epigenetic regulation during early life (Prakash et al., 2006; Millstein and Holmes, 2007).

CONCLUSIONS

Overall, our studies demonstrate that the innately anxious BALB/c mice were more sensitive to social defeat stress than C57BL/6 mice, being affected by both a short and a more severe stress protocol. While C57BL/6 mice did display stress-induced alterations, these were to a lesser extent than BALB/c mice, and were only observed following the long stress procedure. To our knowledge, this has not been investigated before, and is consistent with previous findings reporting BALB/c animals were more sensitive than C57BL/6 mice to chronic mild stress (Palumbo et al., 2009; Uchida et al., 2011). BALB/c and C57BL/6 mice may display different physiological responses to stress as well as differential stress coping strategies (Sgoifo et al., 2005), due to differential central nervous system activation. These data highlight the fundamental role genetic factors play on stress susceptibility. Moreover, these social defeat models may constitute useful tools to investigate underlying mechanisms of stress resilience and thus aid in further our understanding of stress-induced disorders such as depression and IBS. The molecular basis of this stress-resilience/susceptibility warrants further investigation and we propose that BALB/c mice maybe an ideal strain for this purpose.

Acknowledgments—The authors would like to thank Colette Manley, Patrick Fitzgerald, Fabian Sweeney, Dr. Marcela Julio-Piper and Dr. Monica Tramullas for technical assistance. The Alimentary Pharmabiotic Centre is a research centre funded by Science Foundation Ireland (SFI), through the Irish Government's National Development Plan. The authors and their work were supported by SFI (grant no.s 02/CE/B124 and 07/CE/B1368). J.F.C. is also funded by European Community's Seventh Framework Programme; Grant Number: FP7/2007-2013, grant agreement 201714. The funding source had no involvement in the study design, in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the paper for publication.

REFERENCES

Anisman H, Merali Z, Stead JD (2008) Experiential and genetic contributions to depressive- and anxiety-like disorders: clinical and experimental studies. Neurosci Biobehav Rev 32:1185–1206.

Anisman H, Zaharia MD, Meaney MJ, Merali Z (1998) Do early-life events permanently alter behavioral and hormonal responses to stressors? Int J Dev Neurosci 16:149–164.

Avgustinovich DF, Gorbach OV, Kudryavtseva NN (1997) Comparative analysis of anxiety-like behavior in partition and plus-maze tests after agonistic interactions in mice. Physiol Behav 61:37–43.

Avgustinovich DF, Kovalenko IL, Kudryavtseva NN (2005) A model of anxious depression: persistence of behavioral pathology. Neurosci Behav Physiol 35:917–924.

Avitsur R, Stark JL, Sheridan JF (2001) Social stress induces glucocorticoid resistance in subordinate animals. Horm Behav 39:247–257.

Barone FC, Barton ME, White RF, Legos JJ, Kikkawa H, Shimamura M, Kuratani K, Kinoshita M (2008) Inhibition of phosphodiesterase type 4 decreases stress-induced defecation in rats and mice. Pharmacology 81:11–17.

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:e4331.

Bartolomucci A, Palanza P, Gaspani L, Limiroli E, Panerai AE, Ceresini G, Poli MD, Parmigiani S (2001) Social status in mice: behavioral, endocrine and immune changes are context dependent. Physiol Behav 73:401–410.

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

Beitia G, Garmendia L, Azpiroz A, Vegas O, Brain PF, Arregi A (2005) Time-dependent behavioral, neurochemical, and immune consequences of repeated experiences of social defeat stress in male mice and the ameliorative effects of fluoxetine. Brain Behav Immun 19:530–539.

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

Bercik P, Wang L, Verdu EF, Mao YK, Blennerhassett P, Khan WI, Kean I, Tougas G, Collins SM (2004) Visceral hyperalgesia and intestinal dysmotility in a mouse model of postinfective gut dysfunction. Gastroenterology 127:179–187.

Berton O, McClung CA, Dileone RJ, Krishnan V, Renthal W, Russo SJ, Graham D, Tsankova NM, Bolanos CA, Rios M, Monteggia LM, Self DW, Nestler EJ (2006) Essential role of BDNF in the mesolim-

bic dopamine pathway in social defeat stress. Science 311:864–868.
Berton O, Ramos A, Chaouloff F, Mormde P (1997) Behavioral reactivity to social and nonsocial stimulations: a multivariate analysis of six inbred rat strains. Behav Genet 27:155–166.
Blanchard RJ, McKittrick CR, Blanchard DC (2001) Animal models of social stress: effects on behavior and brain neurochemical systems. Physiol Behav 73:261–271.
Browne CA, Clarke G, Dinan TG, Cryan JF (2011) Differential stress-induced alterations in tryptophan hydroxylase activity and serotonin turnover in two inbred mouse strains. Neuropharmacology 60:683–691.
Cervo L, Canetta A, Calcagno E, Burbassi S, Sacchetti G, Caccia S, Fracasso C, Albani D, Forloni G, Invernizzi RW (2005) Genotype-dependent activity of tryptophan hydroxylase-2 determines the response to citalopram in a mouse model of depression. J Neurosci 25:8165–8172.
Coates MD, Johnson AC, Greenwood-Van Meerveld B, Mawe GM (2006) Effects of serotonin transporter inhibition on gastrointestinal motility and colonic sensitivity in the mouse. Neurogastroenterol Motil 18:464–471.
Connor TJ, Leonard BE (1998) Depression, stress and immunological activation: the role of cytokines in depressive disorders. Life Sci 62:583–606.
Cordero MI, Sandi C (2007) Stress amplifies memory for social hierarchy. Front Neurosci 1:175–184.
Crawley JN (2008) Behavioral phenotyping strategies for mutant mice. Neuron 57:809–818.
Cryan JF, Holmes A (2005) The ascent of mouse: advances in modelling human depression and anxiety. Nat Rev Drug Discov 4:775–790.
Cryan JF, Slattery DA (2007) Animal models of mood disorders: recent developments. Curr Opin Psychiatry 20:1–7.
de Kloet ER, Sibug RM, Helmerhorst FM, Schmidt MV (2005) Stress, genes and the mechanism of programming the brain for later life. Neurosci Biobehav Rev 29:271–281.
Dow HC, Kreibich AS, Kaercher KA, Sankoorikal GM, Pauley ED, Lohoff FW, Ferraro TN, Li H, Brodkin ES (2011) Genetic dissection of intermale aggressive behavior in BALB/cJ and A/J mice. Genes Brain Behav 10:57–68.
Engler H, Engler A, Bailey MT, Sheridan JF (2005) Tissue-specific alterations in the glucocorticoid sensitivity of immune cells following repeated social defeat in mice. J Neuroimmunol 163:110–119.
Fairless AH, Dow HC, Toledo MM, Malkus KA, Edelmann M, Li H, Talbot K, Arnold SE, Abel T, Brodkin ES (2008) Low sociability is associated with reduced size of the corpus callosum in the BALB/cJ inbred mouse strain. Brain Res 1230:211–217.
Fano E, Sanchez-Martin JR, Arregi A, Castro B, Alonso A, Brain P, Azpiroz A (2001) Social stress paradigms in male mice: variations in behavior, stress and immunology. Physiol Behav 73:165–173.
Feder A, Nestler EJ, Charney DS (2009) Psychobiology and molecular genetics of resilience. Nat Rev Neurosci 10:446–457.
Griebel G, Belzung C, Perrault G, Sanger DJ (2000) Differences in anxiety-related behaviours and in sensitivity to diazepam in inbred and outbred strains of mice. Psychopharmacology (Berl) 148:164–170.
Gryazeva NI, Shurlygina AV, Verbitskaya LV, Mel'nikova EV, Kudryavtseva NN, Trufakin VA (2001) Changes in various measures of immune status in mice subject to chronic social conflict. Neurosci Behav Physiol 31:75–81.
Holmes A, Murphy DL, Crawley JN (2002) Reduced aggression in mice lacking the serotonin transporter. Psychopharmacology (Berl) 161:160–167.
Jacobson LH, Cryan JF (2007) Feeling strained? Influence of genetic background on depression-related behavior in mice: a review. Behav Genet 37:171–213.
Jacobson LH, Cryan JF (2010) Genetic approaches to modeling anxiety in animals. Curr Top Behav Neurosci 2:161–201.
Julio-Pieper M, Hyland NP, Bravo JA, Dinan TG, Cryan JF (2010) A novel role for the metabotropic glutamate receptor-7: modulation of faecal water content and colonic electrolyte transport in the mouse. Br J Pharmacol 160:367–375.
Kalueff AV, Tuohimaa P (2005) The Suok ("ropewalking") murine test of anxiety. Brain Res Brain Res Protoc 14:87–99.
Keeney A, Jessop DS, Harbuz MS, Marsden CA, Hogg S, Blackburn-Munro RE (2006) Differential effects of acute and chronic social defeat stress on hypothalamic-pituitary-adrenal axis function and hippocampal serotonin release in mice. J Neuroendocrinol 18:330–338.
Kimball ES, Palmer JM, D'Andrea MR, Hornby PJ, Wade PR (2005) Acute colitis induction by oil of mustard results in later development of an IBS-like accelerated upper GI transit in mice. Am J Physiol Gastrointest Liver Physiol 288:G1266–G1273.
Kinsey SG, Bailey MT, Sheridan JF, Padgett DA, Avitsur R (2007) Repeated social defeat causes increased anxiety-like behavior and alters splenocyte function in C57BL/6 and CD-1 mice. Brain Behav Immun 21:458–466.
Koob GF (1999) Stress, corticotropin-releasing factor, and drug addiction. Ann N Y Acad Sci 897:27–45.
Koolhaas JM, de Boer SF, Buwalda B, van Reenen K (2007) Individual variation in coping with stress: a multidimensional approach of ultimate and proximate mechanisms. Brain Behav Evol 70:218–226.
Krishnan V, Han MH, Graham DL, Berton O, Renthal W, Russo SJ, Laplant Q, Graham A, Lutter M, Lagace DC, Ghose S, Reister R, Tannous P, Green TA, Neve RL, Chakravarty S, Kumar A, Eisch AJ, Self DW, Lee FS, Tamminga CA, Cooper DC, Gershenfeld HK, Nestler EJ (2007) Molecular adaptations underlying susceptibility and resistance to social defeat in brain reward regions. Cell 131:391–404.
Krishnan V, Nestler EJ (2008) The molecular neurobiology of depression. Nature 455:894–902.
Krishnan V, Nestler EJ (2010) Linking molecules to mood: new insight into the biology of depression. Am J Psychiatry 167:1305–1320.
Kudryavtseva NN, Bakshtanovskaya IV, Koryakina LA (1991) Social model of depression in mice of C57BL/6J strain. Pharmacol Biochem Behav 38:315–320.
Leonard B (2000) Stress, depression and the activation of the immune system. World J Biol Psychiatry 1:17–25.
Leonard BE, Song C (1996) Stress and the immune system in the etiology of anxiety and depression. Pharmacol Biochem Behav 54:299–303.
Mayer EA, Naliboff BD, Chang L, Coutinho SVV (2001) Stress and irritable bowel syndrome. Am J Physiol Gastrointest Liver Physiol 280:G519–G524.
McEwen BS (2000) The neurobiology of stress: from serendipity to clinical relevance. Brain Res 886:172–189.
Merlot E, Moze E, Dantzer R, Neveu PJ (2004) Cytokine production by spleen cells after social defeat in mice: activation of T cells and reduced inhibition by glucocorticoids. Stress 7:55–61.
Michaud K, Matheson K, Kelly O, Anisman H (2008) Impact of stressors in a natural context on release of cortisol in healthy adult humans: a meta-analysis. Stress 11:177–197.
Miczek KA, de Wit H (2008) Challenges for translational psychopharmacology research—some basic principles. Psychopharmacology (Berl) 199:291–301.
Miczek KA, Yap JJ, Covington HE 3rd (2008) Social stress, therapeutics and drug abuse: preclinical models of escalated and depressed intake. Pharmacol Ther 120:102–128.
Millstein RA, Holmes A (2007) Effects of repeated maternal separation on anxiety- and depression-related phenotypes in different mouse strains. Neurosci Biobehav Rev 31:3–17.
Murphy JM, Horton NJ, Laird NM, Monson RR, Sobol AM, Leighton AH (2004) Anxiety and depression: a 40-year perspective on relationships regarding prevalence, distribution, and comorbidity. Acta Psychiatr Scand 109:355–375.

O'Mahony CM, Sweeney FF, Daly E, Dinan TG, Cryan JF (2010) Restraint stress-induced brain activation patterns in two strains of mice differing in their anxiety behaviour. Behav Brain Res 213: 148–154.

O'Mahony SM, Marchesi JR, Scully P, Codling C, Ceolho AM, Quigley EM, Cryan JF, Dinan TG (2009) Early life stress alters behavior, immunity, and microbiota in rats: implications for irritable bowel syndrome and psychiatric illnesses. Biol Psychiatry 65:263–267.

Palumbo ML, Zorrilla Zubilete MA, Cremaschi GA, Genaro AM (2009) Different effect of chronic stress on learning and memory in BALB/c and C57BL/6 inbred mice: involvement of hippocampal NO production and PKC activity. Stress 12:350–361.

Popova NK (2008) From gene to aggressive behavior: the role of brain serotonin. Neurosci Behav Physiol 38:471–475.

Prakash P, Merali Z, Kolajova M, Tannenbaum BM, Anisman H (2006) Maternal factors and monoamine changes in stress-resilient and susceptible mice: cross-fostering effects. Brain Res 1111:122–133.

Reber SO, Birkeneder L, Veenema AH, Obermeier F, Falk W, Straub RH, Neumann ID (2007) Adrenal insufficiency and colonic inflammation after a novel chronic psycho-social stress paradigm in mice: implications and mechanisms. Endocrinology 148:670–682.

Reber SO, Obermeier F, Straub RH, Falk W, Neumann ID (2006) Chronic intermittent psychosocial stress (social defeat/overcrowding) in mice increases the severity of an acute DSS-induced colitis and impairs regeneration. Endocrinology 147:4968–4976.

Renthal W, Maze I, Krishnan V, Covington HE 3rd, Xiao G, Kumar A, Russo SJ, Graham A, Tsankova N, Kippin TE, Kerstetter KA, Neve RL, Haggarty SJ, McKinsey TA, Bassel-Duby R, Olson EN, Nestler EJ (2007) Histone deacetylase 5 epigenetically controls behavioral adaptations to chronic emotional stimuli. Neuron 56:517–529.

Savignac HM, Hyland NP, Dinan TG, Cryan JF (2011a) The effects of repeated social interaction stress on behavioural and physiological parameters in a stress-sensitive mouse strain. Behav Brain Res 216:576–584.

Savignac HM, Dinan TG, Cryan JF (2011b) Resistance to early-life stress in mice: effects of genetic background and stress duration. Front Behav Neurosci 5:13.

Sgoifo A, Costoli T, Meerlo P, Buwalda B, Pico'-Alfonso MA, De Boer S, Musso E, Koolhaas J (2005) Individual differences in cardiovascular response to social challenge. Neurosci Biobehav Rev 29: 59–66.

Sheridan JF, Stark JL, Avitsur R, Padgett DA (2000) Social disruption, immunity, and susceptibility to viral infection. Role of glucocorticoid insensitivity and NGF. Ann N Y Acad Sci 917:894–905.

Uchida S, Hara K, Kobayashi A, Otsuki K, Yamagata H, Hobara T, Suzuki T, Miyata N, Watanabe Y (2011) Epigenetic status of Gdnf in the ventral striatum determines susceptibility and adaptation to daily stressful events. Neuron 69(2):359–372.

Vialou V, Robison AJ, Laplant QC, Covington HE 3rd, Dietz DM, Ohnishi YN, Mouzon E, Rush AJ 3rd, Watts EL, Wallace DL, Iniguez SD, Ohnishi YH, Steiner MA, Warren BL, Krishnan V, Bolanos CA, Neve RL, Ghose S, Berton O, Tamminga CA, Nestler EJ (2010) DeltaFosB in brain reward circuits mediates resilience to stress and antidepressant responses. Nat Neurosci 13:745–752.

Wang L, Gourcerol G, Yuan PQ, Wu SV, Million M, Larauche M, Tache Y (2010) Peripheral peptide YY inhibits propulsive colonic motor function through Y2 receptor in conscious mice. Am J Physiol Gastrointest Liver Physiol 298:G45–G56.

Wurtman RJ (2005) Genes, stress, and depression. Metabolism 54:16–19.

Young SN, Leyton M (2002) The role of serotonin in human mood and social interaction. Insight from altered tryptophan levels. Pharmacol Biochem Behav 71:857–865.

Zhang X, Beaulieu JM, Sotnikova TD, Gainetdinov RR, Caron MG (2004) Tryptophan hydroxylase-2 controls brain serotonin synthesis. Science 305:217.

(Accepted 25 April 2011)
(Available online 27 May 2011)