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Multifaceted strain-specific effects in a mouse model of depression and of antidepressant reversal

Yadira Ibarguen-Vargas [a,b,1], Alexandre Surget [a,b,1], Chadi Touma [c], Rupert Palme [d], Catherine Belzung [a,b,*]

[a] U930 FRE CNRS 2448, Inserm, Tours, France
[b] Faculté des Sciences et Techniques, Université François Rabelais, Bât O Parc de Grandmont, 37200 Tours, France
[c] Department of Behavioral Neuroendocrinology, Max Planck Institute of Psychiatry, Munich, Germany
[d] Institute of Biochemistry, Department of Natural Sciences, University of Veterinary Medicine, Vienna, Austria

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Summary Etiopathogenesis of depression and the cause of insensitivity to treatment remain poorly understood, although genetic makeup has been established as a contributing factor. The isogenicity of inbred mouse strains provides a useful tool for investigating the link between genes and behavior or drug response. Hence, our aim was to identify inbred mouse strains (among A/J, BALB/c, C3H, C57BL/6, CBA, DBA and FVB) sensitive to a 9-week period of unpredictable chronic mild stress (UCMS) and, from the fifth week onward, to the reversal effect of an antidepressant (AD) (imipramine, 20 mg/kg/day i.p.) on various depression-related changes: physical, behavioral and neuroendocrine states. UCMS induced a significant deterioration of the coat state (in all the strains), blunted emotional reactivity in the novelty-suppressed feeding (NSF) test (A/J, BALB/c, C57BL/6), and changes in the level of fecal corticosterone metabolites (BALB/c, C57BL/6, DBA, FVB). Imipramine treatment reversed the UCMS-induced alterations of the coat state (BALB/c, DBA), in the NSF test (A/J, BALB/c, C57BL/6) and in fecal corticosterone metabolites (BALB/c, C57BL/6). C3H, CBA and FVB mice were irresponsive to imipramine treatment. It is noteworthy that UCMS-induced physical or behavioral changes occurred without hypothalamo—pituitary—adrenal (HPA) axis alterations in some strains (A/J, C3H, CBA), although the AD-induced reversal of these changes in BALB/c and C57BL/6 was associated with HPA axis normalization. Finally, UCMS is shown to discriminate various alterations and to replicate in a strain-dependent manner diverse profiles reminiscent of human disease subtypes. UCMS may thus enable the selection of strains suitable for investigating specific depression-related features and could be an appropriate model for identifying genetic factors associated with increased vulnerability, specific symptoms of affective disorders, and AD resistance.

* Corresponding author at: Faculté des Sciences et Techniques, Université François Rabelais, Bât O Parc de Grandmont, 37200 Tours, France. Tel.: +33 2 47 36 69 94; fax: +33 2 47 36 72 85.
E-mail address: catherine.belzung@univ-tours.fr (C. Belzung).
[1] These Authors contributed equally to the work.

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1. Introduction

Major depressive disorder (MDD) is one of the most common and serious health problems of western societies (Murray and Lopez, 1997). MDD is not a well-defined syndrome as it encompasses various subtypes with different patterns of symptoms. While the etiology of MDD is multifactorial and far from perfectly understood, chronic stress or stressful events have been identified among the major environmental factors precipitating depression (Kendler et al., 1999; Riso et al., 2002; Hammen, 2005). This is corroborated by the frequent occurrence of neuroendocrine stress system disturbances in MDD, such as hypercortisolemia and negative feedback impairments of the hypothalamo–pituitary–adrenal (HPA) axis (Arborelius et al., 1999; Holsboer, 2000; Gold and Chrousos, 2002; Barden, 2004). However, an adverse experience does not automatically trigger depressive episodes, but vulnerability is related to the individual's history of stressful life events as well as developmental, genetic and epigenetic factors (Caspi et al., 2003; Craddock and Forty, 2006; Goldberg, 2006; Mill and Petronis, 2007). Likewise, the heterogeneous pattern of symptoms, the presence of HPA disturbances and the frequently observed insensitivity to antidepressants (AD) could stem from the genetic makeup. Knowledge of the genetic basis of these individual differences could help to unravel the source of vulnerability, MDD subtypes and AD resistance. Animal models can be useful to facilitate the discovery of candidate genes.

Studies on inbred mouse strains can provide a powerful tool for understanding the influence of genes in normal and disordered brain function. Interestingly, variability in the response of different inbred mouse strains has been observed in various paradigms such as the forced swimming test (FST) (Bai et al., 2001; Lucki et al., 2001; David et al., 2003), the tail suspension test (TST) (Bai et al., 2001; Liu and Gershenfeld, 2001; Ripoll et al., 2003; Crowley et al., 2005; Liu et al., 2007) or the unpredictable chronic mild stress (UCMS) (Pothion et al., 2004; Ducottet and Belzung, 2005; Mineur et al., 2006). However, sorting strains according to their propensity to develop depression-like behaviors or AD response would appear to be vain in view of the discrepant results of studies. This discrepancy could be due to the diversity of paradigms used. Their common feature is sensitivity to ADs, but they differ in the theoretical background with which they are constructed. The most widely used paradigms are the FST and the TST. Both tests are based on exposure to a single aversive and inescapable situation which induces a behavioral shift from struggling to immobility. A single AD administration can decrease the duration of immobility (Porsolt et al., 1977; Steru et al., 1985; Cryan and Holmes, 2005). The utilization of these tests for AD drug detection gained popularity as pharmacological screening assays, but have been increasingly used for studying neurobiology and pathophysiology as well as for identifying genes causing depression. However, the fact that MDD is a chronic disease and that ADs are only clinically active after a minimum of three weeks treatment makes the validity of such paradigms questionable, particularly when examining the mechanisms involved in the etiology, maintenance and treatment of MDD. Chronic models of depression, such as the UCMS paradigm, could provide an alternative method, avoiding such drawbacks. UCMS is based on subjecting mice to a period (generally five to nine weeks) of mild socio-environmental stressors. This procedure replicates several depression-related behavioral and physiological impairments which can be reversed by chronic, but not acute, AD treatment (Belzung and Surget, 2008): decreased sucrose consumption (interpreted as anhedonia), increased fearfulness/anxiety-related behaviors, altered weight gain, deterioration of the coat (interpreted as the loss of interest in performing customary tasks).

A survey of inbred mouse strains in the UCMS paradigm could be a step toward identifying genes involved in vulnerability to stress exposure, the development of different MDD-associated symptoms, and insensitivity to AD. The major goal of the present study was to identify inbred mouse strains sensitive to the UCMS procedure and in which ADs can reverse various depression-related changes. Mice from seven different strains (A/J, BALB/c, C3H, C57BL/6, CBA, DBA and FVB) were subjected to a 9-week UCMS regimen. From the fifth week onward, vehicle or imipramine (20 mg/kg) was administered i.p. daily. The effects of UCMS and of imipramine treatment were assessed using physical measures (coat state, weight), behavioral tests (novelty-suppressed feeding [NSF] test and actimeter), and the level of fecal corticosterone metabolites was measured to assess HPA axis functioning.

2. Methods

2.1. Animals

Male mice from seven inbred strains (A/J, BALB/cByJ, CBA/J, C3H/HeJ, C57BL/6J, DBA/2J, FVB/NJ) were obtained from the Centre d'élevage Janvier (Le Genest Saint Isle, France) and Harlan (Gannat, France). They were aged seven weeks on arrival in our lab. Before the onset of the experiments, all animals were housed in groups of 5 and were maintained under standard laboratory conditions with a 12/12 h light/dark cycle (lights on at 20:00 h), 22 ± 2 °C, food and water *ad libitum*. The treatment of the animals complied with the European Community Council directive 86/609/EEC.

2.2. Drugs

Imipramine hydrochloride (Sigma–Aldrich) was used in this study. Imipramine was prepared as solutions in physiological saline (NaCl 0.9%). Concentration was adjusted to administer a final volume of 10 ml/kg.

2.3. General procedure

On arrival, mice were kept in the laboratory for two weeks before the onset of the experiments. A 9-week UCMS procedure was then conducted. UCMS-exposed mice (2/3) were maintained under standard laboratory conditions but were isolated in small individual cages (24 cm × 11 cm × 12 cm), while non-stressed control mice (1/3) were housed in groups of 4 or 5 in standard laboratory cages (42 cm × 28 cm × 18 cm). The first four weeks of the UCMS regimen were drug-free, and treatment began from the fifth week of UCMS and continued to the end of behavioral testing. Vehicle (0.9% NaCl) or imipramine (20 mg/kg/day) was administered i.p. once a day. The dose was chosen on the basis of previous experiments showing

that the compound is active at this concentration (Santarelli et al., 2003; Surget et al., 2008a; Yalcin et al., 2008). Each strain of mice was divided into three groups: control/vehicle, UCMS/vehicle and UCMS/imipramine. Each group consisted of 9—13 animals. Body weight and coat state were assessed weekly until the end of UCMS. The day after the last body weight and coat state evaluation, behavioral tests were carried out as follows: NSF test on the first day and locomotor activity using an actimeter on the fourth day. Control animals were isolated 2 days before and until the end of the actimeter session. To avoid experimental bias, the cages of stressed mice were changed at the same time. One week after the last body weight and coat state evaluation, mice were sacrificed by CO_2 asphyxiation (from 10:00 h to 15:00 h), and feces were then collected. Testing and feces collection were always carried out during the dark period. The experimental design is illustrated in Fig. 1.

2.4. Unpredictable Chronic Mild Stress (UCMS)

Chronic mild stress procedures were initially developed in rats (Willner et al., 1992). The stress regimen used in this study is a variant of the UCMS procedure used with mice in our lab (Santarelli et al., 2003; Surget et al., 2008a). Mice were subjected to various and repeated unpredictable stressors for a period of nine weeks. The stressors were: altered bedding (change or removal of sawdust, damp sawdust, substitution of sawdust with 21 °C water), cage tilting (45°), predator sounds (15 min), cage exchange (mice were placed in the empty cage of another male), altered length and time of light/dark cycle. Body weight and coat state were assessed once a week throughout the nine weeks. The total score for coat state was the sum of the scores obtained from seven different body parts: head, neck, dorsal coat, ventral coat, tail, forepaws and hindpaws. For each body area, a score of 0 was given for a well-groomed coat and 1 for an unkempt coat. This index has been pharmacologically validated in previous studies using BALB/c mice (Ducottet et al., 2003; Yalcin et al., 2005, 2008; Surget et al., 2008a).

2.5. Novelty-suppressed feeding (NSF) test

The NSF Test was similar to the version used by Surget et al. (2008a,b). The testing apparatus consisted of a wooden box, 33 cm × 33 cm × 30 cm, with an indirect red light. The floor was covered with 2 cm sawdust. Twelve hours before the test, food was removed from the cages. At the time of testing, a single pellet of food (regular chow) was placed on a white paper platform in the center of the box. An animal was placed in a corner of the test box. The latency to start consuming the pellet was recorded within a 3 min period. This test induces a conflict between the drive to eat the food pellet and the fear of venturing into the center of the arena. This version has been shown to reveal that antidepressants reverse the UCMS-induced increase in latency in UCMS but not control mice (Surget et al., 2008b). As antidepressants are known to have various effects on appetite, and to control for this potentially confounding factor, the feeding drive of each animal was assessed by returning it to the familiar environment of the home cage immediately after the test and measuring the amount of food consumed over a period of 5 min (home food consumption).

2.6. Actimeter

The actimeter assessed locomotor activity. The mice were tested in their home cage to exclude the possibility of biasing the results due to novelty-induced anxiety. The home cage was placed in the centre of the actimeter which consisted of a 20 cm × 20 cm square plane with two light beams crossing the plane from midpoint to midpoint of opposite sides, thus dividing the plane into four quadrants. The device automatically detects the movement of an animal when it crosses a beam. Each movement is scored; the more the mouse moves, the higher the score. Testing was carried out from 11:00 h and lasted 2 h to give a better estimation of the basal locomotor activity.

2.7. Collection of feces

Feces were collected at the time of euthanasia between 10:00 h and 15:00 h, which was during the dark period, and later directly from the colon by dissection. Fecal boli of each mouse were immediately put into microcentrifuge tubes and then stored at −20 °C until extraction. In this way, urine contamination of the feces samples was avoided.

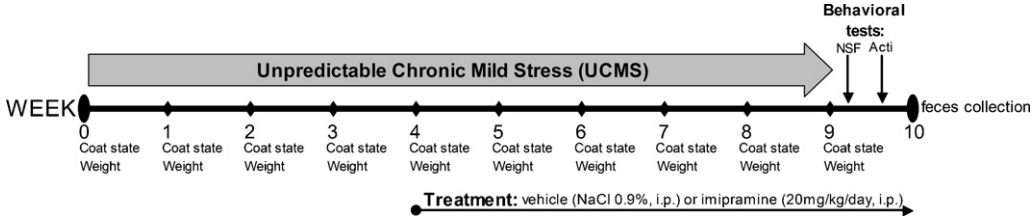

Figure 1 Experimental design. Seven strains were used (A/J, BALB/c, C3H, C57BL/6, CBA, DBA and FVB). The Unpredictable Chronic Mild Stress (UCMS) regimen lasted nine weeks. Three groups per strain (n = 9—13 mice/group) were used: Control/Vehicle, UCMS/Vehicle and UCMS/imipramine. Each week, the coat state was evaluated and the body weight measured by two experimenters blind to the treatment. The first four weeks of UCMS regimen were drug-free. Imipramine or vehicle treatments began after four weeks of UCMS and continued until the end of the experiment (week 10). Imipramine (20 mg/kg/day) or vehicle was administered intraperitoneally once a day. The week following the end of the UCMS regimen, the Novelty-Suppressed Feeding (NSF) test and actimeter (acti) were carried out. At the end, feces were collected at the time of euthanasia (Sac.) in the cage and directly in the colon (4—6 mice/group). These feces samples were processed to evaluate the level of fecal corticosterone metabolites.

2.8. Extraction procedure and analysis of fecal steroid metabolites

The collected fecal samples were analyzed for immunoreactive corticosterone metabolites (CM) using a 5α-pregnane-3β,11β,21-triol-20-one enzyme-immunoassay (EIA). Details of development, biochemical characteristics and biological validation of this assay have been described by Touma et al. (2003, 2004). Before EIA analysis, the fecal samples were dried and homogenized, and aliquots of 0.05 g were extracted with 1 ml of 80% methanol. A detailed description of the extraction procedure and the assay performance has been published elsewhere (Touma et al., 2003). The intra- and inter-assay coefficients of variation were 9.1% and 14.0%, respectively.

2.9. Statistics

Two-way repeated measure ANOVAs were performed for the analysis of the coat state and body weight in each strain by using week of UCMS (from 0 to 9) and group (control/vehicle, UCMS/vehicle and UCMS/imipramine) as main factors. One-way ANOVAs were carried out to analyze latency in the NSF test, locomotor activity and level of fecal corticosterone metabolites in each strain (3 groups: control/vehicle, UCMS/vehicle and UCMS/imipramine). The analysis of variance was followed by a Fisher *post hoc* analysis when required ($p < 0.05$).

3. Results

3.1. Evaluation of coat state

The coat state was evaluated before the onset of the UCMS regimen and then once a week for nine weeks until the end of UCMS (Fig. 1). The total score was the sum of the scores obtained from seven different body parts (see Section 2); the higher the score, the worse the coat state (Fig. 2).

For the A/J strain, ANOVA revealed significant differences (week, $F_{9315} = 37.18$, $p < 0.001$; group, $F_{2315} = 33.27$, $p < 0.001$; week × group, $F_{18,315} = 11.51$, $p < 0.001$); the 9-week UCMS protocol gave rise to a deterioration of the coat state, with the difference between control/vehicle and UCMS/vehicle mice reaching significance from week 3 to the end (weeks 3–9, $p < 0.001$); imipramine treatment did not reverse this effect, as *post hoc* analysis revealed no significant difference between UCMS/vehicle and UCMS/imipramine groups.

For the BALB/c strain, the UCMS regimen induced a deterioration of the coat state (week, $F_{9279} = 51$, $p < 0.001$; group,

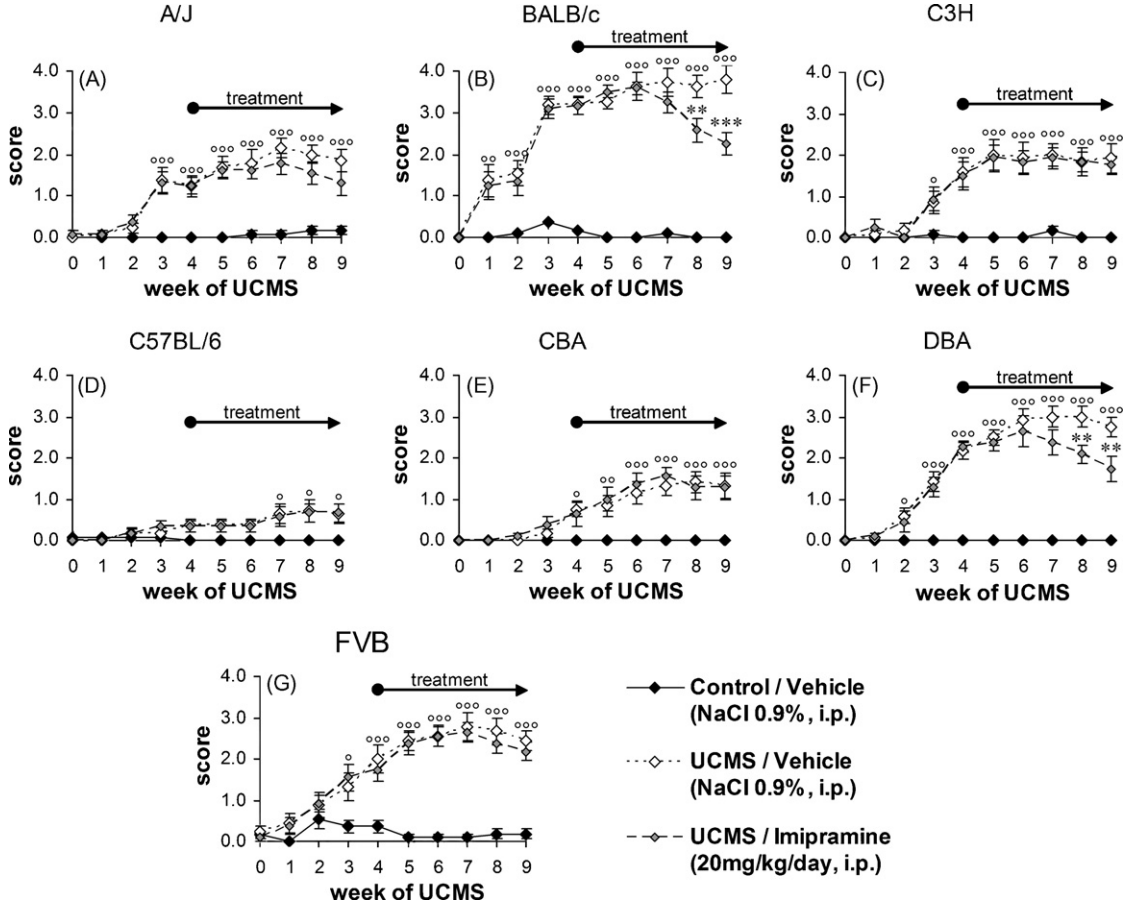

Figure 2 Effect of Unpredictable Chronic Mild Stress (UCMS) and of imipramine treatment on the coat state in A/J, BALB/c, C3H, C57BL/6, CBA, DBA and FVB mice. $N = 9–13$ mice/group. Data represent mean ± S.E.M. $^{\circ}p < 0.05$, $^{\circ\circ}p < 0.01$ and $^{\circ\circ\circ}p < 0.001$ between control/vehicle and UCMS/vehicle. $^{**}p < 0.01$ and $^{***}p < 0.001$ imipramine-treated vs. vehicle-treated mice.

$F_{2279} = 155.27$, $p < 0.001$; week × group, $F_{18,279} = 13.84$, $p < 0.001$); UCMS/vehicle mice differed significantly from control/vehicle mice after just one week of UCMS (week 1, $p < 0.01$; week 2–9: $p < 0.001$). Chronic imipramine treatment counteracted the UCMS-induced deterioration of the coat state after four weeks ($p < 0.01$), and this effect was upheld for the last coat state evaluation ($p < 0.001$).

The C3H strain mice were also sensitive to the UCMS-induced deterioration of the coat state (week, $F_{9297} = 31.36$, $p < 0.001$; group, $F_{2297} = 22.34$, $p < 0.001$; week × group, $F_{18,297} = 7.98$, $p < 0.001$). Significant differences appeared after three weeks of the UCMS regimen and were maintained until the end (control/vehicle vs. UCMS/vehicle, week 3, $p < 0.05$; week 4–9, $p < 0.001$). No significant reversal effect of imipramine treatment was found for this strain.

For the C57BL/6 strain, the ANOVA found significant differences (week, $F_{9288} = 7.54$, $p < 0.001$; group, $F_{2288} = 3.35$, $p < 0.05$; week × group, $F_{18,288} = 2.97$, $p < 0.001$): the UCMS procedure induced a relatively low increase of the coat state scores, reaching statistical significance for the final 3 coat state evaluations (control/vehicle vs. UCMS/vehicle, week 7–9, $p < 0.05$). However, this deterioration was not significantly reversed by imipramine treatment.

For the CBA strain, the ANOVA revealed significant differences (week, $F_{9288} = 19.19$, $p < 0.001$; group, $F_{2288} = 21.96$, $p < 0.001$; week × group, $F_{18,288} = 5.63$, $p < 0.001$). The coat state deteriorated significantly after four weeks of UCMS and this continued until the final evaluation (control/vehicle vs. UCMS/vehicle, week 4, $p < 0.05$; week 5, $p < 0.01$; week 6–9, $p < 0.001$). The imipramine treatment failed to reverse this UCMS-induced deterioration.

For the DBA strain, statistical analysis showed significant differences (week, $F_{9288} = 76.5$, $p < 0.001$; group, $F_{2288} = 76.18$, $p < 0.001$; week × group, $F_{18,288} = 22.22$, $p < 0.001$), with a UCMS-induced deterioration of the coat state from the third to the final evaluation (control/vehicle vs. UCMS/vehicle, week 2, $p < 0.05$; week 3–9, $p < 0.001$). Imipramine treatment improved the coat state significantly during the last two weeks ($p < 0.01$).

For the FVB strain, ANOVA showed significant differences (week, $F_{9252} = 29.71$, $p < 0.001$; group, $F_{2252} = 55.42$, $p < 0.001$; week × group, $F_{18,252} = 8.94$, $p < 0.001$). The coat state scores of UCMS mice increased throughout the UCMS procedure, becoming significant at the fourth evaluation (control/vehicle vs. UCMS/vehicle, week 3, $p < 0.05$; week 4–9, $p < 0.001$). No significant difference due to the imipramine treatment was found in the UCMS groups.

Overall, the 9-week UCMS protocol induced a gradual deterioration of the coat state in all strains, but with strain-specific differences in the kinetics as well as amplitude of alteration. BALB/c and DBA strains were the most sensitive to UCMS. The C57BL/6 strain displayed the least responsiveness, with low sensitivity to the UCMS effect on coat state, while A/J, C3H, CBA and FVB produced intermediate profiles.

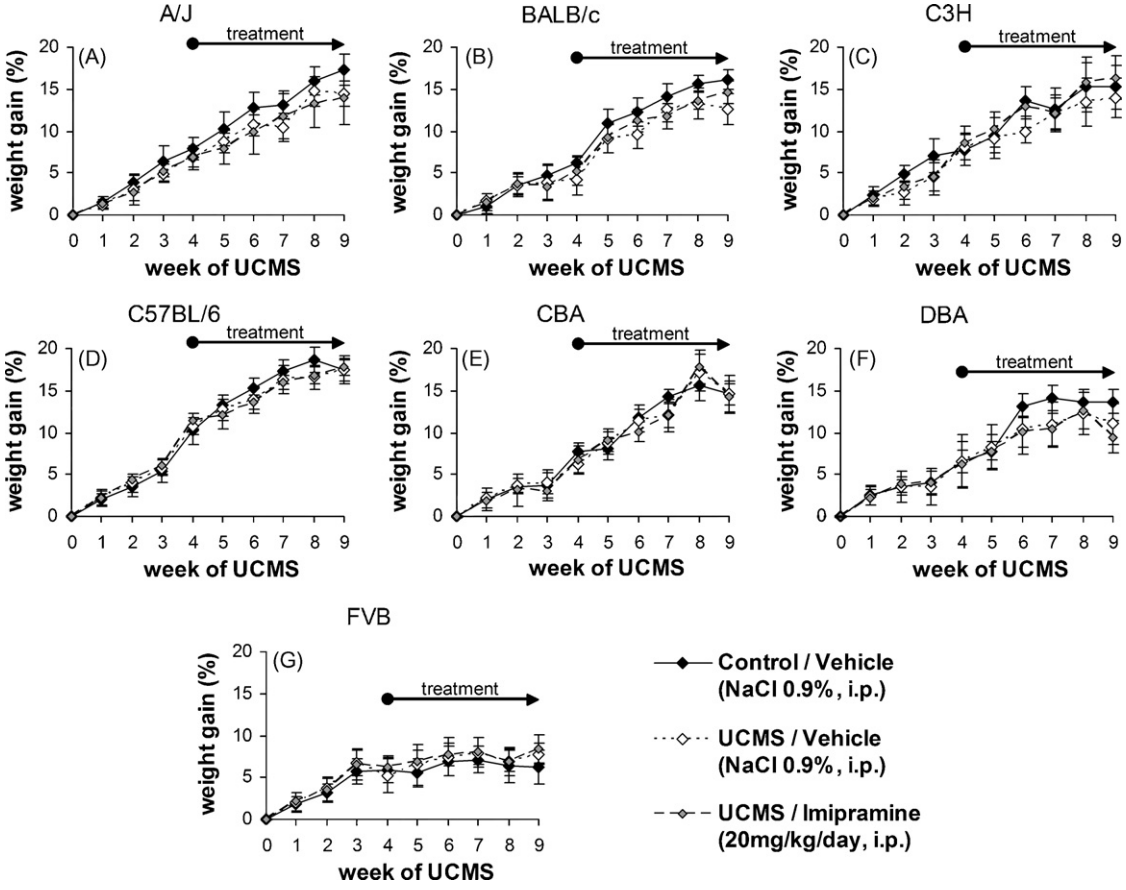

Figure 3 Effect of Unpredictable Chronic Mild Stress (UCMS) and of imipramine treatment on body weight gain in A/J, BALB/c, C3H, C57BL/6, CBA, DBA and FVB mice. $N = 9–13$ mice/group. Data represent mean ± S.E.M.

Finally, chronic imipramine treatment only counteracted the UCMS effect on coat state significantly in the BALB/c and DBA strains.

3.2. Body weight

Body weight was measured before the onset of the UCMS regimen and then weekly until the end of the UCMS procedure nine weeks later (Fig. 1). ANOVA revealed significant effects of ''week'' as main factor in all strains, but no significant modifications due to UCMS exposure or imipramine treatment (Fig. 3): A/J strain (week, $F_{9315} = 72.96$, $p < 0.001$; group, $F_{2315} = 0.45$, $p = 0.64$; week × group, $F_{18,315} = 0.33$, $p = 0.99$.); BALB/c strain (week, $F_{9279} = 80.19$, $p < 0.001$; group, $F_{2279} = 0.53$, $p = 0.59$; week - × group, $F_{18,279} = 0.51$, $p = 0.95$); C3H strain (week, $F_{9297} = 48.06$, $p < 0.001$; group, $F_{2297} = 0.24$, $p = 0.79$; week × group, $F_{18,297} = 0.39$, $p = 0.99$); C57BL/6 strain (week, $F_{9288} = 232.84$, $p < 0.001$; group, $F_{2288} = 0.027$, $p = 0.97$; week × group, $F_{18,288} = 0.69$, $p = 0.82$); CBA strain (week, $F_{9288} = 62.85$, $p < 0.001$; group, $F_{2288} = 0.028$, $p = 0.97$; week × group, $F_{18,288} = 0.33$, $p = 0.99$); DBA strain (week, $F_{9288} = 52.31$, $p < 0.001$; group, $F_{2288} = 0.17$, $p = 0.85$; week × group, $F_{18,288} = 0.75$, $p = 0.76$) and FVB strain (week, $F_{9252} = 18.05$, $p < 0.001$; group, $F_{2252} = 0.14$, $p = 0.87$; week × group, $F_{18,288} = 0.13$, $p = 0.99$).

3.3. NSF test

In this test, mildly food-deprived mice were exposed to a novel environment in which a food pellet was placed in the centre of the test apparatus. The NSF test is thought to assess emotional reactivity toward a new environment and induces competition between motivational states (drive to eat vs. fear of venturing into the centre of the arena). It was first used to identify anxiolytic-like effects of drugs such as benzodiazepines or chronic administration of antidepressants in healthy normal mice (Dulawa and Hen, 2005). This test could thus reveal a UCMS-induced increase in anxiety-related behaviors. It was performed after 9 weeks of UCMS and 5 weeks of imipramine treatment, the day after the final coat state and body weight evaluation (Fig. 1). The latency to start eating was recorded within a 3 min period (Fig. 4A).

For the A/J strain, ANOVA revealed significant differences ($F_{2,35} = 3.49$, $p < 0.05$), with UCMS mice showing greater latency to consume the pellet than controls ($p < 0.05$). Imipramine treatment reversed this effect ($p < 0.05$).

For the BALB/c strain, ANOVA found significant differences ($F_{2,31} = 3.4$, $p < 0.05$); latency in the UCMS/vehicle groups was significantly higher than that of controls ($p < 0.05$). Further, this strain was responsive to the AD treatment, as a reversal effect of imipramine was found in UCMS-subjected mice ($p < 0.05$).

For the C3H strain, no significant effects of UCMS or treatment were found ($F_{2,33} = 0.05$, $p = 0.95$).

For the C57BL/6 strain, the ANOVA showed significant differences ($F_{2,32} = 5.94$, $p < 0.01$), UCMS significantly increasing latency (control/vehicle vs. UCMS/vehicle, $p < 0.01$), while imipramine treatment counteracted this effect ($p < 0.05$).

For CBA strain, the ANOVA indicated no significant modification of latency in due to UCMS or imipramine ($F_{2,32} = 0.46$, $p = 0.63$).

For DBA strain, no significant difference were highlighted by ANOVA ($F_{2,32} = 0.18$, $p = 0.83$).

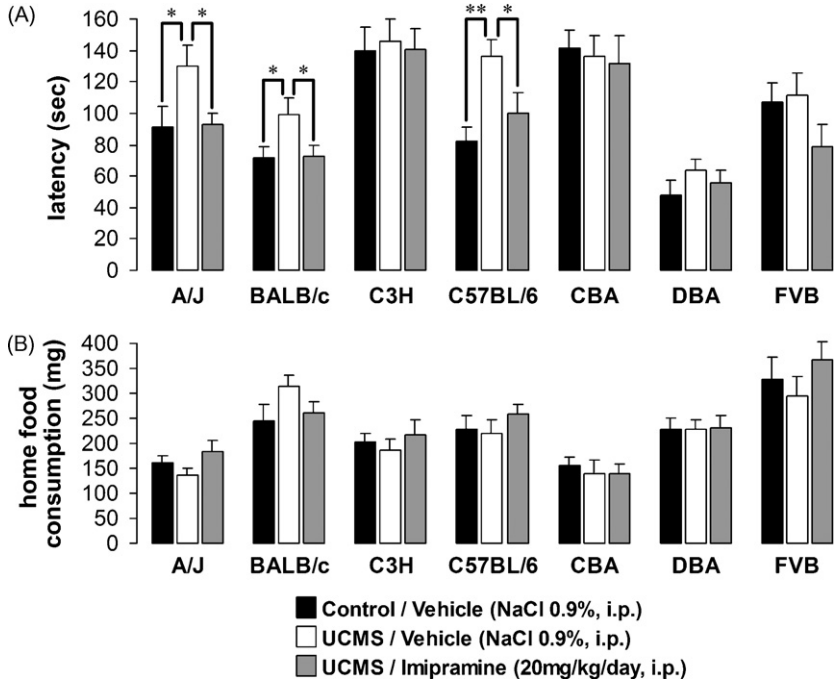

Figure 4 Effect of Unpredictable Chronic Mild Stress (UCMS) and of imipramine treatment in the Novelty-Suppressed Feeding (NSF) test with A/J, BALB/c, C3H, C57BL/6, CBA, DBA and FVB mice: (A) on the latency to chew the pellet and (B) on home food consumption in the 5 min following the test. $N = 9–13$ mice/group. Data represent mean ± S.E.M. $^*p < 0.05$ and $^{**}p < 0.01$ between line-connected groups.

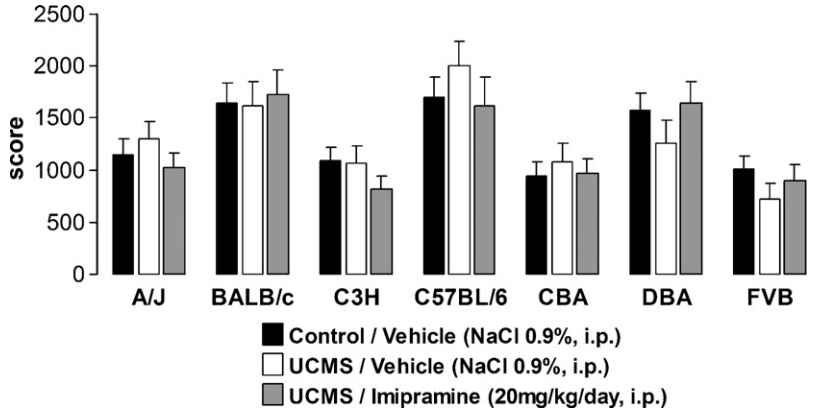

Figure 5 Effect of Unpredictable Chronic Mild Stress (UCMS) and of imipramine treatment in the actimeter with A/J, BALB/c, C3H, C57BL/6, CBA, DBA and FVB mice. $N = 9–13$ mice/group. Data represent mean ± S.E.M.

For FVB strain, neither UCMS nor imipramine treatment had an effect on the latency to feed in the NSF test ($F_{2,28} = 1.75$, $p = 0.19$).

None of these results can be explained by changes in hunger or motivation to feed due to the UCMS or treatment, as no significant differences were found in any of the strains and treatment groups for the 5 min home food consumption assessed immediately after the test (Fig. 4B): A/J ($F_{2,35} = 1.94$, $p = 0.15$), BALB/c ($F_{2,31} = 1.76$, $p = 0.19$), C3H ($F_{2,33} = 0.37$, $p = 0.69$), C57BL/6 ($F_{2,32} = 0.65$, $p = 0.53$), CBA ($F_{2,32} = 0.22$, $p = 0.8$), DBA ($F_{2,32} = 0.003$, $p = 0.99$), FVB ($F_{2,28} = 0.88$, $p = 0.42$).

In summary, three strains (A/J, BALB/c and C57BL/6) were both sensitive to the UCMS-induced detrimental effect and responsive to AD treatment in the NSF test, while no change due to UCMS or imipramine treatment occurred in the other four strains (C3H, CBA, DBA and FVB). These results are not confounded by differences in feeding drive.

3.4. Actimeter

The actimeter measured the locomotor activity of mice in their home cage and was performed 5 days after the final coat state and body weight evaluation, i.e. after five weeks plus 4 days of imipramine administration (Fig. 1). The more the mouse moved, the higher the score. The ANOVA failed to reveal significant differences in any strain (Fig. 5): A/J ($F_{2,35} = 0.83$, $p = 0.44$), BALB/c ($F_{2,31} = 0.07$, $p = 0.93$), C3H ($F_{2,33} = 1.17$, $p = 0.32$), C57BL/6 ($F_{2,32} = 0.68$, $p = 0.51$), CBA ($F_{2,32} = 0.23$, $p = 0.79$), DBA ($F_{2,32} = 1.13$, $p = 0.34$), FVB ($F_{2,28} = 0.99$, $p = 0.38$).

3.5. Fecal corticosterone metabolites

Feces were collected one week after the final coat state and body weight evaluations, i.e. after six weeks of imipramine administration (Fig. 1), over a period of 5 h during the dark phase. Corticosterone metabolite concentrations are expressed in [ng/50 mg feces]. Results are shown in Fig. 6.

For the A/J strain, neither UCMS nor imipramine treatment were found to have an effect on fecal corticosterone metabolites ($F_{2,11} = 0.01$, $p = 0.99$).

For the BALB/c strain, the ANOVA revealed significant changes in fecal corticosterone metabolites ($F_{2,12} = 6.8$, $p < 0.01$), with UCMS inducing a significant increase ($p < 0.01$). This effect was reversed by imipramine treatment (UCMS/vehicle vs. UCMS/imipramine: $p < 0.05$).

For the C3H strain, the ANOVA revealed no significant difference due to UCMS or imipramine treatment in the level of fecal corticosterone metabolites ($F_{2,11} = 1.36$, $p = 0.3$).

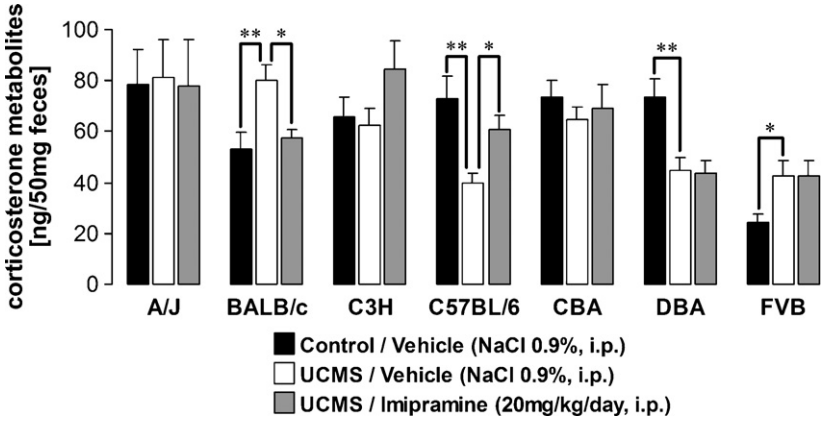

Figure 6 Effect of Unpredictable Chronic Mild Stress (UCMS) and of imipramine treatment on the level of fecal corticosterone metabolites in A/J, BALB/c, C3H, C57BL/6, CBA, DBA and FVB mice. $N = 4–6$ mice/group. Data represent mean ± S.E.M. *$p < 0.05$ and **$p < 0.01$ between line-connected groups.

For the C57BL/6 strain, ANOVA revealed significant changes ($F_{2,13} = 5.99$, $p < 0.05$). The pattern was the reverse of that found in BALB/c, with the UCMS regimen significantly decreasing fecal corticosterone metabolites ($p < 0.01$), and ADs reversing this change ($p < 0.05$).

For the CBA strain, no significant change was shown ($F_{2,12} = 0.34$, $p = 0.72$).

For the DBA strain, statistical analysis found significant differences ($F_{2,11} = 8.1$, $p < 0.01$) with a significant decrease in fecal corticosterone metabolites in the UCMS/vehicle group compared to controls ($p < 0.01$); however, imipramine did not counteract this change.

For the FVB strain, ANOVA revealed significant modifications ($F_{2,12} = 5.3$, $p < 0.05$). Like C57BL/6 mice, FVB mice showed a significant decrease in fecal corticosterone metabolites induced by the UCMS procedure ($p < 0.05$), but were not responsive to the reversal effect of imipramine.

Overall, the UCMS model induced alterations in the concentration of fecal corticosterone metabolites in 4 strains (BALB/c, C57BL/6, DBA and FVB), while imipramine restored concentrations to control levels in UCMS-subjected mice in only two of these strains (BALB/c and C57BL/6).

4. Discussion

This study found strain differences in sensitivity to UCMS, a chronic and naturalistic model of depression, and in response to AD treatment. All seven strains were sensitive to at least one of the UCMS-induced changes, while four strains (A/J, BALB/c, C57BL/6 and DBA) were responsive to at least one of the AD reversal effects. Interestingly, our results revealed different profiles of alterations and imipramine response within the strains (summarized in Table 1). Therefore, each UCMS- and imipramine-sensitive variable can be independent of the others, and could thus model an alteration related to a particular symptom of MDD. Three fields of possible MDD-associated alterations were tested in this study: physical state (coat state, body weight), behavior (locomotor activity, anxiety-related behaviors using the NSF test), and neuroendocrine measures (fecal corticosterone metabolites). BALB/c and C57BL/6 were the only strains exhibiting UCMS-induced alterations in all 3 fields, with significant changes in coat state (although weak for C57BL/6), the NSF test and the concentration of fecal corticosterone metabolites. These two strains were also the most responsive to AD treatment, as imipramine reversed these alterations (except for coat state in C57BL/6). A/J, DBA and FVB were each sensitive to two parameters of the UCMS model: coat state and the NSF test for A/J, and coat state and level of fecal corticosterone metabolites for DBA and FVB. Imipramine was found to have a reversal effect on only one parameter for A/J and DBA: the NSF test for A/J and coat state for DBA. No reversal effect was observed for FVB. Lastly, C3H and CBA were the sole strains exhibiting similar profiles. They were the least sensitive to the detrimental effects of UCMS, with only coat state showing significant deterioration; both strains were also irresponsive to imipramine treatment. Although differences in inbred mouse strains are not exclusively related to genetic background but are also influenced by epigenetic factors and parental behavior (Calatayud et al., 2004), the existence of these six different profiles underlines the role of inheritable factors in sensitivity to stress exposure and to the effects of ADs and could thus provide important information for understanding the genetic basis of stress vulnerability, MDD-associated alterations and resistance to AD treatment.

4.1. Strain-dependent changes in physical state

Our results show that a 9-week UCMS protocol induced a progressive deterioration of coat state in all strains, but with strain-specific kinetics and amplitude. BALB/c and DBA were the most sensitive to the effect of UCMS on coat state, since significant deterioration occurred faster and with higher scores in these strains. The C57BL/6 strain displayed a different profile with a weak effect of UCMS on coat state, while A/J, C3H, CBA and FVB exhibited intermediate profiles. These results confirm those of Mineur et al. (2006) who found a significant deterioration of the coat state after four weeks of UCMS in BALB/c, C57BL/6, DBA and FVB. Another UCMS study found significant deterioration in CBA but not in C57BL/6 and DBA (Pothion et al., 2004); however, assessment of coat deterioration in this study is questionable since no scoring was used, which could account for the discrepancy.

Chronic imipramine treatment only counteracted the UCMS effect on coat state in the BALB/c and DBA strains. It is noteworthy that these strains were the ones most affected by UCMS for this measure; imipramine thus counteracted the detrimental effects of UCMS on this index when deterioration was relatively high. It is therefore possible that the lack of effect of imipramine in the other five strains was

Table 1 Summary of the effects of a nine-week Unpredictable Chronic Mild Stress (UCMS) regimen and of the imipramine treatment

Strain	Effects of UCMS and imipramine reversal				
	Coat state score	Weight	NSF test latency	Locomotor activity	[CORT]
A/J	+	0	+/R	0	0
BALB/c	+/R	0	+/R	0	+/R
C3H	+	0	0	0	0
C57BL/6	+	0	+/R	0	−/R
CBA	+	0	0	0	0
DBA	+/R	0	0	0	−
FVB	+	0	0	0	+

"+" means a significant increase in the measure due to UCMS; "−" indicates a significant decrease in the measure due to UCMS; "0" indicates a lack of significant change; "R" indicates a significant reversal by imipramine treatment. NSF, Novelty-Suppressed Feeding; [CORT], level of fecal corticosterone metabolites.

due to floor effects rather than ineffectiveness. This could be the case for the C57BL/6 strain which was highly responsive to imipramine in all the other UCMS-induced alterations investigated. Alternatively, this measure might not be appropriate for assessing the effect of UCMS on mice of this strain due to their black coat which makes it difficult to assess coat state. To date, the UCMS model has not been used to compare the effect of ADs in different inbred mouse strains, only BALB/c mice were previously tested with fluoxetine, imipramine, desipramine, maprotiline, CRF_1 antagonists or V_{1b} antagonist (Griebel et al., 2002a,b; Santarelli et al., 2003; Alonso et al., 2004; Surget et al., 2008a,b; Yalcin et al., 2008). These compounds were shown to block or reverse the UCMS-induced deterioration of the coat state.

Body weight was measured each week from the onset of the UCMS regimen to the end. No change in body weight due to UCMS or imipramine was found in any of the strains used in this experiment. This result is in line with previous studies using BALB/c mice in a 5-week UCMS condition (Santarelli et al., 2003; Yalcin et al., 2005; Surget et al., 2008a). Nevertheless, recent experiments in our laboratories found significant reductions in body weight gain in BALB/c mice and recovery following fluoxetine treatment after a minimum of five weeks of UCMS in more intensive protocols in terms of stressor occurrence and combination (Surget et al., 2008b). Although body weight disruption was not found in any strain in this experiment, it can be induced, at least in BALB/c mice, by the UCMS model in a protocol-dependent manner.

4.2. Strain-dependent changes in the behavioral tests

The behavioral effects of UCMS and imipramine treatment were assessed by the NSF test and the actimeter. The NSF was used to identify any UCMS-induced increase in anxiety-related behaviors. While the behavior of C3H, CBA, DBA and FVB in the NSF test was not altered by either UCMS or by imipramine treatment, UCMS/vehicle mice of the A/J, BALB/c and C57BL/6 strains displayed a significant augmentation of latency in the NSF test, suggesting a UCMS-related exaggeration of emotional reactivity. Imipramine treatment restored latency to the control level in these 3 strains. Confounding effects of feeding drive or activity can be excluded, as food consumption and locomotion in the home cage remained unchanged.

4.3. Strain-dependent changes in the level of fecal corticosterone metabolites

As HPA axis disturbances such as hypercortisolemia and blunted negative feedback response are among the most consistent biological markers of MDD (Holsboer, 2000), the level of fecal corticosterone metabolites was measured in order to investigate first whether the UCMS model induces increased levels of corticosterone, secondly whether these changes are strain-dependent, and thirdly whether they are related to particular UCMS-induced symptoms and could be reversed by imipramine treatment. UCMS induced alterations in the concentration of fecal corticosterone metabolites in 4 strains (BALB/c, C57BL/6, DBA and FVB), but this effect was restored to control levels by imipramine in only two of these strains (BALB/c and C57BL/6). Furthermore, these changes were strongly strain-specific, as different profiles were obtained in each of these four strains. As expected, BALB/c and FVB mice displayed an increase in fecal corticosterone metabolites following UCMS. Imipramine treatment restored this increase to control levels in BALB/c, but not in FVB. As physical and behavioral changes due to UCMS were reversed by imipramine in BALB/c but not in FVB, the changes in corticosterone metabolites in these two strains are consistent with clinical data showing that, when MDD is associated with HPA disturbances, clinical remission parallels the normalization of the HPA axis (Nemeroff, 1996). On the other hand, a significant UCMS-induced reduction in the level of fecal corticosterone metabolites was found in C57BL/6 and DBA, but imipramine only reversed this diminution in C57BL/6 mice. This result is reminiscent of the atypical subtype of MDD characterized by reduced HPA axis activity, in contrast to the HPA overdrive seen in the melancholic subtype (Gold and Chrousos, 2002; Antonijevic, 2006). Lastly, A/J, C3H and CBA showed no significant change in fecal corticosterone metabolites due to UCMS or imipramine treatment. This result shows that the physical and/or behavioral UCMS-induced symptoms, at least for those investigated in this study, are not necessarily associated with disturbances of corticosterone levels. This is clinically relevant, since MDD can also occur without HPA axis dysfunction (Watson et al., 2002; Gervasoni et al., 2004).

However, it should be noted that the level of fecal corticosterone metabolites was only analyzed at one time point in our study. Since glucocorticoids such as corticosterone display regular circadian variations as well as an episodic pulsatile secretion pattern (Axelrod and Reisine, 1984; Touma et al., 2004), corticosterone levels at only one time point could be unrepresentative of HPA dysfunction as a whole. It cannot therefore be excluded that A/J, C3H and CBA strains experienced significant changes in corticosterone levels at other time points and that the non-reversal HPA profiles of the FVB and DBA strains were specific to this particular time point.

Besides investigating other time points, future studies should use the combined dexamethasone suppression/CRF stimulation test which assesses HPA feedback integrity, in order to identify HPA axis dysfunctions more precisely, since HPA axis disturbances in patients might be associated more with blunted negative feedback than hypercortisolemia (Swaab et al., 2005).

4.4. Strain differences are clinically relevant

Our results emphasize how the UCMS model of depression can generate several profiles which mimic subtypes of MDD-associated syndromes, using inbred mouse strains. BALB/c and C57BL/6 displayed physical and behavioral alterations, associated with up- or down-regulation of the HPA axis respectively, and they were responsive to ADs. DBA mice displayed UCMS-induced physical and neuroendocrine modifications, and imipramine reversed the physical but not the HPA changes. The A/J strain demonstrated physical and behavioral alterations following UCMS, and was AD-responsive in the NSF test, but the HPA axis was not dysregulated. FVB mice displayed physical alterations, the HPA axis was up-regulated, but modifications were resistant to ADs. UCMS induced

physical alterations in C3H and CBA mice, but their neuroendocrine pattern remained unchanged and the modifications were resistant to ADs.

MDD encompasses at least two major subtypes, melancholic and atypical depression, which display contrasting psychological and neurovegetative symptoms; in particular, HPA disturbances have been found in both subtypes but inversely: up-regulated in melancholia and down-regulated in atypical depression (Gold and Chrousos, 2002; Antonijevic, 2006). It is noteworthy that the UCMS model induced similarly divergent biological alterations, with HPA overdrive and hypocortisolemia, reminiscent of the melancholic and atypical conditions respectively. Our results show that a single etiological factor (chronic stress) induced contrasting patterns of alterations depending on the genetic background. Overall, we can speculate that genetic makeup contributes more than environmental factors to the different clinical phenotypes. In any case, these findings confirm that the UCMS model is an appropriate experimental tool for identifying the genetic factors associated with particular subtypes of depression-related syndromes.

4.5. Limitations

In brief, we established that four of the seven strains investigated in this study were responsive to imipramine treatment on at least one measure: DBA on coat state, A/J in the NSF test, C57BL/6 in the NSF test and fecal corticosterone metabolites, and BALB/c on all three measures, while C3H, CBA and FVB were not responsive on any measure (Table 1). Among all the tested strains, BALB/c and C57BL/6 were the only ones in which imipramine reversed at least two parameters, which might limit the usefulness of this model, particularly for genetically modified animals which are bred on other backgrounds. However, the range of behavioral changes investigated was relatively limited (emotional reactivity and locomotor activity). It is therefore possible that other strains would be sensitive to imipramine reversal in other depression-related behaviors (anhedonia, despair, sleep disturbance, appetite, social behavior). Moreover, this shortcoming could be an advantage for investigating the genetic factors involved in vulnerability, pathophysiology or AD resistance.

This model has other significant drawbacks, such as its length and its labor intensive procedure. Overall, the procedure seems to be difficult to carry out in a new laboratory, and there are some inconsistencies in the alterations and AD response between laboratories and even within the same laboratory (protocol-dependent changes in body weight in our lab or rat sucrose consumption in Willner's lab) (for a review, see Willner, 1997). Although the source of these discrepancies is not well understood, the chronic stress models of depression differ between laboratories in terms of duration, nature and combination of stressors, unpredictability, species, strains, treatment and measures. Standardization of the procedure might therefore attenuate the discrepancies. However, despite these concerns, UCMS is a useful model of depression as it encompasses unique features (i.e. various kinds of long-lasting alterations and their reversal by ADs) which can help resolve questions that are not accessible using other approaches.

5. Conclusion

Chronic models of depression such as the UCMS could be suitable for investigating the neurobiological mechanisms involved in the pathophysiology and treatment of MDD. The UCMS model reproduces features of the human disease and the clinical time-course of AD action. Moreover, our results show that it is possible to distinguish various UCMS-induced alterations which replicate specific symptoms and even subtypes of MDD-associated syndromes, reminiscent of the melancholic or atypical features of the human disease. UCMS may indeed make it possible to choose strains for specific aspects of MDD. For example, some strains may be relevant to model MDD associated with HPA dysfunction (strains sensitive to physical, behavioral and neuroendocrine alterations), others to model AD resistance (i.e. strains sensitive to UCMS but insensitive to ADs). The next step is to investigate other MDD-associated symptoms to determine inbred strain profiles more precisely, such as other relevant behavioral changes (anhedonia, sleep disturbance) or physiological measures (Dex/CRF test, brain monoamines level, hippocampal neurogenesis). Given that mice have been shown to display distinct patterns of alterations in a strain-dependent manner, UCMS is a potential model for identifying the genetic factors associated with vulnerability, particular symptoms of affective disorders, and AD resistance.

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Y. Ibarguen-Vargas, A. Surget, C. Touma and R. Palme declare that, other than income received from their primary employer, no financial support or payment has been received from any individual or corporate entity over the past three years for research or professional services, and there are no personal financial holdings that could be perceived as constituting a potential conflict of interest. C. Belzung is paid as a consultant for Takeda.

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