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Research report

Deficit in BDNF does not increase vulnerability to stress but dampens antidepressant-like effects in the unpredictable chronic mild stress

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ABSTRACT

Despite growing evidences of an association between brain-derived neurotrophic factor (BDNF) and antidepressant effects, the neurotrophic hypothesis of depression is challenged by the paucity of direct links between BDNF deficit and depressive-like behaviors. The unpredictable chronic mild stress (UCMS) paradigm might take our understanding a step further by examining whether a decrease in *bdnf* expression can lead to enhanced vulnerability to stress and prevent antidepressant efficacy in all or specific UCMS-induced alterations. Wild-type $bdnf^{+/+}$ and heterozygous $bdnf^{+/-}$ mice were exposed to an 8-week UCMS regimen and, from the third week onward, treated with either vehicle or imipramine (20 mg/kg/day, ip). Physical, behavioral and biological (plasma corticosterone levels, *bdnf* expression in the dentate gyrus) measures were further analyzed regarding to the genotype and the treatment. Heterozygous $bdnf^{+/-}$ mice displayed hyperactivity and increase of body weight but no enhancement of the sensitivity to stress exposure in all the measures investigated here. In contrast, while imipramine treatment reduced anxiety-like behaviors in the novelty-suppressed feeding test in both genotypes, it decreased aggressiveness in the resident/intruder test and immobility in the tail suspension test in wild-type but not in heterozygous mice. Furthermore, imipramine induced a twofold increase of *bdnf* expression in the dentate gyrus in both genotypes, while $bdnf^{+/-}$ mice displayed roughly half-reduced level for the same treatment. In summary, we demonstrate here that depletion in BDNF dampened the antidepressant effects in several behaviors but failed to increase vulnerability to chronic stress exposure.

1. Introduction

Extensive studies over the last decade have highlighted disruptions of neural plasticity and of neurotrophic equilibrium in a growing number of psychiatric diseases, such as major depressive disorders [1,2]. The "neurotrophic hypothesis of depression" emerged from these findings, based on a causal association between BDNF levels and depression-like states or antidepressant response: a decrease in BDNF synthesis and signaling is thought to generate morphological and functional brain changes precipitating depression, while its increase could underlie antidepressant action.

Clinical correlates, as well as basic research, support the involvement of BDNF in antidepressant response. An increase in BDNF immunoreactivity was reported in post-mortem hippocampal tissues of antidepressant-treated patients compared to the one obtained from untreated patients [3]. Administration of BDNF itself or genetically enhanced signaling of BDNF/TrkB receptor produce antidepressant-like effects in rodent models of depression [4–7]. Moreover, heterozygous $bdnf^{+/-}$ mice, which have roughly half-reduced levels of BDNF in the brain, and mice with inducible *bdnf* deletion in the forebrain exhibited blunted antidepressant-like responses in the forced swim test (FST) [8,9].

Nevertheless, the "neurotrophic hypothesis" is challenged by inconsistent linkages between BDNF dysfunction and depressive-like behaviors. Although some observations indicate that corticosterone or stress, a factor known to precipitate depression, are associated with a decrease in BDNF levels in the rodent adult hippocampus [10–13], various strategies reducing brain BDNF levels or signaling failed to demonstrate clear-cut involvements of BDNF in depression-related symptoms. For example, $bdnf^{+/-}$ mice display hyperphagia, obesity, hyperactivity, aggressiveness and learning deficits, but neiher anxiety- nor depression-like behaviors [8,14–19]. In contrast to inducible *bdnf* knockout mice [9], forebrain conditional *bdnf* knockout mice were reported to exhibit

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depressive-like phenotypes, but these results are lacking consistency: despair-like behaviors were only obtained with females in the FST in one study [20], while only in the tail suspension test (TST) and with an opposite pattern in the FST in another [21]. Another possibility is that deficiency in BDNF activity yields to an enhanced vulnerability to stress rather than directly impacting depression-like behaviors. The previous studies are probably irrelevant to address this point considering that they were all performed using tests of behavioral despair (FST and TST) in "normal" rather than in "depressed" mice.

Moreover, Berton et al. [22] showed that a social defeat stress paradigm increased BDNF levels in the ventral tegmental area and that a specific knockdown of *bdnf* in this brain area caused an antidepressant-like effect. Accordingly, the involvement of BDNF in depression-related states and antidepressant-like effect appears to be more complex than a single up- or down-change of the total BDNF levels in the brain. A reduction of BDNF level could thus be associated to vulnerability or resilience to chronic stress according to the brain region where it occurred. Interestingly, such an outcome could perhaps account for the lack of depressive-like phenotype of $bdnf^{+/-}$ mice.

In order to explore the role of BDNF deficit in vulnerability to stress exposure, in specific depression-related alterations and in antidepressant response, we exposed heterozygous $bdnf^{+/-}$ mice to the unpredictable chronic mild stress (UCMS) and to imipramine, a tricyclic antidepressant. Heterozygotous mice were chosen here, as most homozygote mutants die within 2 days after birth, a small fraction living for 2–4 weeks with severe ataxia and sensorial deficits [23,24] rendering them unavailable for behavioral experiments in adult animals. The effects of an 8-week UCMS paradigm along with a chronic imipramine treatment from the third week onward in wild-type and heterozygous $bdnf^{+/-}$ mice were assessed with physical measures (coat state, body weight), in feeding (24 h-home food consumption), locomotor activity (home-cage actimeter), aggressiveness (resident/intruder – R/I – test), anxiety (novelty-suppressed feeding – NSF – test), despair (TST), plasma corticosterone levels and *bdnf* mRNA levels (dentate gyrus of the hippocampus). Our study shows that, although the $bdnf^{+/-}$ mice did not display a higher vulnerability to UCMS regimen, they failed to respond effectively to antidepressant treatment in various, but not all measures.

2. Methods

2.1. Animals

Production of the gene-targeting construct was previously reported [25]. Wild-type $bdnf^{+/+}$ and littermate heterozygous mutant $bdnf^{+/-}$ mice were bred on a mixed S129/Sv × C57BL/6 genetic background. All the animals were genotyped by Polymerase Chain Reaction (PCR). Male mice of between 4 and 5 months of age were used in the study presented here. Before the beginning of the experiment, all animals were housed in groups of 4/5 and were maintained under standard laboratory conditions under a 12/12 h light/dark cycle (lights on at 20:00 h), 22 ± 2 °C, food and water *ad libitum*. The production of transgenic mice and all of the animal experiments were done in accordance to the guidelines 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 (0.9% NaCl). The different solutions were diluted to administer a final volume of 10 ml/kg.

2.3. General procedure

An 8-week UCMS regimen was conducted. Mice were maintained under the same standard laboratory conditions but were isolated in small individual cages (24 × 11 × 12 cm). The first 2 weeks of UCMS regimen were drug-free and treatment began from the third week of UCMS until the day of euthanasia. Imipramine (20 mg/kg/day) or vehicle was administered intraperitoneally (ip) once a day for 6 weeks. They were randomly assigned to the different treatment groups. The antidepressant dose was chosen on the basis of previous experiments showing that the compound is active at this dose in mice [26]. The body weight and the state of the coat were assessed weekly until the end of UCMS. During the eighth week of stress regimen, behavioral testing was performed according to the following sequence: 24 h-home food consumption, actimeter, R/I test, NSF test and TST. Two days after the TST, mice were killed by CO_2 asphyxia for brain sampling. The full experimental design is depicted in Fig. 1.

2.4. UCMS regimen

Chronic unpredictable stress procedures were initially developed in rats by Willner et al. [27]. The stress regimen used has been previously described [26,28,29]. Mice were repeatedly subjected to various stressors according to a "random" schedule for a total period of 8 weeks. The different stressors were: altered bedding (sawdust change, removal or damp; substitution of sawdust with 21 °C water, rat or cat feces directly placed in the home cage); cage tilting (45°) or shaking (2 × 30 in.); cage exchange (mice positioned in the empty cage of another male); induced defensive posture (repeated slight grips on the back until the mouse showed a defensive posture) and altered length and time of light/dark cycle. Various combinations of the different various stressors could occur, for example: without sawdust + cage tilting, without sawdust + cat feces, damp sawdust + cage exchange. Imipramine treatment started at the beginning of the third week of UCMS exposure. The body weight and the state of the fur were assessed once a week until the end of the 8 weeks. The total score of the state of the fur resulted from the sum of the score of seven different body parts: head, neck, dorsal fur, ventral fur, tail, forepaws and the hindpaws. For each of the seven body areas, a score of 0 was given for a well-groomed fur and 1 for an unkempt fur. This index has been pharmacologically validated in previous studies [26,28–30].

2.5. 24 h-home food consumption

The food intake of each animal was assessed by measuring the amount of food consumed over a period of 24 h in the home cage. The food consisted in the regular chow. Food was weighted and presented to the mice at 12:00 h.

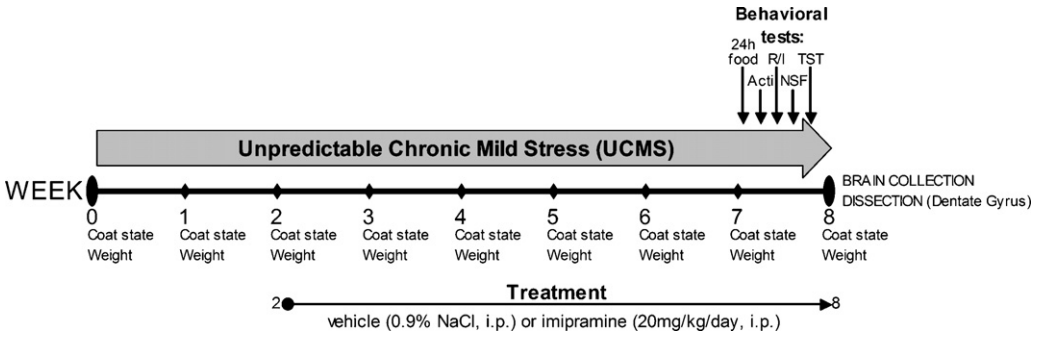

Fig. 1. Experimental design. The Unpredictable Chronic Mild Stress (UCMS) regimen lasted 8 weeks. Each week, the coat state was evaluated and the body weight measured. The first 2 weeks of UCMS regimen were drug-free. Imipramine or vehicle treatments began after 2 weeks of UCMS and continued until the end of the experiment (week 8). Imipramine (20 mg/kg/day) or vehicle (0.9% NaCl) were administered intraperitoneally once a day. The week before the end of the UCMS regimen, behavioral tests were carried out once a day according to the following sequence (n = 11–14 per group): 24 h-home food consumption (24 h food), actimeter (Acti), resident/intruder test (R/I), novelty-suppressed feeding test (NSF) and tail suspension test (TST). Towards the end of the UCMS regimen, dentate gyrus was collected for analysis of the *bdnf* expression (n = 4–5).

Twenty-four hours later, food was weighted and the amount of food consumed calculated.

2.6. Actimeter

The actimeter allowed the assessment of the activity of mice in their home cage, thus excluding the possibility of biasing the results due to novelty-induced anxiety. The home cage was placed in the centre of a device, 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, automatically detecting the movement of the animal when it crossed through, allowing to establish a score. The higher the score, the more the mouse moved. Cages were placed in the device 15 minutes before the beginning of testing. Testing was carried out in the nocturnal period from 11:00 h and lasted 2 h allowing a better estimation of the basal locomotor activity.

2.7. R/I test

R/I test is a variant of the procedure previously used in our lab [29]. Isolated mice were tested against an A/J intruder, known for its high passivity and lack of aggression [31]. The opponent was placed into the cage of the test animal (resident) in such a way that mice were placed in opposite corners and the test started immediately, lasting for a maximum of 5 min. Tests were stopped two minutes after the first attack occurred. We recorded the latency of the first attack as well as the number of attacks for 2 min following the first attack.

2.8. NSF test

The NSF Test is known to examine anxiety-like behaviors and to be sensitive to both anxiolytic and chronic antidepressant treatment [28]. It was performed as previously described [26]. 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 of sawdust. Six hours before the test, the 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 positioned 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 induced a conflicting motivation between the drive to eat the food pellet and the fear of venturing into the center of the arena. This version was shown to be able to specifically reveal antidepressant effects only in UCMS-treated, but not in control mice through a reversal effect of the UCMS-induced increase in the latency [29]. Moreover, antidepressants are known to be able to induce suppressant effects on appetite [32,33]. To control this potential 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).

2.9. TST

The procedure followed in this study was derived from the protocol previously described [34]. Mice were suspended by the tail (approximately 1 cm from the tip of the tail) using adhesive tape to a rod at 80 cm above the floor. Four animals were tested simultaneously. The trials were conducted for a period of 6 min, and were recorded by a video camera positioned directly in front of the mice while the experimenter observed the session in an adjacent room. Videotapes were subsequently scored blind by a highly trained observer. The behavioral measure scored from videotape was the duration of immobility. Mice were considered immobile only when they hung motionless.

2.10. Corticosterone radioimmunoassay

Mice were sacrificed by CO_2 asphyxiation and decapitated. While brains were removed for dissection (see below), trunk blood was collected and centrifuged at 1500 g for 12 min. Plasma was stored at −20 °C and analyzed for total corticosterone levels using a ^{125}I-labeled corticosterone double-antibody radioimmunoassay kit (MP Biomedicals, NY). All samples were run in a single assay; the intra-assay variability was 8.26%. The percentage of cross reactivity with steroids was corticosterone 100%, desoxycorticosterone 0.34%, testosterone 0.1%, cortisol 0.05%, aldosterone 0.03%, progesterone 0.02%, androstenedione 0.01%, 5α-dihydrotestosterone 0.01%, others < 0.01%.

2.11. Brain areas sampling

Mice were killed by CO_2 asphyxiation and decapitated. Brains were rapidly removed and placed in ice-cold slurry of 0.9% NaCl. Two millimeters rostro-caudal sections were quickly obtained on a brain tissue blocker. Two consecutive sections roughly from Bregma −0.6 to Bregma −4.6 were immediately transferred to wells containing RNAlater (Ambion Inc., Austin, TX). Then, each section was microdissected with the assistance of a brain atlas [35]. Dentate gyrus samples were dissected from the first and second sections. The samples were stored in RNAlater solution at −80 °C until further analysis.

2.12. Extraction of RNA and real time PCR

Total RNA was extracted using Trizol (Invitrogen, Cergy Pontoise, France) according to manufacturer's instructions and treated with DNAseI (Invitrogen, 1 μL/μg RNA). Reverse transcription reaction was performed at 42 °C 45 min in a final volume of 50 μL containing 500 ng of total RNA, 100 ng random hexamers, 1 mM dNTP, 10 mM DTT, 1× buffer, 10 U superscript II reverse transcriptase (Invitrogen). The reaction mixture for real time quantitative PCR consisted in 10 ng cDNA from reverse transcription reaction, 12.5 μL of Platinium SYBR Green qPCR SuperMix UDG (Invitrogen), 0.4 pmol/μL of both forward and reverse primers. GAPDH F: 5′-ctgcaccaccaactgcttag-3′, R: 5′-gtcttctgggtggcagtgat-3′; BDNF [36]. Threshold cycle (Ct) values were obtained and relative levels of transcription were calculated by the $2^{-\Delta\Delta Ct}$ method (GADPH).

2.13. Statistics

When the assumptions for parametric statistics were ensured (normality and homogeneity of variances), two-way ANOVAs were performed by using genotype (*bdnf*$^{+/+}$ vs. *bdnf*$^{+/-}$) or treatment (vehicle vs. imipramine) as main factors, followed by a Fisher *post hoc* analysis when required (i.e., $p < 0.05$). Otherwise, data were analyzed using non-parametric procedures: an analysis was done using the Kruskal–Wallis test, followed by a Mann–Whitney test including corrections for multiple comparisons when required (i.e., $p < 0.05$). The Friedman test, a non-parametric "ANOVA by ranks" for repeated measures, was used to examine the changes of the coat state over the 8-week UCMS regimen. Moreover, a Spearman's correlation was performed to analyze the relationship between locomotor activity and body weight in heterozygous mice.

3. Results

3.1. Evaluation of the coat state

The UCMS procedure induced a significant deterioration of the state of the coat in all the groups independently of the genotype or of the drug treatment (Fig. 2A; Friedman test: *bdnf*$^{+/+}$-vehicle, $\chi^2_{(14,8)} = 33.42$, $p < 0.001$; *bdnf*$^{+/+}$-imipramine, $\chi^2_{(14,8)} = 29.66$, $p < 0.001$; *bdnf*$^{+/-}$-vehicle, $\chi^2_{(13,8)} = 35.04$, $p < 0.001$; *bdnf*$^{+/-}$-imipramine, $\chi^2_{(14,8)} = 29.83$, $p < 0.001$). Kruskal–Wallis test failed to establish any differences between the four groups throughout the UCMS regimen (weeks 0–8: $H_{3,55} \leq 4.6$, $p \geq 0.2$). Accordingly, heterozygous *bdnf*$^{+/-}$ mice did not show a higher sensitivity to stress exposure in this measure and chronic imipramine treatment was unable to counteract the worsening effect of UCMS on the coat state in any groups.

3.2. Body weight and food intake

ANOVA revealed a significant effect of the genotype on the body weight, but not of the drug treatment or of interaction factors (Fig. 2B). This genotype effect occurred intermittently (weeks 0, 2, 4, 6–8: $F_{1,51} \geq 4.16$, $p < 0.05$; weeks 1, 3, 5: $F_{1,51} \leq 2.69$, $p \geq 0.11$). *Post hoc* analysis revealed frequent strong trends ($p < 0.1$), but no significant difference between both genotypes for the same treatment in paired comparisons.

The 24 h-home food consumption was evaluated at the beginning of the eighth week of UCMS (Fig. 1). Neither genotype ($F_{1,48} = 3.07$, $p = 0.086$), nor drug treatment ($F_{1,48} = 0.03$, $p = 0.86$) nor interaction ($F_{1,48} = 0.8$, $p = 0.37$) had a significant effect on food intake, although a trend ($p < 0.1$) for a genotype effect on this measure was found (Fig. 2C).

These results demonstrate an elevated body weight in heterozygous *bdnf*$^{+/-}$ mice compared to wild-type mice, which can perhaps be explained by increases in food intake.

3.3. Behavioral testing

Behavioral testing occurred on the eighth week of the UCMS regimen according to the following sequence: actimeter, R/I test, NSF test and TST (Fig. 1).

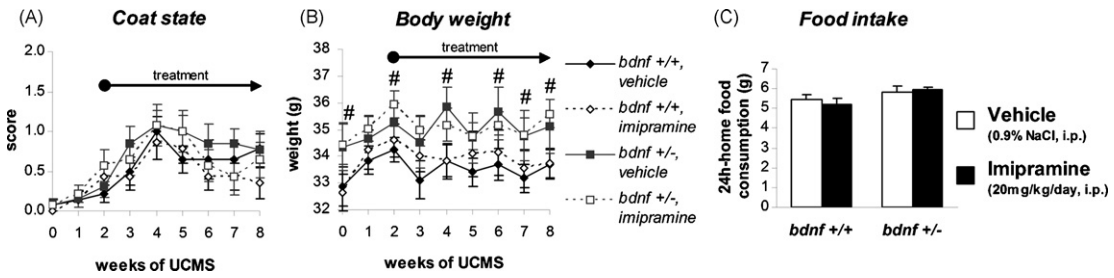

Fig. 2. Effect of the heterozygous *bdnf*$^{+/-}$ system and of the imipramine treatment on the coat state, body weight and food intake in 8-week UCMS-exposed mice. (A) The UCMS model induced a significant deterioration of the coat state in all the groups, but no significant effect of genotype or of drug treatment was observed ($n = 13$–14 per group). (B) Increase in the body weight of the *bdnf*$^{+/-}$ mice was found in several time points ($p < 0.05$, weeks 0, 2, 4, 6, 7, 8). No effect of imipramine was shown ($n = 13$–14 per group). (C) No significant change in the 24 h-home food consumption was found ($n = 12$–14 per group). Data represent mean ± SEM. $^{\#}p < 0.05$ between *bdnf*$^{+/+}$ and *bdnf*$^{+/-}$ mice.

In the actimeter (Fig. 3A), *bdnf*$^{+/-}$ mice were more active than *bdnf*$^{+/+}$ mice (genotype: $F_{1,42} = 6.05$, $p < 0.05$), while no effect of treatment ($F_{1,42} = 0.25$, $p = 0.62$) or genotype-treatment interaction ($F_{1,42} = 0.009$, $p = 0.93$) was reported. However, *post hoc* analysis revealed no significant difference in paired comparisons, only a trend ($p < 0.1$) for a higher activity in heterozygous *bdnf*$^{+/-}$ mice compared with the wild-type for the same treatment (i.e. vehicle or imipramine). As a previous study found an inverse relationship between locomotor activity and body weight in heterozygous *bdnf*$^{+/-}$ mice [17], we analyzed our data with the Spearman's correlation but no significant correlation was found between these two parameters ($R = 0.099$), which suggests that enhanced body weight does not preclude hyperlocomotor activity in heterozygous *bdnf*$^{+/-}$ mice.

The R/I test consisted to test the aggressiveness by introducing an A/J mouse in the home cage of the tested mouse. The number of attacks for 2 min after the first attacks (Fig. 3B) was recorded in a maximum of 5-min period (Kruskal–Wallis test: $H_{3,55} = 9.47$, $p < 0.05$). While the number of attacks was similar in the vehicle-treated mice for both genotypes, imipramine was able to reduce this parameter but only in the wild-type *bdnf*$^{+/+}$ mice ($p < 0.05$).

To examine anxiety-related behaviors, a 6 h food-deprived mouse was exposed to the NSF test. The latency to chew the pellet was similar in wild-type *bdnf*$^{+/+}$ mice and heterozygous *bdnf*$^{+/-}$ mice for both vehicle and imipramine treatments (Fig. 3C; ANOVA: genotype, $F_{1,51} = 0.001$, $p = 0.97$; treatment, $F_{1,51} = 9.22$, $p < 0.01$; interaction, $F_{1,51} = 0.0001$, $p = 0.99$). Imipramine treatment yielded a significant decrease in the latency to feed ($p < 0.05$), indicating an antidepressant-induced decrease in anxiety-related behaviors independently of the genotype. These results cannot be explained by changes in motivation to feed as no significant differences were found for the home food consumption assessed immediately after the test (Fig. 3D; ANOVA: genotype, $F_{1,51} = 1.6$, $p = 0.21$; treatment, $F_{1,51} = 0.024$, $p = 0.88$; interaction, $F_{1,51} = 0.038$, $p = 0.85$).

To investigate whether the deficit of BDNF level exerts an effect on the manifestation of despair behaviors following UCMS and chronic antidepressant treatment, we tested wild-type *bdnf*$^{+/+}$ and

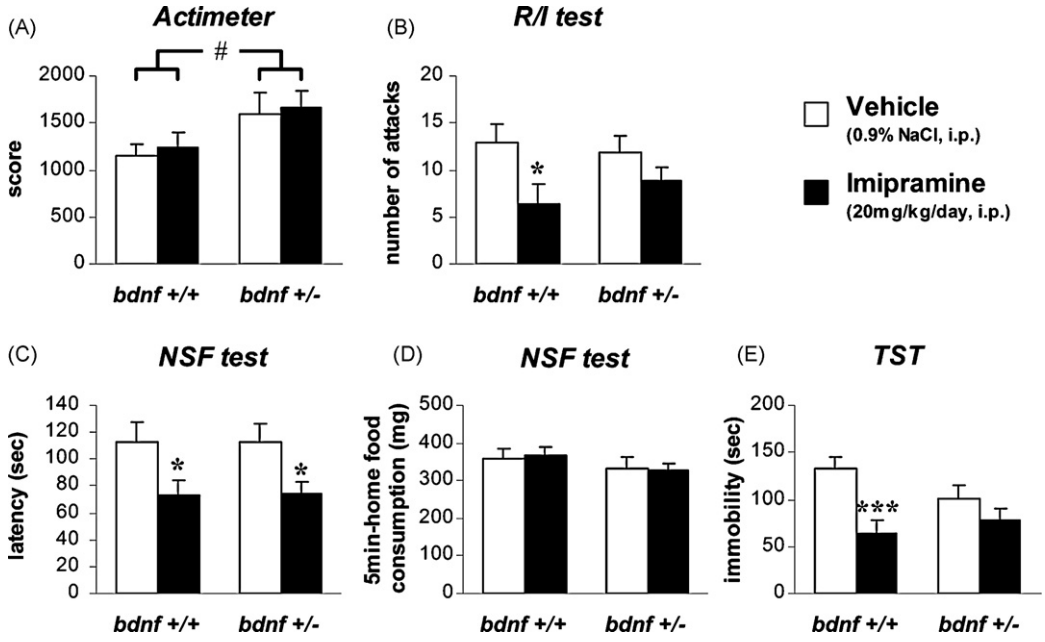

Fig. 3. Effect of the heterozygous *bdnf*$^{+/-}$ system and of the imipramine treatment on the behavior in 8-week UCMS-exposed mice. (A) Heterozygous *bdnf*$^{+/-}$ mice displayed an enhancement of the locomotor activity in the actimeter ($p < 0.05$). No effect of imipramine was found ($n = 11$–12 per group). (B) In the resident/intruder (R/I) test, the treatment with imipramine was able to reduce the number of attacks in *bdnf*$^{+/+}$ ($p < 0.05$) but not in *bdnf*$^{+/-}$ mice ($n = 13$–14 per group). (C) In the novelty-suppressed feeding (NSF) test, imipramine reduced the latency to chew the food pellet in both the *bdnf*$^{+/+}$ ($p < 0.05$) and the *bdnf*$^{+/-}$ mice ($p < 0.05$; $n = 13$–14 per group). (D) The latter result was shown to be independent of a confounding effect due to variations in feeding drive as no difference in the 5min-home food consumption directly measured immediately after the NSF test. (E) In the tail suspension test (TST), duration of immobility was decreased after imipramine treatment in *bdnf*$^{+/+}$ ($p < 0.001$) but not in *bdnf*$^{+/-}$ mice ($n = 12$–14 per group). Data represent mean ± SEM. $^{\#}p < 0.05$ between *bdnf*$^{+/+}$ and *bdnf*$^{+/-}$ mice. $^{*}p < 0.05$ and $^{***}p < 0.001$ between vehicle- and imipramine-treated mice or between line-connected groups.

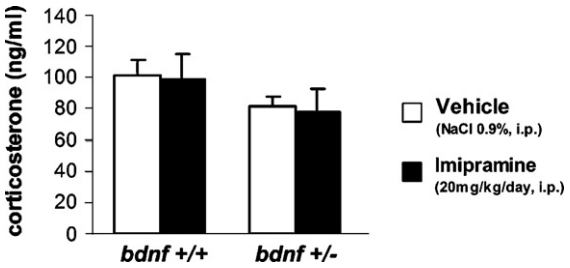

Fig. 4. Effect of the heterozygous *bdnf*$^{+/-}$ system and of imipramine treatment on the plasmatic corticosterone level in 8-week UCMS-exposed mice. No significant difference in the plasmatic corticosterone level was found ($n = 9$–10 per group). Data represent mean ± SEM.

heterozygous *bdnf*$^{+/-}$ mice in the TST (Fig. 3E). Mice were suspended by the tail to a rod and the time past immobile (interpreted as behavioral despair) was recorded during a unique 6-min trial (ANOVA: genotype, $F_{1,48} = 0.452$, $p = 0.5$; treatment, $F_{1,48} = 12.29$, $p < 0.001$; interaction, $F_{1,48} = 3.05$, $p = 0.08$). No significant difference was found between the vehicle-treated groups, although the wild-type *bdnf*$^{+/+}$ mice showed a trend ($p < 0.1$) for a greater immobility time than heterozygous *bdnf*$^{+/-}$ mice. Imipramine reduced the immobility time in wild-type *bdnf*$^{+/+}$ mice ($p < 0.001$), but not in heterozygous *bdnf*$^{+/-}$ mice.

3.4. Plasmatic corticosterone level

Since changes in hypothalamo–pituitary–adrenal axis activity is often associated to depression and antidepressant response [37], we measured by radioimmunoassay plasma corticosterone levels in mice after 8-week UCMS (Fig. 4). No significant difference due to genotype ($F_{1,34} = 2.07$, $p = 0.16$), treatment ($F_{1,34} = 2.07$, $p = 0.16$) or their interaction ($F_{1,34} = 2.07$, $p = 0.16$) was found.

3.5. bdnf mRNA level

Because the dentate gyrus is thought to be essential in mediating central effects of BDNF on depression-related states and antidepressant-like effect [38], we analyzed the total amount of *bdnf* mRNA in the dentate gyrus by quantitative RT-PCR (Fig. 5; Kruskal–Wallis test: $H_{3,17} = 13.12$, $p < 0.01$). In vehicle-treated animals, the *bdnf*$^{+/-}$ mice exhibited roughly a 50% lower *bdnf* expression in the hippocampus than the *bdnf*$^{+/+}$ mice ($p < 0.05$). Imipramine treatment led to double the *bdnf* mRNA level in both genotypes compared with vehicle-treated mice ($p < 0.05$). The difference between wild-type and heterozygous was thus maintained after antidepressant treatment ($p < 0.05$).

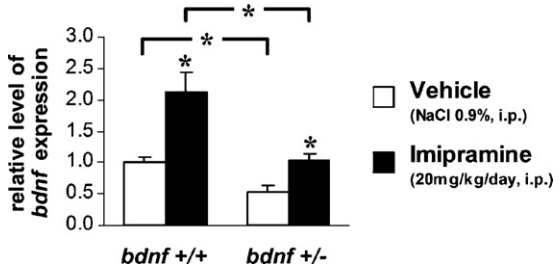

Fig. 5. Effect of the heterozygous *bdnf*$^{+/-}$ system and of imipramine treatment on the total *bdnf* mRNA expression level in the dentate gyrus of 8-week UCMS-exposed mice. Imipramine induced a twofold increase of *bdnf* expression in the dentate gyrus of *bdnf*$^{+/+}$ mice ($p < 0.05$) as well as of *bdnf*$^{+/-}$ mice ($p < 0.05$). Compared with wild-type, *bdnf*$^{+/-}$ mice displayed roughly a half reduction of *bdnf* expression in both the vehicle- ($p < 0.05$) and imipramine-treated mice ($p < 0.05$; $n = 4$–5 per group). Data represent mean ± SEM. *$p < 0.05$ between vehicle- and imipramine-treated mice or between line-connected groups.

4. Discussion

We investigated the consequences of the hemizygous inactivation of the *bdnf* gene in an 8-week UCMS paradigm and considered the results in light of the "neurotrophic hypothesis of depression". Indeed, the previous reports did not investigate the propensity of BDNF to mediate depression-like behaviors or antidepressant efficacy in chronic paradigms. However, these models are very informative as they mimic the role of etiological factors leading to depressive episodes. Hence, we examined whether the heterozygous *bdnf*$^{+/-}$ mice display, first, an enhanced vulnerability to UCMS and, second, an alteration of the antidepressant efficacy. We demonstrate here that depletion of BDNF leads to a dampening of the antidepressant-like effects of imipramine in several behaviors but fails to increase vulnerability to chronic stress exposure.

Findings from the present study are consistent with previous data showing an association of BDNF level with modifications of the body weight and locomotor activity. In line with a large set of studies with heterozygous *bdnf*$^{+/-}$ mice [16,17,19,39], we found an elevated body weight in heterozygous mutants compared to wild-types. Despite a trend for a genotype effect, this result arose in our study without a significant elevation of food intake. Although suggesting BDNF-related changes in metabolic regulation, the latter result must be carefully interpreted since it contrasts with previous studies describing hyperphagia as the primary cause of obesity in these mice [16,39]. In addition, we have to consider that we presented just one snapshot at a precise moment which could be unrepresentative of the overall food intake. In any case, it has been proposed that the elevation of body weight could originate from the direct action of BDNF in ventromedial and dorsomedial hypothalamus [40], and from the intricate relationship between BDNF and the serotoninergic brain system [41–43] which is involved in the neural regulation of appetite and body weight [44]. Moreover, we found an association between the deficit in BDNF and an increase in locomotor activity which is convergent with many other studies [16,17,45]. While Kernie et al. [17] reported that hyperactivity was inversely correlated to obesity in heterozygous *bdnf*$^{+/-}$ mice, this correlation is contradicted by our data and others [21] which demonstrated that mice with increased body weight can also be hyperactive.

Because the UCMS paradigm recapitulates the role of socio-environmental stressors precipitating depressive episodes and induces a syndrome reminiscent of depressive symptoms (for a review see [46], this model is particularly well-suited to investigate causal factors involved in vulnerability to stress or in development of distinct alterations. Considering measures particularly relevant for this study, UCMS was previously shown to deteriorate the coat state, to decrease the body weight gain, to elicit anxiety-like behaviors in the NSF test (increased latency), disturbances in the R/I test (increased aggressiveness), behavioral despair in the TST (increased immobility) and modification of plasma corticosterone levels. All of these alterations were shown to be reversed by chronic exposure to antidepressant treatments in a time course which parallels the clinical feature of these drugs. Regarding the paucity of a direct involvement of the decrease in BDNF level in the emergence of depression-like behaviors, the first goal of this study was to examine whether a depletion of BDNF makes these mutant mice more vulnerable to chronic stress exposure, in respect to the "neurotrophic hypothesis of depression". If heterozygous *bdnf*$^{+/-}$ mice are more susceptible to UCMS, we expected a worsening effect in term of amplitude and/or kinetic of the depression-like syndrome in these mutants compared with wild-type controls. However, no evidence for an association between a deficit in BDNF and enhanced vulnerability to UCMS arose from our data. Vehicle-treated mice were indistinguishable whether they are *bdnf*$^{+/+}$ or *bdnf*$^{+/-}$. Indeed, the deterioration of the coat state, the number of attacks in the

R/I test, the latency to eat in the NSF test and the levels of plasma corticosterone were similar in both genotypes. Differences in the body weight at several time points were revealed; however, since it occurred before the onset of the UCMS regimen, together with the fact that similar differences were previously found in non-stressed mice (see above), these differences cannot be attributed to a superior susceptibility for the UCMS procedure. Our results even demonstrated a trend for a decrease of immobility in the TST in mice lacking one *bdnf*$^{+}$ allele, which could be *a priori* interpreted as a resilient phenotype to despair behavior. Nevertheless, this latter result is mitigated by the fact that these mice were hyperactive in the actimeter, which can account for the decrease in immobility. Lastly, we found no evidence for an anxiety-like phenotype of heterozygous *bdnf*$^{+/-}$ mice, which is consistent with previous reports [15,47–49] despite one contradictory result [50].

We recently provided evidence from a large-scale gene expression survey that antidepressant effects vary greatly between control and UCMS-treated animals [29]. This result challenges the assumption on which the models of behavioral despair are constructed and which postulates that the clinically relevant antidepressant effects occur independently whether animals are in "normal" or "depression-like" states. These outcomes promote the utilization of the UCMS model when examining the neurobiological mechanisms of this disorder and the association between genes and antidepressant responses. Hence, the second purpose of our study was to evaluate whether BDNF mediates antidepressant efficacy in the measures sensitive to the UCMS model. In accordance with previous studies [46], imipramine significantly decreased the number of attacks in the R/I test, the latency to eat in the NSF test and the duration of immobility in the TST in wild-type mice. Imipramine was able in heterozygous *bdnf*$^{+/-}$ mice to diminish the latency to eat in the NSF test, but was ineffective to reduce the number of attacks in the R/I test and the immobility in the TST. Together, our results highlighted a lessened antidepressant efficacy in the heterozygous *bdnf*$^{+/-}$ mice in contrast to wild-type *bdnf*$^{+/+}$ mice; particularly, the antidepressant was ineffective in the heterozygous mutants to decrease aggressiveness during social encounter and despair behavior in the TST. These results are consistent with earlier studies indicating a requirement of BDNF in the behavioral effects of the antidepressants although limited to models of behavioral despair in normal mice [8,9,20]; we provide here further evidences extended to social disturbances and to "depressed" UCMS-treated mice.

In contrast, we reported no change in the coat state and in the plasma corticosterone levels due to genotype or to drug treatment. We previously found that the antidepressant-induced recovery of the coat state and the change in corticosterone occurs in a strain-dependent manner [51]. Therefore, these results may well be linked, at least partly, to the genetic background of these mice which could be less sensitive to these measures [46]. Particularly, the failure of imipramine to reverse the UCMS-induced coat deterioration might be due to a weak magnitude of the stress-induced effect on the coat state which is rather lower in this strain when compared to our previous experiments with other strains. [30,51].

Since it has been hypothesized that the level of *bdnf* expression in the dentate gyrus of the hippocampus might be related to a depressive-like state or to the antidepressant effect, we examined the total *bdnf* mRNA in the dentate gyrus. As expected, heterozygous *bdnf*$^{+/-}$ mice exhibited roughly half-reduced level when compared with the wild-type *bdnf*$^{+/+}$ mice. Consistent with previous findings, treatment with imipramine induced more than a twofold increase in the *bdnf* expression in the dentate gyrus in wild-type mice. Interestingly, a similar augmentation was also observed in the *bdnf*$^{+/-}$ mice. It is noteworthy that imipramine-treated *bdnf*$^{+/-}$ mice display a similar *bdnf* expression than the vehicle-treated *bdnf*$^{+/+}$ mice, while they thoroughly differ in term of behaviors and states. This result is interesting as it shows that the level of *bdnf* expression in the dentate gyrus could not be the driver of the animals' state and performances, but only contributes to particular antidepressant-like effects.

Finally, all these data combined can be extrapolated to the studies investigating the human *bdnf* Val_{66}Met polymorphism. The replacement of Val_{66} by Met_{66} produces no effect on *bdnf* expression *per se*, but it disrupts cellular processing, trafficking and activity-dependant secretion of BDNF which might result in local deficits in available BDNF [52,53]. The existence of these $BDNF_{Met}$ variants has attracted much interest whether this polymorphism may represent a risk factor for depression or altered antidepressant response. Although the *bdnf* Val_{66}Met polymorphism was not shown to be reliably related with occurrence of depression [54–57], it seems more consistently associated with modification of antidepressant efficacy [55,58,59], which is reminiscent of the outcomes from heterozygous *bdnf*$^{+/-}$ mice.

In summary, we have demonstrated that brain depletion in *bdnf* expression fails to increase vulnerability to a chronic stress procedure which mimics etiological factors for depression; however this deficit dampens some aspects of the antidepressant efficacy. This result suggests that a low BDNF level does not contribute per se to vulnerability for depression or depressive-like phenotypes while BDNF might be essential for specific antidepressant action. Future studies should unravel whether the involvement of BDNF in these phenotypes is due to its role during development or in the adult brain as well as the possibility of compensatory mechanisms evolving in chronically heterozygous mutant mice by comparing heterozygous *bdnf*$^{+/-}$ mice with conditional or inducible knockout mice in chronic models of depression.

Conflicts of interest

Y. Ibarguen-Vargas, A. Surget, S. Leman, P. Vourc'h, C. Andres and A.M. Gardier declare that, except for income received from our primary employer, no financial support or compensation has been received from any individual or corporate entity over the past three years for research or professional service and there are no personal financial holdings that could be perceived as constituting a potential conflict of interest. C. Belzung receives compensation as a consultant for Takeda.

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