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Abstract
Two mild stress paradigms were used in the present study: acute (i.e. three injections of saline over 24 h) and subchronic (i.e. single daily injection of saline for 7 d). These mild stress procedures did not alter the behaviour of wild-type mice in the forced swim test. However, male BDNF+/− mice exhibited increased immobility in the forced swim test after mild stress. This genotypic difference in stress responsivity was also evident in plasma corticosterone levels after a single injection of saline. The behaviour of female mice of either genotype was not altered by mild stress, and there was no genotypic difference in the corticosterone response of female mice to a single saline injection. Male BDNF+/− mice should be a useful model in which to examine behavioural and neurochemical consequences of interactions among genetic and environmental factors implicated in stress-related affective disorders, such as major depression.

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Introduction
Brain-derived neurotrophic factor (BDNF) plays a fundamental role in determining the functional architecture of neurons in the adult brain. In limbic structures, most notably the hippocampus and cortex, antidepressant treatments increase the expression of BDNF (e.g. see Duman & Monteggia, 2006) and result in auto-phosphorylation and activation of TrkB, the primary receptor for BDNF (Rantamäki et al. 2007; Saarelainen et al. 2003). Infusion of BDNF into the midbrain or hippocampus results in an antidepressant-like effect in two standard behavioural models: learned helplessness and the forced swim test. In contrast, repeated administration of corticosterone and a variety of types of stress reduce BDNF mRNA expression in the hippocampus and cortex (see Duman & Monteggia, 2006; Dwivedi et al. 2006). BDNF may therefore play a fundamental role in the pathophysiology of stress-related mood disorders such as major depression.

A frequent single nucleotide polymorphism at nucleotide 196, which produces an amino-acid substitution (valine to methionine) at codon 66 (Val66Met), results in impaired cellular processing and secretion of BDNF (Egan et al. 2003). The Met allele is associated with poorer episodic memory and abnormal hippocampal activation in human subjects (Egan et al. 2003). The Val66Met polymorphism has also been implicated in geriatric depression (Hwang et al. 2006; Kim et al. 2007). Clinical studies have revealed a significant BDNF gene × serotonin transporter gene interaction conveying vulnerability to depression, which is exacerbated by stressful life events (e.g. Kaufman et al. 2006; Kim et al. 2007). The s allele of the serotonin transporter gene (5-HTTLPR) results in decreased serotonin transporter expression, and is associated with major depression in those who have experienced traumatic or stressful life events (Caspi et al. 2003; Kendler et al. 2005). We have recently shown that serotonin transporter function is attenuated in BDNF+/− mice (Daws et al. 2007). Thus, BDNF+/− mice provide a unique system in which to model the interaction between environmental (i.e. stress) and genetic factors (i.e. deficiency in BDNF and attenuated serotonin transporter function) that predispose individuals to psychiatric disorders such as major depression.

Although BDNF+/− mice show unaltered emotional behaviour and appear normal in a variety of behavioural tests compared with wild-type mice (Chourbaji et al. 2004; MacQueen et al. 2001), we hypothesized that BDNF+/− mice are more vulnerable to
stress than wild-type mice and will exhibit behavioural depression-like behaviour after mild stress. We used intraperitoneal (i.p.) injection of saline as a mild stressor (Ryabinin et al. 1999) to which wild-type mice easily habituate (Ryabinin et al. 1999). Two mild stress paradigms were used: acute, which consisted of three injections of saline over 24 h, and subchronic, which consisted of a single daily injection of saline for 7 d. Behavioural depression was assessed in the forced swim test. We also measured plasma levels of corticosterone in wild-type and BDNF+/– mice, which provided a physiological measure of the responsiveness or reactivity of these mice to mild stress.

**Methods**

**Animals**

Group-housed, male and female mice aged 4–6 months were used for the present study. Mice were bred at the University of Texas Health Science Center–San Antonio. Breeding pairs consisted of wild-bred at the University of Texas Health Science Center–San Antonio. Breeding pairs consisted of wild-type female (C57BL/6J) and heterozygous male mice. Two mild stress paradigms were used: acute, which consisted of three injections of saline over 24 h, and subchronic, which consisted of a single daily injection of saline for 7 d. Behavioural depression was assessed in the forced swim test. We also measured plasma levels of corticosterone in wild-type and BDNF+/– mice, which provided a physiological measure of the responsiveness or reactivity of these mice to mild stress.

**Mild stress paradigms**

We used i.p. injection of saline as a mild stress. Saline injections were administered in a volume of 10 ml/kg. Acute mild stress consisted of three saline injections over 24 h, administered 24 h, 4 h and 1 h before assessing behaviour in the forced swim test. Subchronic mild stress consisted of a single daily injection of saline for 7 d. The last injection was administered 1 h before behavioural testing. To obtain a physiological indication of the responsiveness or reactivity of these mice to mild stress, a separate group of animals was administered a single injection of saline and plasma corticosterone levels were then determined from animals sacrificed 5, 15, 30 or 45 min after injection.

**Forced swim test**

Mice were placed in individual acrylic cylinders [45 cm high x 21 cm diameter, filled with water (23–25 °C) to a depth of 15 cm] for 6 min. Behaviour in the forced swim test was recorded using a video camera placed above the cylinder. The water in each cylinder was changed after each swim test. Two trained observers, blind to genotype, gender and treatment, scored the videos for duration of immobility during the last 4 min of the test period. A mouse was judged to be immobile when making only those movements necessary to keep its head above water (Lucki et al. 2001). The average of the two scores (in seconds) is reported.

**Determination of plasma corticosterone**

Animals were rapidly sacrificed by decapitation. Trunk blood was collected in chilled 1.8-ml Eppendorf microcentrifuge vials containing 50 µl of 0.3 M EDTA. The vials were kept on ice, and centrifuged at 4 °C, 6000 rpm for 20 min. Plasma aliquots were stored at −80 °C. Plasma corticosterone was measured using a commercially available radioimunoassay kit with [125I]corticosterone (MP Biomedicals, USA; sensitivity 3 ng/ml).

**Data analysis**

Data for the forced swim test were analysed separately for each gender by two-way ANOVA, with genotype and experimental condition as factors. Data for the time-course of plasma corticosterone were analysed separately for each gender by two-way ANOVA, with genotype and time as factors. F values reaching significance (p < 0.05) were evaluated further by post-hoc analysis using the Tukey–Kramer test (NCSS software, USA).

**Results**

The effect of mild stress on the behaviour of wild-type and BDNF+/– mice in the forced swim test was assessed by measuring the time spent immobile. For male mice, two-way ANOVA revealed a significant effect of genotype (F1,46 = 17.45, p < 0.001) and handling condition (F2,46 = 16.78, p < 0.001), and significant interaction between these two factors (F2,46 = 7.18, p < 0.002). We found no difference in time spent immobile in the forced swim test between BDNF+/– and
Vulnerability of BDNF+/- mice to mild stress

Fig. 1. The time spent immobile in the forced swim test was measured for group-housed (a) male or (b) female wild-type (□) and BDNF+/- (■) mice under three experimental conditions: unhandled, acute mild stress, or subchronic mild stress. The duration of immobility (in seconds) was assessed for the last 4 min of the 6-min test. Plotted values are mean ± S.E.M. Experimental group sizes for male mice: unhandled, wild-type (n = 7), BDNF+/- (n = 8); acute mild stress, wild-type (n = 8), BDNF+/- (n = 7); subchronic mild stress, wild-type (n = 13), BDNF+/- (n = 9). Experimental group sizes for female mice: unhandled, wild-type (n = 10), BDNF+/- (n = 9); acute mild stress, wild-type (n = 5), BDNF+/- (n = 9); subchronic mild stress, wild-type (n = 8), BDNF+/- (n = 9). * p < 0.05, Tukey–Kramer multiple comparisons test. However, in contrast to what was observed for male mice, female BDNF+/- mice did not exhibit any change in time spent immobile when compared to wild-type mice following the acute or subchronic mild stress procedure (Fig. 1b).

At baseline, there was no difference between genotypes in plasma corticosterone levels for either male (Fig. 2a) or female (Fig. 2b) mice. Plasma corticosterone levels were also determined from animals sacrificed 5, 15, 30 and 45 min after a single injection of saline. Plasma corticosterone levels peaked 30 min after saline injection in male wild-type and BDNF+/- mice. Two-way ANOVA revealed a significant effect of genotype (F_{1,53} = 9.74, p < 0.03) and time (F_{4,53} = 5.49, p < 0.001), but no significant interaction (F_{4,53} = 0.82, p = 0.52). Male BDNF+/- mice exhibited higher corticosterone levels than male wild-type mice in response to a single injection of saline (Fig. 2a). In contrast, plasma corticosterone levels peaked 15 min after saline injection in female wild-type and BDNF+/- mice, and there was no difference between genotypes in plasma corticosterone levels in response to a single

wild-type mice that were unhandled except for routine transfer to clean cages (Fig. 1a). However, following an acute mild stress procedure, which consisted of three i.p. injections of saline 24 h, 4 h and 1 h before the forced swim test, male BDNF+/- mice exhibited a marked increase in time spent immobile. An increase in time spent immobile in the forced swim test was also observed for male BDNF+/- mice following a subchronic mild stress procedure consisting of a single daily i.p. injection of saline for 7 d. These mild stress conditions did not alter the behaviour of wild-type mice in the forced swim test (Fig. 1a).

As observed for male mice, there was no difference between genotypes in the time previously unhandled female mice spent immobile in the forced swim test (Fig. 1b). Two-way ANOVA revealed no significant effect of genotype (F_{1,41} = 0.38, p = 0.541), a significant effect of handling condition (F_{3,41} = 3.94, p = 0.027), but no significant interaction between these two factors (F_{3,41} = 1.18, p = 0.316). When data were collapsed across genotype, subchronic but not acute mild stress significantly increased immobility in the forced swim test compared to unhandled controls (p < 0.05, post-hoc Tukey–Kramer multiple comparison test). However, in contrast to what was observed for male mice, female BDNF+/- mice did not exhibit any change in time spent immobile when compared to wild-type mice following the acute or subchronic mild stress procedure (Fig. 1b).
injection of saline (main effect of genotype: \( F_{4.45} = 0.31, \ p < 0.57 \); main effect of time: \( F_{4.45} = 7.08, \ p < 0.001 \); genotype \times time interaction: \( F_{4.45} = 0.71, \ p = 0.59 \) (Fig. 2b).

Discussion

For mice that were unhandled except for routine transfer to clean cages, we found no difference in genotype, or gender in the duration of immobility in the forced swim test. Our data are in agreement with previous studies showing that in a panel of behavioural tests, BDNF\(^{+/-}\) mice are indistinguishable from wild-type littermates (Chourbaji et al. 2004; MacQueen et al. 2001). This is in contrast to what has been observed for conditional BDNF knockout mice in which the BDNF gene is deleted selectively in the forebrain, i.e. male conditional knockouts exhibit no increase in immobility in the forced swim test, whereas female conditional knockouts display increased immobility (Monteggia et al. 2007). Thus, under baseline conditions the constitutive deficiency in BDNF does not result in apparent changes in behaviour.

However, the mild stress of handling and repeated injection of saline increased the duration of immobility of male BDNF\(^{+/-}\) mice in the forced swim test. The behaviour of wild-type mice was not altered by these handling procedures. Increased immobility in the forced swim test is commonly used as a measure of depression-like behaviour or 'behavioural despair' in rats and mice (see Forsolt, 2000). Our data indicate that male BDNF\(^{+/-}\) mice are markedly vulnerable to mild stress, which does not alter the behaviour of their wild-type counterparts.

Male BDNF\(^{+/-}\) wild-type mice also exhibited depression-like behaviour in the forced swim test after being subjected to subchronic mild stress. Saline injection constitutes a simple, mild stressor to which mice easily habituate (Ryabinin et al. 1999). Because BDNF promotes activity-dependent synaptic plasticity, BDNF is expected to play a role in the modulation of responses to repeated stress. Thus, it is perhaps not unexpected that BDNF\(^{+/-}\) mice did not habituate to repeated mild stress.

To examine the generality of this effect of mild stress in BDNF\(^{+/-}\) mice, we assessed another measure of stress reactivity, specifically plasma corticosterone levels. This provided us with a physiological indication of the responsiveness or reactivity of these mice to mild stress. At baseline, there was no difference between genotypes in plasma corticosterone levels for either male or female mice, in agreement with previous studies (Chourbaji et al. 2004). However, following a single injection of saline, plasma levels of corticosterone were higher in male BDNF\(^{+/-}\) vs. wild-type mice. Our data are in consistent with those of Ren-Patterson et al. (2005), who found that the stress of a single injection of saline results in a marked (2.2-fold) increase in plasma ACTH in male SERT\(^{+/-}\) \times BDNF\(^{+/-}\) mice, but not in wild-type mice. In contrast, we and others have found no difference between genotypes in the neuroendocrine response of female mice to an acute stress (Chourbaji et al. 2004; present study). Taken together these findings indicate that male BDNF\(^{+/-}\) mice exhibit an enhanced response to
mild stress when compared to their wild-type counterparts.

For both male and female mice, regardless of genotype, the saline injection was stressful as indicated by increased plasma corticosterone levels above baseline. However, the behaviour in the forced swim test was not altered in female BDNF+/− mice. In many studies, female rodents appear to be less vulnerable to the effects of stress on behaviour. For example, we have shown that following the stress of post-weaning social isolation, male but not female C57Bl/6j mice show an increase in ethanol preference (Advani et al. 2007). In male rats this period of social isolation between weaning and adulthood is associated with anxiety-like behavioural profiles (e.g. Weiss et al. 2004; Wright et al. 1991) and enhanced neuroendocrine responses following stress (Weiss et al. 2004). However, female rats do not show anxiogenic behaviours in the elevated plus-maze or neuroendocrine changes following post-weaning social isolation (Weiss et al. 2004). A reason for these gender differences in response to stress may be in part due oestrogen’s modulation of BDNF expression. Oestrogen positively modulates BDNF mRNA and protein within the hippocampus and cortex (Singh et al. 1995; Sohrabji et al. 1995; Zhou et al. 2005). Hippocampal BDNF levels change across the oestrous cycle, accompanied by neurophysiological responses that resemble the effects of BDNF (Scharffman et al. 2003). Oestradiol induces synaptogenesis in the hippocampus by enhancing BDNF release (Sato et al. 2007). Thus, oestrogen may serve to protect females from the deleterious effects of stress through increases in BDNF expression and function. Female rats have significantly higher levels of BDNF protein in the CA3 region of the hippocampus, relative to male rats, following immobilization stress (Franklin & Perrot-Sinal, 2006). Interestingly, the behavioural phenotype of BDNF+/− mice may be dependent on housing conditions. In a very recent study, Chourbaji et al. (2008) have found that under group-housing conditions BDNF+/− mice display increased anxiety-like behaviour. These behavioural indices of increased anxiety are prevented by enriched housing conditions (Chourbaji et al. 2008). Group housing has been reported to constitute a stress for male mice under certain conditions, altering behaviour in tests of anxiety and depression (Karolewicz & Paul, 2001; Palanza et al. 2001). In the present study, housing conditions may have been a factor influencing the behaviour of male BDNF+/− mice in the forced swim test.

In summary we have found that a deficiency in BDNF makes male mice vulnerable to mild stress, increasing signs of behavioural despair in the forced swim test and plasma corticosterone levels. The behaviour of male wild-type mice and the behaviour of female mice of either genotype were not altered. In the present study we have used one model of mild stress, i.e. injection of saline. However, given that genotypic differences were observed in the behaviour and corticosterone response of males after such a mild form of stress, it will be of interest to determine the effects of other types of stressors in future studies. Male BDNF+/− mice may prove to be a useful model in which to examine not only the behavioural, but also the neurochemical consequences of interactions between stress and reductions in BDNF.

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Statement of Interest

None.

References


