Gender-Specific Impact of Brain-Derived Neurotrophic Factor Signaling on Stress-Induced Depression-Like Behavior

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Background: Major depressive disorder is a leading debilitating disease known to occur at a two-fold higher rate in women than in men. The neurotrophic hypothesis of depression suggests that loss of brain-derived neurotrophic factor (BDNF) may increase susceptibility for depression-like behavior, although direct evidence is lacking.

Methods: Using the chronic unpredictable stress (CUS) paradigm, we investigated whether male and female mice with inducible BDNF deletion in the forebrain were more susceptible to depression-related behavior.

Results: We demonstrate that in certain behavioral measures the loss of BDNF lowers the threshold for female mice studied at random throughout estrus to display anxiogenic and anhedonic behaviors after chronic stress compared with wild-type female mice. However, the loss of BDNF in forebrain does not increase the susceptibility to depression-like behavior in male mice.

Conclusions: These gender differences suggest a role for BDNF in mediating some aspects of depression-related behavior in females.

Key Words: Animal model, BDNF, behavior, depression, gender, stress

Major depressive disorder (MDD) is a leading debilitating disease in the United States and affects about 14.8 million Americans over age 18 each year. The clinical presentation of MDD has a spectrum of symptoms, including anxiety, anhedonia, loss of appetite, and sleep disturbances as set forth in the *Diagnostic and Statistical Manual of Mental Disorders* (1). Notably, MDD occurs twice as often in women than in men, though the cause is currently unknown (2,3).

Recent work suggests an important role for neurotrophins in psychiatric diseases including MDD (4,5). Brain-derived neurotrophic factor (BDNF), the most prevalent growth factor in the brain, may underlie depression-related behavior and mediate the therapeutic action of antidepressants. The neurotrophic hypothesis of depression suggests that loss of BDNF from hippocampus contributes to neuroanatomical and functional alterations that underlie aspects of depression-related behavior, while antidepressants may mediate therapeutic effects, in part, by increasing levels of BDNF in this brain region (6). Recent studies demonstrate that BDNF heterozygous mice, mice with inducible BDNF deletion in forebrain (inducible knockouts [KOs]), and conditional BDNF knockouts display attenuated responses to antidepressants in forced swim test (7–9), a paradigm that predicts antidepressant efficacy and by analogy depression-related behavior (10,11). Indeed, we have recently extended these findings to show that BDNF in dentate gyrus of hippocampus is required for antidepressant efficacy in this paradigm (12).

While these studies demonstrated that loss of BDNF produces alterations in antidepressant responses, BDNF heterozygous mice, inducible BDNF KOs, and dentate gyrus specific BDNF KOs were indistinguishable from wild-type littermate control mice in baseline depression-related behavior; this suggests that loss of BDNF per se is not sufficient to mediate depression-like behavior (8,12,13). However, it is possible that loss of BDNF may increase vulnerability to particular chronic perturbations.

To investigate this possibility, we exposed BDNF inducible KOs to a chronic unpredictable stress (CUS) paradigm that is known to induce alterations in depression-related behaviors in rodents. We examined BDNF inducible KOs, since this line has a regionally restricted forebrain specific deletion of BDNF compared with our conditional line, to gain a direct assessment of the neurotrophic hypothesis of depression. Since previous work demonstrated that loss of BDNF produces gender-specific effects, we examined both male and female inducible KO mice in depression-related behavior following CUS.

Methods and Materials

Mice

The inducible BDNF knockout mice were generated from a trigenic cross of neuron specific enolase (NSE)-tetracycline transactivator (tTA), tetracycline operator sequences (TetOp)-Cre recombinase, and floxed BDNF mice as previously described (8). For all behavior testing, male and female mice were age- (3 months–6 months) and weight-matched and groups were balanced by genotype. Eight experimental groups of 7 to 14 animals were tested; male and female BDNF knockouts or wild-type littermate controls (CTLs), nonstressed or stressed (Supplement 1). The order of behavior tests was performed from least to most stressful and blind to group and genotype (Figure S1A in Supplement 2). For more information, refer to Materials and Methods in Supplement 3.

Chronic Unpredictable Stress Model

Our CUS model was adapted from Muscat *et al.* (14) and Monleon *et al.* (15). Mice were exposed to one or two stressors for a period of 4 hours to 12 hours during each 24-hour period over 52 days, though animals were not stressed within 8 hours of behavioral testing. Stressors consisted of food or water deprivation, periods of overnight illumination, 45° cage tilt, single housing, and bedding soiled with water or rat feces (Table 1).

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Table 1. Chronic Unpredictable Stress Paradigm

Week\Day	M	T	W	Th	F	Sa	Su
1	A3	B1, F2	C1, G	D1, B3	E	E	E, G
2	C1	B3, F1	B2	A2	D2	E	B3, E
3	D2, G	B3	C1	A2, B3	D2	E	E
4	A2	C1	F1	C1	F1	A2	G
5	B3	C1, F2	A1, A4	A5, B1	A4, F1	B3	B2
6	C1, G	B3	F2	D2	A4, B1	E	C3, E
7	B1, G	D2	C1, G	D2, F2	B3	A2, D2	C2
8	G	B1, E					

Stressor type coding is as follows: (A) water deprivation; (B) 45° cage tilt; (C) food deprivation; (D) rat feces in bedding; (E) single housing; (F) soiled bedding; (G) overnight illumination. Stressor period coding is as follows: (1) 4 hours; (2) 7 hours; (3) 12 hours; (4) 14 hours; (5) 17 hours.

Locomotor Activity

Mice were placed in cages and locomotor activity was recorded for 2 hours under red light by photocell beams linked to computer acquisition software (San Diego Instruments, San Diego, California).

Open Field

Mice were assessed for activity in a 72 × 72-cm open field (OF) arena at 40 lux for 5 min. Movement was tracked by video (Ethovision 3.0 Noldus, Leesburg, Virginia) for time spent in center (14 cm × 14 cm) and peripheral zones (5 cm around perimeter).

Fur State Assessment

Mouse fur state was rated on a 4-point scale with another point each for either hunched posture or redness around eyes (6 points total). The fur scoring scale (Materials and Methods in Supplement 3) was adapted from Mineur *et al.* (16).

Sucrose Consumption Test

Sucrose consumption test (SCT) protocol was adapted from Gourley *et al.* (17). Mice were habituated to 1% sucrose solution and water deprivation periods followed by 1 hour of sucrose access. On test day, mice accessed sucrose solution for 1 hour and the following day accessed water. We measured percent sucrose intake compared with total volume consumed in both trials. For more information, refer to Materials and Methods in Supplement 3.

Novelty Suppressed Feeding

The novelty suppressed feeding (NSF) task was performed as previously described (18). Detailed methods are listed in Supplement 3.

Tail Suspension Test

The tail suspension test (TST) was performed as previously described (19) and detailed methods are listed in Supplement 3.

Forced Swim Test

The forced swim test (FST) was performed as previously described (10) and detailed methods are available in the Materials and Methods in Supplement 3.

Corticosterone Measure

Blood serum was isolated from trunk blood samples by centrifugation. A high-sensitivity corticosterone (CORT) enzyme immunoassay (EIA) was performed according to manufacturer's instructions (Immunodiagnostic Systems Ltd, Fountain Hills, Arizona).

Quantitative Reverse Transcription Polymerase Chain Reaction

Fresh whole hippocampi were dissected and total RNA was extracted using Trizol reagent (Invitrogen, Carlsbad, California) according to manufacturer's instructions. Conditions for complementary DNA (cDNA) synthesis, amplification, and primer sequences were described previously (20). Fold change in BDNF expression was normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

Statistical Analysis

Weight and locomotor data were analyzed with repeated measures analysis of variance (ANOVA) using SAS software (Cary, North Carolina) to determine statistical significance ($p < .05$). The fur score data were analyzed by logistical regression analysis followed with a Mantel-Haenszel test for frequency comparison between groups. Anxiety data, SCT, NSF, FST, TST, CORT, and BDNF expression data were analyzed by a two-way ANOVA followed with multiple comparisons using a Bonferroni *t* test to assess the difference among groups. Data are presented as mean ± SEM.

Results

Weight

Mouse weights were monitored at early, mid, and late time points during the period of CUS (days 5, 21, and 40) (Figure S1B in Supplement 2). In female mice, there was a significant stress effect [$p < .0001$, $F(1,11) = 36.42$], while there was no significant knockout effect [$p = .2722$, $F(1,11) = 1.34$] or interaction effect [$p = .2075$, $F(1,10) = 1.82$]. Also for male mice, there was a significant stress effect [$p = .0135$, $F(1,8) = 9.96$], while there was no significant knockout effect [$p = .3016$, $F(1,7) = 1.24$] or interaction effect [$p = .3256$, $F(1,7) = 1.12$]. Inducible BDNF KO mice have normal weight compared with littermate CTLs, similar to data previously reported (8). Our CUS paradigm was found to significantly impact weight of the animals over the course of the experiment. However, loss of BDNF in either sex did not further contribute to a change in weight following CUS.

Locomotor Activity

Previous studies showed that a reduction in locomotor activity after CUS correlates to depression-like behaviors (21). Examining 2 hours of locomotor activity in female mice following CUS revealed a significant stress effect [$p = .0335$, $F(1,42) = 4.83$] and knockout effect [$p = .0461$, $F(1,42) = 4.22$], while there was no significant interaction effect [$p = .1104$, $F(1,42) = 2.66$]. Multiple comparisons using a Bonferroni *t* test indicated that stressed KOs were significantly hypoactive compared with the other groups ($^*p < .05$) (Figure 1A). To gain a better understanding of this difference in female mice, data were analyzed in 5-min epochs (Figure 1A); there was a significant main effect of stress [$p < .0001$, $F(1,11) = 62.00$] and a significant main effect of genotype [$p < .0001$, $F(1,9) = 57.91$] and the number of beam breaks significantly decreased over time [$p < .0001$, $F(23,299) = 52.56$] with a significant interaction between stress and genotype [$p < .0001$, $F(1,9) = 50.76$] and no other significant interaction effects ($^*p < .05$). Total locomotor activity in male mice during a 2-hour period following CUS revealed a significant stress effect [$p = .0092$, $F(1,30) = 7.75$], with no significant knockout effect [$p = .1796$, $F(1,30) = 1.89$] or interaction effect [$p = .9385$, $F(1,30) = .01$]. Multiple comparisons using a Bonferroni *t* test indicated that under nonstressed conditions BDNF KOs are significantly hyperactive compared with wild-type mice and that following stress CTLs show a significant decrease in locomotor activity compared with nonstressed CTLs ($^*p < .05$) (Figure 1B). For male mice, locomotor data were analyzed in 5-min epochs (Figure 1B); there was a significant main effect of stress [$p < .0001$, $F(1,8) = 86.32$],