Variant Brain-Derived Neurotrophic Factor Val66Met Polymorphism Alters Vulnerability to Stress and Response to Antidepressants

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Brain-derived neurotrophic factor (BDNF) plays important roles in cell survival, neural plasticity, learning, and stress regulation. However, whether the recently found human *BDNF* Val66Met (*BDNF*_{Met}) polymorphism could alter stress vulnerability remains controversial. More importantly, the molecular and structural mechanisms underlying the interaction between the *BDNF*_{Met} polymorphism and stress are unclear. We found that heterozygous *BDNF*^{+/Met} mice displayed hypothalamic-pituitary-adrenal axis hyperreactivity, increased depressive-like and anxiety-like behaviors, and impaired working memory compared with WT mice after 7 d restraint stress. Moreover, *BDNF*^{+/Met} mice exhibited more prominent changes in BDNF levels and apical dendritic spine density in the prefrontal cortex and amygdala after stress, which correlated with the impaired working memory and elevated anxiety-like behaviors. Finally, the depressive-like behaviors in *BDNF*^{+/Met} mice could be selectively rescued by acute administration of desipramine but not fluoxetine. These data indicate selective behavioral, molecular, and structural deficits resulting from the interaction between stress and the human genetic *BDNF*_{Met} polymorphism. Importantly, desipramine but not fluoxetine has antidepressant effects on *BDNF*^{+/Met} mice, suggesting that specific classes of antidepressant may be a more effective treatment option for depressive symptoms in humans with this genetic variant BDNF.

Introduction

Stress is a well known risk factor for the development of psychiatric diseases, such as depression and anxiety disorders (Roozendaal et al., 2009; McEwen and Gianaros, 2011), but stress responses vary greatly among individuals. It has been well established that both genetic and environmental factors, such as age, gender, and nutrition, influence the endocrine stress responses (Pittenger and Duman, 2008; Feder et al., 2009; Gillespie et al., 2009; Lupien et al., 2009). Identification of susceptibility genes that interact with stress that influences the onset or severity of psychiatric diseases will help to inform the underlying mechanisms of stress regulation and treatment of these disorders. The growth factor, brain-derived neurotrophic factor (BDNF), has been postulated to be a key mediator of stress-induced neural atrophy, cell loss, and inhibition of neurogenesis in the hippocampus (HPC) and prefrontal cortex (PFC) (Duman and

Monteggia, 2006; Magariños et al., 2011). Furthermore, BDNF expression in the HPC and PFC has been shown to be decreased by stress exposure (Calabrese et al., 2009; Taliaz et al., 2011), and BDNF administration (peripheral or central) attenuates the behavioral effects of chronic unpredictable mild stress (Schmidt and Duman, 2010; Ye et al., 2011). These findings suggest that BDNF may play an important role in the stress regulation and stress-induced behaviors. When BDNF heterozygous knock-out mice (*BDNF*^{+/-}) were exposed to chronic isolation stress, a significant depressive-like behavioral phenotype was revealed (Duman et al., 2007). Another study has shown that the mild stress of handling and repeated injections of saline increased the duration of immobility of male *BDNF*^{+/-} but not WT mice in the forced swim test (FST), demonstrating a synergistic interaction between stress and BDNF deficit (Advani et al., 2009).

Recently, a common single-nucleotide polymorphism (SNP) in the human *BDNF* gene, which leads to a valine-to-methionine change at position 66 (Val66Met) in the prodomain of *BDNF*, was found to decrease activity-dependent BDNF secretion (Egan et al., 2003; Chen et al., 2004) and to be associated with increased susceptibility to neuropsychiatric disorders including depression, and anxiety-related disorders (Sen et al., 2003; Verhagen et al., 2010). Human studies attempting to investigate the stress response in *BDNF*_{Met} allele carriers have resulted in mixed results. In one study, an interaction between early life stress and the *BDNF*_{Met} polymorphism was found to predict elevated neuroticism and higher anxiety (Gatt et al., 2009). However, another study showed that subjects with the *BDNF*_{Val} genotype showed higher harm avoidance with recent negative stressors (Kim et

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al., 2010). Because of the complicated genetic background and reliance on self-report questionnaires to evaluate emotional status, human studies have had difficulty in clarifying the interaction between stress and the *BDNF*_{Met} polymorphism, as well as the molecular and structural mechanisms underlying this interaction.

A variant *BDNF*_{Met} knock-in mouse, which reproduces the phenotypic hallmarks of humans with this BDNF SNP, was recently generated (Chen et al., 2006). In the present study, we used the *BDNF*_{Met} knock-in mice to investigate the role of the *BDNF*_{Met} polymorphism in vulnerability to stress exposure. The neuroendocrine and behavioral responses of heterozygous *BDNF*^{+/Met} mice to restraint stress, its potential mechanism, and pharmacological intervention are investigated.

Materials and Methods

Animals. Adult male *BDNF*^{+/Met} mice and littermate WT mice (3–4 months old) derived from WT × *BDNF*^{+/Met} crossed parents. All animals were kept on a 12 h light/dark cycle at 18°C–22°C with food and water available *ad libitum* unless noted otherwise. All procedures were in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the institutional animal care and use committee of Shandong University.

Restraint stress procedure. Male *BDNF*^{+/Met} and littermate WT mice were divided randomly into stress groups and home cage controls. Restraint stress was performed on the stress groups during the morning (8:00 A.M.–10:00 A.M.). For restraint stress, mice were restrained daily 2 h in well ventilated polypropylene restrainers without access to food and water for consecutive 7 d. At the end of the stress session, mice were returned to the home cage. Home cage controls were handled for 2 min and then returned to the home cage.

Hormone assays. To determine the activity of hypothalamic-pituitary-adrenal (HPA) axis at various times during the circadian cycle, home cage mice were decapitated without prior disturbance at 7:00 A.M., 1:00 P.M., and 7:00 P.M. Stress mice were decapitated immediately after stress (10:00 A.M.) on day 7. Home cage mice were also rapidly removed from their cages and decapitated at 10:00 A.M. on day 7. Trunk blood (0.5 ml) from each mouse was collected in iced tubes containing 5 μ l of 0.5 M EDTA, and plasma was separated by centrifugation and stored at –80°C until assay. Corticosterone levels were measured using the corticosterone ELISA kit (Assay Designs) as per the manufacturer's instructions. Adrenocorticotropin hormone (ACTH) levels were determined by radioimmunoassay using ACTH RIA Kit (IDS) following the protocol provided by the company. Hypothalamic corticotropin-releasing hormone (CRH) levels were determined by quantitative real-time reverse transcriptase (RT)-PCR.

Behavioral procedures. Behavioral testing was performed during the light phase of the cycle between 9:00 A.M. and 4:00 P.M. All behavioral testing began at day 8 and mice were allowed 2 h to habituate to the testing rooms before test. Different batches of mice were used for different behavioral tests. For correlation analyses, mice were killed after the behavioral tests, and brains were removed with one hemisphere for quantitative RT-PCR and another hemisphere for Golgi staining.

Sucrose preference test. Sucrose preference was measured by slightly modified procedure described previously (Duncko et al., 2001). Mice were habituated to sucrose over a 3 d period by replacing water bottles with bottles containing sucrose solution (1%). The test consisted of 23 h water deprivation after which animals were given free access to two bottles, one containing tap water and the other containing 1% sucrose solution. After 1 h, the weights of bottles were measured and fluid consumption was calculated. Sucrose preference was determined as follows: Sucrose preference (%) = sucrose intake/(sucrose intake + water intake) × 100. Sucrose preference was assessed for 2 d. The position of the sucrose and water bottles was alternated daily to avoid spurious effects from a side-bias.

Forced swim test. To assess depressive-like behavior, mice were placed into a glass cylinder (25 cm height, 10 cm diameter), which was filled with water (22°C) up to a height of 18 cm, as described earlier (Zörner et al., 2003). A testing period of 6 min was used to determine the percentage of

time spent immobile (Porsolt et al., 1977). Immobility was defined as being stationary with only enough motion of the tail or forepaws to keep the head above water. The forepaws usually remained at the animals' sides. Swimming was defined as active use of the forepaws with forward movement, in the center or along the sides of the cylinder, which did not involve lifting the paws above the surface of the water. The body was usually oriented parallel to the sides of the cylinder. Climbing was defined as active pawing of the side of the cylinder, lifting the paws above the surface of the water. The body was oriented with the head toward the wall and the body oriented perpendicularly to the side of the cylinder.

Open field test. Spontaneous exploratory activity and anxiety-like behavior were assessed in the open field test. The open field test consisted of 40 cm × 40 cm area divided into central (20 cm × 20 cm) and peripheral areas with 35 cm high walls. For the test, mice were placed in the center of the field and behavior was recorded for 10 min. A videotracking system (Smart) was used to score the distance traveled and total time spent in the central and peripheral areas. The total distance moved in the arena of 10 min was recorded as a measure of locomotor activity. Time spent in a central of the open field used as a measure of anxiety-like behavior.

Elevated plus maze. The elevated plus maze was conducted as described previously (Holmes et al., 2003) and has been accordingly modified for use in our laboratory. Briefly, the apparatus was constructed of black stainless steel and consisted of four arms (30 cm length × 5 cm width): two closed arms with high, black walls (30 cm high) and two open arms with a small raised lip (0.5 cm). All four arms were connected by a center platform (5 cm × 5 cm). The maze was elevated to a height of 50 cm above the ground. Each mouse was placed on the center platform facing an open arm to initiate the test session of 5 min. A videotracking system (Smart) was used to score the time spent in open arms and the number of entries into the open and closed arms (an arm entry was defined as all 4 paws in an arm).

Novelty-induced hypophagia test (NIH). The NIH was conducted as described previously (Dulawa and Hen, 2005). Mice were singly housed, and trained to drink a diluted solution of sweetened condensed milk (3:1, tap water to milk). Training consists of presenting the milk in a 10 ml serological pipette through the lid of the cage to each mouse for 30 min per day for 3 d. On the fourth day, the mice are tested in their home cage for latency to drink the milk consumed over a 30 min period. On the fifth day, mice are retested in a novel cage, which has no bedding and is more brightly lit. Anxiety-like behavior was measured by the latency to drink in the home cage and novel cage.

Morris water maze. Spatial learning, one of the best characterized hippocampal functions, was assessed using the Morris water maze test. The apparatus consisted of a circular water tank (120 cm diameter, 40 cm height) filled with water (22°C) to a depth of 25 cm, and the water was made opaque by the addition of nontoxic white powder paint. A circular escape platform (6 cm in diameter) was placed 1 cm below the water surface. A full experiment consisted of a learning period (platform in place) with four trials per day for 5 consecutive days and a probe test (platform moved) on the sixth day. In the learning period, the platform was always located in the center of the same quadrant (target quadrant) for all animals. Each trial consisted of a maximum of 60 s starting from one of the four quadrants with the animal facing the wall. If an animal did not reach the platform after 60 s, it was guided to the platform. After reaching the platform, animals were allowed to remain there for 30 s, and then mice were quickly dried with a towel and put under a heating lamp set at exactly 37°C between each trial to avoid hypothermia. In the learning period, the escape latencies for a single day were averaged to produce a daily mean. At day 6, the platform was removed, and mice were allowed to swim for 60 s. The time spent in the target quadrant for each mouse was recorded with a videotracking system (Smart).

T-maze test. The T-maze task used to test working memory is based on spontaneous alternation behavior, which does not require the use of food reinforcement to emerge. The tests were performed in a T-maze constructed of black stainless steel walls. Stem and arms were 35 cm long, 6 cm wide and 50 cm high. The start box (6 cm × 10 cm) was separated from the stem by a vertical sliding door. Vertical sliding doors were also placed at the entrance of each arm. An alternation response was considered each time the subject entered the arm opposite to the one visited on

the immediately previous trial. Alternation rate was expressed in percentage relative to the maximal alternation rate of 100% (obtained when the subject never returned into the same arm over two consecutive trials). For all the animals, the following procedure was used: on day 1, all mice were allowed to freely explore the apparatus 10 min in the morning and 10 min in the afternoon, in order for them to become familiar with the experimental conditions. Between both sessions, animals were replaced in their individual cage in the animal room. From day 2 to day 4, the mice had to complete two daily training sessions of spontaneous alternation, to foster the development of the alternation behavioral pattern and to familiarize them with the opening and/or closing of the doors over successive runs. Each training session included 6 trials, separated by a 30 s intertrial delay. On day 5 (the day of the test), all the mice were exposed to 6 successive trials (intertrial delay: 30 s).

Quantitative RT-PCR. Mice from stress groups were killed immediately after stress (10:00 A.M.) on day 7. Home cage controls were also killed at 10:00 A.M. on day 7. Brains were removed after decapitation and then coronal sections (1 mm thick) were obtained using a mouse brain slicer (Braintree Scientific). Seven different brain regions from each mouse [HPC, PFC, amygdala, striatum, hypothalamus, dorsal raphe nucleus (DRN), and locus ceruleus (LC)] were punched out using a blunt-end 17 gauge syringe needle (1 mm inner diameter) and frozen in liquid nitrogen followed by RNA extraction. Total RNA was isolated using TRNzol-A⁺ RNA isolation reagent (Tiangen) according to the manufacturer's protocol. Purified total RNA (500 ng) was reverse-transcribed to cDNA (Fermentas). Quantitative real time RT-PCR was performed in a Cyclar (Roche) using SYBR-Green (TaKaRa). Primer sequences used were as follows: BDNF forward primer, 5'-TAA ATG AAG TTT ATA CAG TAC AGT GGT TCT ACA-3', and reverse primer: 5'-AGT TGT GCG CAA ATG ACT GTT T-3'; CRH forward primer, 5'-ATC TCA CCT TCC ACC TTC TGC G-3', and reverse primer: 5'-CCC GAT AAT CTC CAT CAG TTT CC-3'; serotonin transporter (5-HTT) forward primer, 5'-AAG CCC CAC CTT GAC TCC TCC-3', and reverse primer, 5'-CTC CTT CCT CTC CTC ACA TAT CC-3'; norepinephrine transporter (NET) forward primer, 5'-ATG CCA GAA AGC CTT GAT GT C-3', and reverse primer, 5'-CGT GAC ACG GAG AAG ATG TG-3'; β -actin forward primer, 5'-CAA CTT GAT GTA TGA AGG CTT TGG T-3', and reverse primer, 5'-ACT TTT ATT GGT CTC AAG TCA GTG TAC AG-3'. Each sample was assayed in duplicate and the mRNA levels were normalized for each well to the β -actin mRNA levels using the $2^{-\Delta\Delta CT}$ method.

BDNF ELISA. The HPC, PFC, amygdala, and striatum were dissected and stored in microcentrifuge tubes at -80°C until analysis. Brain tissue samples were homogenized in ice-cold lysis buffer containing (in mM): 137 NaCl, 20 Tris-HCl, pH 8.0, 1% NP-40, 10% glycerol, 1 phenylmethylsulfonyl fluoride, 10 mg/ml aprotinin, 1 mg/ml leupeptin, and 0.5 sodium vanadate. The tissue homogenate solutions were centrifuged at $14,000 \times g$ for 5 min at 4°C . The supernatants were collected and used for quantification of total protein and BDNF levels. BDNF levels were assessed in each region using a commercially available assay kit from Pro-

Figure 1. *BDNF*^{+/Met} mice displayed increased HPA axis reactivity to stress. **A, B**, The plasma corticosterone (**A**) and ACTH (**B**) levels at the indicated time points in home cage mice. **C–E**, Restraint stress resulted in higher plasma corticosterone (**C**), plasma ACTH levels (**D**), and hypothalamus CRH mRNA levels (**E**) in *BDNF*^{+/Met} mice than in WT mice. All values ($n = 6–10$ per group) are presented as mean $\pm$ SEM (** $p < 0.01$, compared with home cage same-genotype mice; # $p < 0.05$, ## $p < 0.01$, compared with stress WT mice).

Figure 2. *BDNF*^{+/Met} mice displayed depressive-like behaviors after stress. **A**, In the sucrose preference test, *BDNF*^{+/Met} but not WT mice showed decreased sucrose intake percentage after stress. **B**, In the FST, *BDNF*^{+/Met} but not WT mice showed increased total immobility time after stress. All values ($n = 10$ per group) are presented as mean $\pm$ SEM (** $p < 0.01$, compared with home cage same-genotype mice; # $p < 0.01$, compared with stress WT mice).

mega. In brief, flat-bottom plates were coated with the BDNF capture antibody. The captured BDNF was bound by a second specific antibody, which was detected using a species-specific antibody conjugated to horseradish peroxidase as a tertiary reactant. All unbound conjugates were removed by subsequent wash steps according to the Promega protocol. After incubation with chromogenic substrate, color change was measured in an ELISA plate reader at 450 nm.

Rapid Golgi impregnation. Golgi impregnation of all brains was conducted using FD Rapid GolgiStain Kit (FD NeuroTechnologies). Golgi-Cox (G–C) solution (mixture of A and B solutions from kit) was mixed a minimum of 12 h before use and stored in a dark place at room temperature. Brains were immersed in G–C solution for 14 d at room temperature (the G–C mixture was changed after the initial 12 h of impregnation). Following 14 d of incubation, brains were transferred to solution C (10 ml/brain), and incubated for 3 d at 4°C , again with the solutions having been changed after the initial 12 h. Brains were then embedded

Figure 3. *BDNF*^{+/-}Met mice displayed heightened anxiety-like behaviors after stress. **A**, In the open field test, all four group mice showed no difference in the locomotor activity. **B**, In the open field test, *BDNF*^{+/-}Met but not WT mice showed decreased percentage of time spent in the center after stress. **C, D**, In the elevated plus maze, *BDNF*^{+/-}Met but not WT mice showed decreased percentage of time spent in the open arms (**C**) and percentage of entries into the open arms (**D**) after stress. **E, F**, In the NIH test, *BDNF*^{+/-}Met but not WT mice showed increased latency to drink diluted milk in the home cages (**E**) and novel cages (**F**) after stress. All values ($n = 8-9$ per group) are presented as mean $\pm$ SEM (** $p < 0.01$, compared with home cage same-genotype mice; ## $p < 0.01$, compared with stress WT mice).

in a 3% agarose solution, blocked, and cut at room temperature (150 μ m sections) on a vibratome (VT1200S, Leica). Serial sections were immediately mounted onto 0.3% gelatin-coated slides. Once on the slides, before complete drying of tissue, sections were brushed with solution C, and allowed to air dry for 48 h. Slides were then immersed in distilled water three times for 5 min each and then transferred into a solution of D & E (Golgi kit) (25 ml of D, 25 ml of E, and 150 ml of distilled water) for 5–10 min at 4°C, and again rinsed three times for 5 min each in double-distilled water. Slides were then dehydrated with ethanol, cleared with Histoclear (three times for 5 min each), and coverslipped with DPX mounting medium.

Estimation of spine density. The analysis was performed on coded Golgi impregnated brain sections containing the PFC or basolateral amygdale (BLA) of seven mice per experimental group and the measurement of spine density was performed as previously described (Magariños et al., 2011). The number of visible spines was counted on the basis of their shapes and spines were classified in the following categories: (1) stubby,

very short spines without a distinguishable neck and head; (2) thin, spines with a long neck and a clearly visible small head; and (3) mushroom, big spines with a well defined neck and a very voluminous head. Other spine shapes considered immature or transitional forms were excluded from the analyses because they were rarely observed or, when detected, difficult to be precisely identified at the light microscopic level. Cells chosen for analyses had to be well impregnated, clearly distinguishable from adjacent cells and have continuous unbroken dendrites. Five pyramidal neurons within layer II/III of medial PFC (mPFC) and five pyramidal neurons of BLA were analyzed per experimental mice. Spines were counted under oil (60 $\times$), using light microscopy (Nikon 80i), and the entire visible dendritic length was measured by Imaging computer program (NIS-Elements BR, Nikon). Spine density was calculated referring to the length of the dendrite.

Drug treatment. The experimental groups were randomly assigned to treatment with saline (2 ml/kg), fluoxetine (10 mg/kg, Sigma-Aldrich) or desipramine (10 mg/kg, Sigma-Aldrich). Drugs were dissolved in saline solution and injected intraperitoneally in a volume of 2 ml/kg were given 30 min before the FST.

Statistical analysis. Data were analyzed with Student's *t* test, one-way or two-way ANOVA, followed by *post hoc* comparisons, where appropriate. Student's *t* test or one-way ANOVA was used for single factor experiments involving two or more than two groups. For experiments comprised of multiple factors a two-way ANOVA, with test for interaction, was used. Pearson correlations were calculated to assess correlations between data. A significance level was set to 0.05 for all statistical analyses, and all values in the text and figures represent means $\pm$ SEM. Data analyses were performed using SPSS statistical program version 10.0.

Results

BDNF^{+/-}Met mice were associated with increased HPA axis response to stress

Dysregulation of the HPA axis is associated with stress vulnerability (de Kloet et al., 2005). Plasma corticosterone and ACTH concentrations are well known to show circadian variation (Sheward et al., 2007). We first measured the circadian fluctuations of plasma corticosterone and ACTH concentrations in the home cage of *BDNF*^{+/-}Met and WT mice during the day. Although there were clear differences between basal levels (7:00 A.M.) and peak levels (7:00 P.M.) of plasma corticosterone in *BDNF*^{+/-}Met and WT mice, two-way ANOVA for the effects of time and genotype indicated an effect of time ($F_{(2,35)} = 88.93$, $p < 0.01$), but not genotype ($F_{(1,35)} = 0.24$, $p = 0.63$), nor any genotype $\times$ time interaction ($F_{(2,35)} = 0.11$, $p = 0.90$) (Fig. 1A). Similarly, plasma levels of ACTH did not differ between *BDNF*^{+/-}Met and WT mice (time $F_{(2,35)} = 13.67$, $p < 0.01$; genotype $F_{(1,35)} = 0.81$, $p = 0.38$; interaction $F_{(2,35)} = 0.16$, $p = 0.85$) (Fig. 1B). Next we measured plasma corticosterone, ACTH levels and hypothalamic CRH mRNA expression immediately after restraint stress to assess the effect of stress on HPA axis reactivity in *BDNF*^{+/-}Met and littermate WT mice. There were significant ge-

nototype $\times$ stress interaction effects in plasma corticosterone, ACTH concentrations and hypothalamic CRH mRNA levels (corticosterone: interaction $F_{(1,35)} = 6.12$, $p < 0.05$; ACTH: interaction, $F_{(1,35)} = 10.51$, $p < 0.01$; CRH: interaction, $F_{(1,35)} = 4.60$, $p < 0.05$) (Fig. 1C–E). *Post hoc* analysis indicated that stress significantly increased plasma corticosterone, ACTH, and hypothalamic CRH mRNA levels compared with home cage controls. Notably, $BDNF^{+/Met}$ mice displayed a more robust elevation of these three hormones in response to stress compared with WT mice, though genotype did not affect the circadian rhythms of these hormones under basal conditions. These data indicated that $BDNF^{+/Met}$ mice had increased HPA axis response to stress.

$BDNF^{+/Met}$ mice displayed depressive-like and anxiety-like behaviors after stress

Having observed a difference in HPA axis reactivity to stress between $BDNF^{+/Met}$ and WT mice, we next examined the behavioral responses of these animals to stress. Sucrose preference test is a paradigm used to measure an animal's anhedonic-like deficits, which is an important feature of major depression. There was a significant genotype $\times$ stress interaction effect ($F_{(1,35)} = 5.01$, $p < 0.05$). Moreover, $BDNF^{+/Met}$ mice subjected to the stress paradigm had a significant decrease in percentage sucrose intake compared with stress WT and home cage $BDNF^{+/Met}$ mice (Fig. 2A). We also performed the FST, which uses increased immobility time as an index of depressive-like behavior in rodents (Porsolt et al., 1977). In the FST, we observed a significant genotype $\times$ stress interaction effect on the immobility time ($F_{(1,39)} = 7.69$, $p < 0.01$). *Post hoc* analysis indicated that genotype did not affect the immobility time in home cage controls. However, following stress, $BDNF^{+/Met}$ but not WT mice, displayed significantly increased immobility time in the FST (Fig. 2B). These findings suggest that $BDNF^{+/Met}$ mice displayed increased depressive-like behaviors following restraint stress.

To examine whether $BDNF^{+/Met}$ mice displayed altered anxiety-like behaviors in response to restraint stress, we performed three standard anxiety-like behavioral paradigms (open field, elevated plus maze, NIH). In the open field test, the total distance traveled by the mouse is a measure of locomotor activity, and we found no effects of genotype, stress, and interaction (genotype, $F_{(1,34)} = 0.05$, $p = 0.83$; stress, $F_{(1,34)} = 0.09$, $p = 0.77$; interaction, $F_{(1,34)} = 0.12$, $p = 0.74$) on locomotor activity (Fig. 3A). In comparison with stress WT and home cage $BDNF^{+/Met}$ mice, stress $BDNF^{+/Met}$ mice spent significantly less time in the center compartment and revealed a significant genotype $\times$ stress interaction effect ($F_{(1,34)} = 13.58$, $p < 0.01$) (Fig. 3B). Stress $BDNF^{+/Met}$ mice also exhibited, in the elevated plus maze test, a significant decrease in the percentage of time spent in the open arms (Fig. 3C) and a significant reduction in the percentage of entries into the open arms (Fig. 3D) compared with stress WT and home cage $BDNF^{+/Met}$ mice. In the NIH test, an increase in the latency to drink suggests an increase in anxiety. *Post hoc* analysis revealed that stress significantly increased

Figure 4. $BDNF^{+/Met}$ mice exhibited impaired spatial memory and working memory after stress. **A, B**, In the Morris water maze, both $BDNF^{+/Met}$ and WT mice showed increased escape latency to find the platform (**A**) and decreased time spent in the target quadrant during the probe test (**B**) after stress. **C**, In the T-maze test, $BDNF^{+/Met}$ but not WT mice showed poor spontaneous alternation performance after stress. All values ($n = 8–10$ per group) are presented as mean $\pm$ SEM (* $p < 0.05$, ** $p < 0.01$, compared with home cage same-genotype mice; ## $p < 0.01$, compared with stress WT mice).

the latency to drink in the $BDNF^{+/Met}$ mice, but not in the WT mice (Fig. 3E,F). All of these tests suggest that $BDNF^{+/Met}$ mice develop increased anxiety-like behaviors following restraint stress.

$BDNF^{+/Met}$ mice exhibited impaired spatial memory and working memory after stress

As stress has been shown to impede learning and memory (Joëls et al., 2006; Roozendaal et al., 2009; Schwabe et al., 2010), we evaluated the effect of stress on spatial and working memory using Morris water maze and T-maze tests in $BDNF^{+/Met}$ and WT mice. Examining 5 d of escape latency in the water maze learning session revealed a significant stress effect, but no significant genotype effect and interaction effect (fifth day: stress, $F_{(1,31)} = 20.26$, $p < 0.01$; genotype, $F_{(1,31)} = 2.34$, $p = 0.14$; interaction, $F_{(1,31)} = 1.13$, $p = 0.30$) (Fig. 4A). *Post hoc* analysis indicated that stress $BDNF^{+/Met}$ and stress WT mice exhibited significantly increased escape latency during learning compared with home cage mice, suggestive of a poorer acquisition of spatial memory. During the probe test, both stress groups spent less time in the target quadrant compared with home cage controls, revealed a significant stress effect, while there was no significant genotype effect or interaction effect (stress, $F_{(1,31)} = 17.23$, $p < 0.01$; genotype, $F_{(1,31)} = 0.37$, $p = 0.55$; interaction, $F_{(1,31)} = 0.89$, $p = 0.35$) (Fig. 4B). These results suggested that restraint stress could impair the acquisition of spatial memory in $BDNF^{+/Met}$ and WT mice.

We further conducted a T-maze version of spontaneous alternation, which is a non-rewarded exploration task and has been used as a test of working memory (Paylor et al., 2001). Two-way ANOVA demonstrated a significant genotype $\times$ stress interaction effect ($F_{(1,38)} = 6.84$, $p < 0.05$) (Fig. 4C). *Post hoc* analysis showed the alternation performance of the stress

Figure 5. BDNF mRNA and protein levels changed after stress in *BDNF*^{+/Met} and WT mice. **A–D**, Quantitative RT-PCR analysis of BDNF mRNA expression in the HPC (**A**), PFC (**B**), amygdala (**C**), and striatum (**D**) in *BDNF*^{+/Met} and WT mice. **E–H**, ELISA analysis of BDNF protein levels in the HPC (**E**), PFC (**F**), amygdala (**G**), and striatum (**H**) in *BDNF*^{+/Met} and WT mice. All values ($n = 8–13$ per group) are presented as mean $\pm$ SEM (* $p < 0.05$, ** $p < 0.01$, compared with home cage same-genotype mice; # $p < 0.05$, ## $p < 0.01$, compared with stress WT mice, + $p < 0.05$, ++ $p < 0.01$, compared with home cage WT mice).

BDNF^{+/Met} mice was poorer than that of stress WT and home cage *BDNF*^{+/Met} mice. These findings suggested that *BDNF*^{+/Met} mice were more susceptible to stress-induced working memory impairments.

Changes of BDNF levels after stress in *BDNF*^{+/Met} and WT mice

We next examined the molecular mechanisms underlying stress vulnerability in *BDNF*^{+/Met} mice. Previous reports indicated that stress could alter BDNF expression in distinct brain regions (Smith et al., 1995; Berton et al., 2006; Fumagalli et al., 2009). Quantitative RT-PCR was used to detect BDNF mRNA levels change after stress in distinct brain regions including HPC, PFC, amygdala and striatum in *BDNF*^{+/Met} and WT mice. In the HPC, both stress groups showed decreased BDNF mRNA levels compared with home cage controls; however, there was no significant genotype or interaction effect (stress, $F_{(1,34)} = 113.91$, $p < 0.01$; genotype, $F_{(1,34)} = 0.37$, $p = 0.55$; interaction, $F_{(1,34)} = 0.03$, $p =$

0.86) (Fig. 5A). In the PFC, there were significant effects of stress, genotype, and interaction (stress, $F_{(1,34)} = 58.21$, $p < 0.01$; genotype, $F_{(1,34)} = 4.41$, $p < 0.05$; interaction, $F_{(1,34)} = 5.67$, $p < 0.05$) (Fig. 5B). Moreover, stress *BDNF*^{+/Met} mice showed significantly decreased BDNF mRNA levels in the PFC compared with stress WT mice. In the amygdala, in contrast to the PFC, we found significantly increased BDNF mRNA levels in stress mice compared with home cage mice. Importantly, stress *BDNF*^{+/Met} mice showed higher levels of BDNF mRNA in the amygdala than stress WT mice (Fig. 5C). In the striatum, there were no significant stress, genotype, or interaction effect (stress, $F_{(1,34)} = 0.78$, $p = 0.38$; genotype, $F_{(1,34)} = 0.14$, $p = 0.71$; interaction, $F_{(1,34)} = 0.04$, $p = 0.84$) (Fig. 5D).

Next, we analyzed the effects of restraint stress on BDNF protein levels in *BDNF*^{+/Met} and WT mice. Significant decreases in BDNF protein levels were found in the HPC and PFC of stress groups compared with home cage controls (HPC, $F_{(1,46)} = 8.47$, $p < 0.01$; PFC, $F_{(1,46)} = 15.40$, $p < 0.01$) (Fig. 5E,F). *Post hoc* analysis showed that stress produced significantly decreased BDNF protein levels in the PFC in *BDNF*^{+/Met} mice compared with that in WT mice. In contrast, BDNF protein levels were significantly increased in the amygdala after stress in both *BDNF*^{+/Met} and WT mice (Fig. 5G). Moreover, stress *BDNF*^{+/Met} mice showed significantly increased BDNF protein levels in the amygdala compared with stress WT mice. In the striatum, similar to the BDNF mRNA levels, BDNF protein levels were unchanged in both stress groups and home cage controls (Fig. 5H). These data suggested more robust changes of BDNF levels in the PFC and amygdala in *BDNF*^{+/Met} mice after restraint stress.

Altered spine density in the PFC and BLA pyramidal neurons after stress in *BDNF*^{+/Met} and WT mice

To further investigate the neuronal morphology change in *BDNF*^{+/Met} and WT mice in response to restraint stress, we examined the spine density in layer II/III of mPFC and BLA pyramidal neurons. Under basal conditions, there were no significant differences in spine density between the home cage *BDNF*^{+/Met} and WT mice. Restraint stress induced decreased spine density in the mPFC distal apical but not basal dendrites in both *BDNF*^{+/Met} and WT mice (Fig. 6A–C). Moreover, stress *BDNF*^{+/Met} mice showed a more significant reduction of spine density in the mPFC distal apical dendrites compared with stress WT mice (Fig. 6A,B). In contrast to the decreased spine density in the mPFC apical dendrites, stress induced a significantly increased spine density in the BLA apical dendrites in *BDNF*^{+/Met} mice compared with stress WT mice (Fig. 6D,E). However, no difference in the BLA basal dendritic spine density was detected among groups (Fig. 6F). Together, these observa-

tions suggested that stress-induced spine remodeling was region specific and selective to apical dendrites and that *BDNF*^{+/-Met} mice were more susceptible to this stress-induced spine remodeling.

We further performed the correlation analyses between BDNF mRNA level or neuronal spine density and behavior performance in the same individual animals. We found positive correlation between BDNF mRNA level or apical spine density in the PFC and working memory performance. In contrast, BDNF mRNA level or apical spine density in the amygdala displayed negative correlation with the time spent in the center in open field test (data not shown). These data suggest that the stress-induced BDNF levels and spine density changes in the PFC and amygdala in *BDNF*^{+/-Met} mice might explain its stress related working memory deficits and increased anxiety-like behaviors.

Desipramine but not fluoxetine could rescue the depressive-like behaviors in *BDNF*^{+/-Met} mice

In an attempt to rescue the stress-induced depressive-like behaviors in *BDNF*^{+/-Met} mice, pharmacological studies were performed with established antidepressants. Mice subjected to the stress paradigm were injected intraperitoneally with either the selective serotonin reuptake inhibitor (SSRI), fluoxetine (10 mg/kg, i.p.), or the selective norepinephrine reuptake inhibitor (NRI), desipramine (10 mg/kg, i.p.). FSTs were performed 30 min later to assess the response to antidepressants. Compared with vehicle treatment, stress WT mice treated with fluoxetine showed significantly reduced immobility duration and increased swimming behavior; however, stress *BDNF*^{+/-Met} mice displayed a blunted response to fluoxetine treatment (Fig. 7A–C). In contrast to fluoxetine, desipramine treatment could significantly decrease the immobility time in both stress WT and stress *BDNF*^{+/-Met} mice (Immobility: genotype, $F_{(1,35)} = 14.13$, $p < 0.01$; treatment, $F_{(1,35)} = 34.17$, $p < 0.01$; interaction, $F_{(1,35)} = 7.21$, $p < 0.05$). To be noted, the main effect of desipramine treatment was to increase the climbing instead of swimming time, which is consistent with previous report (Cunningham et al., 2008; Drugan et al., 2010). These data suggested that desipramine but not fluoxetine was able to rescue the stress-induced depressive-like behaviors in *BDNF*^{+/-Met} mice.

To further investigate the molecular mechanism underlying the differential response to antidepressants in *BDNF*^{+/-Met} mice, we measured the mRNA levels of 5-HTT and NET in different brain regions by quantitative RT-PCR in home cage WT and *BDNF*^{+/-Met} mice. There were significantly reduced 5-HTT mRNA levels in the DRN and increased NET mRNA levels in the LC in *BDNF*^{+/-Met} mice compared with WT mice ($p < 0.05$, Fig. 8A,B), which may explain, in part, the differential response of *BDNF*^{+/-Met} mice to fluoxetine

Figure 6. Altered spine density in the PFC and BLA pyramidal neurons after stress in *BDNF*^{+/-Met} and WT mice. **A**, Spine density in apical dendrites of PFC pyramidal neurons in *BDNF*^{+/-Met} and WT mice. **B**, Representative photomicrographs of apical dendritic spines from PFC pyramidal neurons. Scale bar, 10 μ m. **C**, Spine density in basal dendrites of PFC pyramidal neurons in *BDNF*^{+/-Met} and WT mice. **D**, Spine density in apical dendrites of BLA pyramidal neurons in *BDNF*^{+/-Met} and WT mice. **E**, Representative photomicrographs of apical dendritic spines from BLA pyramidal neurons. Scale bar, 10 μ m. **F**, Spine density in basal dendrites of BLA pyramidal neurons in *BDNF*^{+/-Met} and WT mice. All values ($n = 7$ per group) are presented as mean $\pm$ SEM ($*p < 0.05$, $**p < 0.01$, compared with home cage same-genotype mice; $\#p < 0.05$, $\#\#p < 0.01$, compared with stress WT mice).

and desipramine treatment. We found that the mRNA levels of 5-HTT and NET in other brain regions (HPC, PFC, amygdala and striatum) were significantly less compared with those in DRN or LC. In addition, there were no significant differences on 5-HTT and NET mRNA levels in the HPC, PFC, amygdala, and striatum between *BDNF*^{+/-Met} mice and WT mice (Fig. 8A,B), which suggested a region-specific regulation of the expression of these two transporters in the *BDNF*^{+/-Met} mice.

Discussion

In the present study, we found an increased stress response associated with *BDNF*_{Met} polymorphism using a knock-in mouse model for this SNP. Only following chronic restraint stress did heterozygous *BDNF*^{+/-Met} mice show increased depressive-like and anxiety-like behaviors and impaired working memory compared with WT mice subjected to the stress paradigm. Moreover, compared with WT mice, stress induced more prominent BDNF

Figure 7. Desipramine but not fluoxetine could rescue the depressive-like behaviors in *BDNF*^{+/Met} mice. **A**, Effects of fluoxetine or desipramine on immobility behavior in *BDNF*^{+/Met} and WT mice after stress. **B**, Effects of fluoxetine or desipramine on swimming behavior in *BDNF*^{+/Met} and WT mice after stress. **C**, Effects of fluoxetine or desipramine on climbing behavior in *BDNF*^{+/Met} and WT mice after stress. All values ($n = 6–10$ per group) are presented as mean $\pm$ SEM (* $p < 0.05$, ** $p < 0.01$, compared with vehicle-treated same-genotype mice; ## $p < 0.01$, compared with vehicle-treated WT mice).

Figure 8. Altered 5-HTT, NET mRNA levels in *BDNF*^{+/Met} mice. **A**, Quantitative RT-PCR analysis of 5-HTT mRNA levels in the DRN, HPC, PFC, amygdala, and striatum in *BDNF*^{+/Met} and WT mice. **B**, Quantitative RT-PCR analysis of NET mRNA levels in the LC, HPC, PFC, amygdala, and striatum in *BDNF*^{+/Met} and WT mice. All values ($n = 7$ per group) are presented as mean $\pm$ SEM (* $p < 0.05$, ** $p < 0.01$, compared with home cage WT mice).

levels and spine density changes in selective brain regions in *BDNF*^{+/Met} mice. Finally, we demonstrated that an NRI, desipramine, but not an SSRI, fluoxetine, could rescue the stress-induced depressive-like behaviors in *BDNF*^{+/Met} mice.

Our data provide several new insights into the stress responses associated with the human *BDNF*_{Met} polymorphism. First, we found the *BDNF*_{Met} polymorphism could modulate stress-induced endocrine and behavioral responses. The baseline plasma corticosterone and ACTH levels were similar in WT and *BDNF*^{+/Met} mice. By contrast, following chronic restraint stress, the increased corticosterone, ACTH and CRH levels were significantly higher in stress *BDNF*^{+/Met} mice compared with stress WT mice, indicating that the *BDNF*_{Met} polymorphism increased HPA axis reactivity to stress. Children who were homozygous for the short 5-HTT linked polymorphic region (5-HTTLPR) and carrying the *BDNF*_{Met} allele displayed a significant increase in cortisol level in response to the laboratory stressors compared with *BDNF*_{Val} allele (Dougherty et al., 2010). However, another healthy male adult study showed HPA axis reactivity to the psychosocial stressor was attenuated in carriers of the *BDNF*_{Met} allele compared with subjects with the *BDNF*_{Val} genotype (Alexander et al., 2010). These human studies are difficult to interpret due to complex genetic backgrounds. In contrast, our mouse studies were performed under identical genetic backgrounds, and provide evidence that the *BDNF*_{Met} allele was associated with increased HPA axis response to stress, which has been postulated to be an important factor contributing to depression and anxiety disorders. In addition, compared with WT mice, we found increased depressive-like and anxiety-like behaviors in the *BDNF*^{+/Met} mice after stress. Previous studies have shown that haploinsufficient *BDNF*^{+/-} mice displayed depressive-like behaviors following stress (Advani et al., 2009; Autry et al., 2009), suggesting BDNF deficiency could induce a depressive-like behavior in stressful contexts. Clinical studies also suggest that *BDNF*_{Met} carriers are sensitive to early stressful life events. Childhood depression scores were assessed by the interaction between *BDNF* Val66Met, 5-HTTLPR polymorphism and the childhood maltreatment, with higher depression scores found in children with the short/short 5-HTTLPR-*BDNF*_{Val/Met} genotype (Kaufman et al., 2006). This finding has been replicated in an adult sample with highest depression scores in the long/short 5-HTTLPR-*BDNF*_{Met} carrier genotype (Wichers et al., 2008).

Second, this is the first study to demonstrate a working memory impairment following stress in *BDNF*^{+/Met} mice. The effects of the *BDNF*_{Met} polymorphism on memory after stress were evaluated in the Morris water maze and T-maze tests. In the water maze test, we found that restraint stress impaired acquisition of hippocampal-dependent spatial memory in *BDNF*^{+/Met} and WT mice, however, with no genotype difference, suggesting that *BDNF*^{+/Met} and WT mice was almost equally vulnerable to alterations in spatial memory by stressful stimuli. Previous studies have shown that different stress paradigms could lead to spatial memory deficits in mice (Luksys and Sandi, 2011; Wang et al., 2011). Our data suggested that the *BDNF*_{Met} polymorphism might not play a significant role in stress-induced spatial memory deficit. Conversely, the effect of BDNF on working memory after stress has been rarely investigated. Spatial alternation in a T-maze demands updating of information, inhibition of a tendency to return to a previously location, and concentration during the delay period, which relies on PFC function (Taylor et al., 1999). Our results showed that chronic stress led to working memory impairments only in *BDNF*^{+/Met} but not in WT mice, suggesting working memory and its relevant PFC function is more susceptible to stress in *BDNF*^{+/Met} mice.

The potential mechanism underlying the stress-induced abnormal behaviors and working memory deficits in *BDNF*^{+/Met} mice was also investigated in our study. Previous studies have

shown that various types of stress stimuli could decrease BDNF levels in the HPC (Smith et al., 1995; Taliaz et al., 2011), which might in part account for the stress-associated depressive-like behaviors and spatial memory deficits. We investigated the role of BDNF in other brain regions in addition to HPC in stress related behavior and memory response. Following restraint stress, we found a significant reduction in BDNF mRNA and protein levels in the HPC and PFC of *BDNF*^{+/Met} and WT mice. Moreover, stress *BDNF*^{+/Met} mice showed decreased BDNF mRNA and protein levels in the PFC compared with stress WT mice. In contrast, stress-induced increased BDNF mRNA and protein levels in the amygdala with more robust effect on *BDNF*^{+/Met} mice. Since BDNF has been shown to regulate neuronal morphology (Magariños et al., 2011), stress-induced alterations in dendritic spine density was investigated in *BDNF*^{+/Met} and WT mice. Dendritic spines are highly dynamic structures and undergo constant remodeling as a function of neuronal activity (Kasai et al., 2003; Yuste and Bonhoeffer, 2004). Effect of BDNF haploinsufficiency on stress-induced remodeling of hippocampal neurons has been reported (Magariños et al., 2011); however, few studies have reported the effect of BDNF on stress-induced spinogenesis in PFC and amygdala except one study showing that chronic stress triggered amygdala spinogenesis was occluded by BDNF overexpression (Govindarajan et al., 2006). After stress, we observed decreased spine density in PFC whereas increased spine density in amygdala in the distal apical dendrites, with more prominent effects in *BDNF*^{+/Met} mice compared with WT mice. Previous studies have shown that stress results in neuronal structural remodeling in both PFC and amygdala (Mittra et al., 2005; Govindarajan et al., 2006; Liston et al., 2006; Goldwater et al., 2009). Our current findings suggest that the *BDNF*_{Met} polymorphism could modulate the stress-induced neuroanatomical remodeling in these two regions. The altered spine density triggered by stress in PFC and amygdala of *BDNF*^{+/Met} mice is consistent with the BDNF mRNA and protein levels change after stress. The clear correlation between BDNF mRNA level or spine density in PFC (or amygdala) and working memory performance (or anxiety) may provide insights into the molecular and structural mechanisms underlying stress-induced behavior phenotype in *BDNF*^{+/Met} mice. Why the stress-induced changes in BDNF levels and spine density changes were in opposite directions in PFC and amygdala requires further investigation.

Finally, we found that an NRI, desipramine, but not an SSRI, fluoxetine was able to rescue the stress-induced depressive-like behaviors in *BDNF*^{+/Met} mice. Our previous study reported that the increased anxiety-like behaviors in *BDNF*^{Met/Met} mice could not be normalized by chronic fluoxetine treatment (Chen et al., 2006). This is the first study showing *BDNF*^{+/Met} mice have a blunted response to acute fluoxetine treatment, but, significant responsiveness to another class of antidepressants. Previous studies have shown that an increase in climbing behavior in the FST is more likely associated with effects on noradrenergic transmission, whereas serotonergic antidepressants selectively increase swimming behavior (Page et al., 1999), which is in agreement with our results. To further investigate the molecular mechanism underlying the differential responsiveness to distinct antidepressants in *BDNF*^{+/Met} mice, 5-HTT and NET levels in different brain regions were examined by quantitative RT-PCR. Compared with WT mice, we found significantly decreased 5-HTT mRNA level in the DRN and increased NET mRNA level in the LC in *BDNF*^{+/Met} mice. Human studies have found that carriers of the *BDNF*_{Met} allele displayed reduced 5-HTT availability in the raphe, insular and anterior cingulate cortex compared with the

*BDNF*_{Val} allele, suggesting that the *BDNF*_{Met} polymorphism influences 5-HTT expression or the density of serotonergic neurons (Henningsson et al., 2009). These data suggest that the altered 5-HTT expression and function associated with the *BDNF*_{Met} polymorphism might account for the blunted response to fluoxetine treatment, which is consistent with the previous study showing that BDNF regulates serotonergic neurotransmission (Hensler et al., 2007). In addition, it has also been demonstrated that antidepressants may differentially regulate targeting of BDNF protein in the synaptic compartment, suggesting that the lack of activity of fluoxetine may also be related to its inability to modulate specific BDNF function (Calabrese et al., 2007). Interestingly, we found the increased NET mRNA levels in the LC in *BDNF*^{+/Met} mice, the influence of the *BDNF*_{Met} polymorphism on noradrenergic transmission needs to be further investigated.

In conclusion, through the use of a mouse model system, we determined that the variant *BDNF*_{Met} allele leads to increased vulnerability to stress. We provide evidence, for the first time to our knowledge, that *BDNF*^{+/Met} mice, carrying only one copy of the variant allele, displayed depressive-like, and anxiety-like behaviors and impaired working memory only following environmental stress. The alterations in these behaviors may be explained by the stress-induced changes in BDNF levels and neuronal spine remodeling in selective brain regions. These studies could potentially guide and inform future human studies on the impact of this common SNP in neuropsychiatric disorders especially in humans carrying one copy of this variant BDNF. Furthermore, we found that desipramine but not fluoxetine was effective in rescuing stress-induced depressive-like behaviors in *BDNF*^{+/Met} mice, suggesting that in humans with this genetic variant BDNF, selective norepinephrine reuptake inhibitors may be a more effective treatment option for depressive disorders.

References

- Advani T, Koek W, Hensler JG (2009) Gender differences in the enhanced vulnerability of BDNF +/− mice to mild stress. *Int J Neuropsychopharmacol* 12:583–588.
- Alexander N, Osinsky R, Schmitz A, Mueller E, Kuepper Y, Hennig J (2010) The BDNF Val66Met polymorphism affects HPA-axis reactivity to acute stress. *Psychoneuroendocrinology* 35:949–953.
- Autry AE, Adachi M, Cheng P, Monteggia LM (2009) Gender-specific impact of brain-derived neurotrophic factor signaling on stress-induced depression-like behavior. *Biol Psychiatry* 66:84–90.
- Berton O, McClung CA, Dileone RJ, Krishnan V, Renthall W, Russo SJ, Graham D, Tsankova NM, Bolanos CA, Rios M, Monteggia LM, Self DW, Nestler EJ (2006) Essential role of BDNF in the mesolimbic dopamine pathway in social defeat stress. *Science* 311:864–868.
- Calabrese F, Molteni R, Maj PF, Cattaneo A, Gennarelli M, Racagni G, Riva MA (2007) Chronic duloxetine treatment induces specific changes in the expression of BDNF transcripts and in the subcellular localization of the neurotrophin protein. *Neuropsychopharmacology* 32:2351–2359.
- Calabrese F, Molteni R, Racagni G, Riva MA (2009) Neuronal plasticity: a link between stress and mood disorders. *Psychoneuroendocrinology* 34 [Suppl 1]:S208–S216.
- Chen ZY, Patel PD, Sant G, Meng CX, Teng KK, Hempstead BL, Lee FS (2004) Variant brain-derived neurotrophic factor (BDNF) (Met66) alters the intracellular trafficking and activity-dependent secretion of wild-type BDNF in neurosecretory cells and cortical neurons. *J Neurosci* 24:4401–4411.
- Chen ZY, Jing D, Bath KG, Ieraci A, Khan T, Siao CJ, Herrera DG, Toth M, Yang C, McEwen BS, Hempstead BL, Lee FS (2006) Genetic variant BDNF (Val66Met) polymorphism alters anxiety-related behavior. *Science* 314:140–143.
- Cunningham JT, Mifflin SW, Gould GG, Frazer A (2008) Induction of c-Fos and DeltaFosB immunoreactivity in rat brain by vagal nerve stimulation. *Neuropsychopharmacology* 33:1884–1895.
- de Kloet ER, Joëls M, Holsboer F (2005) Stress and the brain: from adaptation to disease. *Nat Rev Neurosci* 6:463–475.

- Dougherty LR, Klein DN, Congdon E, Canli T, Hayden EP (2010) Interaction between 5-HTTLPR and BDNF Val66Met polymorphisms on HPA axis reactivity in preschoolers. *Biol Psychol* 83:93–100.
- Drugan RC, Macomber H, Warner TA (2010) Intermittent and continuous swim stress-induced behavioral depression: sensitivity to norepinephrine- and serotonin-selective antidepressants. *Psychopharmacology (Berl)* 212: 85–91.
- Dulawa SC, Hen R (2005) Recent advances in animal models of chronic antidepressant effects: the novelty-induced hypophagia test. *Neurosci Biobehav Rev* 29:771–783.
- Duman CH, Schlesinger L, Kodama M, Russell DS, Duman RS (2007) A role for MAP kinase signaling in behavioral models of depression and antidepressant treatment. *Biol Psychiatry* 61:661–670.
- Duman RS, Monteggia LM (2006) A neurotrophic model for stress-related mood disorders. *Biol Psychiatry* 59:1116–1127.
- Duncko R, Kiss A, Skultetyová I, Rusnák M, Jezová D (2001) Corticotropin-releasing hormone mRNA levels in response to chronic mild stress rise in male but not in female rats while tyrosine hydroxylase mRNA levels decrease in both sexes. *Psychoneuroendocrinology* 26:77–89.
- Egan MF, Kojima M, Callicott JH, Goldberg TE, Kolachana BS, Bertolino A, Zaitsev E, Gold B, Goldman D, Dean M, Lu B, Weinberger DR (2003) The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. *Cell* 112:257–269.
- Feder A, Nestler EJ, Charney DS (2009) Psychobiology and molecular genetics of resilience. *Nat Rev Neurosci* 10:446–457.
- Fumagalli F, Caffino L, Racagni G, Riva MA (2009) Repeated stress prevents cocaine-induced activation of BDNF signaling in rat prefrontal cortex. *Eur Neuropsychopharmacol* 19:402–408.
- Gatt JM, Nemeroff CB, Dobson-Stone C, Paul RH, Bryant RA, Schofield PR, Gordon E, Kemp AH, Williams LM (2009) Interactions between BDNF Val66Met polymorphism and early life stress predict brain and arousal pathways to syndromal depression and anxiety. *Mol Psychiatry* 14:681–695.
- Gillespie CF, Phifer J, Bradley B, Ressler KJ (2009) Risk and resilience: genetic and environmental influences on development of the stress response. *Depress Anxiety* 26:984–992.
- Goldwater DS, Pavlides C, Hunter RG, Bloss EB, Hof PR, McEwen BS, Morrison JH (2009) Structural and functional alterations to rat medial prefrontal cortex following chronic restraint stress and recovery. *Neuroscience* 164:798–808.
- Govindarajan A, Rao BS, Nair D, Trinh M, Mawjee N, Tonegawa S, Chattarji S (2006) Transgenic brain-derived neurotrophic factor expression causes both anxiogenic and antidepressant effects. *Proc Natl Acad Sci U S A* 103:13208–13213.
- Henningsson S, Borg J, Lundberg J, Bah J, Lindström M, Ryding E, Jovanovic H, Saijo T, Inoue M, Rosén I, Träskman-Bendz L, Farde L, Eriksson E (2009) Genetic variation in brain-derived neurotrophic factor is associated with serotonin transporter but not serotonin-1A receptor availability in men. *Biol Psychiatry* 66:477–485.
- Hensler JG, Advani T, Monteggia LM (2007) Regulation of serotonin-1A receptor function in inducible brain-derived neurotrophic factor knockout mice after administration of corticosterone. *Biol Psychiatry* 62:521–529.
- Holmes A, Lit Q, Murphy DL, Gold E, Crawley JN (2003) Abnormal anxiety-related behavior in serotonin transporter null mutant mice: the influence of genetic background. *Genes Brain Behav* 2:365–380.
- Joëls M, Pu Z, Wiegert O, Oitzl MS, Krugers HJ (2006) Learning under stress: how does it work? *Trends Cogn Sci* 10:152–158.
- Kasai H, Matsuzaki M, Noguchi J, Yasumatsu N, Nakahara H (2003) Structure-stability-function relationships of dendritic spines. *Trends Neurosci* 26:360–368.
- Kaufman J, Yang BZ, Douglas-Palumberi H, Grasso D, Lipschitz D, Houshyar S, Krystal JH, Gelernter J (2006) Brain-derived neurotrophic factor-5-HTTLPR gene interactions and environmental modifiers of depression in children. *Biol Psychiatry* 59:673–680.
- Kim SJ, Cho SJ, Jang HM, Shin J, Park PW, Lee YJ, Cho IH, Choi JE, Lee HJ (2010) Interaction between brain-derived neurotrophic factor Val66Met polymorphism and recent negative stressor in harm avoidance. *Neuropsychobiology* 61:19–26.
- Liston C, Miller MM, Goldwater DS, Radley JJ, Rocher AB, Hof PR, Morrison JH, McEwen BS (2006) Stress-induced alterations in prefrontal cortical dendritic morphology predict selective impairments in perceptual attentional set-shifting. *J Neurosci* 26:7870–7874.
- Luksys G, Sandi C (2011) Neural mechanisms and computations underlying stress effects on learning and memory. *Curr Opin Neurobiol* 21:502–508.
- Lupien SJ, McEwen BS, Gunnar MR, Heim C (2009) Effects of stress throughout the lifespan on the brain, behaviour and cognition. *Nat Rev Neurosci* 10:434–445.
- Magariños AM, Li CJ, Gal Toth J, Bath KG, Jing D, Lee FS, McEwen BS (2011) Effect of brain-derived neurotrophic factor haploinsufficiency on stress-induced remodeling of hippocampal neurons. *Hippocampus* 21:253–264.
- McEwen BS, Gianaros PJ (2011) Stress- and allostasis-induced brain plasticity. *Annu Rev Med* 62:431–445.
- Mitra R, Jadhav S, McEwen BS, Vyas A, Chattarji S (2005) Stress duration modulates the spatiotemporal patterns of spine formation in the basolateral amygdala. *Proc Natl Acad Sci U S A* 102:9371–9376.
- Page ME, Detke MJ, Dalvi A, Kirby LG, Lucki I (1999) Serotonergic mediation of the effects of fluoxetine, but not desipramine, in the rat forced swimming test. *Psychopharmacology (Berl)* 147:162–167.
- Paylor R, Zhao Y, Libbey M, Westphal H, Crawley JN (2001) Learning impairments and motor dysfunctions in adult Lhx5-deficient mice displaying hippocampal disorganization. *Physiol Behav* 73:781–792.
- Pittenger C, Duman RS (2008) Stress, depression, and neuroplasticity: a convergence of mechanisms. *Neuropsychopharmacology* 33:88–109.
- Porsolt RD, Le Pichon M, Jalfre M (1977) Depression: a new animal model sensitive to antidepressant treatments. *Nature* 266:730–732.
- Roozendaal B, McEwen BS, Chattarji S (2009) Stress, memory and the amygdala. *Nat Rev Neurosci* 10:423–433.
- Schmidt HD, Duman RS (2010) Peripheral BDNF produces antidepressant-like effects in cellular and behavioral models. *Neuropsychopharmacology* 35:2378–2391.
- Schwabe L, Wolf OT, Oitzl MS (2010) Memory formation under stress: quantity and quality. *Neurosci Biobehav Rev* 34:584–591.
- Sen S, Nesse RM, Stoltenberg SF, Li S, Gleiberman L, Chakravarti A, Weder AB, Burmeister M (2003) A BDNF coding variant is associated with the NEO personality inventory domain neuroticism, a risk factor for depression. *Neuropsychopharmacology* 28:397–401.
- Sheward WJ, Maywood ES, French KL, Horn JM, Hastings MH, Seckl JR, Holmes MC, Harmar AJ (2007) Entrainment to feeding but not to light: circadian phenotype of VPAC2 receptor-null mice. *J Neurosci* 27:4351–4358.
- Smith MA, Makino S, Kvetnansky R, Post RM (1995) Stress and glucocorticoids affect the expression of brain-derived neurotrophic factor and neurotrophin-3 mRNAs in the hippocampus. *J Neurosci* 15:1768–1777.
- Taliaz D, Loya A, Gersner R, Haramati S, Chen A, Zangen A (2011) Resilience to chronic stress is mediated by hippocampal brain-derived neurotrophic factor. *J Neurosci* 31:4475–4483.
- Taylor JR, Birnbaum S, Ubriani R, Arnsten AF (1999) Activation of cAMP-dependent protein kinase A in prefrontal cortex impairs working memory performance. *J Neurosci* 19:RC23(1–5).
- Verhagen M, van der Meij A, van Deuren PA, Janzing JG, Arias-Vásquez A, Buitelaar JK, Franke B (2010) Meta-analysis of the BDNF Val66Met polymorphism in major depressive disorder: effects of gender and ethnicity. *Mol Psychiatry* 15:260–271.
- Wang XD, Rammes G, Kraev I, Wolf M, Liebl C, Scharf SH, Rice CJ, Wurst W, Holsboer F, Deussing JM, Baram TZ, Stewart MG, Müller MB, Schmidt MV (2011) Forebrain CRF modulates early-life stress-programmed cognitive deficits. *J Neurosci* 31:13625–13634.
- Wichers M, Kenis G, Jacobs N, Mengelers R, Derom C, Vlietinck R, van Os J (2008) The BDNF Val(66)Met $\times$ 5-HTTLPR $\times$ child adversity interaction and depressive symptoms: an attempt at replication. *Am J Med Genet B Neuropsychiatr Genet* 147B:120–123.
- Ye Y, Wang G, Wang H, Wang X (2011) Brain-derived neurotrophic factor (BDNF) infusion restored astrocytic plasticity in the hippocampus of a rat model of depression. *Neurosci Lett* 503:15–19.
- Yuste R, Bonhoeffer T (2004) Genesis of dendritic spines: insights from ultrastructural and imaging studies. *Nat Rev Neurosci* 5:24–34.
- Zörner B, Wolfer DP, Brandis D, Kretz O, Zacher C, Madani R, Grunwald I, Lipp HP, Klein R, Henn FA, Gass P (2003) Forebrain-specific trkB-receptor knockout mice: behaviorally more hyperactive than “depressive.” *Biol Psychiatry* 54:972–982.