Variant Brain-Derived Neurotrophic Factor (Valine66Methionine) Polymorphism Contributes to Developmental and Estrous Stage-Specific Expression of Anxiety-Like Behavior in Female Mice

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Background: Most anxiety and depressive disorders are twice as common in women compared with men, and the sex difference in prevalence typically emerges during adolescence. Hormonal changes across the menstrual cycle and during the postpartum and perimenopausal periods are associated with increased risk for anxiety and depression symptoms. In humans and animals, reduced brain-derived neurotrophic factor (*BDNF*) has been associated with increased expression of affective pathology. Recently, a single nucleotide polymorphism (SNP) in the *BDNF* gene (*BDNF* Valine66Methionine [Val66Met]), which reduces *BDNF* bioavailability, has been identified in humans and associated with a variety of neuropsychiatric disorders. Although *BDNF* expression can be directly influenced by estrogen and progesterone, the potential impact of the *BDNF* Val66Met SNP on sensitivity to reproductive hormone changes remains an open question.

Methods: As a predictive model, we used female mice in which the human SNP (*BDNF* Val66Met) was inserted into the mouse *BDNF* gene. Using standard behavioral paradigms, we tested the impact of this SNP on age and estrous-cycle-specific expression of anxiety-like behaviors.

Results: Mice homozygous for the *BDNF* Val66Met SNP begin to exhibit increased anxiety-like behaviors over prepubertal and early adult development, show significant fluctuations in anxiety-like behaviors over the estrous cycle, and, as adults, differ from wild-type mice by showing significant fluctuations in anxiety-like behaviors over the estrous cycle—specifically, more anxiety-like behaviors during the estrus phase.

Conclusions: These findings have implications regarding the potential role of this SNP in contributing to developmental and reproductive hormone-dependent changes in affective disorders in humans.

Key Words: Anxiety, *BDNF*, behavior, development, estrous, female, Val66Met

The development of anxiety and depressive disorders peaks during adolescence and early adulthood, with females being at significantly greater risk than males (1–4). Specifically, females show a significant increase in the expression of affective disorders that often coincides with the onset of ovarian cycling (4). Periods of reproductive hormone flux, including the menstrual cycle, the postpartum period and perimenopause are associated with exacerbations in mood and anxiety symptoms (5–12). Significant questions remain regarding the mechanisms through which changes in estrogen and progesterone could affect mood and anxiety and the reasons why only a subset of women demonstrate particularly robust changes in emotional state in response to reproductive events (9). Twin studies and family studies suggest that genetic factors may contribute to susceptibility to reproductive-related affective illness (13–16).

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Brain-derived neurotrophic factor (*BDNF*) has been implicated in the development as well as treatment of affective disorders in both humans and animal models (17). In rodent and humans, decreased *BDNF* expression is associated with the development of affective pathology (18–21). In the rodent brain and human serum, the expression of *BDNF* is augmented by nearly every antidepressant regimen tested to date (reviewed in Duman and Monteggia [17]). In animal models, the direct administration of *BDNF* into the brain has antidepressant effects (22,23). Furthermore, the increase in *BDNF* expression and signaling in response to antidepressants appears to be a key step in decreasing the expression of anxiety and depressive-like behaviors on established marker tasks (24,25). Interestingly, the expression or release of *BDNF* is significantly increased in response to estrogen (26–28) with progesterone serving to counteract the effects of estrogen on *BDNF* expression (29). The modulation of *BDNF* by estrogen and progesterone has been proposed to contribute to alterations in cognitive and emotional functioning across the estrous cycle and during the transitional period of perimenopause (16,30,31).

Recently, a common single nucleotide polymorphism (SNP) in the human *BDNF* gene was identified (*BDNF* Valine66Methionine) (32). This SNP leads to a change from a Valine (Val) to a Methionine (Met) at position 66 within the prodomain of *BDNF* (thus *BDNF* Val66Met). Approximately 30% of the Caucasian population carry the Met allele, with approximately 4% being homozygous (33). The Met allele leads to a reduction in the activity dependent release of *BDNF* (32,34,35) and has been associated with subtle changes in memory function (36) as well as with a variety of neuropsychiatric disorders. Human association studies have begun to investigate the

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potential role of this SNP in the development of affective disorders with somewhat mixed results (17,37,38). To reduce the potential variability related to environmental factors and other mood-related genetic variations in humans, we have developed an animal model of the *BDNF* Val66Met polymorphism by inserting the analogous SNP into an inbred strain of mice. Mice homozygous for the Met allele recapitulate the hallmark effects that have been reported in human Met allele carriers (35) and serves as a translational tool to assess the potential effects of this SNP on cognitive and emotional functioning (39).

Here, we used this animal model to investigate whether the previously demonstrated association of the Met allele insertion with anxiety-like behaviors is evident in female mice and whether the expression of these behaviors varies across development or the estrous cycle. We find here that the Met allele is associated with increased expression of anxiety- and depressive-like behaviors in female adults; that anxiety-like behaviors increase over the course of early development in Met allele carriers but not wild-type mice, and the anxiogenic effects of the Met allele are most apparent during the stage of the estrous cycle in which estrogen has just rapidly fallen. These findings have potential relevance for understanding genetic factors that may contribute to the increased expression of affective pathology in women during times of hormonal cycling (puberty and premenopausal years) and during reproductive events associated with declines in reproductive hormones (postpartum and perimenopause).

Methods and Materials

Animals

All animal procedures were approved by the Institutional Animal Care and Use Committees of Weill Cornell Medical College and were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. All female mice aged 3 to 22 weeks were housed in standard shoebox caging on a 12-hour light–dark cycle (lights on 7 AM) with food and water available ad libitum. *BDNF* Val66Met mice were generated by using a knock-in allele with a point mutation (G to A at position 196) in the coding region of the mouse *BDNF* gene as described in Chen *et al.* (36). This mutation changes the valine at position 66 to a methionine (e.g., Val66Met). Mice were generated from heterozygous Val/Met mothers, were weaned and sex segregated at postnatal Day 21, and in nearly all cases Val/Val, Val/Met, and Met/Met females were mixed housed in a single cage. This allowed testing of Val/Val and Met/Met mice during the same testing session. The mice were crossed onto a C57BL/6J background (13th generation backcross at the time of these experiments). All mice were bred at Weill Cornell Medical College, underwent tail-tipping for collection of DNA at the time of weaning (postnatal Day 20), were then sex segregated and were genotyped using previously described methods (35).

Estrous Cycling

Adult (9- to 30-week old) animals in which cycle status was assessed, received a single vaginal swabbing following the completion of behavioral testing. Swabs were taken within 10 min of the end of testing and occurred between the hours of 9:30 AM and 12:30 PM. Vaginal cytology was observed under a microscope after staining with the Hema 3 Stain Set from Fisher Scientific (Pittsburgh, Pennsylvania). Cycle stage was determined as proestrus, estrus, metestrus, or diestrus according to previously published criteria (40,41). Any animals in which cycle status could not be definitively identified were excluded from the experiment (5 mice).

Behavioral Testing

Elevated Plus Maze. The elevated plus-maze was constructed of white Lexan, raised 70 cm above the floor, and consisted of two opposite enclosed arms with 14-cm-high opaque walls and two opposite open arms of the same size (30 cm × 5 cm). The maze was set up under an infrared sensitive digital camera connected to a video recorder and computer under the control of Ethovision software (Noldus, Leesburg, Virginia). A single testing session lasted 10 min and was carried out under low light (~4 Lux). To begin a trial, the test animal was placed in the center of the plus-maze facing an open arm, and their behavior was recorded for 10 min. The maze was cleaned with a 70% ethanol solution and dried after each trial to eliminate possible odor cues left by previous subjects. The time spent in both the open and enclosed arms was recorded and analyzed using Ethovision software. Measures of anxiety-like behavior were assessed by calculating the relative amount of time spent in the open arms relative to the enclosed arms. Mice were tested only once on the elevated plus maze task.

Open Field. The open-field apparatus consists of a (40 cm × 40 cm × 49 cm) white Lexan arena with a white floor. With the aid of tracking software (Noldus EthoVision XT), the arena was digitally divided into 12 equal quadrants. The arena was set up in a dim room (~4 Lux) under a digital camera and connected to a video recorder and a computer under the control of the EthoVision software (Noldus). A single mouse was placed into the center of the arena, and its behavior was recorded over a 10-min session. Measures of anxiety-like behavior included the relative amount of time spent exploring the center quadrants relative to those located adjacent to the walls of the arena. Mice were tested only once on the open field task.

Forced Swim. The forced swim apparatus consisted of a 2-L beaker filled with 1500 mL of room-temperature water. Beakers were set up in isolation cubicles and positioned in front of a digital camera connected to a computer running Noldus Ethovision tracking software. Mice were placed in the water, and their behavior was recorded for 5 min. Using the mobility measurement function on EthoVision software, we calculated immobility of the mice on this task. Measures of depressive-like behavior included the total amount of time spent immobile during the 5-min test.

Statistics. To assess the effect of genotype on the expression of anxiety-like and depressive-like behavior in female mice independent of estrous status, a simple Student's *t* test was used. To assess the correlation between age and levels of anxiety-like behavior, we used the Pearson correlation test. For comparisons of the potential contribution of estrous status to the differential expression of anxiety-like behavior in *BDNF* Val66Met mice, we used analysis of variance (ANOVA) to assess within genotype effects and Tukey's least significant difference (LSD) for post hoc comparisons, controlling for multiple tests. For comparisons of genotype by estrous stage, due to power limitations comparison's of anxiety-like behavior between genotype were carried out using independent planned *t* tests for each stage of the estrous cycle, with Bonferroni corrections for multiple tests, and an adjust alpha of $<.01$. Consistent with standard statistical practices, any data point that was greater than 2 SD from the group mean was eliminated (one instance). For all statistics alpha was set at $<.05$. SPSS software (IBM, Armonk, New York) was used for all analyses.

Results

Female *BDNF* Val66Met Display Increased Anxiety-Like and Depressive-Like Behavior

To investigate the potential contribution of the *BDNF* Val66Met SNP to the expression of anxiety- and depressive-like behavior in