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BDNF Val66Met Genotype Interacts With a History of Simulated Stress Exposure to Regulate Sensorimotor Gating and Startle Reactivity

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Reduced expression of Brain-Derived Neurotrophic Factor (BDNF) has been implicated in the pathophysiology of schizophrenia. The BDNF Val66Met polymorphism, which results in deficient activity-dependent secretion of BDNF, is associated with clinical features of schizophrenia. We investigated the effect of this polymorphism on Prepulse Inhibition (PPI), a translational model of sensorimotor gating which is disrupted in schizophrenia. We utilized humanized BDNF^{Val66Met} (hBDNF^{Val66Met}) mice which have been modified to carry the Val66Met polymorphism, as well as express humanized BDNF in vivo. We also studied the long-term effect of chronic corticosterone (CORT) exposure in these animals as a model of history of stress. PPI was assessed at 30 ms and 100 ms interstimulus intervals (ISI). Analysis of PPI at the commonly used 100 ms ISI identified that, irrespective of CORT treatment, the hBDNF$^{Val/Met}$ genotype was associated with significantly reduced PPI. In contrast, PPI was not different between hBDNF$^{Met/Met}$ and hBDNF$^{Val/Val}$ genotype mice. At the 30 ms ISI, CORT treatment selectively disrupted sensorimotor gating of hBDNF$^{Val/Met}$ heterozygote mice but not hBDNF$^{Val/Val}$ or hBDNF$^{Met/Met}$ mice. Analysis of startle reactivity revealed that chronic CORT reduced startle reactivity of hBDNF$^{Val/Val}$ male mice by 51%. However, this was independent of the effect of CORT on PPI. In summary, we provide evidence of a distinct BDNF^{Val66Met} heterozygote-specific phenotype using the sensorimotor gating endophenotype of schizophrenia. These data have important implications for clinical studies where, if possible, the BDNF$^{Val/Met}$ heterozygote genotype should be distinguished from the BDNF$^{Met/Met}$ genotype.

Key words: brain-derived neurotrophic factor/BDNF/Val66Met/G196A/rs6265/schizophrenia/psychosis/prepulse inhibition/sensorimotor gating/stress/glucocorticoid hormones

Introduction

Brain-Derived Neurotrophic Factor (BDNF) is a key molecular mediator of neuronal development, differentiation and plasticity. Reduced expression of BDNF has been observed in the post-mortem brain[1,2] and serum[3–5] of schizophrenia patients, and the BDNF gene has been extensively screened for association with schizophrenia. One genetic alteration, termed the Val66Met polymorphism, results in deficient subcellular translocation and defective activity-dependent secretion of BDNF,[6] reducing the secretion of BDNF by approximately 18% in cells transfected with one BDNF^{66Met} allele and 29% in those transfected with 2.[7] The BDNF^{66Met} allele has been associated with impaired memory function and reduced hippocampal volumes,[6–9] and has been implicated as a locus of risk for several psychiatric disorders including schizophrenia.[10,11] Clinical studies have found that the BDNF$^{Met/Met}$ genotype is more frequent in patients reporting more positive than negative symptoms,[12] while another study reported that schizophrenia patients of BDNF$^{Met/Met}$ genotype had significantly more delusional symptoms than patients carrying the BDNF^{66Val} allele.[13] However, the role of the BDNF^{Val66Met} polymorphism within this disorder remains controversial.[14]

Sensorimotor gating refers to the latent ability to inhibit a motor response given a preceding sensory stimulus. Sensorimotor gating deficits are observed amongst schizophrenia patients[15,16] and are typically quantified using the Prepulse Inhibition (PPI) paradigm; where a blunted startle response to an auditory pulse stimulus is

observed when preceded by the presentation of a weaker auditory prepulse. As PPI deficits are observed during acute phases of illness[17] and are a correlate of positive symptom presentation[18] amongst schizophrenia patients, PPI is commonly viewed as an endophenotype of relevance to psychosis.[19] PPI deficits are not necessarily specific to psychosis itself, as evidenced by their persistence during treatment[20] and occasional observation amongst other psychiatric disorders,[21] but their presence in unaffected relatives of schizophrenia patients[22] classifies PPI as a schizophrenia endophenotype that can be used to dissect the molecular genetic architecture of the disorder. A strength of the PPI paradigm is its translational merit, with both rodent and human versions of the test available. This can be exploited for the preclinical modeling of schizophrenia endophenotypes,[23] as genetic and environmental heterogeneity can be controlled for in rodents allowing for the detection of subtle genomic modifiers of PPI. While the Val66Met polymorphism's regulation of PPI has not been assessed, studies in BDNF heterozygote knockout mice with a global reduction in BDNF expression of approximately 50%, have revealed reduced PPI,[24,25] including sex-specific disruptions at varying prepulse intensities and interstimulus intervals (ISI).[24]

The aim of the current study was to assess the role of the $BDNF^{Val66Met}$ polymorphism in PPI. We utilized humanized $BDNF^{Val66Met}$ ($hBDNF^{Val66Met}$) mice genetically modified to (1) carry the Val66Met polymorphism and (2) express human BDNF via endogenous mouse promoters. Given that the Val66Met genotype determines stress sensitivity,[26] and that in healthy samples both mild social stress[27] and childhood abuse[28] result in an increase in the expression of psychosis-related symptom expression in $BDNF^{66Met}$ allele carriers, a secondary aim was to explore whether a chronic corticosterone (CORT) treatment administered in late adolescence/young adulthood, a critical period associated with the psychosis prodrome, may interact with BDNF genotype to determine adult PPI.

Method

$hBDNF^{Val66Met}$ Mice and Housing

$hBDNF^{Val66Met}$ mice were generated as previously described.[29] Mice used in the present study were offspring from $hBDNF^{Val/Met} \times hBDNF^{Val/Met}$ breeding pairs. There were 12 groups comprising 3 genotypes ($hBDNF^{Val/Val}$, $hBDNF^{Val/Met}$ and $hBDNF^{Met/Met}$), 2 treatment groups (CORT and control) and 2 sexes (male and female). Group sizes ranged from 11 to 15 mice per group, for a total sample size of 152 mice. All animals were of a C57Bl/6 genetic background. Animals were group-housed with same-sex littermates (n = 2–6 per box) in individually-ventilated cages (IVC; Techniplast) under standard lighting conditions (ie, an automated 12-h light cycle), and had ad libitum access to standard pellet food and water. IVC cages contained woodchip bedding and enrichment in the form of tissue paper and a small amount of "furl." Mice were checked daily by animal house staff, and cages were changed as necessary when soiled. Mice were observed and weighed at least weekly to track health, and the effects of our stress treatment. Housing and experimental procedures were carried out with approval from the Florey Institute of Neuroscience's animal ethics committee.

Chronic CORT Protocol

Chronic adolescent stress was simulated by treating mice with 25 mg/L of the mouse stress hormone, CORT, which was dissolved in the animal's drinking water.[30] Treatment occurred between weeks 6 to 9, a period we have previously established to mimic late adolescence/early adulthood[31] that is also commonly associated with the emergence of the psychosis prodome. The CORT solution was changed every 3 days over the treatment period. Control animals received unaltered water over this period. A 2-week washout period followed the treatment period before behavioral experimentation commenced, so that long-term behavioral adaptation to the chronic CORT treatment could be examined.

Prepulse Inhibition Protocol

Automated SR-Lab startle boxes (San Diego Instruments) were used to assess PPI. The test session consisted of 104 stimulus presentations as previously described.[32,33] Briefly, test sessions began and ended with a block of 8 presentations of a 40 ms 115 dB pulse, while prepulse-pulse trials consisted of a single 115 dB pulse that was preceded 100 ms or 30 ms by a 20 ms prepulse of variable intensity (2, 4, 8, or 16 dB over baseline). For all trials, 65 dB of background noise was used to prevent startle by exogenous stimuli. Prepulse inhibition was quantified as the difference between stimulus responses during prepulse-pulse and pulse-only trials, and expressed as a ratio of pulse-alone responses. Four blocks of 8 115 dB pulses of 40 ms duration were used to generate a startle habituation curve.

Data Analysis

Data analysis was undertaken using the IBM Predictive Analytical Software (PASW) and Graphpad Prism packages. A 3 (genotype) × 2 (sex) × 2 (treatment) ANOVA was used to analyze data for within-subject and between-subject comparisons. Analyses were considered significant at $P < .05$. Sidak's correction was applied to all post hoc comparisons, while the Greenhouse-Geisser correction was applied per Mauchley's Test of Sphericity. If no significant interaction involving the sex of the animals was observed, then data from male and female mice were analyzed together to increase power. This analysis

strategy is consistent with clinical research given the potentially small effect sizes generated by the Val66Met polymorphism,[34] as well as our previous report utilizing this mouse line.[30]

Results

No Effect of hBDNF^{Val66Met} Genotype on Body Weight Across Development

Body weight was recorded across development (weeks 4 to 14) and behavioral testing to rule out any confounding effect of genotype-mediated body weight differences on startle amplitudes. A mixed model ANOVA revealed a significant main effect of week ($F(9,1368) = 1438.17$, $P < .001$) and a week × sex interaction ($F(9,1368) = 70.48$, $P < .001$), reflecting that body weight increased between weeks 4 to 14 as a function of time and that female mice weighed less than males. There was no main effect of hBDNF^{Val66Met} genotype, suggesting no major effect on food intake or metabolism.

hBDNF^{Val66Met} Genotype Interacts With a History of CORT Exposure to Regulate PPI in an ISI-Specific Manner

A Mixed-Model ANOVA revealed a significant between-subject effect of hBDNF^{Val66Met} genotype ($F(2,140) = 5.2$, $P = .007$), as well as a significant hBDNF^{Val66Met} genotype × CORT interaction ($F(2,140) = 3.68$, $P = .028$) suggesting differences in PPI between the genotypes depending on CORT treatment. However, there was also a significant effect of ISI ($F(1,140) = 22.57$, $P < .001$), as well as an ISI × hBDNF^{Val66Met} genotype × CORT treatment interaction ($F(2,140) = 4.32$, $P = .015$), reflecting that such genotype-dependent changes in PPI differed between the 100 ms and 30 ms ISI and as a function of CORT treatment. Because of this complex interaction, we split our PPI dataset according to ISI to further dissect the effect of hBDNF^{Val66Met} genotype.

Analysis of PPI at the commonly used 100 ms ISI, revealed a significant between-subjects main effect of genotype ($F(2,140) = 4.94$, $P = .008$), where the hBDNF$^{Val/Met}$ genotype was associated with lower PPI than the hBDNF$^{Val/Val}$ group ($P < .01$; figure 1A). The hBDNF$^{Val/Val}$ and hBDNF$^{Met/Met}$ genotypes did not significantly differ from one another. No other between-subjects or repeated-measures main effects or interactions emerged from this analysis implying that PPI did not significantly differ between groups in its direction across PP intensities (supplementary figure 1) nor did it depend on sex or CORT treatment (figure 1A).

Analysis of PPI at the 30 ms ISI yielded a similar but divergent behavioral outcome. Specifically, a significant main effect of hBDNF^{Val66Met} genotype was once more detected ($F(2,140) = 4.02$, $P = .02$), independent of sex.

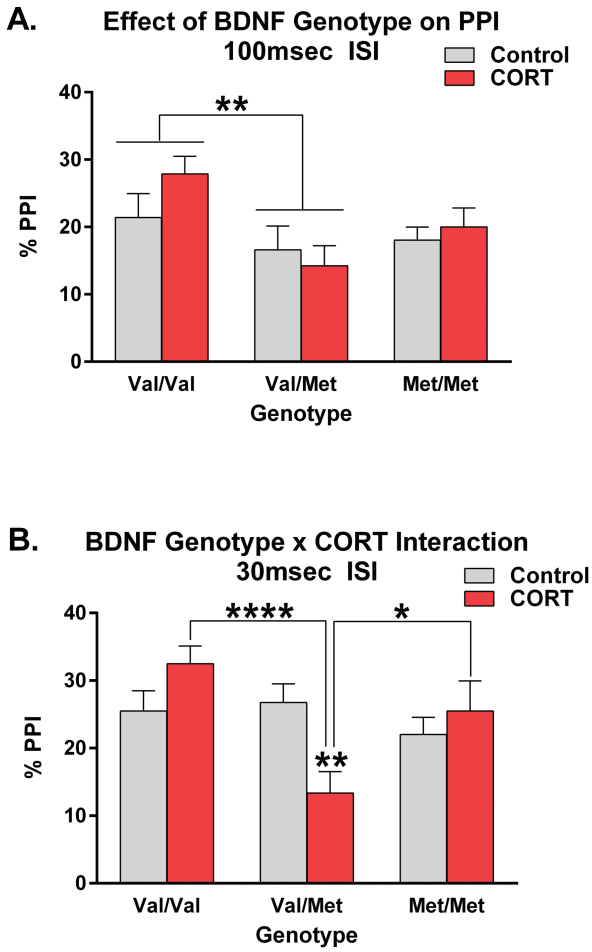

Fig. 1. Human Brain-Derived Neurotrophic Factor (BDNF) variant Val66Met modulates Prepulse Inhibition (PPI). At the 100 ms interstimulus interval (ISI) (panel A), no effect or interaction comprising corticosterone (CORT) treatment was observed but the hBDNF$^{Val/Met}$ genotype group had significantly lower PPI than the hBDNF$^{Val/Val}$ "wildtype" group. However, at the 30 ms ISI (panel B), a significant hBDNF^{Val66Met} genotype × CORT treatment interaction was observed, where chronic glucocorticoid hormone exposure unmasked an effect of the hBDNF$^{Val/Met}$ heterozygote genotype on PPI. Specifically, CORT-treated hBDNF$^{Val/Met}$ mice had significantly lower PPI than hBDNF$^{Val/Val}$ and hBDNF$^{Met/Met}$ homozygote mice. No sex differences were detected. All data presented as mean ± SEM; *$P < .05$, **$P < .01$, ****$P < .0001$, corrected for multiple comparisons. Per group, $n = 23$–27.

However, unlike at the 100 ms ISI, a significant hBDNF^{Val66Met} genotype × CORT interaction ($F(2,140) = 5.93$, $P = .003$) was found at this ISI (figure 1B). Further analysis of this interaction revealed that following CORT exposure in late adolescence, hBDNF$^{Val/Met}$ mice had significantly lower PPI than both hBDNF$^{Val/Val}$ ($P < .0001$) and hBDNF$^{Met/Met}$ ($P < .05$) homozygotes, who did not significantly differ from one another (figure 1B). Analysis of control-treated mice alone failed to reveal an effect of genotype, further implicating that a phenotype at this ISI only emerges following CORT treatment.

Aside from these between-subject effects, ANOVA also revealed a significant interaction comprising

hBDNF^{Val66Met} genotype and PP Intensity at the 30 ms ISI (F(5.09,356.302) = 2.36, P = .039). Splitting the analysis by prepulse intensity revealed that there was an inhibitory effect of CORT treatment on the hBDNF$^{Val/Met}$ genotype group (F(1,196) = 19.20, P < .0001) across the PP intensities studied. Analyzing for genotype-mediated differences across individual PP intensities identified a significant effect of hBDNF^{Val66Met} genotype at PP intensities of 4 db (F(2,140) = 5.23, P = .006) and 16 db (F(2,140) = 6.45, P = .002), which was underscored by the deficient PPI of the hBDNF$^{Val/Met}$ group. Interactions between hBDNF^{Val66Met} genotype and chronic CORT treatment were also observed at PP intensities of 2db (F(2,140) = 4.73, P = .01) and 8db (F(2,140) = 5.26, P = .006), which once more reflected a selective effect of prior CORT treatment on the PPI of the hBDNF$^{Val/Met}$ group (supplementary figure 1).

Corticosterone Interacts With hBDNF^{Val66Met} Genotype to Regulate Startle

A Mixed-Model ANOVA revealed a strong trend for a hBDNF^{Val66Met} genotype × CORT interaction (F(2,140) = 3.01, P = .053) for startle reactivity. Because of a significant hBDNF^{Val66Met} genotype × CORT × sex interaction (F(2, 140) = 4.37, P = .014), we split our post hoc analysis according to sex. Amongst the male-only data (figure 2A), a hBDNF^{Val66Met} genotype × CORT interaction was once more observed (F(2,72) = 5.63, P = .005). hBDNF$^{Met/Met}$ male mice had significantly lower startle amplitudes than hBDNF$^{Val/Val}$ "wildtype" male mice at baseline (P < .05). Splitting the post-hoc analysis for an effect of chronic adolescent CORT exposure revealed that the hBDNF$^{Val/Val}$ group was the only genotype group to significantly respond to such (P < .01). Specifically, chronic adolescent CORT exposure was found to reduce the average startle amplitude of this group by 51.4% when compared to nontreated controls (figure 2A). In contrast, analysis of the female-only dataset revealed that chronic CORT slightly increased startle amplitudes irrespective of hBDNF^{Val66Met} genotype (F(1,68) = 4.06, P = .048; figure 2B). This result suggests that in a sex-dependent fashion, hBDNF^{Val66Met} genotype confers vulnerability to alterations in startle reactivity both at baseline and following exposure to chronic CORT in adolescence.

We next verified whether alterations in startle reactivity mediate the effects of CORT and hBDNF^{Val66Met} genotype on PPI—especially amongst our hBDNF$^{Val/Val}$ genotype group. To explore this hypothesis we ran an ANCOVA, with average startle amplitude included as our primary covariate. After controlling for average startle, the multivariate model retained the hBDNF^{Val66Met} × chronic CORT interaction observed in the overall analysis (F(2,139) = 4.30, P = .015), as well as at the 30 ms ISI (F(2,139) = 6.44, P = .002). These results confirm that, irrespective of any changes in average startle amplitude between groups, the alterations in PPI induced by the hBDNF^{Val66Met} genotype × CORT interaction represent changes that are specific to the sensorimotor gating circuitry.

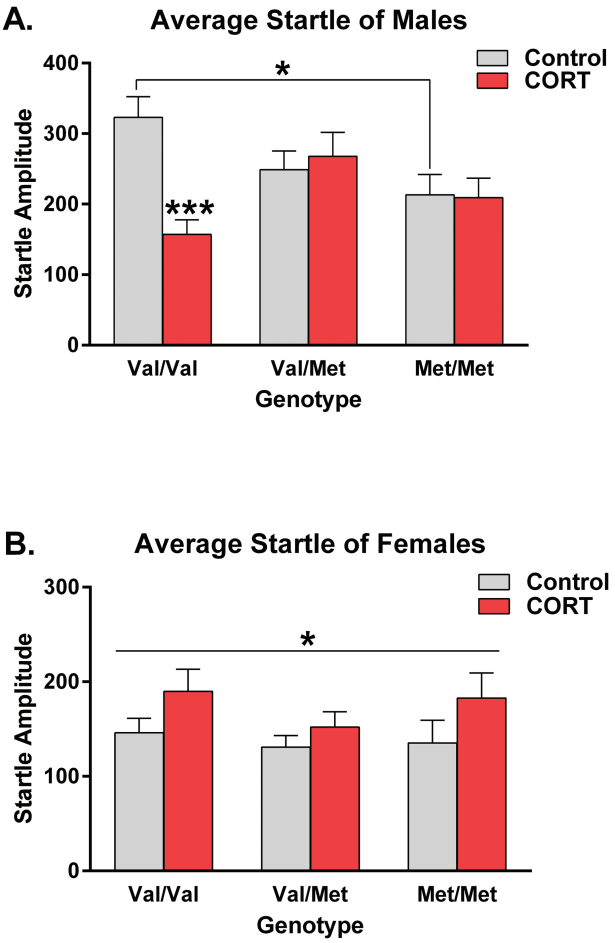

Fig. 2. hBDNF^{Val66Met} genotype, history of corticosterone (CORT) exposure and sex interact to determine startle reactivity. Analysis of average startle revealed a significant hBDNF^{Val66Met} genotype × adolescent CORT treatment × sex interaction. Splitting the analysis according to sex revealed that chronic adolescent CORT exposure reduced startle reactivity in hBDNF$^{Val/Val}$ male mice by 51.4% (A). In the female dataset (B), chronic CORT increased startle reactivity irrespective of hBDNF^{Val66Met} genotype. All data presented as mean ± SEM; *P < .05, ***P < .001, corrected for multiple comparisons. Per group, n = 11–15.

As expected, mice habituated to repeated startle stimulus presentations (F(3,2.73) = 23.68, P < .001). However, analysis with a Mixed-Model ANOVA, with startle block included as a repeated-measures factor, failed to produce evidence of an effect of hBDNF^{Val66Met} genotype either alone or via interaction with adolescent CORT treatment or sex (supplementary figure 2). This result implicates that the hBDNF^{Val66Met} genotype × CORT × sex interaction observed for average startle is a change in overall amplitude and is not a secondary effect of a genotype-mediated difference in habituation.

Discussion

The current study sought to determine the role of the BDNF^{Val66Met} polymorphism in sensorimotor gating, a translational endophenotype of schizophrenia. We report evidence of a distinct PPI deficit amongst hBDNF$^{Val/Met}$ mice that is sensitive to chronic CORT treatment, and that startle reactivity is regulated via complex interactions including susceptibility to glucocorticoid stress hormones and sex of the animals. These results have important implications for the clinical schizophrenia literature focused on BDNF, which has implicated that the BDNF^{66Met} allele may alter clinical features such as the positive symptoms of schizophrenia.[14]

Translational Merit and Model Validity

To study the effect of the Val66Met polymorphism on PPI, we utilized a novel transgenic mouse line that carries this gene variant, as well as an extended sequence which humanizes the BDNF peptide in vivo.[29] This pre-clinical, bottom-up, approach was used due to the documented effects of ethnicity, sampling bias, medication history and other environmental factors which may confound clinical investigations of BDNF functionality.[10,14] In support of the construct and predictive validity of Val66Met transgenic mice, both humans and mice show deficient hippocampus-dependent memory function,[6,7,30] extinction learning,[35] as well as altered anxiety-related behavior in mice[7] and traits in humans.[36] In this respect, BDNF^{Val66Met} knock-in mice arguably have face, construct, and predictive validity[10] making preclinical investigations that utilize this model inherently of translational value. On the other hand, chronic CORT exposure is an established model of chronic stress that targets glucocorticoid receptors independent of other factors involved in the stress response. A major advantage of this simulation of chronic stress is that it removes the need for animal handling, movement or novel environment exposure, which may impact later behavior. Low doses of chronic CORT (25–100 mg/L) in drinking water have been shown to induce a long-lasting anhedonia-like phenotype in mice that persists following treatment termination but can be rescued by antidepressant therapeutics.[37] Likewise, low-dose chronic CORT has also been shown to mimic the long-term decrease in hippocampal phosphorylation of CREB and BDNF's cognate receptor, TrkB, much like environment-based models of stress.[37] In this respect, chronic CORT as a simulation of chronic stress also has face, construct and predictive validity, and confers numerous methodological advantages in modeling psychiatric endophenotypes.

Defining a Novel hBDNF$^{Val/Met}$ PPI Phenotype

Adapting these genetic and stress models revealed a deficient PPI phenotype selectively amongst the hBDNF$^{Val/Met}$ genotype group at the commonly used 100 ms ISI in the total dataset, and a modulatory effect of glucocorticoid stress hormones on the PPI of this genotype at the 30 ms ISI. Collectively, these experimental results suggest that hBDNF$^{Val/Met}$ mice, in general, have poor information handling and relay efficiency of sensory inputs similar to what is observed in schizophrenia patients. Interestingly, no significant PPI deficit was found amongst hBDNF$^{Met/Met}$ mice in any of our PPI analyses. While the 66Met allele has been previously shown to exert a gene-dosage effect on hippocampus-dependent behavior,[7] the data presented here do not support that this gene-dosage effect occurs for the deficient sensorimotor gating endophenotype. Similar heterozygote-specific effects have been reported for the COMT Val158Met variant in the regulation of PPI amongst schizophrenia patients,[38] as well as several other gene variants in affective disorders[39] and for antidepressant response.[40] Importantly, there is evidence to suggest that the BDNF$^{Val/Met}$ heterozygote genotype may also elicit other biological effects that are gene dosage-independent. Specifically, the BDNF$^{Val/Met}$ genotype may alter gray matter volumes,[41] cortical morphology,[42] and brain development[43] to produce distinct phenotypic divergence from both BDNF$^{Val/Val}$ wildtype and BDNF$^{Met/Met}$ homozygotes. Our data build upon these reports and reinforce the need for clinical research to sample and stratify all 3 BDNF genotypes where possible.[10]

The mechanism underscoring this heterozygote-specific PPI phenotype, however, remains unclear. The underlying PPI circuitry includes a number of brainstem and pontine nuclei, which receive modulatory input from several forebrain regions.[44–46] While it is likely that there are partly independent pathways for PPI at short vs long ISI delays in mice, an exact definition of each respective pathway remains elusive.[45,47] In previous studies, we and others have observed differential effects of treatments depending on the ISI,[32,48–50] similar to the effect of the hBDNF$^{Val/Met}$ genotype and chronic CORT treatment in this study. A mechanism for this effect, given a lack of defined circuitry, remains difficult to assess. However, a biochemical clue is a recent report which shows that the 66Met-containing BDNF prodomain binds with altered affinity to the SorCS2 receptor[51]—which plays an important role in dopaminergic wiring[52]—to elicit biological effects.[53] Given this role of SorCS2 on the development of dopaminergic innervation[51,52] in the brain, and the established role of dopamine in schizophrenia[54] and on PPI,[55–57] it is possible that hBDNF$^{Val/Met}$ mice show altered catecholamine innervation of the PPI circuitry, rendering the PPI performance of this genotype group more vulnerable to environmental factors such as stress. The hBDNF$^{Met/Met}$ group may be spared from this phenotype due to intrinsic compensation given the greater degree of perturbed activity-dependent BDNF release.[6] Further research to explore this hypothesis is, however, required and will be the topic of further mechanistic investigation.

Role of the BDNF^{Val66Met} Variant on Startle Reactivity

Aside from PPI, an effect of both glucocorticoid stress hormone exposure and hBDNF^{Val66Met} genotype also emerged for startle reactivity via a modulatory effect of sex of the animals. Amongst females, no effect of genotype emerged but prior CORT treatment was found to slightly increase startle reactivity. Contrary to this, amongst male mice, a significant genotype × CORT treatment interaction was observed for startle amplitudes. It should be noted that this sex-specific divergence in phenotype—or rather lack of genotype or interaction effect amongst female mice—may be influenced by a relative floor effect in range of startle amplitudes, as female mice have much lower startle amplitudes than male mice. In any case, amongst male mice, it was observed that, following chronic CORT exposure, the startle reactivity of the hBDNF$^{Val/Val}$ group was markedly reduced. Given that our previous studies utilizing BDNF$^{+/-}$ heterozygous mice have failed to detect CORT-induced reductions in startle amongst wildtype littermates,[25] the effect of chronic CORT treatment on the startle amplitudes of hBDNF$^{Val/Val}$ mice suggests that this phenotype is likely specific to this genotype and transgenic line and should therefore be the subject of further investigation—especially given the relevance of startle reactivity to other psychiatric conditions such as post-traumatic stress disorder.

Conclusion

The genomic influence of the BDNF^{Val66Met} polymorphism in schizophrenia has been a controversial topic within the clinical literature. Our study provides support for reports that the BDNF^{66Met} allele may play a role in the pathophysiology of schizophrenia, specifically stress-sensitive clinical aspects such as positive-related symptomology[12,13] neurocognitive function[58,59] and age of onset,[60–63] and that some of this involvement is likely to occur as a result of differential susceptibility to glucocorticoid signaling during critical periods such as adolescence/young adulthood. Clinical studies assessing a genomic role of BDNF in schizophrenia should therefore consider stratifying their analyses for all 3 Val66Met genotypes, as well as for an effect of adversity, early life stress, trauma or related measures of stress exposure, which may play a role in phenotype determination.

Supplementary Material

Supplementary material is available at http://schizophreniabulletin.oxfordjournals.org.

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The authors have declared that there are no conflicts of interest in relation to the subject of this study.

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