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The Interaction of *BDNF* Val66Met, PTSD, and Child Abuse on Psychophysiological Reactivity and HPA Axis Function in a Sample of Gulf War Veterans

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Abstract

Introduction—While the BDNF Val66Met polymorphism has been linked to various psychological disorders, limited focus has been on its relationship to posttraumatic stress disorder (PTSD) and early traumas such as child abuse. Therefore, we assessed whether Val66Met was associated with fear potentiated psychophysiological response and HPA axis dysfunction and whether PTSD status or child abuse history moderated these outcomes in a sample of Veterans.

Methods—226 and 173 participants engaged in a fear potentiated acoustic startle paradigm and a dexamethasone suppression test (DST) respectively. Fear conditions included no, ambiguous, and high threat conditions. Psychophysiological response measures included electromyogram (EMG), skin conductance response (SCR), and heart rate. The Clinician Administered PTSD Scale (CAPS) and the Trauma History Questionnaire (THQ) were used to assess PTSD status and child abuse history respectively.

Results: Met allele carriers exhibited greater SCR magnitudes in the no and ambiguous threat conditions ($p<0.01$ and $p<0.05$ respectively). Met carriers with PTSD exhibited greater physiological response magnitudes in the ambiguous (SCR, $p<0.001$) and high threat conditions (SCR and heart rate, both p 0.005). Met carrier survivors of child abuse exhibited blunted heart rate magnitudes in the high threat condition ($p<0.01$). Met allele carries with PTSD also exhibited greater percent cortisol suppression ($p<0.005$).

Limitations—Limitations included small sample size and the cross-sectional nature of the data.

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Conclusions—The Val66met may impact PTSD susceptibility differentially via enhanced threat sensitivity and HPA axis dysregulation. Child abuse may moderate Val66Met's impact on threat reactivity. Future research should explore how neuronal mechanisms might mediate this risk.

Keywords

Val66Met; PTSD; child abuse; dexamethasone suppression test; psychophysiological response

1. Introduction

Brain derived neurotropic factor (BDNF), is expressed widely throughout the central and peripheral nervous systems and is involved in neurogenesis, cell survival, differentiation, and synapse formation (Martinowich and Lu, 2008). BDNF is concentrated in neural structures critical to learning and memory (e.g. hippocampus, cerebellum; Conner et al., 1997). Earlier research has focused on whether BDNF levels (e.g. serum or plasma) were associated with trauma-related disorders such as posttraumatic stress disorder (PTSD). While some findings have been contradictory, research suggests a relationship between BDNF levels and PTSD such that people with PTSD show lower levels of BDNF (see Suliman et al., 2013 for a review). Nonetheless, it remains unclear how BDNF impacts brain function after trauma exposure. Research linking genes that modulate BDNF, such as *BDNF* Val66Met, to markers associated with posttraumatic stress symptoms have attempted to resolve some of these inconsistencies (Rakofsky et al., 2012).

Val66Met is a common single nucleotide polymorphism (SNP) that results in a substitution of methionine (Met) for valine (Val) at codon 66 in the pro-domain of the human BDNF protein (Egan et al., 2003). Research has shown that this methionine substitution results in impaired BDNF intercellular packaging and secretion regulation, which may impact the stress response, possibly through hippocampal (Egan et al., 2003) and hypothalamic dysregulation (Tapia-Arancibia et al., 2004). Given the common occurrence of this SNP, investigations have attempted to link Val66Met to PTSD and other stress-related disorders (Frielingsdorf et al., 2010). Evidence suggests the Met allele may be linked to neuroticism (a possible risk factor for PTSD), anxiety, and depression (Engelhard and van den Hout, 2007; Gatt et al., 2009). Met allele carriers also appear to be at a greater risk for PTSD, possibly due to BDNF overexpression (Zhang et al., 2014). In fear conditioning paradigms, met allele carriers exhibit increased activity in neural structures (e.g. the insula, amygdala, and hippocampus), which are in turn responsible for the regulation of the hypervigilance and heightened startle symptoms associated with PTSD (Lonsdorf et al., 2014).

Recent studies exploring the Val66Met link to PTSD have focused on the fear-potentiated startle response, the largely unconscious defensive psychophysiological response to sudden or threatening stimuli (Ramirez-Moreno and Sejnowski, 2012) and hypothalamic-pituitary-adrenal (HPA) axis reactivity. However, results have been equivocal. For example, one novel investigation using a virtual reality based fear conditioning paradigm revealed that Met allele carriers had difficulty differentiating between fear and safety cues and had stronger startle responses to novel stimuli compared to Val-Val carriers (Mühlberger et al., 2014). Conversely, a recent study that used an acoustic startle paradigm found homozygous Val-Val

participants had greater startle magnitudes compared to Met allele carriers in a sample of healthy adults and children (Armbruster et al., 2016). Similarly, one group found Met allele carriers to have an attenuated cortisol response to social stress (Alexander et al., 2010) where others found the opposite (Armbruster *et al*, 2016).

Early trauma such as child abuse impacts brain development across the life span (Dannlowski et al., 2012) and is a strong risk factor for PTSD (Duncan et al., 1996). Key structures in the limbic system that are rich with BDNF continue to develop throughout childhood (Giedd et al., 1996). Animal models suggest neural BDNF expression is adversely affected by early environmental insults (Ognibene et al., 2008). Thus, lower BDNF levels as a result of early trauma and genetic predisposition may increase the risk of adverse psychological outcomes in humans. In a sample of depressed participants, Met allele carriers who were exposed to child abuse had lower BDNF serum levels compared to Val-Val carriers. In contrast Met allele carriers without child abuse exposure had higher BDNF levels compared to Val-Val counterparts (Elzinga et al., 2011). While evidence suggests adult survivors of child abuse exhibit greater startle response magnitudes compared to individuals who were not exposed to child abuse (Jovanovic et al., 2009), it remains unclear how Val66Met might impact startle response within the context of early trauma. Thus, exploring a possible Val66Met x child abuse interaction on psychophysiological response and HPA axis reactivity would move us closer to understanding the etiology of PTSD as well as differential posttraumatic stress responses in trauma-exposed individuals.

We investigated whether the *BDNF* Val66Met polymorphism is associated with psychophysiological reactions to startling sounds over successive trials across three different threat conditions in a sample of Veterans. We also assessed whether this SNP was associated with HPA axis reactivity to a dexamethasone suppression test (DST). We hypothesized Met allele carriers would exhibit greater psychophysiological response magnitudes compared to homozygous Val-Val carriers. We also hypothesized Met allele carriers would exhibit greater dexamethasone suppression compared to Val-Val allele carriers. In addition, we examined whether Val66Met interacts with either child abuse or PTSD diagnosis on psychophysiological response and cortisol levels after DST.

2. Materials and methods

2.1. Participants

We conducted secondary data analyses on Veterans from a cross-sectional study of the effects of Gulf War deployment on the brain. Gulf War Veterans were recruited between 2002 and 2007 through contacts with physicians at VA clinics in Northern California using methods described elsewhere (Apfel et al., 2011). Inclusion criteria for the broader study was being a US veteran of the First Persian Gulf War; exclusion criteria included severe physical impairment or medical illness, current or lifetime history of psychosis or of suicidal or homicidal ideation, and a history of neurological or systemic illness affecting central nervous system functioning (for a complete list of exclusion criteria please see Apfel et al., 2011). The University of California San Francisco and Veterans Administration Committees on Human Research and the Department of Defense Human Subjects Research Review Board approved all research. This research was carried out in accordance with The Code of

Ethics of the World Medical Association and all participants provided consent to be included in this study. Of the 369 Veterans from the original sample, 226 of them engaged in the psychophysiological response task and provided a blood plasma sample from which we extracted and analyzed DNA. 149 Veterans were Val-Val carriers, 67 were Val-Met carriers, and 10 were homozygous Met-Met carriers in the overall sample. A subsample of 173 participants provided blood plasma samples and engaged in the DST. Of those, 119 were Val-Val carriers, 46 were Val-Met carriers, and 8 were Met-Met carriers. Both the overall samples and the DST subsample conformed to the Hardy-Weinberg equilibrium ($\chi^2 = 0.90$; $p = 0.63$ and $\chi^2 = 1.07$; $p = 0.26$ respectively) and there were no significant differences between the minor allele frequencies of the three most representative races in our sample regarding this particular SNP ($\chi^2 = 0.26$; $p = 0.61$; National Institutes of Health HapMap Project, Bethesda MD).

Demographic variables were obtained by self-report as prior literature has linked them to differential traumatic stress response (Neylan et al., 2005). Current PTSD symptoms were evaluated by a Ph.D. level clinical interviewer using the Clinician Administered PTSD Scale (CAPS; Blake et al., 1995). Criterion A was used to assess whether participants had experienced any traumatic event in adulthood (DSM-IV-TR 4th ed., text rev.; APA, 2000). Participants were diagnosed with PTSD based upon frequency and severity of their CAPS scores (e.g. the "1, 2" rule) and the DSM-IV-TR algorithm. Of the ninety-four participants who were exposed to adult trauma, seventy-four endorsed military-related trauma exposure. Twenty participants reported non-military/civilian trauma exposure and of those, only two had PTSD and were retained in subsequent analyses. All subjects with PTSD had combat-related trauma exposure. Twenty-six participants reported paramilitary adult trauma exposure but none of these individuals had PTSD. Exposure to child abuse occurring prior to the age of 16 years old was assessed using the last six items of the Trauma History Questionnaire (Green, 1996).

2.2. Psychophysiological Assessment

Three indices of psychophysiological response were collected by trained technicians, who were blind to participants' psychometric status. The participant's left eye blink electromyogram (EMG) activity, skin conductance response (SCR) level, and heart rate electrocardiograph EKG were assessed during a two-minute baseline period. Participants were fitted with headphones and told that they would hear potentially startling sounds. They were asked to focus their eyes on a monitor in front of them. A Coulbourn Instruments Lablinc V Modular System binaurally presented 106-dB(A), 40 millisecond white noise bursts with 0-millisecond rise and fall times separated by inter-trial intervals of between 30 and 50 seconds in each threat condition. In the "no threat" condition, participants were instructed that they would not be shocked until later in the study. They were then exposed to ten startling sounds. Only their last five responses were retained. In the "ambiguous threat" condition, participants were fitted with a Coulbourn Instruments Transcutaneous Aversive Finger Stimulator but were told that they would not be shocked. Five additional startling sounds were presented. In the "high threat" condition, Veterans wore the finger stimulator and were told that shocks were imminent. Then five additional startling sounds were presented followed by an annoying (but not painful) 2.5 mA shock. Each condition lasted

approximately 4 minutes and was separated by about 1 minute. The medium and high threat conditions were counterbalanced to minimize carry-over effects between these conditions. All physiological signals were sampled at 2 Hz during the resting baseline and at 1000 Hz during the acoustic presentations, digitized, and stored for off-line analysis. EMG, measured in microvolts was captured using three, 4-mm (sensor diameter) In Vivo Metrics Ag/AgCl surface electrodes filled with electrolyte paste according to published specifications published elsewhere (Blumenthal et al., 2005). SCR was measured in microsiemens by sending a constant 0.5 V through 9-mm (sensor diameter) InVivo Metrics Ag/AgCl electrodes filled with isotonic paste and placed on the hypothenar surface of the medial phalanges of the middle and index fingers of the non-dominant hand. heart rate was measured in beats per minute and recorded via electrodes attached in a Type-I EKG configuration. Human Startle Software (Coulbourn Instruments, Allentown, PA) automatically calculated mean psychophysiology at baseline and during the one second prior to each stimulus onset. It also calculated the peak post-stimulus levels within 21 to 200 milliseconds for eyeblink EMG and within 1 to 4 seconds for SCR and EKG. Data were inspected for artifact and rejected accordingly. No minimum response threshold was designated for any physiological measure. Each measurement of psychophysiological response was recorded at five trials prior to exposure to the startle stimulus and five time points after the startle stimulus for each measurement of psychophysiological response within each threat condition. Participants needed at least four (of five) valid responses with a trial for all three physiological measures to be included in the study.

2.3. Measurement of Salivary Cortisol and DST

Participants collected saliva using methods used in previous research (Pruessner et al., 1997). Four saliva samples were collected at 1, 30, 45 and 60-minute increments with sample collection occurring upon the subject's awakening on day one of the procedure. Patients were given instructions to take the 0.5 mg of dexamethasone 15 hours after awakening on day 1. A second set of saliva samples were collected upon the subject's awakening on day 2 using the same incremental pattern described for day one. We relied on the subjects to inform us about the time of awakening and samples were time-stamped on both collection days to ensure accuracy. All participants included in this study were compliant with taking the dexamethasone. Samples were collected using Salivettes (Sarstedt, Inc., Newton, NC), and deep-frozen (−78°C) until assay. Because dexamethasone bioavailability may affect cortisol levels and to check compliance with the protocol, we included dexamethasone levels to assess covariance in our analysis of cortisol levels (O'Sullivan et al., 1997). Salivary cortisol and dexamethasone were assayed and measured by Salimetrics Saliva Laboratory Services, L.L.C.

2.4. Genotyping

Genomic DNA was extracted using the Promega Wizard Genomic DNA Purification Kit (Promega Biosystems, Sunnyvale, CA, USA). Samples were genotyped at the University of California, San Francisco Genomics Core Facility, using the ABI 3730×l (Applied Biosystems Inc., Foster City, CA, USA). Sequencer DNA Sequence Analysis Software (Gene Codes Corporation, Ann Arbor, MI) was used to analyze the Val66Met alleles.

2.5. Data Analyses

Based upon previous research concerning Val66Met (Armbruster *et al*, 2016; Marusak *et al*, 2016; Mühlberger *et al*, 2014), a dominant (versus recessive) genetic model was used where we combined both Val-Met and Met-Met carriers and compared them to Val-Val carriers in all analyses of this study. Descriptive statistics and initial pairwise comparisons were generated using pairwise t and χ^2 tests. EMG, SCR, and heart rate response outcome were assessed by using within trial square root post- minus pre-psychophysiological responses. HPA axis regulation was assessed by using the log transformed percentage of suppression of cortisol (for a review on percentage cortisol suppression computation, see Pruessner et al., 2003). A series of repeated measures linear mixed models were used to assess the main effects of Val66Met on EMG, SCR, and heart rate in each (no, ambiguous, high) threat condition. Val66Met x PTSD x diagnosis s trial and Val66Met x child abuse x trial interaction terms were also included in each of these models to assess whether PTSD status or child abuse moderated any potential effects of Val66Met on EMG, SCR, and/or heart rate in no, ambiguous, and high threat conditions. Age, race (white vs. non-white), exposure to adult trauma, and sex (female vs. male) were included as covariates for all models. The order that participants were exposed to the ambiguous and high threat conditions was included as a covariate for mixed models assessing val66met relationship on psychophysiological response in ambiguous and high threat conditions. Linear regression models were used to assess the Val66Met x child abuse and Val66Met x PTSD status relationship on cortisol suppression while controlling for the covariates described above. Due to the bio-availably of dexamethasone being a potential confounding factor, percent salivary dexamethasone level was also included in each of the multiple linear regression models (Neylan *et al*, 2005). Based on recommendations by Keller (2014), we also assessed whether any pairwise Val66Met risk allele x covariate and child abuse x covariate relationships existed. Then, risk allele x covariate and/or child abuse x covariate interaction terms were created based upon the significance of their pairwise relationships and included in the subsequent repeated mixed and linear regression models. Cohen's f^2 and R^2 were used to assess proportion of model variance explained in repeated measures mixed models and linear regressions respectively (Draper and Smith, 2014; Selya et al., 2012). Post hoc marginal analyses were used to assess the significance of the interaction terms in both the repeated measures and linear regression models. Stata 15.0 was used to conduct all statistical analyses (StataCorp LP, 2013 College Station, TX).

3. Results

3.1. Demographics

Demographics are described in Table 1. Our sample was predominantly White and male with a mean age of 44. Approximately 60% of participants had been exposed to traumatic events during adulthood, 31% of participants had experienced child abuse, and 26% of them met criteria for PTSD at the time of the study (see Table 1.). A significant child abuse x sex interaction was observed ($\chi^2 = 16.78$; $p = 0.002$) was uncovered and female Veterans in the sample were at greater odds of being exposed to child abuse compared to their male Veteran counterparts ($OR = 3.59$; $p < 0.001$). Thus, a child abuse x sex covariate was included in subsequent analyses (Keller, 2014).

3.2. No Threat Condition

There were significant model effects for SCR ($Wald \chi^2 = 67.68$; $p < 0.001$) but not EMG or heart rate. Post hoc marginal analyses revealed a significant main effect x trial interaction for the SCR model where participants who were Met allele carriers exhibited greater mean within-trial SCR magnitudes compared to non-Met allele carriers ($\chi^2 = 13.71$; $f^2 = 0.16$; $p = 0.008$; see Figure 1a.). A marginally significant Val66Met x PTSD x trial interaction was also observed where Met allele carriers diagnosed with PTSD had greater SCR mean magnitudes but this was not significant within trials ($\chi^2 = 8.80$; $f^2 = 0.07$; $p = 0.066$; see Figure 2a.).

3.3. Ambiguous Threat Condition

There were significant model effects for SCR and heart rate ($Wald \chi^2 = 60.75$; $p < 0.002$) but not EMG and heart rate. Post hoc marginal analyses revealed a significant main effect x trial interaction where Met allele carriers exhibited greater mean within-trial SCR magnitudes compared to non-Met allele carriers ($\chi^2 = 8.47$; $f^2 = 0.14$; $p = 0.015$; see Figure 1b.). There was no relationship observed between Val66Met on EMG or heart rate as a main effect (see Table 2.). Post hoc marginal analyses also uncovered a significant Val66Met x PTSD x trial interaction where Met carriers diagnosed with PTSD had greater mean within-trial SCR magnitudes compared to other participants ($\chi^2 = 10.88$; $f^2 = 0.18$; $p < 0.001$; see Figure 2b.).

3.4. High Threat Condition

There were significant model effects for SCR and heart rate ($Wald \chi^2 = 65.62$; $p < 0.001$ and $Wald \chi^2 = 53.34$; $p < 0.008$ respectively) but not EMG. Post hoc marginal analyses revealed a significant Val66Met x PTSD x trial interaction on both SCR and heart rate where Met allele carriers diagnosed with PTSD exhibited greater mean within-trial SCR and heart rate magnitudes compared to other participants ($\chi^2 = 11.86$; $f^2 = 0.14$; $p = 0.005$ and $\chi^2 = 9.82$; $f^2 = 0.17$; $p = 0.003$ respectively, see Figures 2c. and 2d. respectively). Post hoc marginal analyses also uncovered a significant Val66Met x child abuse x trial interaction on heart rate magnitude where Met allele carriers with a history of child abuse had lower mean within-trial heart rate magnitudes compared to others in the sample ($\chi^2 = 9.71$; $f^2 = 0.16$; $p = 0.008$ see Figures 3.). No significant main effect by trial or other interactions were observed in any threat condition.

3.5. Dexamethasone Suppression Test

Regression models that included Val66Met as a main effect were not significant in predicting cortisol suppression ($F_{6,163} = 0.86$; $p = 0.528$; $R^2 = 0.01$) and the Met allele was not significant in these models ($b = 0.90$; $p = 0.863$); PTSD was not significant in predicating suppression ($b = -9.07$; $p = 0.089$). However, the model predicting the Val66Met x PTSD interaction was significant ($F_{6,163} = 4.15$; $p = 0.008$; $R^2 = 0.20$); Met allele carriers who were diagnosed with PTSD exhibited significantly greater cortisol suppression compared to others in the sample ($b = 36.88$; $p = 0.008$). Post hoc analyses confirmed that Met allele carriers with a PTSD diagnosis exhibited greater percent cortisol suppression

($F_{6,163} = 9.64$; $p = 0.002$; see Figure 4.). Models predicting child abuse as a main effect were not significant and no Val66Met x child abuse interaction was observed on cortisol.

4. Discussion

Met allele carriers had *greater* mean within-trial SCR magnitudes compared to their Val-Val counterparts in the no and ambiguous threat conditions of a fear-potentiated psychophysiological response paradigm. We also found that Met carriers with PTSD had greater mean within-trial SCR magnitudes in the ambiguous threat condition, greater mean within-trial SCR and heart rate magnitudes in the high threat condition, and greater cortisol suppression after the DST. These findings extend previous research that suggests the Val66Met SNP may be associated with psychological impairment and HPA axis dysregulation in clinical populations (Elzinga et al., 2011; Schüle et al., 2006). Additionally, we observed a possible gene by environment interaction in the high threat condition whereby Met allele carriers who endorsed a history of child abuse exhibited *blunted* heart rate patterns across trials in the high threat condition compared to other participants.

The pattern of psychophysiological response magnitudes that Met allele carriers displayed in the no and ambiguous threat conditions and Met allele carriers with PTSD displayed in the no (albeit marginally) and ambiguous conditions may be explained by a deficit in habituation. Habituation is the reduction of a response to a stimulus over several presentations due to the organism learning that the stimulus is biologically and/or behaviorally irrelevant (Rankin et al., 2009). Within this context, habituation should reduce the psychophysiological response to the acoustic stimulus over time as the stimulus loses its threat value. The Met allele carriers in our sample exhibited greater psychophysiological magnitudes and delayed psychophysiological response inhibition, which implies sensitization rather than habituation. Animal models suggest sensitization to a stressor may be associated with neurobehavioral changes linked to PTSD (Servatius et al., 1995) and earlier studies using various populations have shown individuals diagnosed with PTSD exhibit exaggerated physiologic response patterns compared to those without PTSD (Orr et al., 1995; Pole et al., 2003; Shalev et al., 1992). Furthermore, a deficit in habituation may be linked to abnormalities in neuroanatomical structures such as the amygdala, which are key to non-associative learning processes (e.g. habituation and sensitization) and rich with BDNF (Conner et al., 1997; Rakofsky et al., 2012). Previous findings suggest a delayed decrement in psychophysiological response may represent an inherent risk factor for PTSD rather than an acquired response pattern gained after trauma exposure (Orr et al., 1995). Moreover, twin studies indicate that genetics may have considerable influence over individuals' habituation patterns (Kotchoubei, 1987; Lykken et al., 1988). Thus, our results suggest Met allele carriers may have a decrement in habituation, which could increase PTSD susceptibility and lead to less than favorable outcomes after trauma exposure. This theory is substantiated by a finding that Met allele patients respond poorly to exposure therapy compared to their Val-Val counterparts (Felmingham et al., 2013).

On the other hand, met allele carriers with PTSD exhibited greater physiological response magnitudes with elevated arousal across the trials with no evidence of habituation in the high threat condition. This indicates, met allele carriers with PTSD are more sensitive to the

threat of shock associated with the high threat condition compared to other participants and may suggest greater threat sensitivity in general. While the current study is unequipped to explore this, it is further possible the observed arousal met carriers with PTSD exhibited in the high threat condition may be linked to disruptions in underlying neurocircuitry. Studies have implicated the met allele in anxiety morbidity in clinical samples (Moreira et al., 2015; Frustaci et al., 2008) and we have previously suggested neurobiologically mediated arousal and threat sensitivity in anxious individuals is associated with a host of adverse outcomes (O'Donovan et al., 2013). While few studies have explored the possible relationship between val66met and neuroanatomy/neurofunctioning on arousal, one found that the met allele is associated with less recruitment of the ventro-medial prefrontal cortex and greater recruitment of the amygdala during extinction learning in both human and animal models (Soliman et al., 2010). Thus, perturbations in cortical and subcortical structures may similarly play a role in mediating the val66met – threat sensitivity relationship, particularly in the context of trauma exposure (Britton et al., 2010).

We also uncovered a Val66Met x child abuse interaction where Met allele carriers who were also survivors of child abuse evidenced *blunted* within-trial heart rate response patterns compared to their study counterparts in the high threat condition. The fact that we only observed this Val66Met x child abuse interaction in the high threat condition is intriguing and may suggest context contingency; that is, the response pattern may have been elicited by the expectation of adverse consequences specific to the high threat condition. Although further investigation is needed, it is plausible that where the no/ambiguous threat conditions may be assessing habituation to the startle stimulus, the high threat condition may be assessing threat reactivity. Specifically, the blunted psychophysiological reactivity observed in Met allele survivors of child abuse may be suggestive of a broader maladaptive conditioning process associated with enduring adverse and inescapable consequences during conditions of perceived threat. Prior research has shown that adult survivors of child abuse tend to exhibit blunted startle responses when exposed to an acoustic startle stimulus (Medina et al., 2001). Evidence also suggests that children and adolescents who were victims of maltreatment exhibit blunted SCR responses to both fear and safety cues during fear conditioning, which also appear to be associated with reduced hippocampal and amygdala volume (McLaughlin et al., 2015). While root cause of this observed Val66Met x child abuse interaction remains unclear, it is possible that Met carriers may be particularly sensitive to the adverse neurological effects of child abuse (Mesa-Gresa and Moya-Albiol, 2011). Recent findings suggest that early trauma may interact with the Met allele to reduce hippocampal volume in children and this may have consequences across the lifespan (Frodl et al., 2014; Marusak et al., 2016). Thus, the Met allele may impart increased neuronal sensitivity to early environmental insults such as child abuse and Met allele carriers who are exposed to child abuse may exhibit a blunted threat response pattern, which could be associated with adverse outcomes such as poor threat/safety discrimination and an increased tolerance for threatening/hostile situations.

A Val66Met x PTSD interaction on response to DST was also observed where Met allele carriers who were diagnosed with PTSD exhibited significantly greater levels of cortisol suppression compared to other participants. To our knowledge, this is the first demonstration of a Val66Met x PTSD interaction on HPA axis dysregulation in a group of Veterans. These

findings are in line with recent evidence suggesting that the Val66Met SNP Met allele may play a role in PTSD susceptibility via altered BDNF levels (Zhang et al., 2014; Zhang et al., 2016), which may, in turn, impact neural structures leading to HPA axis dysregulation (Marusak et al., 2016). Animal research indicates BDNF is widely expressed in hypothalamic neurons (Tapia-Arancibia et al., 2004) and that BDNF may be a stress-responsive intercellular messenger as externally administered BDNF is associated with increased HPA axis response after extended stress (Givalois et al., 2004). Therefore, HPA axis dysregulation found in our study may be due, in part, to inhibited activity-induced BDNF release in the HPA axis, possibly modulated by the Met allele and the exposure to trauma (Egan *et al*, 2003; Matsuda *et al*, 2009). On the contrary, we found no evidence that a history of child abuse impacted separately or interacted with Val66Met to influence cortisol suppression, which is inconsistent with previous findings of child abuse being linked to HPA axis dysregulation (Heim et al., 2008). This may stem from child abuse having a differential physiological impact on this Veteran sample compared other populations where the child abuse-HPA axis relationship has been investigated.

Our results extend Mühlberger *et al*, 's (2014) findings that the Val66Met SNP Met allele is associated poor safety cue discrimination and the over-generalization of threat cues. However, our findings are in contrast with Armbruster *et al*, 's (2016) finding that the Val-Val allele, rather than the Met allele is associated with elevated startle response. One major difference between our study and the latter is that we employed a Veteran sample with relatively high PTSD risk, whereas Armbruster *et al*'s were comprised of a healthy community sample of children and adults. Our findings suggest the inclusion of both clinical and community samples while controlling for early trauma may be important in further understanding the Val66Met-PTSD relationship.

There are several limitations of note. First, our sample size was comparatively small for a gene by environment study. Although other recent studies that have published on Val66Met with similar or smaller sample sizes (Frodl *et al*, 2014; Marusak *et al*, 2016), other studies with larger sample sizes are needed to assess the reliability of our findings. This is further substantiated by the fact that our effect sizes were relatively modest. Secondly, our focus was limited to Val66Met and outcomes associated with PTSD. Future studies should investigate whether Val66Met might interact with other genes and child abuse and if they are associated with other adverse outcomes including but not limited to PTSD. Thirdly, since this study is cross-sectional and we did not have access to a replication sample, we can make no causal inferences from the present findings. Additionally, our sample was comprised of mostly while male Veterans, which further limits the generalizability of our findings outside of this population. We were also not able to explore whether Val66Met genotype was associated with differing BDNF levels in this sample, which may have been particularly informative. Finally, although we controlled for percent dexamethasone salivary cortisol, we have no way of knowing whether participants took the dexamethasone dose exactly 15 hours after awaking. Thus, it is possible that our results could have been associated with the variability in terms of the time that the dexamethasone was taken.

In conclusion, our results suggest that the *BDNF* Val66Met SNP may increase PTSD susceptibility and be associated with negative outcome after trauma exposure due to deficits

in psychophysiological habituation and HPA axis functioning along with increased threat sensitivity. Our results also indicate Met allele carrier survivors of child abuse appear to exhibit blunted stress responses during situations of perceived threat. Based upon the interpretation of our results, child abuse may adversely impact neurological structures in Met allele carriers, which may lead to adverse outcomes such as a decreased sensitivity to threatening stimuli/situations and poor threat/safety discrimination. However, our findings warrant further investigation to explore the intermediary neuronal mechanisms associated with these observed interactions along with how other forms of learning might be impacted by Val66Met.

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Highlights

- Val66Met was associated with greater SCR in no and ambiguous threat conditions.

- Val66Met interacted with PTSD on SCR in ambiguous and high threat conditions.

- Val66Met interacted with PTSD on HR in the high threat condition.

- Val66Met interacted with child abuse on HR in the high threat condition.

- Val66Met interacted with PTSD on percent cortisol suppression.
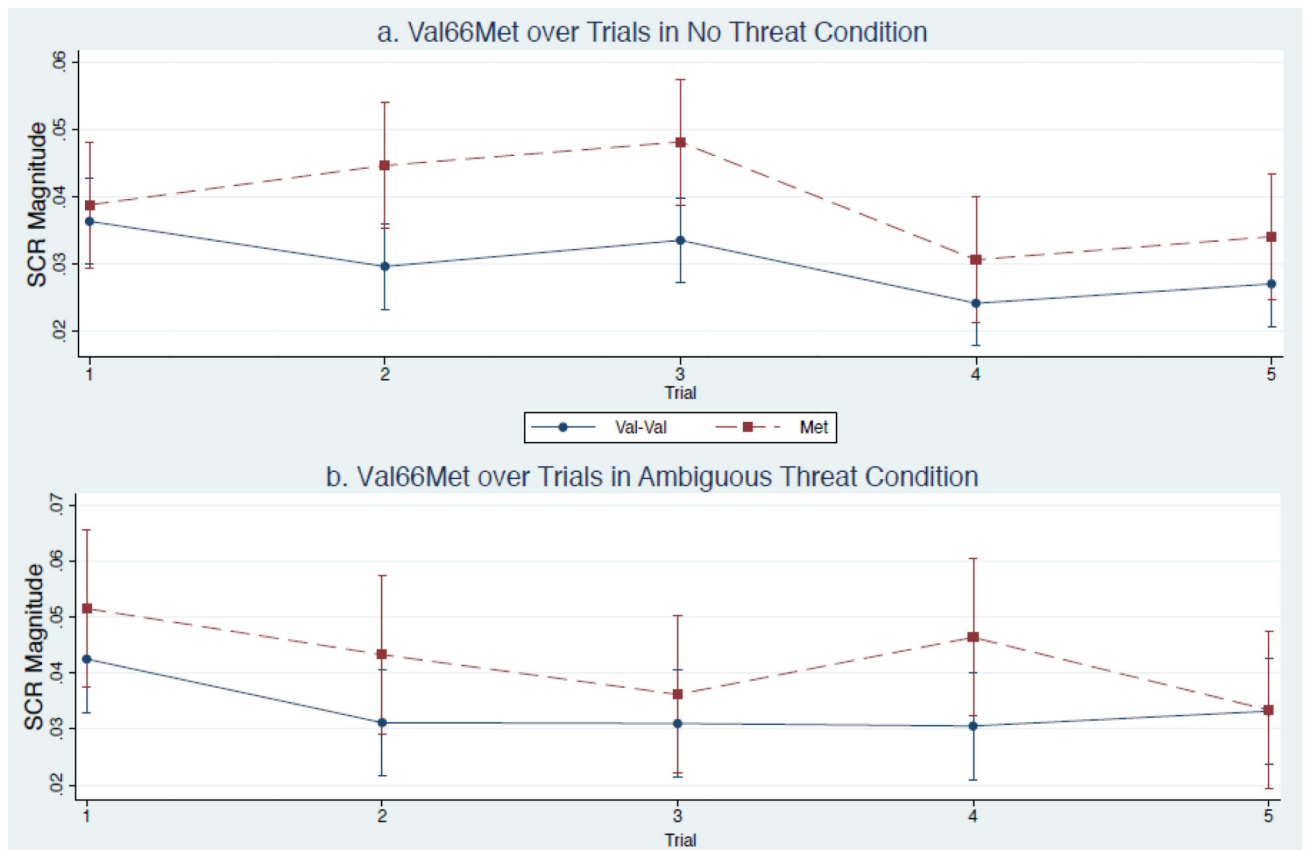
Figure 1.
n = 226. Note : SCR magnitude is given in μV;m odel covariates included age, race, years of education, adult trauma exposure, high threat condition exposure order (in ambiguous threat), and a female x child abuse interaction term.
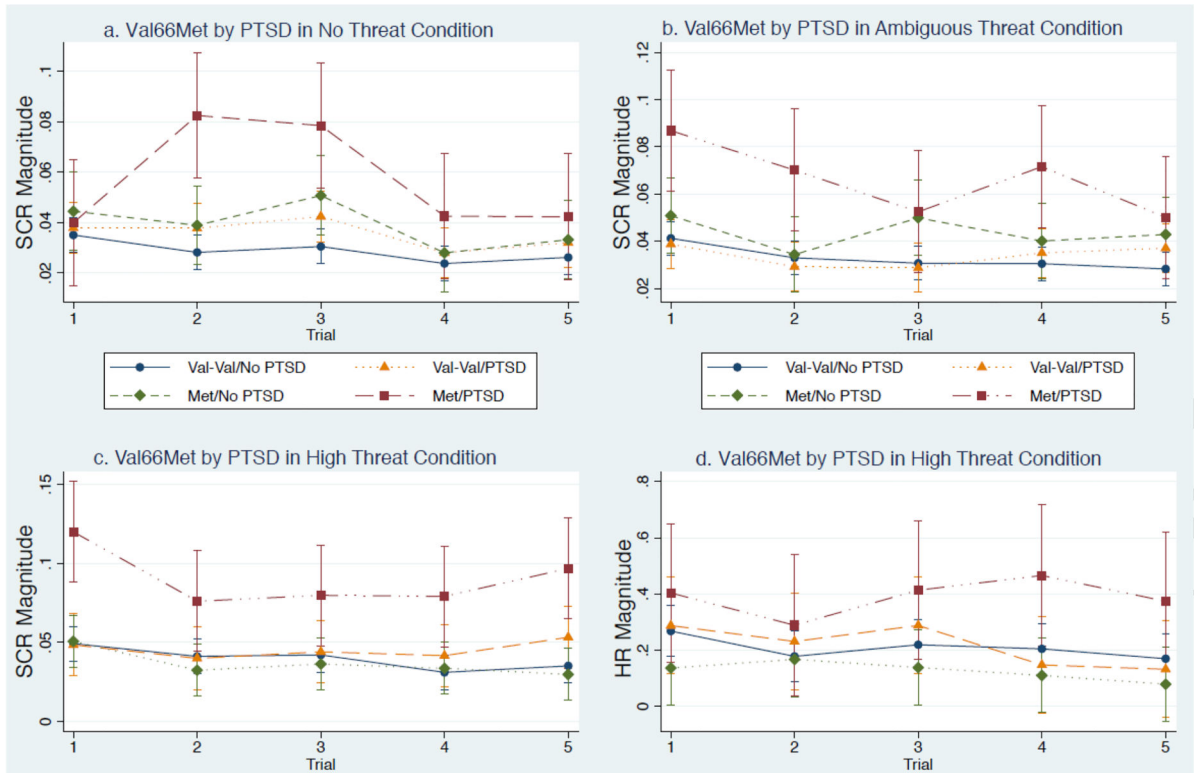
Figure 2.
n = 226. Note : SCR and HR magnitude are given in μV and BPM respectively; these models included the following covariates: age, race, years of education, the order that participants were exposed to the high threat condition, and a female x child abuse interaction term.
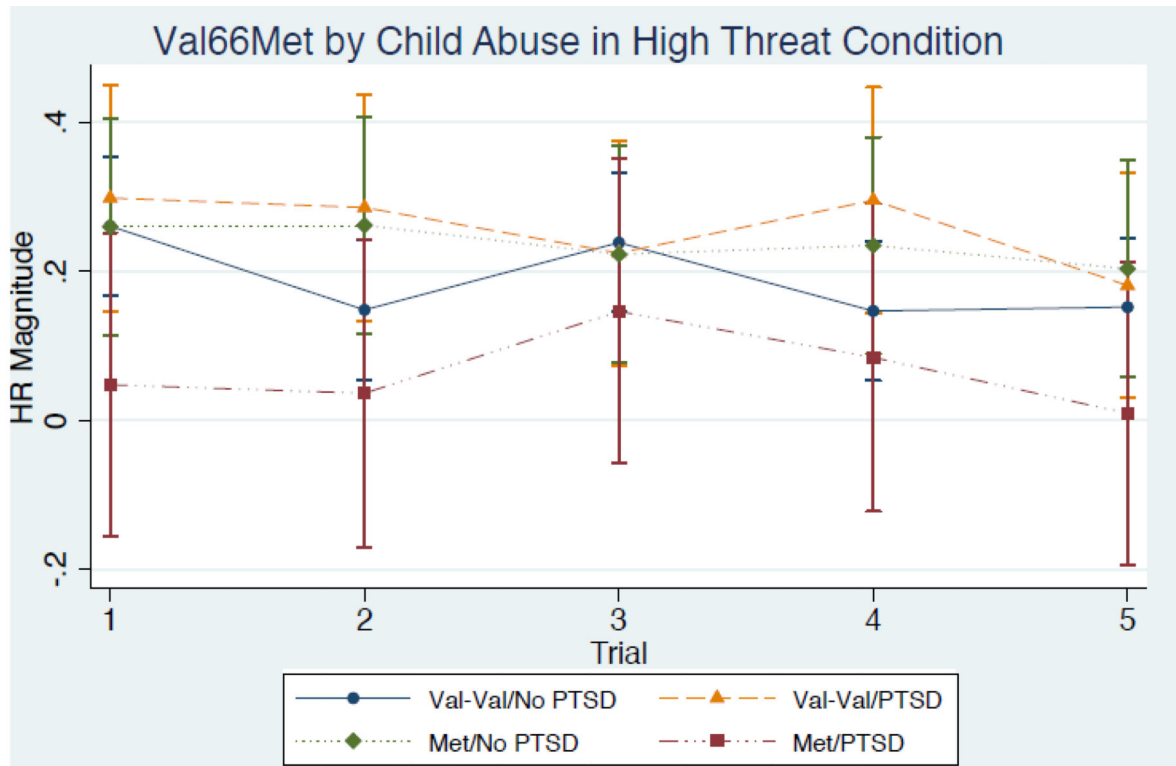
Figure 3.
n = 226; Note : HR magnitude are given in BPM; model covariates included age, race, years of education, adult trauma exposure, high threat condition exposure order, and a female × child abuse interaction term.
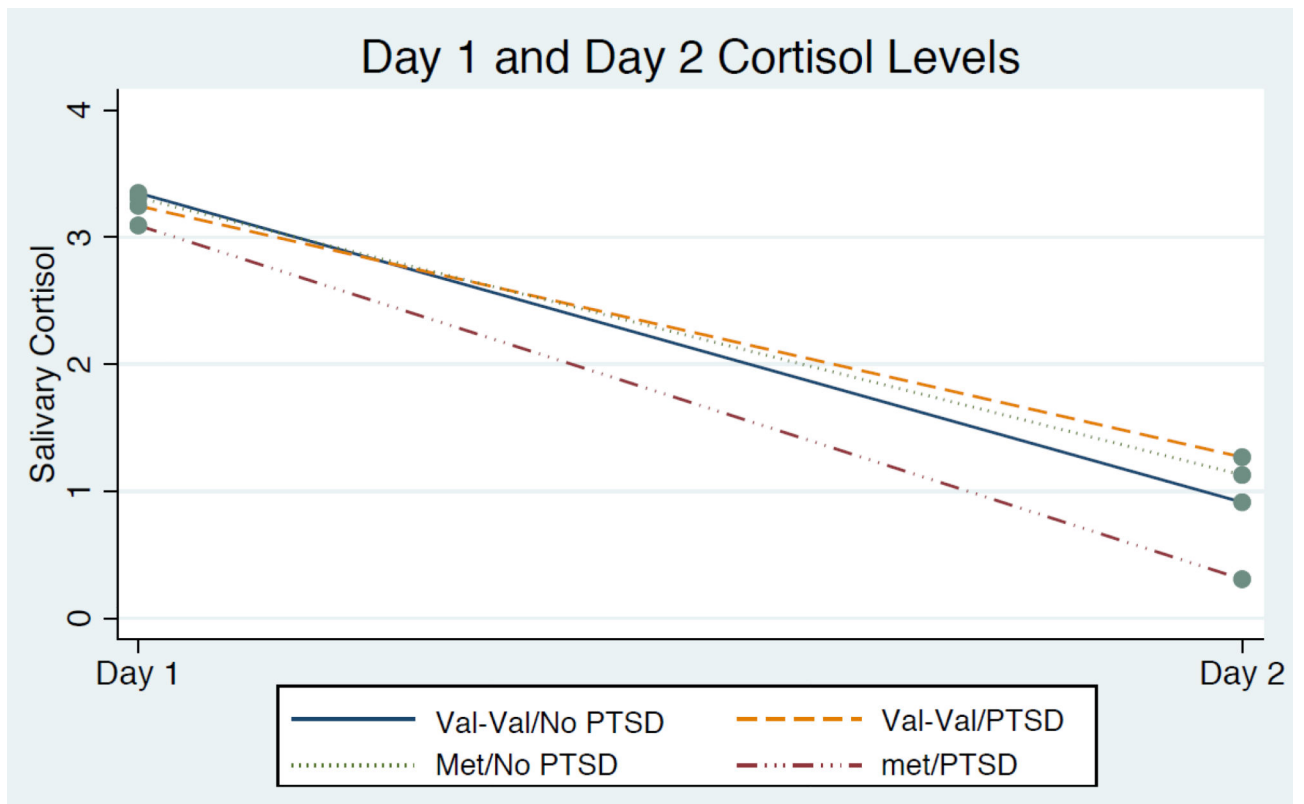
Figure 4.
n = 173; Note: Salivary cortisol is given in [log(μg/dL) × 100]; circles represent mean salivary cortisol levels; this model included the following covariates: age, race, years of education, adult trauma exposure, % dexamethasone suppression, and a female x child abuse interaction term.

Table 1.

Sample descriptive statistics by Val66Met allele (N = 226)

Characteristics		Val-val	Val-met/met-met	Total
N (%)		149 (65.93)	77 (34.07)	226 (100)
Male		128 (56.64)	68 (30.09)	196 (86.73)
Female		21 (9.29)	9 (3.98)	30 (13.27)
Race				
	Asian/PI	4 (1.77)	12 (5.31)	16 (7.08)
	Black	33 (14.60)	7 (3.10)	40 (17.70)
	Latino	18 (7.96)	3 (1.33)	21 (9.29)
	White	90 (39.82)	46 (20.35)	136 (60.18)
	Other	4 (1.77)	9 (3.98)	13 (5.75)
Exposed to adult trauma		94 (41.59)	41 (18.14)	135 (59.73)
Survivors of child abuse		40 (17.70)	30 (13.27)	70 (30.97)
Positive PTSD diagnosis		36 (15.93)	22 (9.73)	58 (25.66)
Mean (SD)				
Age		44.46 (9.77)	43.17 (9.98)	44.83 (9.53)
Education		14.51 (1.95)	15.05 (1.96)	14.63 (2.42)
CAPS score[+]		17.54 (1.77)	16.24 (2.39)	17.10 (1.42)
% Cortisol suppression (n=173)		72.92 (35.04)	71.49 (28.49)	64.06 (34.08)

Note: SD = standard deviation

[+]standard error was reported in place of SD as an index of CAPS score variance; no significant val66met × bivariate interactions were observed.

Table 2.

Mixed Models on Psychophysiological Response

Measure	Predictors	Threat Condition					
		No		Ambiguous		High	
		χ^2	f^2	χ^2	f^2	χ^2	f^2
EMG	Met allele × trial	2.18	0.01	1.22	0.00	1.31	0.00
	Met allele × PTSD × trial	2.29	0.01	4.67	0.01	6.33	0.01
	Met allele × child abuse × trial	1.15	0.00	1.86	0.00	4.62	0.00
SCR	Met allele × trial	13.71**	0.16	8.47*	0.14	1.31	0.00
	Met allele × PTSD × trial	8.80+	0.07	10.88***	0.18	11.86**	0.14
	Met allele × child abuse × trial	2.54	0.03	2.21	0.02	3.58	0.01
Heart rate	Met allele × trial	2.19	0.01	2.86	0.01	1.83	0.00
	Met allele × PTSD × trial	6.11	0.03	6.13	0.02	9.82**	0.17
	Met allele × child abuse × trial	1.03	0.00	7.62	0.05	9.71**	0.16

Note: All models included the following covariates: age, race, years of education, the order that participants were exposed to the high threat condition, and a female × child abuse interaction term

+ $p < 0.10$

* $p < 0.05$

** $p < 0.01$

*** $p < 0.001$.