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The *BDNF* Val66Met Polymorphism Moderates the Relationship between Posttraumatic Stress Disorder and Fear Extinction Learning

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Abstract

The low expression Met allele of the *BDNF* Val66Met polymorphism is associated with impaired fear extinction in healthy controls, and poorer response to exposure therapy in patients with Posttraumatic Stress Disorder (PTSD). Given that fear extinction underlies exposure therapy, this raises the question of the impact of *BDNFVal66Met* polymorphism on fear extinction in PTSD, yet this question has not yet been examined. One hundred and six participants (22 PTSD, 46 trauma-exposed controls (TC) and 38 non-trauma exposed controls (NTC)) completed a fear conditioning and extinction task and saliva samples were taken for DNA extraction and genotyped for the *BDNF* Val66Met polymorphism. Moderation analyses using PROCESS examined whether *BDNF* genotype (Val-Val vs Met carriers) moderated the relationship between PTSD symptom severity (and diagnostic status) and skin conductance response (SCR) amplitude during fear extinction. The PTSD group displayed significantly slower fear extinction learning compared to TC and NTC in the early extinction phase. The *BDNF* Val66Met polymorphism moderated the relationship between PTSD and fear extinction learning, such that poorer fear extinction learning was associated with greater PTSD symptom severity (and PTSD diagnostic status) in individuals with the low-expression Met allele, but no relationship was demonstrated in individuals with the Val-Val allele. This study reveals that impaired fear extinction learning is particularly evident in individuals with PTSD who carry the low-expression *BDNF* Met allele and importantly not in those with the Val-Val allele. This provides novel evidence of a link between BDNF and impaired fear extinction learning in PTSD, which may contribute to poorer response to exposure therapy.

Keywords: BDNF, fear extinction, PTSD, Posttraumatic Stress Disorder, Brain Derived Neurotrophic Factor

Introduction

Brain-Derived Neurotrophic Factor (BDNF) is a neurotrophin that has an important role in neural development as well as synaptic plasticity and long-term potentiation (Andero & Ressler, 2005). It is integral to learning and memory and therefore along with its high affinity tyrosine kinase-B (TrkB) receptor, is concentrated in brain regions associated with mnemonic functions, such as the amygdala, prefrontal cortex and hippocampus (Minichiello, 2009). *BDNF* gene expression in these regions is essential for the formation of emotional memories (Andero & Ressler, 2005; Rattiner, Davis, French et al., 2004). Accumulating evidence indicates that *BDNF* gene expression is linked with fear conditioning and extinction (Andero & Ressler, 2005). Fear conditioning is a form of associative learning centered in the basolateral amygdala, whereas fear extinction involves new inhibitory learning centered in ventromedial prefrontal cortical, regions that are thought to inhibit amygdala activity (Milad & Quirk, 2012). Notably genetic and pharmacological studies in animals reveal that the binding of BDNF to its high affinity TrkB receptors in the basolateral amygdala is critical for the acquisition and consolidation of fear (Rattiner et al., 2004; Chhaatwal, Stanek-Rattiner, Davis et al., 2006; Karpova, Pickenhagen, Lindholm et al., 2011)

Regions of the ventromedial prefrontal cortex (and infralimbic cortex in rats) are also implicated in extinction learning and in the consolidation of extinction memory (Milad & Quirk, 2012). In animal models, consolidation of extinction memories is at least partially dependent on protein synthesis in the infralimbic prefrontal cortex (Santini, Ge, Ren et al., 2004). Animal studies reveal that BDNF signaling regulates aversive memories by enhancing plasticity in the medial PFC which promotes top-down regulation of the amygdala (Ninan, 2014; Heldt, Stanek, Chhatwal et al., 2007; Pattwell, Bath, Perez-Castro et al., 2013). Further animal research reveals that direct infusion of BDNF into infralimbic and hippocampal regions enhances fear extinction (Perez, Dieppa-Perea, Melendez et al., 2010).

Human BDNF research is typically operationalized by examining the influence of specific polymorphisms in the *BDNF* gene. A single nucleotide polymorphism (SNP) in the human *BDNF* gene

produces a functional Valine (Val) to methionine (Met) substitution at codon 66 (Val66Met; Egan, Kojima, Callicott et al., 2003).Met substitution reduces BDNF trafficking and activity-dependent release of this neurotrophin (Egan et al., 2003). Human fear conditioning studies reveal that inheritance of particular *BDNF* gene variations is implicated in conditioned fear learning (Lonsdorf, Weike, Golkar et al., 2010; Hajcak, Castille, Olvet et al., 2009) and the generalization of fear conditioning (Muhlberger, Andreatta, Ewald et al., 2014), however other studies have failed to find a relationship between fear conditioning and the BDNF Val66met polymorphism (Lonsdorf, Golkar, Lindstrom, et al., 2015; Soliman, Glatt, Bath et al., 2010). One translational study found no effect of *BDNF* genotype on fear conditioning, but reduced BDNF expression was associated with impaired fear extinction learning in mice and humans (Soliman, Glatt, Bath et al., 2010). The low-expression Met allele of the *BDNF* Val66Met polymorphism was associated with impaired fear extinction learning (higher skin conductance response (SCR) amplitude) and reduced activation in ventral prefrontal brain regions which mediate fear extinction learning compared to Val homozygotes (Soliman et al., 2010). It should be noted that this study employed a reversal learning manipulation prior to extinction, which may have influenced their findings. However, a recent fMRI-SCR study using a 2-day differential fear conditioning and extinction recall task also found that participants with the Met allele of the Val66Met polymorphism had greater amygdala activity and reduced ventromedial prefrontal activity (in subgenual anterior cingulate cortex) during extinction, but no differences were observed in the conditioning phase or in SCR (Lonsdorf, Golkar, Lindstrom et al., 2015). Further studies are required to replicate these findings.

Impaired fear extinction learning is posited to be a key mechanism underlying the development and maintenance of PTSD (Pitman, Rasmussen, Koenen et al., 2012; Zuj, Palmer, Lommen et al., 2016) - a proposition supported by evidence that fear extinction learning is associated with the risk of developing and maintaining PTSD (Guthrie & Bryant, 2006; Orr, Lasko, Macklin et al., 2012).Recent review papers and a meta-analysis revealed an association between the BDNFVal66Met genotype and PTSD status (Notaras,

Hill, Van Den Buuse, 2015; Bruenig et al., 2016, but see Wang, 2015). On the basis that fear extinction is a key mechanism underlying exposure-based treatment (Graham & Milad, 2011), a recent study examined the relationship between response to exposure therapy in PTSD and *BDNF* genotype. Poorer response to exposure therapy for PTSD was found in individuals carrying the low-expression *BDNF* Met relative to patients with the Val-Val genotype (Felmingham, Dobson-Stone, Schofield et al., 2013). This suggests that reduced BDNF expression led to impaired fear extinction, resulting in slower responses to exposure therapy. However, no studies have examined the relationship between the *BDNF* Val66Met polymorphism and fear extinction learning in participants with PTSD.

Accordingly, in this study we examined whether *BDNF* Val66Met genotype moderates the relationship between PTSD and fear extinction learning. It was predicted that the association between impaired fear extinction and PTSD would be particularly strong in individuals with the low-expression *BDNF* Met allele compared to Val homozygotes.

Materials and Methods

Participants

One hundred and six participants of white European ancestry were recruited from community health centres and university student populations. Participants were aged 18-61 (*M* = 24.8 years, *SD* = 8.7 years, 38 males and 68 females) and comprised three groups: PTSD (*n* = 22), trauma-exposed controls (TC; *n* = 46), and trauma non-exposed controls (NTC; *n* = 38). Participants were classified on the basis of exposure to a DSM-5 criterion A stressor that threatened physical integrity using the Traumatic Events Questionnaire (TEQ; Vrana & Lauterbach, 1994). The PTSD Checklist-Civilian version (PCL: Weathers, Litz, Husker et al., 1994) was used to assess PTSD symptom severity - participants who displayed minimal PTSD symptoms were classified as TC, and participants with PCL scores >40 were

classified as PTSD (or subsyndromal PTSD, in accordance with guidelines from the National Center for PTSD). Trauma-exposed participants were exposed to a variety of environmental and interpersonal traumas including combat exposure (3%), accident (21%), natural disaster (18%), witness violence (19%), assaulted or molested (30%), threatened or held captive (6%), and tortured or terrorist victim (3%). Mean years since trauma was 10.5 years (*SD* = 11.8 years). Participants who reported no experience of a traumatic event were classified as NTC. Participants completed the Depression Anxiety Stress Scale-21 item version (DASS: Lovibond & Lovibond, 1995). This study was approved by the Tasmanian Medical Human Research Ethics Committee, and all participants gave full informed consent.

Fear conditioning and extinction paradigm

A standardized differential fear conditioning and extinction paradigm was adapted from previous studies (Orr, Metzger, Lasko et al., 2000) in which a colored circle is paired with an aversive unconditioned stimulus (electric shock) during fear acquisition (CS+), and a second colored circle is presented during the fear acquisition phase and is never followed by the electric shock (CS-). This conditioned fear response is extinguished in the extinction phase where the CS+ is no longer followed by an electric shock. The unconditioned stimulus (US) was a 500ms mild electric shock delivered to the first interosseous muscle of the dominant hand, and set to a level considered “highly annoying, but not painful” by each participant prior to the task. Conditioned stimuli were red and blue circles presented individually for 12s on a computer screen. Four experimental phases were presented in the task in a single testing session: *habituation*, *acquisition*, *early extinction*, and *late extinction*. During *habituation*, participants were exposed to four trials of each colored circle (eight trials in total). During *acquisition*, one of the colored circles (CS+) was followed by the US on all five trials (100% reinforcement schedule) while the other colored circle was never followed by an electric shock (CS-; ten trials in total). The color of the CS+ was randomized and counterbalanced between groups, and the order of CS+ and CS- trials

was randomized. The *early extinction* phase consisted of five trials of the CS+ and five trials of the CS- (ten trials in total). Following a short rest period of approximately 1-minute, participants completed the *late extinction* phase, which replicated the early extinction phase. Trial order was pseudo-randomized, with no more than two consecutive CS+ or CS- trials.

Skin conductance response

Skin conductance level was measured through a 22mV$_{rms}$, 75Hz constant-voltage coupler (FE116, ADI Instruments, Sydney, Australia) with bipolar electrodes placed on the intermediate phalange of the first and third fingers of the non-dominant hand, sampled at 512Hz and stored at 64Hz, and recorded in micro-Siemens (μS). SCR to the CS+ and CS- was calculated by subtracting the mean SCL during the 2s prior to stimulus onset from the maximum SCL during the 12s stimulus duration. SCR values were square-root transformed, and the absolute value of negative scores was transformed and the negative sign replaced.

US-expectancy ratings

During the 12s stimulus presentation, participants were asked to rate their threat expectancy of the US on a 0-100 visual analogue scale (VAS: 0 “certain no electrical stimulus”; 100 “certain electrical stimulus.”

Salivary Genomic Analysis

Extraction and purification of DNA was performed following standard methods using saliva samples collected from participants (DNA Genotek Inc., 2012). Genotypes of the *BDNF* Val66Met polymorphism were identified using an established polymerase chain reaction (PCR) method (Sheikha, Hayden, Krisky et al., 2011). PCR amplifications were conducted using a ten microliter reaction volume

containing approximately 50 ng of genomic DNA. PCR amplicons were resolved on a 2% agarose gel, where one band present at 253 base pairs represented a Val homozygote, a Met homozygote showed one band present at 201 base pairs, and bands present at both 201 and 253 base pairs showed a heterozygote. Genotyping was repeated to ensure accuracy, with the proportion of concordance >99%.

Data Analysis

Demographic and clinical data were analysed using one-way analyses of variance (ANOVA) for the PTSD, TC and NTC groups. *BDNF* Val66Met genotype was analysed in two groups by combining Val/Met and Met/Met genotypes to represent Met carriers, and compared against Val-Val homozygous genotypes. To determine if there were differences between genotype groups on screening and demographic variables (age, sex, years of prior education, DASS anxiety score and DASS depression score). A chi-square goodness of fit test was also conducted to confirm genotypes aligned with the Hardy-Weinberg equilibrium. The *BDNF* Val66Met genotype distribution in this study was 67.4% Val homozygote and 32.6% Met carrier, which did not differ significantly from Hardy-Weinberg equilibrium, χ^2 (1) = 0.07, p = .79. To examine the impact of group on fear conditioning and extinction, a series of 3 (Group: PTSD, TC, NTC) x 2 (Condition: CS+/CS-) x 5 (Trial) were conducted on SCR amplitude data for each phase of the experimental task. For the habituation and acquisition phases, four trials were examined (the initial acquisition trial was not analysed as the initial shock – circle association was only made on the initial trial). For the early and late extinction phases, five trials were examined. Given the low frequency of the *BDNF*Val66Met genotype and to enhance statistical power, a moderation analysis was conducted to explore the association between BDNF, PTSD severity and fear extinction learning.

To examine whether *BDNF* Val66Met genotype moderated the relationship between PTSD and fear extinction learning, a moderation analysis was conducted using the PROCESS Macro (Hayes, 2012)

in SPSS 23 with PTSD symptom severity (PCL total score) as the predictor (X) variable, *BDNF*Val66Met genotype as the moderator (M) variable, and an index of fear extinction learning (average SCR amplitude across the initial three trials of the fear extinction phase) was used as the outcome (Y) variable. Separate analyses were conducted for the SCR amplitude to the CS+ and CS- for the early extinction phase. Moderation analyses were also completed on SCR responses during late extinction phases (data presented in Supplementary Tables 3 and 4). Moderation analyses were also conducted examining whether *BDNF* genotype moderated the relationship between PTSD diagnostic status (in this analysis, the trauma-exposed and non-trauma exposed control groups were collapsed to form a dichotomous predictor variable (PTSD group vs controls) as the two control groups did not differ significantly on fear extinction learning) and fear extinction learning. All moderation analyses controlled for age and weight, which are factors known to influence BDNF expression (Lommatzsch, Zingler, Schuhbaeck et al., 2005). Analyses were conducted with SPSS v23 (IBM, Armonk, NY, USA), an alpha value of $p<.05$ was used for all analyses, Sidak posthoc tests were used where required, and effect sizes are reported.

Results

Clinical and Demographic Data

Table 1 presents the means and standard deviations for clinical and demographic data for the groups. The PTSD group was significantly older than the NTC group, but there were no significant differences between PTSD and TC. There were no significant differences in gender distribution, alcohol use (AUDIT), and hours-since-waking at test. As expected, the PTSD group had significantly greater total scores on the PTSD Checklist, the DASS depression, anxiety and stress scales than the control groups, which did not significantly differ.

BDNF and Clinical and Demographic Data

Overall, there were no significant differences in age, gender distribution, alcohol use, depression, group status or PTSD total symptoms (PCL score) between the participants with the Val-Val genotype and Met allele carriers (see Supplementary Tables 1 and 2).

Fear Conditioning and Extinction Data

A repeated measures ANOVA for the habituation phase revealed a significant main effect of Trial, $f\ (3,39) = 4.35$, $p = .005$, $\dot{\eta} = .04$, which showed a reduction in SCR amplitude across the trials in the habituation phase. There were no significant effects of group, condition, or any significant interactions involving group, condition or trial.

For the fear acquisition phase, a significant condition main effect, $f\ (1,103) = 89.8$, $p = .001$, $\dot{\eta} = .47$ revealed significantly greater SCR amplitude to CS+ (M=.91, SD=.05) than the CS- (M=.49, SD=.05) reflecting effective fear conditioning. A significant effect of Trial, $f\ (4,412) = 21.9$, $p = .001$, $\dot{\eta} = .175$, revealed a reduction in SCR amplitude across trials in the acquisition phase. A significant Condition x Trial interaction, $f\ (4,412) = 3.28$, $p = .018$, $\dot{\eta} = .03$, revealed that the initial trial was significantly greater than trial 4 for CS+, and for trial 3 for CS-. There was also a marginally significant Group x Condition interaction, $f\ (2,92) = 3.39$, $p < .05$, $\dot{\eta} = .07$, however there were no significant group differences when examined by posthoc tests.

In early extinction, a significant Condition effect, $f\ (1,103) = 5.4$, $p = .022$, $\dot{\eta} = .05$ revealed that SCR amplitude was greater to the CS+ (M=.59, SD=.04) than CS- (M+.51, SD=.04). A significant Trial main effect, $f\ (4,412) = 37.6$, $p = .001$, $\dot{\eta} = .27$ revealed that SCR amplitude decreased significantly across trials during the early extinction phase. A significant Group x Trial interaction, $f\ (8,412) = 2.2$, $p = .032$, $\dot{\eta} = .04$, revealed that the PTSD group had slower fear extinction learning across the initial trials of extinction than the control groups collapsed across CS+ and CS-. This effect can be seen in Figure 1.

In late extinction, there were no significant main effects or interactions except a significant main effect of Trial, *f (4,408) = 21.2, p =.001, ή =.17* which revealed a significant reduction in SCR amplitude across trials in the late extinction phase, pooled across the CS+/-.

Moderation Analysis: Moderating Effects of BDNF Genotype

To examine whether the *BDNF* Val66met genotype moderated the relationship between the severity of PTSD symptoms (indexed by the PCL Total score) and fear extinction learning, a second moderation analysis was conducted using the PROCESS MACRO (Model 1). With PCL total score as the predictor variable, *BDNF* genotype as the moderator, and the averaged SCR amplitude from trial 1 to trial 3 (where the maximal fear extinction occurred) to the CS+ in the early extinction phase, and age and weight entered as covariates, *BDNF* genotype was found to significantly moderate the relationship between PTSD symptom severity and fear extinction learning (see Table 2), and simple slopes analyses revealed that as PTSD symptoms increase in severity, there is poorer fear extinction learning (indexed by greater averaged SCR amplitude to the CS+ averaged over trials 1 to 3 of early extinction). *BDNF* genotype and PCL total scores were non-significant individual predictors of fear extinction learning (as were age and weight), but the interaction between PCL symptom severity and fear extinction learning was significant and independently contributed 5.21% of variance in the model (R^2=.0408, p=.025). Simple slopes analysis revealed no significant association between group status and fear extinction learning for individuals with the Val-Val genotype (p=.385), while the association between group status and fear extinction learning significantly differed from zero for Met allele carriers (β = .385, 95% CI[.02, 0.75], *t* = 2.10, *SE* = 0.1, 8 *p.* =.039). The significant positive relationship between PCL total score and SCR amplitude across the first three trials of early extinction in the Met allele carrier group revealed that increased severity of PTSD symptoms was related to greater impaired fear extinction (indexed by higher mean SCR amplitude across the early extinction trials: see Figure 2).

A second moderation analysis was run to examine whether *BDNF* Val66Met genotype moderated the relationship between PTSD diagnostic status and fear extinction learning. Group status was coded as PTSD vs Controls (collapsing control groups) given the lack of differences between control groups in the fear extinction measures, and to avoid difficulties with non-dichotomous categorical predictors in moderation analyses (Hayes. 2012). With Group status (PTSD, Controls) as the predictor variable, *BDNF* genotype as the moderator, and the averaged SCR amplitude from trial 1 to trial 3 (where the maximal fear extinction occurred) to the CS+ in the early extinction phase, and age and weight entered as covariates, a significant overall model was found $F_{(6,82)} = 2.41$ *p*. =.044, $R^2 = .082$ (see Table 3 for details of moderation analysis). No significant independent associations were found between Group status (p=.78) or *BDNF* genotype (p=.21) and fear extinction outcome, but there was a significant interaction between Group x *BDNF* genotype on fear extinction outcome ($t_{(82)} = -2.5587$, *p*. = .005: Table 3) which added 6.6% of independent variance to the model (R^2=.0664, p=.005). Simple slopes analysis revealed no significant association between group status and fear extinction learning for individuals with the Val/Val genotype, while the association between group status and fear extinction learning significantly differed from zero for individuals with a Met allele (β = -0.46, 95% CI[-.80, -0.13], *t* = -2.77, *SE* = 0.24, *p*. =.007). The significant negative relationship between group status (PTSD group 1, controls group 2) and fear extinction learning in the Met allele group revealed that PTSD status was related to greater impaired fear extinction (indexed by higher mean SCR amplitude across the early extinction trials: see Figure 3).

Discussion

This study investigated the impact of the BDNF Val66met polymorphism on fear extinction learning in PTSD, and tested the prediction that impaired fear extinction would be related to greater

PTSD symptom severity particularly in those individuals with the low-expression BDNF met allele. Participants with PTSD displayed slower extinction learning compared to controls, and the BDNF Val66Met polymorphism was found to moderate the relationship between PTSD symptoms and fear extinction learning. Specifically, individuals with PTSD who carried the low-expression *BDNF* Met allele revealed less fear extinction (reflected in greater mean SCR across early extinction trials), but those who were Val-Val homozygotes did not. This finding is important given previous evidence that individuals with PTSD who carried the Met-allele of the *BDNF* Val66Met polymorphism had poorer response to exposure therapy (Felmingham et al., 2013). Given that fear extinction is thought to be a key underlying mechanism of exposure therapy (Graham & Milad, 2011), it was hypothesized in the previous study that the poorer response to exposure therapy in individuals with the *BDNF* Met-allele may have been due to lower BDNF levels leading to impaired fear extinction learning (Felmingham et al., 2013). To our knowledge, no previous studies have examined how the *BDNF* Val66Met polymorphism may influence fear extinction learning in the context of PTSD. The current findings reveal that the *BDNF* Met-allele is associated with impaired fear extinction learning in PTSD.

This finding accords with robust animal evidence that BDNF in prefrontal and hippocampal regions influences fear extinction learning (Peters et al., 2010; Soliman et al., 2010)and with human evidence that the *BDNF* Met-allele is associated with impaired fear extinction learning and concurrent reduced activity in the ventromedial prefrontal cortex (Lonsdorf et al., 2015; Soliman et al., 2010). A possible mechanism for this effect is that BDNF enhances synaptic plasticity and long-term potentiation in these regions to consolidate fear extinction learning (Andero & Ressler, 2005; Ninan, 2014).

The fear conditioning and extinction task in the current study elicited robust fear conditioning and extinction. Confirming previous findings, no significant differences were observed in the acquisition of fear conditioning between PTSD groups and controls and there appeared a specific

deficit in fear extinction learning for PTSD (Pitman et al., 2012). Previous studies in healthy controls have revealed a relationship between the Met-allele of the *BDNF* Val66Met polymorphism and impaired fear conditioning (Hajcak et al., 2009; Lonsdorf et al., 2010) or fear generalization (Muhlberger et al., 2014), but others reveal no relationship between *BDNF* Val66Met genotype and fear conditioning (Lonsdorf et al., 2015; Soliman et al., 2010). Group differences in early extinction were not specifically found to the CS+, but manifested as slower SCR decline across initial fear extinction trials to the CS+ and CS-. This finding is in line with recent evidence that individuals with PTSD display impaired discrimination of danger and safety signals (CS-), and often display enhanced fear responses (indexed by startle) to safety signals. Of note, the moderating effect of the *BDNF* genotype was found specifically to the CS+.

The relatively small group difference in early extinction learning may reflect the impact of the *BDNF* genotype (as individuals with different genotypes were collapsed within groups) but may also have been influenced by methodological factors such as the use of a 100% reinforcement schedule during fear conditioning, which can result in rapid fear extinction. More robust evidence has been found for deficits in the recall of fear extinction memories in PTSD (Milad, Orr, Lasko et al, 2008; Pitman et al, 2012),suggesting that the consolidation of fear extinction memories is particularly impaired in PTSD. Given that BDNF is critically involved in synaptic plasticity and long-term potentiation (Andero & Ressler, 2005), processes that are integral to the consolidation of fear extinction memories, it may be expected that the moderating effects of the *BDNF* Val66Met polymorphism in PTSD would be even stronger when examining fear extinction recall. Future research is needed to replicate these initial findings using 2-day fear extinction recall paradigms with partial reinforcement schedules.

To definitively relate BDNF, fear extinction deficits and poorer response to exposure therapy in PTSD, future research needs to examine fear extinction learning before and after exposure therapy

stratified by *BDNF* genotype. The relatively modest sample size of the present study limited our capacity to examine potential polygenic influences on fear extinction learning. The serotonin transporter gene (5HHTLTP) has been associated with fear extinction learning (Hartley, McKenna, Salman et al., 2012; Lonsdorf & Kalish, 2011) and the serotonin transporter gene has been shown to influence response to exposure therapy in PTSD (Bryant, Felmingham, Falconer et al., 2010). Further, the *COMT* Val58Met polymorphism which acts on dopamine metabolism, and FKBP5 which influences glucocorticoid receptor sensitivity, have been shown to influence fear extinction learning (Lonsdorf & Kalish, 2011; Sawamura, Klengel, Armaris et al., 2015), have been implicated in gene x environmental interactions to predict PTSD (Xie, Kranzler, Poling et al., 2010) and FKBP5 gene expression has increased following exposure-treatment for PTSD (Yehuda, Daskalaskis, Desarnaud et al., 2013; Wilker, Pfeiffer, Kolassa et al., 2014). It should be noted that whilst our sample size is relatively modest, this project was not designed as a gene x environment study but is a hypothesis-driven candidate genotype study and the current sample size is larger than several comparable candidate genotype studies.

Evidence suggests that glucocorticoids modulate BDNF expression(Gray, Milner, McEwen, 2013; Suri & Vaidya, 2013) with a recent rodent study suggesting that contextual fear dependent memory is increased in Met-carriers following chronic corticosterone administration (Notaras, Hill, Gogos et al., 2015). Given the dysregulation in cortisol function in PTSD (Pitman et al., 2012)and the relationship between cortisol and fear extinction learning (De Quervain, Bentz, Michael et al., 2011), it is of particular relevance to examine the impact of *BDNF* genotype on fear extinction learning in PTSD. Further, growing evidence highlights the influence of estrogen and sex (Hill, 2012), as well as glucocorticoids (Gray et al., 2013), on BDNF expression, and both cortisol and estrogen facilitate fear extinction inhumans (De Quervain et al., 2011; Hill, 2012). Future research should employ sufficient sample sizes to model the interactive effects of *BDNF* genotype and estrogen, and *BDNF* genotype and cortisol on fear extinction learning. This is of particular relevance, given the notable female

vulnerability for developing PTSD and other stress-related psychiatric disorders (Olff, Langeland & Draijer et al., 2007).

This study highlights that the low expression Met allele of the *BDNF* Val66Met genotype may be an important risk factor in the development and maintenance of PTSD, and an important influence on response to exposure therapy via affecting fear extinction learning. Taking together the findings from prospective studies that impaired fear extinction learning prior to trauma is a predictor of later PTSD [21-23] and the current evidence that the met allele is associated with impaired fear extinction learning in PTSD, this implicates the low expression Met allele of the *BDNF* Val66Met polymorphism as a risk factor for the development of PTSD. Recent studies have revealed a significant association between this *BDNF* Val66met allele and PTSD, with risk associated with the met allele in one systematic review paper (Notaras et al., 2015), and the Val/Val allele to be a protective factor for PTSD in a case-control study and meta-analysis (Bruenig et al., 2016), however a third meta-analysis found no significant association between the BDNFVal66met polymorphism and PTSD risk (Wang, 2015)

The current findings highlight that specific genetic factors are important influences on fear extinction, which is involved in the development, maintenance and treatment of PTSD. Furthermore, these findings increase the potential for a more targeted and personalized treatment approach as identifying individuals with the *BDNF* Met-allele may alert clinicians to the risk of greater propensity to develop PTSD following trauma exposure and impaired fear extinction learning and slower response to exposure therapy. Future prospective and treatment-related research needs to examine the *BDNF* Val66Met polymorphism alongside other known genetic risk factors for PTSD (FKBP5) in conjunction with the capacity for fear extinction learning and the retention of fear extinction.

Conclusion

In conclusion, the current study provides novel initial evidence that the *BDNF* genotype moderates the relationship between PTSD symptoms and fear extinction learning. This suggests that

low expression met allele of the BDNFVal66met polymorphism may be associated with impaired fear extinction learning in PTSD, which in turn may contribute to a poorer response to exposure therapy in PTSD therapy.

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Conflict of Interest

Gin S Malhi has received grant or research support from National Health and Medical Research Council, NSW Health, Ramsay Health, American Foundation for Suicide Prevention, AstraZeneca, Eli Lilly & Co, Organon, Pfizer, Servier, and Wyeth; has been a speaker for AstraZeneca, Eli Lilly & Co, Janssen Cilag, Lundbeck, Pfizer, Ranbaxy, Servier, and Wyeth; and has been a consultant for AstraZeneca, Eli Lilly & Co, Janssen Cilag, Lundbeck, and Servier. Professor's Felmingham, Bryant, and Vickers research is funded by the National Health and Medical Research Council (NHMRC) and Dr Palmer by the Australian Research Council, but these funding bodies have no involvement in the planning, acquisition and analysis, or publication of findings. There are no other financial conflicts or considerations to declare.

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Table 1: Clinical and Demographic Data

Variable	**PTSD**	**TC**	**NTC**	**F**	**P**	**Effect size**
Age	33 (14.4)	25.9 (8.8)	23.5 (8.5)	6.36	.002	.110
Gender	8 M, 14 F	23M, 23 F	13M, 25 F	2.44	.295	
PCL Tot	52.9 (11.2)	24 (4.9)	20.9 (4.2)	190	.000	.79
DASS Dep	9.63 (5.7)	2.2 (2.3)	1.6 (2.2)	48.2	.000	.49
DASS Anx	8.14 (4.2)	2.02 (1.8)	1.92 (2.3)	47.7	.000	.48
DASS Stress	13.7 (6.2)	4.4 (2.9)	2.8 (2.3)	66.1	.000	.56
Hour awake	6.2 (2.4)	6.9 (2.5)	6.3 (2.2)	.602	.55	.018
AUDIT	7 (5.3)	5.8 (3.9)	6 (3.9)	.578	.56	.011

Table 2: Moderation Analysis Summary – Impact of PTSD Symptom Severity moderated by BDNFVal66Met Genotype on SCR amplitude to CS+ During Early Extinction

Prediction of Fear Extinction Learning by PTSD Symptom Severity Moderated by BDNF Genotype

Predictor	*B*	SE	T	P	95% CI	
					Lower	Upper
Constant	.625	.258	2.418	.018	.111	1.14
BDNF	.113	.103	1.099	.275	-.091	.317
PCL total	.040	.121	.333	.740	-.199	.280
BDNF x PCL Total	.514	.226	2.28	**.025**	.066	.963
Age	.001	.004	-.003	.998	-.008	.008
Weight	.001	.003	.097	.923	-.006	.007

Conditional Effect of PTSD Symptom Severity on Fear Extinction Learning at Values of BDNF Genotype

BDNF Genotype	*B*	SE	T	P	95% CI	
					Lower	Upper
ValVal	-.129	.148	-.847	.385	-.424	.165

Met	.385	.183	2.10	**.039**	.02	.750

Table 3: Moderation Analysis Summary – Impact of BDNF Genotype on the relationship between Group Status (PTSD vs Controls) and Fear Extinction (SCR amplitude to CS+)

Prediction of Fear Extinction Learning by Group Status (PTSD vs Controls) Moderated by BDNF Genotype

Predictor	*B*	SE	T	P	95% CI	
					Lower	Upper
Constant	.594	.252	2.360	.021	.093	1.096
BDNF	.128	.102	1.260	.211	-.074	.331
Group	-.029	.106	-.272	.786	-.339	.181
BDNF x Group	-.63	.22	-2.87	**.005**	-1.06	-.193
Age	-.001	.004	-.249	.810	-.009	.007
Weight	.001	.003	.341	.734	-.006	.008

Conditional Effect of Group on Fear Extinction Learning at Values of BDNF Genotype

BDNF Genotype	*B*	SE	T	P	95% CI	
					Lower	Upper

ValVal	.178	.119	1.497	.138	-.059	.415
Met	-.451	.192	-2.34	**.022**	-.833	-.067

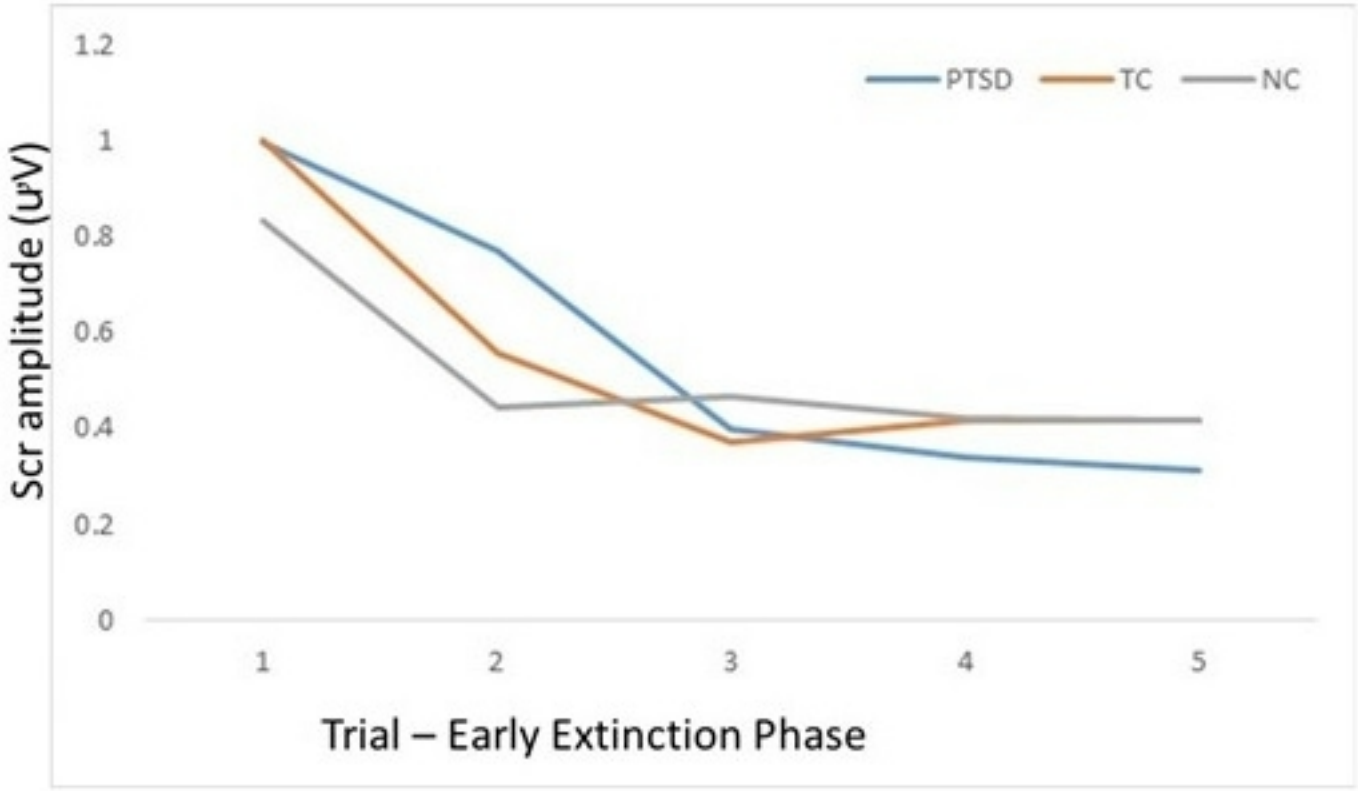
.

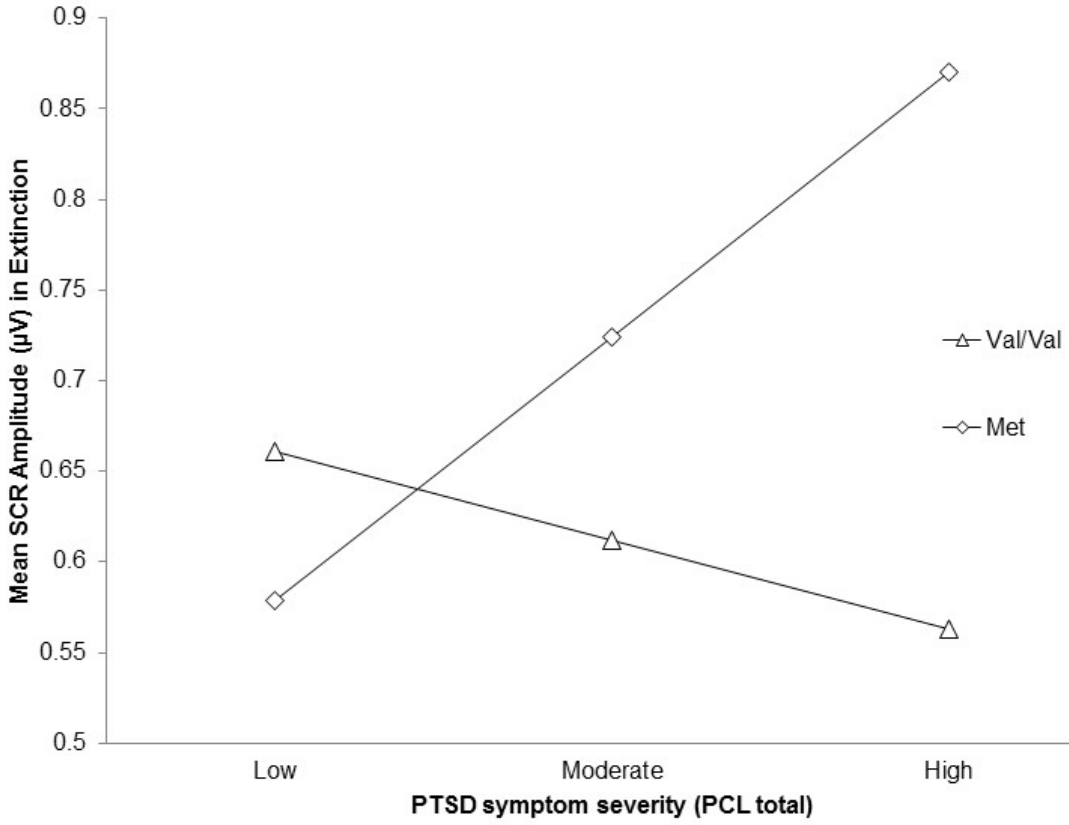
Figure Legends

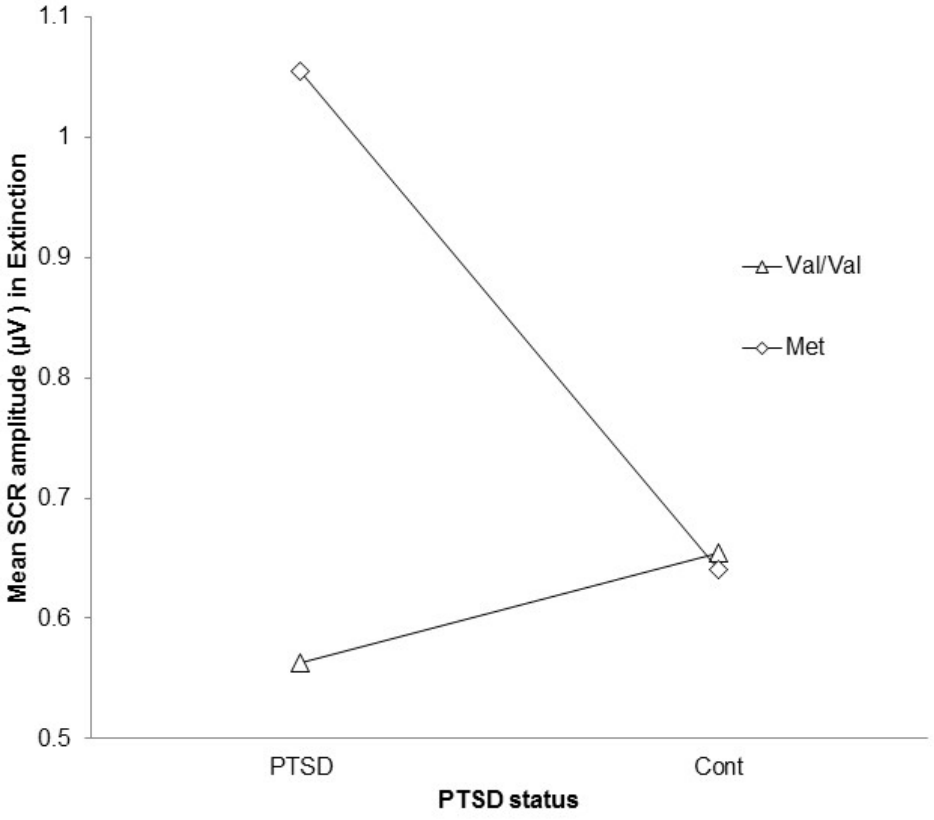
Figure 1: Averaged SCR Amplitude across CS+ and CS- for Group (PTSD, TC, NC) across Early Extinction Trials

Figure 2: Moderating Effect of BDNF Val66Met Genotype on PTSD Symptom Severity (PCL Total) and Fear Extinction (Averaged SCR amplitude during Early Extinction)

Figure 3: Moderating Effect of BDNFVal66Met Genotype on PTSD Status (PTSD vs Controls) and Fear Extinction (Averaged SCR amplitude during Early Extinction)


PTSD
TC
NC
Scr amplitude (uV)
1.2
1
0.8
0.6
0.4
0.2
0
1
2
3
4
5
Trial – Early Extinction Phase


0.9
0.85
0.8
0.75
0.7
0.65
0.6
0.55
0.5
Mean SCR Amplitude (μV) in Extinction
Low
Moderate
High
PTSD symptom severity (PCL total)
Val/Val
Met


1.1
1
0.9
0.8
0.7
0.6
0.5
Mean SCR amplitude (µV) in Extinction
PTSD
Cont
PTSD status
Val/Val
Met

Supplementary Table 3: Moderation Analysis Summary for Late Extinction CS- (minus)

Prediction of Fear Extinction Learning by Group Status Moderated by BDNF Genotype

Predictor	*B*	SE	T	P	95% CI	
					Lower	Upper
BDNF	.05	.13	.34	.73	-.22	.32
Group	-.07	.17	-.39	.69	-.39	.27
BDNF x Group	-.21	.42	-.49	.62	-1.05	.63

Conditional Effect of Group on Fear Extinction Learning at Values of BDNF Genotype

BDNF Genotype	*B*	SE	T	P	95% CI	
					Lower	Upper
ValVal	.01	.16	.02	.98	-.31	.31
Met	-.21	.39	-.52	.60	-.98	.57

Supplementary Table 1: Effect of BDNF genotype on clinical and demographic data

Variable	BDNF Met	BDNF Val	F	P	Effect size
Age	26.5 (11.2)	26.3 (10.4)	.006	.94	.000
Gender	15M, 15F	21M,40F	X=2.04	.18	
Group Status	6 PTSD, 16 TC, 8 NC	12 PTSD, 23 TC, 26 NC	X =2.52	.28	
PCL Tot	29.2 (12.8)	27.3 (12.1)	.48	.49	.005
DASS Dep	4.3 (5.3)	2.9 (3.9)	1.81	.18	.02
DASS Anx	3.1 (3)	3.0 (3.6)	.04	.85	.000
DASS Stress	6.4 (6.1)	5.2 (5.1)	.88	.35	.01
Hour awake	6.06 (2.2)	6.26 (2.2)	.12	.73	.002
AUDIT	5.4 (3.4)	6.5 (4.5)	1.27	.26	.014

Supplementary Table 2: Moderation Analysis Summary for Late Extinction CS+ plus

Prediction of Fear Extinction Learning by Group Status Moderated by BDNF Genotype

Predictor	*B*	SE	T	P	95% CI	
					Lower	Upper
BDNF	.08	.13	.06	.95	-.25	.27
Group	-.11	.09	-1.21	.23	-.27	.07
BDNF x Group	-.09	.21	-.41	.68	-.49	.32

Conditional Effect of Group on Fear Extinction Learning at Values of BDNF Genotype

BDNF Genotype	*B*	SE	T	P	95% CI	
					Lower	Upper
ValVal	-.08	.09	-.82	.41	-.26	.10
Met	-.16	.18	-.88	.37	-.52	.20

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