Influence of the BDNF Val66Met Polymorphism on the Development and Persistence of Post-Traumatic Stress Disorder in Military Personnel

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Abstract

The prevalence of post-traumatic stress disorder (PTSD) tends to be higher among military personnel and veterans compared to the general civilian population. PTSD is characterized by impaired cognitive functioning as it affects learning and memory. Brain-derived neurotrophic factor (BDNF) modulates cognition and is involved in neuroprotection and memory consolidation. The BDNF Val66Met polymorphism was found to influence cognitive functions negatively and is potentially related to PTSD. Despite previous research, the precise link between BDNF genotype and PTSD remains unclear. Therefore, this study focuses on the question of whether BDNF Val66Met polymorphism influences either the prevention or recovery of PTSD symptoms. The study is based on longitudinal research from war veterans deployed to Afghanistan (N = 517) and data from war veterans with PTSD (N = 51). Besides focusing on the prevention and recovery of PTSD symptoms, the study also examined the influence of the BDNF Val66Met polymorphism on hippocampal volume using Magnetic Resonance Imaging (MRI) data. No significant results are found for the influence of Val66Met on PTSD symptom development or PTSD treatment recovery. Additionally, Val66Met has no significant effect on hippocampal volume. Taken together, the BDNF Val66Met polymorphism does not influence the development of PTSD symptoms or recovery from PTSD. Future research is required to investigate the underlying causes of the differences in hippocampal volume and whether this poses a risk for the persistence of PTSD.

Keywords: post-traumatic stress disorder, military, deployment, BDNF Val66Met polymorphism, hippocampal volume

Plain language summary

Worldwide, many people go through stressful and traumatic experiences. These experiences can cause anxiety, trauma, and stress-related disorders. Around 8% of the general population ends up developing post-traumatic stress disorder (PTSD). The risk of developing PTSD is higher in environments with an increased risk of experiencing a stressful or traumatic event, such as in the military. The prevalence PTSD is therefore almost twice as high among military personnel and veterans as in the general population. PTSD can cause symptoms like flashbacks, avoidance behavior, and negative thoughts.

Chronic stress, such as experienced in PTSD, can affect the brain. A brain region called the hippocampus, which is essential for memory and learning, is especially vulnerable to chronic stress. Previous studies have shown that chronic stress can reduce the size of this brain region, and researchers are exploring how genetics might be involved in this process. However, the relationship between genetics, PTSD, and the size of the brain region remains unclear. A possible way to investigate this is by looking at the brain-derived neurotrophic factor (BDNF) gene. This gene might be involved since it plays an important role in learning and memory. A mutation of this gene can change the composition to a variation called BDNF Val66Met polymorphism. This variation might affect how well people respond to PTSD treatments and their risk of developing PTSD symptoms. This study aims to explore whether this genetic variation increases the risk of PTSD and affects treatment outcomes in Dutch military personnel after deployment**.**

To understand how this gene variation might affect PTSD in Dutch veterans, this study used data from two research projects. The first research project tracked military personnel who were deployed to Afghanistan to see how their experiences affected their mental health over time. The study started in 2005 and followed the military from the Dutch Armed Forces. For this research, data from 517 participants were analyzed, focusing on their PTSD symptoms before deployment and six months after returning home. The second research project focused on treatment recovery of military personnel and veterans with PTSD. With data from 51 participants, we explored how genetic

variation might influence the recovery from PTSD in Dutch military veterans. This is done by looking at recovery from PTSD symptoms before and after treatment, and by looking at the size of the specific brain region involved in learning and memory.

This research found that the BDNF gene variation does not influence whether military personnel develops PTSD symptoms after deployment, nor does it affect their recovery from trauma-related symptoms. Additionally, the study found no link between gene variation and changes in the size of the brain region.

These findings contributes to the understanding of the impact of genetics on the development and recovery of PTSD symptoms. Uncovering the genetic influence and the specific role of the brain region could lead to improved treatment for anxiety, trauma, and stress-related disorders in the future.

Introduction

Nearly 90% of the general population experiences a potentially traumatic event during their lifetime, of which 8% develops post-traumatic stress disorder (PTSD) (Bremner, 2006; Judkins et al., 2020). The prevalence of PTSD is almost twice as high among military personnel and military veterans compared to the general population (Judkins et al., 2020; Van Der Wal et al., 2020). The higher prevalence of PTSD among military personnel and veterans may be attributed to their increased risk of experiencing combat-related stressors, such as sniper fire or improvised explosive devices. Additionally, they also might develop PTSD symptoms from non-combat-related stressors. These stressors include life-threatening situations, serious injury, or sexual violence. Symptoms of PTSD include intrusive memories, flashbacks, avoidance behavior, and negative cognitions (Judkins et al., 2020). Increased exposure to traumatic stressors inherent to combat and the operational environment increases a service member's risk of developing chronic stress and potentially stressor-related disorders such as PTSD (Judkins et al., 2020).

Chronic stress causes damage and neuronal loss in the hippocampus and impairs the process of neurogenesis (Bremner, 2006; Krugers et al., 2010). The hippocampus, an important brain region involved in learning and memory, is vulnerable to changes from prolonged stress. These stress-induced changes can result in reduced hippocampal volume, a condition that has been consistently observed in patients with PTSD (Bremner, 2006; Van Rooij et al., 2015). However, the relationship between genetics, PTSD treatment response, and hippocampal volume remains unclear. A possible way to investigate this is by looking at the brain-derived neurotrophic factor (BDNF).

BDNF is a neurotrophin highly expressed in the hippocampus and commonly associated with learning and memory formation (Bruening et al., 2016). This neurotrophin mediates synaptic plasticity and emotional memory consolidation (Bruenig et al., 2016; Nicholson et al., 2023). BDNF can be examined in various ways, such as blood levels or genetics. Changes within the BDNF gene, caused by a single nucleotide polymorphism (SNP), can impair the functioning of this gene (Felmingham et al., 2018). This impairment arises from the substitution of the amino acid Methionine

(Met) for Valine (Val) at position 66 in the BDNF gene, resulting in what is known as the Val66Met polymorphism (Nicholson et al., 2023). Evidence indicates that BDNF gene expression is linked with fear conditioning and extinction, of which the Val66Met polymorphism is related to impaired fear extinction learning (Felmingham, 2018). This polymorphism can impair fear extinction learning, a key mechanism underlying the development and maintenance of PTSD (Felmingham et al., 2018).

BDNF Val66Met is associated with this key mechanism of PTSD since BDNF expression is important for emotional memory formation, including fear memory (Nicholson et al., 2023). Prior research has established a relationship between the response to exposure therapy in individuals with PTSD and their BDNF genotype. Specifically, individuals carrying the Met allele tend to exhibit a diminished response to exposure therapy compared to Val homozygotes (Felmingham et al., 2018; Mestrovic et al., 2020). This difference in response suggests a link between PTSD treatment response and BDNF genotype. Moreover, the Val66Met polymorphism was associated as a risk factor for developing PTSD (Bruenig et al., 2016). Despite the knowledge of this association, the precise link between BDNF genotype and PTSD remains unclear.

This study aimed to investigate the influence of BDNF Val66Met on PTSD among Dutch military personnel post-deployment, an area that has not been extensively explored. Specifically, this study examined whether the BDNF Val66Met polymorphism serves as a risk factor for developing PTSD symptoms and whether Met allele carriers exhibit different responses to PTSD treatment. Additionally, the study explored the potential impact of the BDNF Val66Met polymorphism on hippocampal volume in PTSD.

Methods

To investigate the impact of the Val66Met polymorphism on PTSD symptoms among Dutch veterans, data from earlier studies is used. To answer the first research question, a longitudinal dataset was used in which military personnel were followed after their deployment in Afghanistan (PRISMO). To examine the impact of the Val66Met polymorphism on PTSD treatment response, data is used from a cohort that was followed during their PTSD treatment (BETTER).

PRISMO

The Prospective Research In Stress-Relation Military Operations (PRISMO) study was initiated in 2005 by the Research Centre of Military Mental Healthcare at the Dutch Ministry of Defense. This study aimed to gain a better understanding of the long-term impact of military deployment on mental health and to map the different biological and psychological factors that contribute to the development of stress-related mental health symptoms (Van Der Wal et al., 2019). The extensive study design of PRISMO is explained in the paper of Van Der Wal (2019): 'Cohort profile: the Prospective research In Stress-Relation Military Operations (PRISMO) study in the Dutch Armed Forces'.

Participants

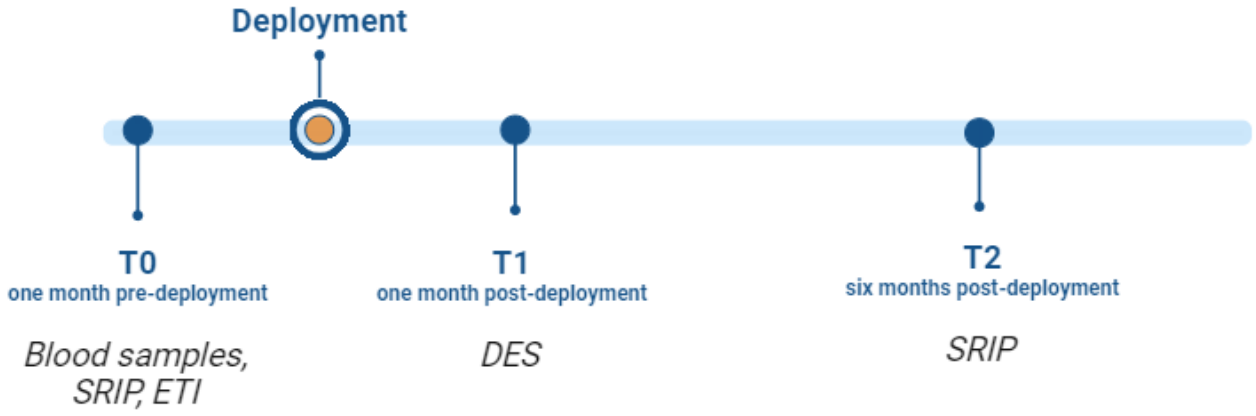
The PRISMO cohort recruited a sample of 963 military men and women who were deployed to Afghanistan between March 2005 and September 2008 as a part of the International Security Assistance Force (ISAF) mission. All participants provided written informed consent after receiving written and verbal explanation of the study. For the current study, a complete dataset of 517 out of the 963 participants was selected for analysis.

Measurements

The longitudinal study lasted ten years post-deployment, during which seven measurements were conducted (Appendix A). For the current study, data from before deployment and six months after deployment were utilized. The baseline measurement (T_0) was conducted approximately one month before deployment at the army base. The follow-up assessment occurred about six months after the military personnel returned home (T_2), also at the army base. The decision to focus solely on these two time points was made to minimize the influence of other potentially traumatic events after deployment on PTSD symptoms and to examine the effect of the Val66Met polymorphism on developing combat-related PTSD. PTSD symptoms were assessed using the Dutch Self-Rating Inventory for PTSD (SRIP; Hovens et al., 1994), and traumatic childhood experiences were measured with the Early Trauma Inventory-Self Report (ETI; Bremner et al., 2007). The Deployment Experience Scale (DES; Reijnen et al., 2015) consist of nineteen questions regarding exposure to combat-related stressors during deployment (Table B1) . Figure 1 illustrates the time points at which the different questionnaires were administered throughout the longitudinal study. The ETI and DES were used as covariates in the analysis to assess their influence on PTSD symptom development. Furthermore, the PRISMO study measured a broad range of biological and psychological covariates (Table C1).

Figure 1

Timeline of the Prospective Research in Stress-Related Military Operations study



Note. Figure 1 illustrates the timeline of the Prospective Research in Stress-Related Military Operations (PRISMO) study. It marks three key time points for data collection used in the current study: T_0 (one month pre-deployment), T_1 (one month post-deployment), and T_2 (six months post-deployment). At T_0, blood samples are collected, and the Self-Rating Inventory for PTSD (SRIP) and Early Trauma Inventory (ETI) are administered. At T_1, the Deployment Experience Scale (DES) is administered, and at T_2, the SRIP is administered again. The figure was created using Biorender.

Genotyping

The genetic variation of the BDNF Val66Met polymorphism is determined via genotyping. Blood samples were collected through venipuncture, and DNA extraction was performed following standard procedures. The concentration and quality of the extracted DNA were examined using Nanodrop (Thermo Fisher Scientific, MA, USA) and genotyping was conducted using Illumina Human OmniExpress 24 v1.1 (Leen, 2024c). In the end, the genetic variations of the BDNF Val66Met polymorphism were extracted using PLINK software version 1.9 (Purcell et al., 2007).

Statistical Analysis

Participants were only included for data analysis when they completed the T_0 and T_2 measurements. Moreover, a complete DES was necessary to control combat exposure. Data from the ETI, DES, and SRIP was imputed if less than 25% of the complete measurement was missing. Imputation was done with the MICE package in R-studio (Van Buuren & Groothuis-Oudshoorn, 2011) in five iterations due to limited computer processing capacity. Afterward, one model was chosen and tested with a student's T-test to examine whether the imputed values were not statistically different from the original data. Moreover, this study controlled for the frequency of combat-related stressors on the DES. More information on the calculation of this score can be found in Table B2. In total, the final analysis for PRISMO is based on 517 individuals. All data was analyzed with JASP statistics (version 0.18.3). A linear regression analysis is used to determine how the Val66Met polymorphism influences PTSD symptom development. The SRIP was used as the dependent variable in the model, with BDNF polymorphism, Time, ETI, and DES as independent variables. The assumptions for the regression analysis are met since we can assume that the data is normally distributed due to the high number of participants. Additionally, the data must meet the assumption of homoscedasticity, which is done with Levene's test ($F_{(1,1032)} = 3.190$, $p = .074$), indicating a constant variance in the regression model. Finally, genetic data must adhere to Hardy-Weinberg Equilibrium (HWE). The PRISMO dataset met

the HWE-criterium, $X^2 = 1.90$, based on the rule of thumb that the HWE must be lower than the critical value of 3.84. More detailed information can be found in Table 1.

BETTER

The aim of the Biological Effects of Traumatic Experiences, Treatment, and Recovery (BETTER) study was to investigate the neurobiological mechanisms of recovery from PTSD. Understanding the neural mechanisms underlying treatment response can contribute to improving response rates. The extensive study design of the different aspects is explained in 'Getting Better' of Van Rooij (2015).

Participants

The study population consists of three different groups: PTSD patients (*n*=58), combat controls (*n*=29), and healthy controls (*n*=26). Veterans with PTSD were recruited from the Military Mental Healthcare outpatient clinics of the Ministry of Defense in The Netherlands. These 58 PTSD patients were included and examined close to the start of their treatment. Twenty-nine healthy veterans (combat controls) were included as a control group for PTSD patients. In addition to veterans, 26 non-military healthy samples were included to control for the effects of deployment. Because the primary focus of this study is on the effects of the BDNF Val66Met polymorphism on PTSD treatment response, only PTSD patients are included. PTSD patients were eligible for the study if they participated in a military deployment for minimally four months, had a PTSD diagnosis according to the DSM-IV, and had a CAPS score higher than 45. To exclude potential geriatric symptomology, only participants between 18 and 60 were included and they all had to provide written consent. Exclusion criteria included substance abuse, severe neurological disorders, claustrophobia, and the presence of a pacemaker or metal objects in the body that would prevent a Magnetic Resonance Imaging (MRI) scan (Appendix D). Due to missing data and drop-outs, the analysis in the current study was performed with data from 51 PTSD patients.

Measurements

Several questionnaires and clinical interviews were used to examine symptom reduction and prior exposure to potentially traumatic events. Participants underwent three assessments to evaluate the effects of treatment over time. For the current study, only the first and third assessment are analyzed. The first assessment took place one week before the start of treatment (T_0), and the third assessment took place six months after treatment began (T_6) (Figure 2). At both T_0 and T_6 , the Clinical-Administered PTSD Scale (CAPS; Hovens et al., 1994) was examined to determine the severity of PTSD-related symptoms and the presence of comorbidity. The Mood and Anxiety Symptom Questionnaire (MASQ; Clark & Watson, 1991) was also administered at T_0 and T_6 to assess depression and anxiety symptoms. The MASQ includes five subscales: Anhedonic Depression (MASQ-AD), Anxious Arousal (MASQ-AA), General Distress Depression (MASQ-GDD), General Distress Anxiety (MASQ-GDA), and General Distress Mixed (MASQ-GDM). For this study, the MASQ-GDD and MASQ-GDA subscales were specifically used to assess depression and anxiety symptoms. Due to missing data and participant dropouts, the analysis for depression and anxiety includes 42 participants. Additionally, the ETI was administered at T_0 to control for traumatic childhood experiences. A detailed overview of the various questionnaires administered at different time points can be found in Table C2.

Genotyping

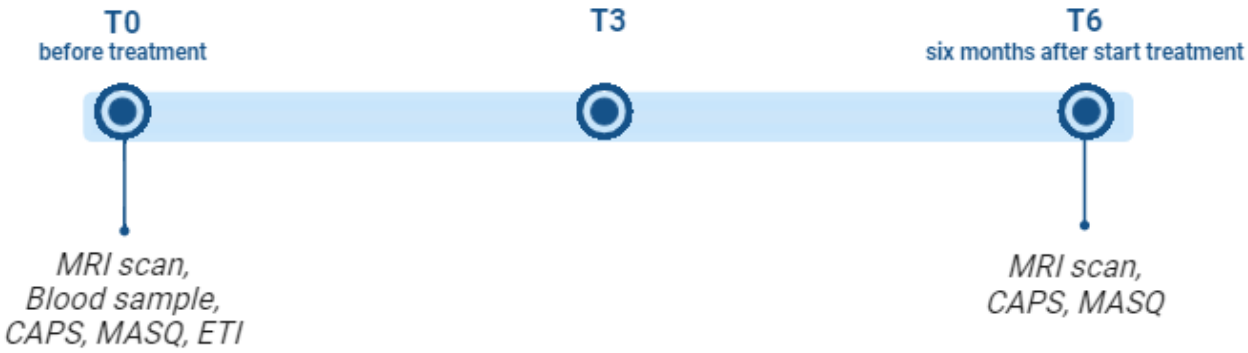
The genotyping process used to determine the genetic variation of the BDNF Val66Met polymorphism in the BETTER study follows the same methodology as for the PRISMO study. Blood samples were collected through venipuncture, and DNA extraction was performed following standard procedures. The concentration and quality of the extracted DNA were assessed using Nanodrop (Thermo Fisher Scientific, MA, USA). Genotyping was conducted using Illumina Human OmniExpress 24 v1.1 (Leen, 2024c). Lastly, the genetic variations of the BDNF Val66Met polymorphism were extracted using PLINK software version 1.9 (Purcell et al., 2007).

Image acquisition and image processing

To examine structural and functional changes in the brain, a 3.0 Tesla MRI scanner (Philips Medical System, Best, the Netherlands) at the University Medical Center Utrecht was used. A T1-weighted image (200 slices, repetition time = 10 ms, echo time = 3.8 ms, flip angle = 8° , field of view = 240 x 240 x 160 mm, matrix of 304 x 299) was used for within-subject registration purposes. To estimate the volumes of the left and right hippocampus, the validated Freesurfer software (Version 5.1.0.) was used. For these volumes, neuroanatomical labels were automatically assigned (Van Rooij et al., 2015). Segmentation of the hippocampus was visually inspected before analysis was performed. Moreover, subcortical labeling was inspected following the standardized ENIGMA protocol (Van Rooij et al., 2015). MRI scans were obtained to examine the effects of treatment outcomes on the brain. Forty-seven male war veterans with PTSD were included. However, one patients was excluded due to poor quality caused by movement, another was excluded due to severe temporal lobe atrophy, and a third patient was excluded because he did not receive treatment in between the two MRI scans. This resulted in a final MRI dataset of 44 PTSD patients.

Figure 2

Timeline of the BETTER study



Note. Figure 2 illustrates the timeline of the Biological Effects of Traumatic Experiences, Treatment, and Recovery (BETTER) study, showing the specific measurement time points. At T_0, participants undergo a MRI scan, provide blood samples, and complete the Clinician-Administered PTSD Scale (CAPS), the Mood and Anxiety Symptom Questionnaire (MASQ), and the Early Trauma Inventory (ETI). At T_6, a second MRI is performed, and the CAPS and the MASQ are administered again. The figure was created using Biorender.

Statistical Analysis

Before conducting the analysis of the BETTER study data, missing data and participant dropout were first excluded. The analysis regarding PTSD recovery after treatment, required the CAPS scores from both pre-treatment and post-treatment assessments (T_0 and T_6). Consequently, the analysis was based on data from 51 patients. Secondary outcomes, such as the impact of Val66Met on depression and anxiety symptoms, were evaluated using completed MASQ assessments, involving 42 patients. Lastly, the effect of the Val66Met polymorphism on hippocampal volume was conducted with data from 44 patients. Information on the BDNF polymorphism of the patients was required for all analysis. Statistical analyses of these data were conducted using JASP statistics (version 0.18.3). A linear regression analysis is used to determine how the Val66Met polymorphism influences the recovery of PTSD symptoms. The CAPS was used as the dependent variable in the model, with BDNF polymorphism, Time, and ETI as independent variables. The assumptions for the regression analysis are met since we can assume that the data is normally distributed due to the high number of participants. Additionally, the data must meet the assumption of homoscedasticity, which is done with Levene's test ($F_{(1,100)} = 0.866$, $p = .354$), indicating a constant variance in the regression model. Finally, genetic data must adhere to Hardy-Weinberg Equilibrium (HWE). The BETTER dataset met the HWE-criterium, $X^2 = 0.14$, based on the rule of thumb that the HWE must be lower than the critical value of 3.84. More detailed information can be found in Table 1.

Table 1

Calculations for Hardy-Weinberg Equilibrium for the PRISMO and BETTER study

	Observed	Allele frequency	Genotype frequency	Expected	Chi-Squared value
PRISMO					$X^2 = 1.899$
Val/Val	334	p = 0.81	$p^2 = 0.656$	341	
Val/Met	171		2pq = 0.308	160	
Met/Met	12	q = 0.19	$q^2 = 0.036$	16	
BETTER					$X^2 = 0.142$
Val/Val	31	p = 0.77	$p^2 = 0.599$	30	
Val/Met	17		2pq = 0.356	18	
Met/Met	3	q = 0.23	$q^2 = 0.051$	3	

Note. Table 1 presents the observed genotype counts, allele frequencies, genotype frequencies, and expected genotype counts for the Prospective Research in Stress-Related Military Operations (PRISMO) and Biological Effects of Traumatic Experiences, Treatment, and Recovery (BETTER) studies, along with the chi-squared (X^2) values. The three different genotypes are Val/Val, Val/Met, and Met/Met. The chi-squared values ($X^2 = 1.899$ for PRISMO and $X^2 = 0.142$ for BETTER) are below the critical value of 3.84, indicating that both study populations are in Hardy-Weinberg Equilibrium (HWE). Here, *p* represents the frequency of the Val allele, and *q* represents the frequency of the Met allele. The expected genotype frequencies were calculated using the Hardy-Weinberg equation (p^2, $2pq$, and q^2).

Results

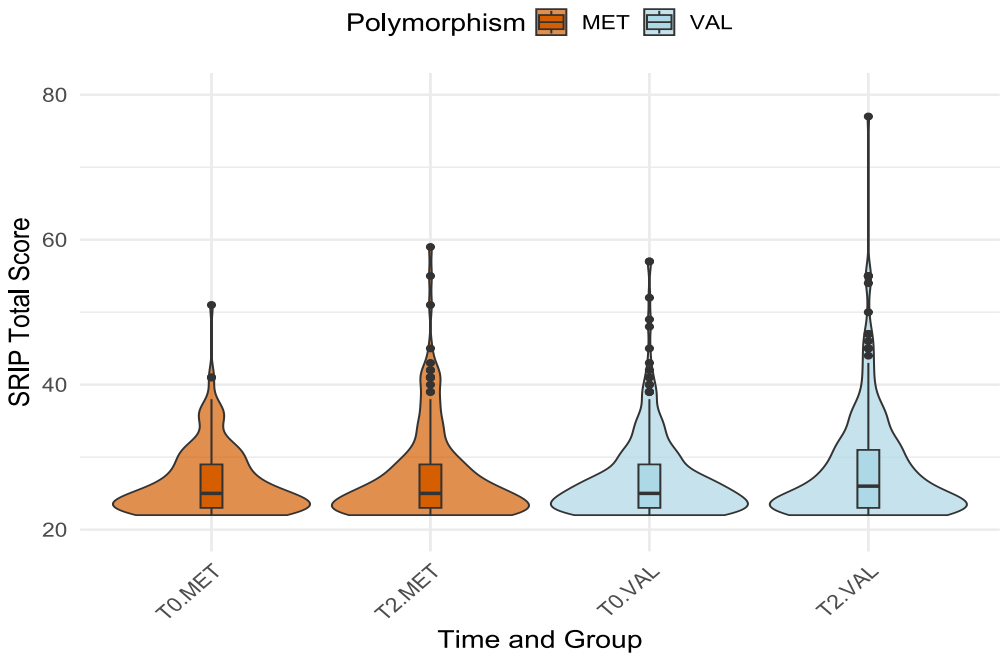
PRISMO

Demographics

Demographic information of the PRISMO data are presented in Table 2. Data were collected from 517 participants, including 469 men and 48 women, with an average age of 28.8 years (range = 18 - 57 years, SD = 9.0 years). The majority of participants (38.1%) held the rank of Private prior to deployment, and nearly half (49.5%) of the 517 participants had no prior deployment experience. Demographic details for the Val and Met groups separately are presented Table E1. A Chi-Square Test indicated no significant differences between the groups with respect to gender, age, rank, and previous deployment. The statistical details are also provided in Table E1.

Figure 3

SRIP scores for the Val and Met group



Note. This figure shows the total score of the Self-Rating Inventory for PTSD (SRIP) questionnaire for the Val and Met group. The SRIP score is represented on the y-axis. On the x-axis, the Val and the Met group are plotted against each other. The difference between the time points is visualized, where T_0 is before deployment and T_2 is six months after the military returned home from deployment. The violin plots show the scatter and the boxplots inside represents more precise the average SRIP score of each group.

Effect of BDNF on PTSD symptoms

Val vs. Met Figure 3 shows the results of PTSD symptom severity before and after deployment to Afghanistan ($F_{(5,1028)}$ = 13.878, p < .001). Significant main effects on PTSD symptom severity are found for the covariates, including the number of deployment experiences (*b* = 0.086, *p* = .001) and early trauma (*b* = 0.458, *p* < .001). However, no significant main effect was found on either time or the Val/Met groups on PTSD symptom severity. Moreover, no interaction between Val/Met and Time was observed for PTSD symptom severity before and after deployment (Table 4).

BETTER

Demographics

Demographics details of the BETTER data are provided in Table 3. The study included data from 51 military personnel diagnosed with PTSD, comprising 50 men and one women. All PTSD patients met the DMS-IV criteria for PTSD, as confirmed by a CAPS score ≥ 45. The average age of the patients was 37 years (range = 21 - 57, SD = 9.7 years). A substantial proportion of the patients (38.8%) had been deployed once. Table E2 presents the demographic information for the Val and Met groups separately. Analysis using a Chi-Square Test revealed no significant differences between the groups in terms of gender, age, rank, and prior deployment. Detailed statistical information can also be found in Table E2.

Effect of BDNF on PTSD treatment recovery

Val66Met and treatment outcome Figure 4A displays the change in PTSD symptoms before treatment and six months after treatment began ($F_{(4,97)}$ = 7.350, *p* < .001). The results of the linear regression analysis are provided in Table 5. The analysis revealed no significant main effect of the Val66Met polymorphism (b = 2.148, *p* = .721), indicating that this polymorphism does not affect symptom recovery. Additionally, no significant main effects of time (b = - 17.437, *p* = .165) or early trauma (b = 0.124, *p* = .767) were found. Moreover, no interaction effect between Val/Met and Time

was found (b = -3.423, p = .686). This suggests that neither the Val66Met polymorphism nor early trauma influences PTSD symptom severity. Figure 4B illustrates the difference between depression and anxiety symptoms before treatment and six months after treatment began ($F_{(4,79)}$ = 1.321, p = .269). No significant main effect of the Val66Met polymorphism on symptom severity was observed (b = 2.943, p = .644), nor was there a significant main effect of time on symptom severity (b = -0.706, p = .958). Additionally, no significant main effect of early trauma or interaction effect between Val/Met and Time was found (b = -5.603, p = .531). These results suggest that neither the Val66Met polymorphism nor time significantly impacts anxiety and depression symptoms. Further analysis aimed to determine whether the Val66Met polymorphism specifically influences anxiety or depression symptoms individually. However, also no significant findings were observed. The violin plot depicting anxiety symptoms is presented in Figure F1A , while the plot for depression symptoms is shown in Figure F1B . Detailed results from the linear regression analysis are provided in Table F1.

Val66Met and hippocampal volume Figure 5 illustrates the differences in hippocampal volume before treatment and six months after the initiation of treatment, with the corresponding statistical outcomes detailed in Table 5. Specifically, no significant increase in hippocampal volume over time was observed for the left hippocampus, $F_{(3,90)}$ = 0.442, p = .723 (Figure 5A). Additionally, no significant main effects were detected for the Val/Met groups (b = -20.498, p = .878) or for time (b = 205.130, p = .458). A similar analysis for the right hippocampus showed no significant main effects for the Val/Met groups (b = -163.885, p = .165) or for time (b = - 44.464, p = .855) (Figure 5B). Lastly, no significant interaction effects between Val/Met and time were found for either the left (b = -119.510, p = .526) or the right hippocampus (b = 13.843, p = .934).

Table 2

Pre-deployment characteristics of PRISMO

Variable	N	%
Gender		
Male	469	90.7
Female	48	9.3
Age[a] (years)		
Mean	28.8 ± 9.0	
Minimum	18	
Maximum	57	
Rank[b]		
1 – Private	197	38.1
2 - Corporal	101	19.5
3 – Non-commissioned officer (NCO)/Sergeant	148	28.6
4 – Junior officer/Subaltern	54	10.4
5 – Senior officer	17	3.3
Genotype		
Val/Val	334	64.6
Val/Met	171	33.1
Met/Met	12	2.3
Previous deployments[c]*		
0	249	49.5
1	129	25.6
2	64	12.7
≥ 3	61	12.1

Note. Table 2 shows pre-deployment characteristics of the Prospective research in Stress-Related Military Operations (PRISMO) cohort (N = 517). The percentages reflect the proportion of each characteristic within the cohort.
[a] The age data are reported as mean ± standard deviation.
[b] Rank is categorized into five levels, which each level representing a specific rank within the military hierarchy.
[c] Previous deployments are categorized into four groups: 0, 1, 2, and 3 or more.
*Sample sizes might not add up to total participants due to missing data in the descriptive values.

Table 3

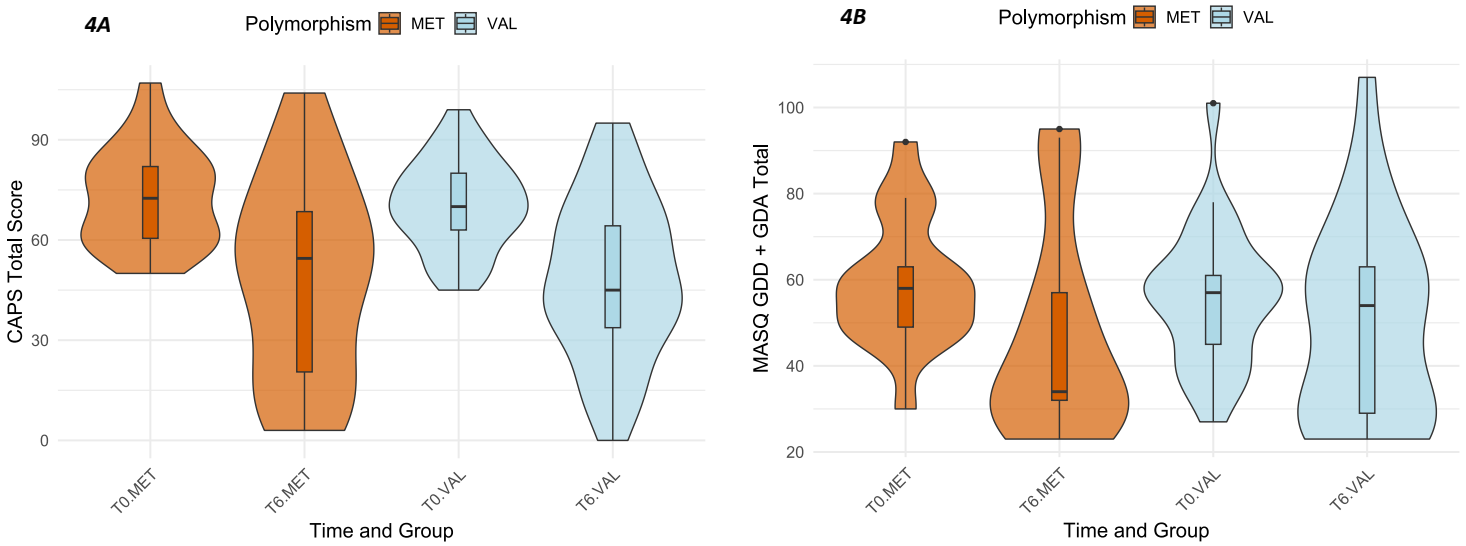
Participant characteristics of BETTER

Variable	N	%
Gender		
Male	50	98
Female	1	2
Age[a] (years)		
Mean	37.0 ± 9.7	
Minimum	21	
Maximum	57	
Rank[b]*		
1 – Private	13	28.9
2 - Corporal	17	37.8
3 – Non-commissioned officer (NCO)/Sergeant	12	26.7
4 – Junior officer/Subaltern	1	2.2
5 – Senior officer	2	4.4
Genotype		
Val/Val	31	60.8
Val/Met	17	33.3
Met/Met	3	5.9
Previous deployments[c]*		
1	19	38.8
2	11	22.4
3	10	20.4
≥ 4	9	18.4

Note. Table 3 shows participant characteristics of the Biological Effects of Traumatic Experiences, Treatment and Recovery study (N = 51). The percentages reflect the proportion of each characteristic within the cohort.
[a] The age data are reported as mean ± standard deviation.
[b] Rank is categorized into five levels, which each level representing a specific rank within the military hierarchy.
[c] Previous deployments are categorized into four groups: 1, 2, 3, and 4 or more.
*Sample sizes might not add up to total participants due to missing data in the descriptive values.

Figure 4

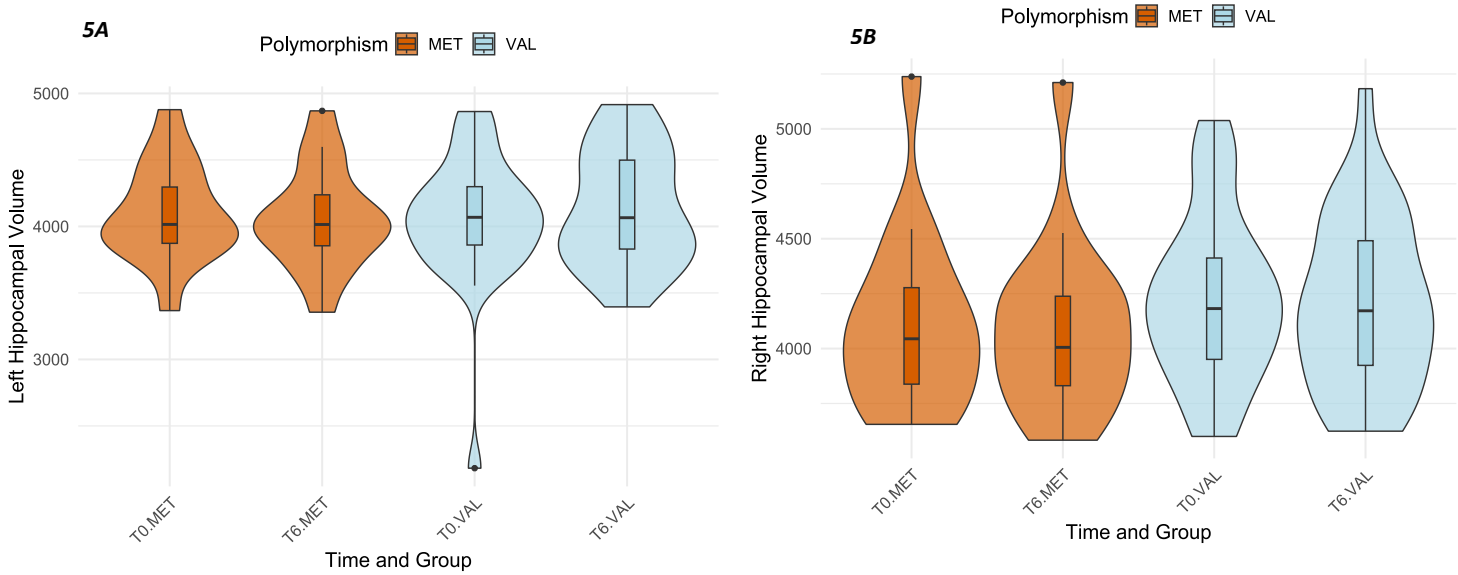
Violin plots from the CAPS and MASQ score of the Val and Met group



Note. Figure 4A shows the total score of the Clinical-Administered PTSD Scale (CAPS) for the Val and Met group. Figure 4B shows the total score from the General Distress Depression and General Distress Anxiety (GDD and GDA) subscales of the Mood and Anxiety Symptom Questionnaire (MASQ) for the Val and Met group. In both figures, the x-axis represents time, labeled as T_0 and T_6 . T_0 is before deployment and T_2 is six months after the military returned home from deployment. Boxplots are incorporated within the violin plots to provide a more detailed overview.

Figure 5

Violin plots from the hippocampal volume of the Val and Met group



Note. Figure 5A shows the volume of the left hippocampus for the Val and Met group. Figure 5B shows the volume of the right hippocampus for the Val and Met group. In both figures, the x-axis represents time, labeled as T_0 and T_6 . T_0 is before deployment and T_2 is six months after the military returned home from deployment. Boxplots are incorporated within the violin plots to provide a more detailed overview.

Table 4

Statistical outcomes of the PRISMO study

			PRISMO		95% CI	
PTSD development	*b*	*S.E.*	*t*	*p*	Lower	Upper
Val/Met	-0.654	0.546	-1.199	.231	-1.725	0.416
Time	1.150	1.108	0.299	.299	-1.023	3.324
DES	0.086	0.026	3.266	***.001****	0.035	0.138
ETI	0.458	0.069	6.620	***< .001****	0.322	0.594
Val/Met * Time	-0.135	0.771	-1.075	.861	-1.649	1.379

Note. The table shows the statistical outcomes of the linear regression analysis for variables related to PTSD symptom development from Prospective Research In Stress-Relation Military Operations (PRISMO). *Val/Met* refers to the Val/Met genotype, *Time* indicates the time point of measurement, and the interaction term *Val/Met * Time* examines whether the effect of the Val/Met genotype on PTSD development changes over time. *DES* refers to Deployment Experiences Scale, and *ETI* refers to the Early Trauma Inventory. The values presented include regression coefficients (*b*), standard errors (*S.E.*), *t*-values, *p*-values, and 95% confidence intervals (Lower and Upper bounds).
Statistical significance is indicated by p-values less than .05. In this analysis, the DES ($p = .001$) and ETI ($p < .001$) are statistically significant. * $p < .05$

Table 5

Statistical outcomes of the BETTER study

			BETTER		95% CI	
CAPS	*b*	*S.E.*	*t*	*p*	Lower	Upper
Val/Met	2.148	5.986	0.359	.721	-9.733	14.028
Time	-17.437	12.460	-1.399	.165	-42.166	7.293
ETI	0.124	0.417	0.297	.767	-0.704	0.952
Val/Met * Time	-3.423	8.446	-0.405	.686	-20.186	13.340
MASQ-GDD + MASQ-GDA						
Val/Met	2.943	6.336	0.464	.644	-9.669	15.554
Time	-0.706	13.248	-0.053	.958	-27.076	25.663
ETI	0.408	0.450	0.908	.367	-0.487	1.303
Val/Met * Time	-5.603	8.904	-0.629	.531	-23.326	12.120

Left Hippocampal Volume	*b*	*S.E.*	*t*	*p*	95% CI Lower	Upper
Val/Met	-45.063	137.944	-0.327	.745	-319.379	229.254
Time	-36.938	155.621	-0.237	.813	-346.408	272.533
Val/Met * Time	121.938	195.082	0.625	.534	-266.004	509.879
Right Hippocampal Volume						
Val/Met	113.027	122.653	0.922	.359	-130.881	356.935
Time	-18.750	138.371	-0.136	.893	-293.915	256.415
Val/Met * Time	-7.714	173.457	-0.044	.965	-352.652	337.224

Note. The table presents the statistical outcomes of the linear regression analysis for variables related to the Biological Effects of Traumatic Experiences, Treatment, and Recovery (BETTER) study. The results are organized by four measures: the Clinical-Administered PTSD Scale (CAPS), the Mood and Anxiety Symptom Questionnaire with the General Distress Depression (MASQ-GDD) and General Distress Anxiety (MASQ-GDA) subscales, left hippocampal volume, and right hippocampal volume.
Val/Met refers to the Val/Met genotype, *Time* indicates the time point of measurement, and the interaction term *Val/Met * Time* examines whether the effect of the Val/Met genotype on PTSD development changes over time. *DES* refers to Deployment Experiences Scale, and *ETI* refers to the Early Trauma Inventory. The values presented include regression coefficients (*b*), standard errors (*S.E.*), *t*-values, *p*-values, and 95% confidence intervals (Lower and Upper bounds).
Statistical significance is indicated by p-values less than .05. This analysis reveals no significant findings.

Discussion

This study investigated the effect of the BDNF Val66Met polymorphism on the development and recovery of PTSD symptoms in Dutch military personnel. Firstly, no differences were found between the Val homozygotes and Met allele carriers in terms of developing PTSD symptoms after deployment to Afghanistan. Although the Val66Met polymorphism does not affect the development of PTSD symptoms, deployment experience and early trauma both influence PTSD symptom severity. Secondly, treatment response is not affected by the Val66Met polymorphism, indicating that Val66Met does not affect recovery of anxiety, depression, or trauma-related symptoms. Lastly, no effects of BDNF Val66Met polymorphism on hippocampal volume were found.

The findings of this study are in line with the results of Pivac and colleagues (2012), who found that BDNF Val66Met does not influence the development of combat-related PTSD symptoms. Moreover, recent meta-analyses have produced conflicting evidence for the association between the BDNF Val66Met polymorphism and the risk of developing PTSD symptoms. While some research found that Met allele carriers do not display elevated risks for PTSD symptom development compared to Val homozygotes, significant findings do emerge when more restrictive inclusion criteria are used (Bountress et al., 2017). For example, after excluding studies that did not meet HWE expectations, Met allele carriers were found to be more likely to have PTSD symptoms compared to Val homozygotes (Bruenig et al., 2016). Additionally, research on BDNF Val66Met and PTSD symptoms in trauma-exposed US veterans found that Met allele carriers displayed higher lifetime PTSD symptom severity (Pitts et al., 2019). However, this study differs methodologically from the current study, in which we examined the prediction of Val66Met polymorphism on recovery and development, whereas Pitts found associations. Another explanation for the lack of effects in our study could be the importance of trauma type in determining Val66Met as a risk factor for the development of PTSD symptoms. La Greca et al. (2013) and Dai and colleagues (2017) demonstrate a gene-environment interaction for a significantly higher occurrence of PTSD symptoms among Met allele carriers in a population exposed to natural disasters. They distinguish several specific natural

disaster stressors that caused PTSD symptoms, indicating that the Val66Met polymorphism is an event-specific biomarker for developing PTSD.

Other emerging evidence that BDNF Val66Met may not be related to the development of PTSD symptoms is confirmed by multiple Genome-Wide Association Studies (GWAS) that are performed to map genetics involved in PTSD. Research among U.S. veterans from the Million Veteran Program (Stein et al., 2021; Gelernter et al., 2019) and Danish veterans (Wang et al., 2019) show specific genes and loci that influence the development of PTSD. Here, the BDNF genotype is not mentioned, indicating that the Val66Met polymorphism has no clear association with the risk of developing PTSD symptoms (Stein et al., 2021; Gelernter et al., 2019; Wang et al., 2019).

In addition to investigating the effect of the BDNF Val66Met polymorphism on PTSD symptom development, we also examined the influence of Val66Met on treatment recovery. Our findings did not indicate a significant effect of Val66Met on PTSD treatment recovery. The absence of an observed effect may be due to differences in the types of trauma treatment administered, which could alter the influence of the Val66Met polymorphism on treatment response. The essential role of the hippocampus in learning and memory processes could potentially explain this relationship with treatment response (van Rooij, 2015). Previous studies by Felmingham et al. (2013) and Nicholson and colleagues (2023) revealed an impaired treatment response to cognitive behavioral therapy (CBT) in PTSD patients carrying the Met allele compared to Val homozygotic patients. CBT, a type of exposure therapy, aims to facilitate the extinction of conditioned fear (Felmingham et al., 2013). However, the Val66Met polymorphism influences cognitive functions like memory and recall ability (Mestrovic et al., 2020). The absence of an effect of Val66Met on PTSD treatment response in our study may be attributable to the type of treatment administered. In contrast to the study by Felmingham et al. (2013), which exclusively focused on CBT, our study did not control for treatment modality, which could explain the discrepancy in findings.

Furthermore, we investigated the influence of the Val66Met polymorphism on depression and anxiety through the MASQ-GDD and MASQ-GDA subscales. However, the results did not indicate

any significant effects. Although animal studies have demonstrated that the Met allele is related to both depression and anxiety-like behaviors (Chen et al., 2006), evidence for associations with trait anxiety or anxiety disorders in humans is less prevalent compared to the associations with depression. Montag et al. (2008) therefore aimed to investigate the association between the BDNF Val66Met polymorphism and the anxiety-related personality trait of harm avoidance. Harm avoidance has been associated with greater severity in patients diagnosed with anxiety disorders. In the study by Montag and colleagues (2018), the impact of Val66Met suggests a link to a disposition to develop generalized anxiety disorder (Montag et al., 2008; Moreira et al., 2015). Although the results of these studies do not align with our findings, several differences may account for this discrepancy. Montag et al. (2008) found stronger associations of Val66Met with certain subscales of anxiety-related personality traits. In our study, the lack of examination of specific anxiety subscales may explain the absence of an association found. Additionally, the MASQ-GDA comprises eleven questions, which may not have provided sufficient information about anxiety to draw a solid conclusion. A similar explanation may apply to the absence of significant findings regarding the effect of BDNF Val66Met on depression. Another possible explanation is the role of comorbid depression with PTSD. The study by Pivac et al. (2012) investigated the impact of comorbidity and found no significant effect of the Val66Met polymorphism on PTSD comorbid with major depressive disorder or anxiety-depressive disorder. The influence of depression and anxiety as comorbid conditions in the current study contributes to the absence of significant findings.

As for the last research question, no significant effects were found regarding the influence of Val66Met on hippocampal volume. This finding is not in line with existing literature, which indicates that total hippocampal volume is smaller in Met allele carriers compared to Val homozygotes (Molendijk et al., 2012). However, evidence regarding the role of Val66Met is inconclusive, with meta-analyses revealing conflicting patterns of results (Miranda et al., 2019). Kambeitz et al. (2012) reported reductions in declarative memory performance, hippocampal activation, and hippocampal volume in carriers of the Met allele compared to Val homozygotes. However, Dodds et al. (2013)

argued that Kambeitz did not accurately quantify the true effect size of the influence of the Val66Met polymorphism. They contend that the hippocampal effects are likely to be substantially smaller than those reported in the meta-analysis by Kambeitz (Dodds et al., 2013; Miranda et al., 2019). The observation that a smaller hippocampal volume may not be a direct cause of traumatic events but rather a risk factor for the persistence of PTSD is also supported by twin studies (Gilbertson et al., 2002). This finding might also explain why hippocampal volumes are not increased after PTSD treatment.

The current study has several limitations. First, it is important to note that for both PRISMO and BETTER, the effect of BDNF Val66Met was not the primary outcome measure. The BETTER study was primarily conducted to observe how patients with PTSD respond to trauma treatment, without focusing on BDNF. This led to missing data for the various research questions within the BETTER study. The same issue applies to PRISMO, where data from over 400 participants could not be used because it was incomplete for our study. Some of these participants may have had severe symptoms that prevented them from completing parts of the research, potentially leading to a distorted perspective. Secondly, no distinction was made between the types of traumatic stressors experienced by veterans. The influence of Val66Met on traumatic exposure and, consequently, on the development of PTSD symptoms may depend on the type of stressors experienced. Lastly, PRISMO predominantly relies on self-report measures, which are inherently subject to biases common in such studies. Although standardized and validated screening instruments were used to assess the prevalence of mental health problems, this approach might have resulted in different prevalence estimates compared to those obtained through clinician diagnoses (Van Der Wal et al., 2019).

Conclusion

Our findings suggest that the BDNF Val66Met polymorphism neither influences the development of PTSD-related symptoms nor impacts treatment response in combat-exposed military personnel.

Additionally, no significant differences were found in hippocampal volume between Met allele carriers and Val homozygotes after PTSD treatment. To build on these results, future research could consider utilizing the ENIGMA study, which combines large-scale neuroimaging and genetic data, to investigate the potential impact of other genetic variations on PTSD symptom development and hippocampal volume. Furthermore, exploring underlying differences in hippocampal volume using such comprehensive data could help determine whether these variations might be a risk factor for the persistence of PTSD.

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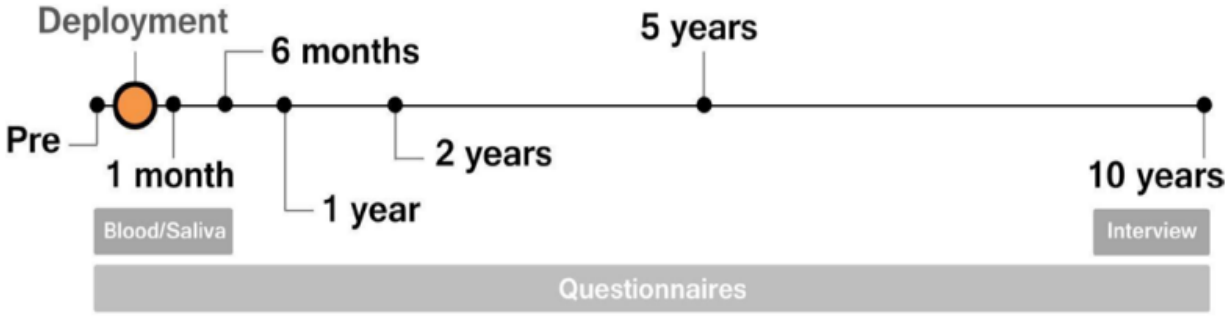
Appendix

APPENDIX A. Timeline PRISMO

The timeline of the Prospective Research in Stress-Related Military Operations (PRISMO) study states the different measurement time points. The longitudinal study lasted for ten years after deployment, in which seven measurements were performed. The baseline measurement (T_0) was carried out approximately one month before deployment and completed at the army base. The first two follow-up assessments were also completed at the army base, approximately one month (T_1) and six months (T_2) after the military personnel returned home. The 1-year (T_3), 2-year (T_4), and 5-year (T_5) assessments were completed at home. Questionnaires were sent in by mail (T_3 and T_4) or were completed online (T_5). The 10-year follow-up (T_6) is conducted at the Research Centre of the Military Mental Healthcare.



Note. Timeline of the Prospective research in Stress-Related Military Operations (PRISMO) study. Adapted from *Cohort profile: the Prospective Research In Stress-Related Military Operations (PRISMO) study in the Dutch Armed Forces*, by S. J. Van der Wal, R. Gorter, A. Reijnen, E. Geuze, & E. Vermetten, 2019, *BMJ Open*, 9, e022670.

APPENDIX B. DES Scoring

The Deployment Experience Scale (DES) includes nineteen questions asking whether someone has experienced a particular event and, if so, how frequently. This appendix provides information on the combat-related stressors and the DES scoring method utilized in the current study.

Table B1

Combat-related stressors

Items	**Items experienced**	
	Counts	*%*
Enemy fire		
Witnessed people suffering		
Witnessed wounded		
Colleague injured or killed		
Incoming fire		
Witnessed dead		
Rejected by locals		
Personal danger		
Witnessed others injured/killed		
Heard people screaming		
Insufficient means to intervene		
Mission experienced as useless		
Insufficient control over situation		
Memories of earlier deployments		
Traffic incident		
Held at gunpoint		
Physical injuries		
Colleague held hostage		
Held hostage		

Note. The nineteen items on the left represent the type of combat-related stressors someone can experience. The table is adapted from *Prevalence of Mental Health Symptoms in Dutch Military Personnel Returning from Deployment to Afghanistan: A 2-year Longitudinal Analysis,* by A. Reijnen, A. Rademaker, E. Vermetten & E. Geuze, 2015, *European Psychiatry, 30*(2), 341-346.

Table B2

Scoring method DES

The answers to how frequently someone has experienced particular events are broadly characterized as follows:

1. No, I have not experienced it (0)
2. Yes, I have experienced it (1). This is further dived into frequency scores:
    a. Once
    b. 2-5 times
    c. 6-10 times
    d. More than 10 times
    e. Not sure
    f. Not sure, but more than once

Upon reviewing the questionnaire, it becomes evident that some individuals have encountered certain events (such as being shot at or coming under fire) quite frequently during their missions. However, the current method only considers the cumulative number of events experienced, without accounting for the frequency of these occurrences, which can range from once to more than 10 times. To address this, it is proposed to adjust for the frequency of occurrences, resulting in the following approach:

	score		**score**
Experienced?	1	**Not experienced?**	0
Once	1		
2-5 times	2		
6-10 times	3		
More than 10 times	4		
Not sure	1		
Not sure, but more than once	2		

An advantage of this approach is that higher scores would indicate not only that an individual has experienced more events but also that they have been exposed to these events more frequently.

APPENDIX C. Questionnaires

Appendix C provides a comprehensive overview of all questionnaires and measurement points used in the Prospective Research in Stress-Related Military Operations (PRISMO) study and the Biological Effects of Traumatic Experiences, Treatment, and Recovery (BETTER) study.

Table C1

Questionnaires PRISMO

	T_0: Pre-deployment	**T_1: 1-month follow-up**	**T_2: 6-month follow-up**	**T_3: 1-year follow-up**	**T_4: 2-year follow-up**	**T_5: 5-year follow-up**	**T_6: 10-year follow-up**
(Epi)Genetics	Plasma	Plasma	Plasma				
Immunology	Plasma	Plasma	Plasma				
Neuroendocrinology	Plasma	Plasma, Salivary	Plasma, Salivary				
Demographic factors	Self-report	Self-report	Self-report	Self-report	Self-report	Self-report	Self-report, interview
General health and psychological mobility							
- Physical health	Self-report	Self-report	Self-report	Self-report	Self-report		Interview
- Psychological symptoms	SCL-90-R	SCL-90-R	SCL-90-R	SCL-90-R	SCL-90-R	BSI	SCL-90-R, M.I.N.I Plus
- Depression				CES-D	CES-D	CES-D	CES-D
- PTSD	SRIP	SRIP	SRIP	SRIP	SRIP	SRIP	SRIP
- Fatigue	CIS-20R	CIS-20R	CIS-20R	CIS-20R	CIS-20R	CIS-20R	CIS-20R
- Alcohol use							AUDIT
- Burnout	UBOS	UBOS	UBOS				
- Quality of life							SF-36
- Healthcare utilization						Self-report	Self-report
- Production losses						SF-HLQ	SF-HLQ
Life events							

- Life events				Self-report	Self-report	Self-report	Self-report
- Early trauma	ETISR-SF						
Personality and coping							
- Hostility	CMHS	CMHS	CMHS	CMHS	CMHS		
- Type-D personality	DS-14	DS-14	DS-14	DS-14	DS-14	DS-14	
- Temperament and character	TCI-SF	TCI-SF	TCI-SF		TCI-SF	TCI-SF	TCI-SF
- Anger							STAXI-2
- Coping style				Brief-COPE	Brief-COPE	Brief-COPE	
Social support							
- General support						SSL-6	SSL-6
- Deployment social support				DRRI-F	DRRI-F		
- Postdeployment support				DRRI-L	DRRI-L		
Deployment experience							
- Combat exposure		DES					Interview
- Traumatic blast						BTBIS	
- Reintegration after deployment				PDRS	PDRS		
- Moral injury							MIQ-M
- Meaning							ZGL

Note. AUDIT, Alcohol Use Disorders Identification Test; Brief-COPE, Brief COPE Inventory; BSI, Brief Symptom Inventory; BTBIS, Brief Traumatic Brain Injury Screen; CES-D, Centre for Epidemiologic Studies Depression Scale; CIS-20R, Checklist Individual Strength; CMHS, Cook-Medley Hostility Scale; DES, Deployment Experience Scale; DRRI, Deployment Risk and Resilience Inventory; DS-14, Type-D Scale; ETISR-SF, Early Trauma Inventory-Self Report; GR, Glucocorticoid receptor; M.I.N.I. Plus, MINI-International Neuropsychiatric Interview-Plus; MIQ-M, Moral Injury Questionnaire-Military version; PBMC, Peripheral blood mononuclear cells; PDRS, PostDeployment Reintegration Scale; SCL-90-R, Symptom CheckList; SF-36, Medical Outcome Study Short-Form Survey; SF-HLQ, Short Form-Health and Labour Questionnaire; SHBG, Sex hormone-binding globulin; SNP, Single nucleotide polymorphism; SRIP, Self-Rating Inventory for PTSD; SSL-6, Social Support List; STAXI-2, State-Trait Anger Expression Inventory-2; TCI-SF, Temperament and Character Inventory-Short Form; UBOS, Utrecht Burnout Scale; ZGL, Zingevingslijst. Adapted from *Cohort profile: the Prospective Research In Stress-Related Military Operations (PRISMO) study in the Dutch Armed Forces,* by S. J. Van der Wal, R. Gorter, A. Reijnen, E. Geuze, & E. Vermetten, 2019, *BMJ Open*, 9, e022670.

Table C2

Questionnaires BETTER

	T_0: before treatment	**T_3: three months after start treatment**	**T_6: six months after start treatment**
Questionnaires			
To correct for potential cofounders			
	TCI		TCI
	BIS/BAS		BIS/BAS
	ETI		ETI
To objectify treatment effect			
	SRS-PTSD	SRS-PTSD	SRS-PTSD
	MASQ	MASQ	MASQ
	COPE	COPE	COPE
	STAI	STAI	STAI
	SDS	SDS	SDS
	WHO-QoL		WHO-QoL

Note. TCI, Temperament and Character Inventory; BIS/BAS, Behavioral Inhibition System – Behavioral Activation System Scales; ETI, Ealy Trauma Inventory; SRS-PTSD, Self-Rating Scale for PTSD; MASQ, Mood and Anxiety Symptoms Questionnaire; COPE, COPE Inventory, STAI, State-Trait Anxiety Inventory; SDS, Self-Rating Depression Scale; WHO-QoL, World Health Organization Quality of Life.

APPENDIX D. Inclusion and Exclusion Criteria BETTER

To participate in the Biological Effects of Traumatic Experiences, Treatment, and Recovery (BETTER) study, participants must meet specific inclusion criteria. The table below outlines the criteria that must be met for each group: PTSD patients, combat controls, and healthy controls. The table also specifies the exclusion criteria. Individuals who meet these criteria are not eligible to participate in the study.

Inclusion Criteria
PTSD patients
- Military personnel or veterans who have participated in a military deployment (minimum of 4 months).
- PTSD according to DSM-IV criteria and confirmed by a diagnostic interview with a CAPS score higher than 45.
- Age between 18-60.
- Written consent
Combat controls
- Military personnel or veterans who have participated in a military deployment (minimum of 4 months).
- No current psychiatric disorder according to DSM-IV criteria, CAPS score lower than 15.
- Age between 18-60
- Written consent
Healthy controls
- Healthy non-military individuals (civilians) who have not participated in a military deployment.
- No current psychiatric disorder according to DSM-IV criteria, CAPS score lower than 15.
- Age between 18-60.
- Written consent
Exclusion criteria
- Alcohol/substance abuse and/or dependence during treatment or at the time of the initial measurements (controls).
- Severe neurological disorders
- Claustrophobia, pacemaker (or other implants), or metal objects in the body preventing a MRI scan.

APPENDIX E. Demographics

Table E1

Participant characteristics of the Val and Met groups in the PRISMO study

Group	Val	Met	Chi-Squared Test
Gender			
Male	299	170	$p = .206$
Female	35	13	$X^2 = 1.599$
Age [a] (years)			
Mean	28.8 ± 8.8	29.0 ± 9.4	$p = .946$
Minimum	18	18	$X^2 = 24.317$
Maximum	57	54	
Rank [b]			
1 – Private	124	73	$p = .444$
2 – Corporal	62	39	$X^2 = 3.726$
3 – Non-commissioned officer (NCO)/Sergeant	96	52	
4- Junior officer/Subaltern	41	13	
5 – Senior officer	11	6	
Genotype			
Val/Val	31	0	
Val/Met	0	17	
Met/Met	0	3	
Previous deployments[c] *			
0	160	89	$p = .203$
1	87	42	$X^2 = 8.515$
2	41	23	
> 3	37	24	

Note. Table E1 shows participant characteristics of the Prospective Research in Stress-Related Military Operations (PRISMO) study (N = 517). The gender, age, rank, and number of previous deployments of the Val and Met group are compared. To assess demographic differences between the groups, a Chi-Squared test was performed. The larger the X^2 value, the greater the discrepancy between the groups The results revealed that for gender, age, rank, and previous deployments, the *p*-values were all greater than .05. This indicates that there are no significant demographic differences between the Val and Met groups.

[a] The age data are reported as mean ± standard deviation.

[b] Rank is categorized into five levels, which each level representing a specific rank within the military hierarchy.

[c] Previous deployments are categorized into four groups: 0, 1, 2, and 3 or more.

*Sample sizes might not add up to total participants due to missing data in the descriptive values.

Table E2

Participant characteristics of the Val and Met groups in the BETTER study

Group	**Val**	**Met**	**Chi-Squared Test**
Gender			
Male	30	20	$p = .417$
Female	1	0	$X^2 = .658$
Age [a] (years)			
Mean	35.0 ± 9.4	40.2 ± 9.5	$p = .300$
Minimum	21	23	$X^2 = 33.520$
Maximum	54	57	
Rank [b]*			
1 – Private	7	6	$p = .212$
2 – Corporal	13	4	$X^2 = 5.835$
3 – Non-commissioned officer (NCO)/Sergeant	7	5	
4- Junior officer/Subaltern	1	0	
5 – Senior officer	0	2	
Genotype			
Val/Val	31	0	
Val/Met	0	17	
Met/Met	0	3	
Previous deployments [c]*			
1	11	8	$p = .292$
2	9	2	$X^2 = 7.322$
3	3	7	
> 4	6	3	

Note. Table E2 shows participant characteristics of the Biological Effects of Traumatic Experiences, Treatment, and recovery study (N = 51). The gender, age, rank, and number of previous deployments of the Val and Met group are compared. To assess demographic differences between the groups, a Chi-Squared test was performed. The larger the X^2 value, the greater the discrepancy between the groups. Results revealed that for gender, age, rank, and previous deployments, the p-values were all greater than .05. This indicates that there are no significant demographic differences between the Val and Met groups.

[a] The age data are reported as mean ± standard deviation.

[b] Rank is categorized into five levels, which each level representing a specific rank within the military hierarchy.

[c] Previous deployments are categorized into four groups: 1, 2, 3, and 4 or more.

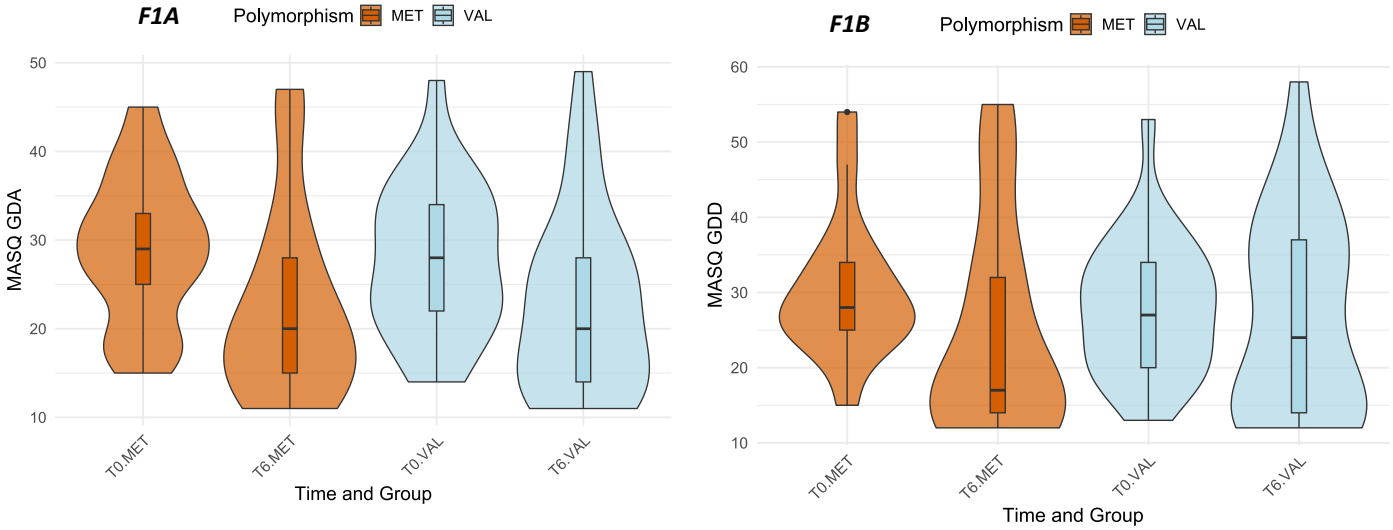
*Sample sizes might not add up to total participants due to missing data in the descriptive values.

APPENDIX F. MASQ-GDA and MASQ-GDD

Additional statistical information about the Mood and Anxiety Symptom Questionnaire (MASQ) is presented in this appendix. The outcomes of the General Distress Anxiety (MASQ-GDA) and General Distress Depression (MASQ-GDD) subscales are shown separately. Violin plots for these subscales are displayed in Figure F1, and the corresponding statistical results are presented in Table F1.

Figure F1

Violin plots of the MASQ-GDA and MASQ-GDD



Note. Figure F1A shows the score on the Mood and Anxiety Symptom Questionnaire General Distress Anxiety (MASQ-GDA) subscale. Figure F1B shows the score on the Mood and Anxiety Symptom Questionnaire General Distress Depression (MASQ-GDD) subscale. The violin plots show the distribution of data for the Val homozygotes and the Met allele carriers. In both figures, the x-axis represents time, labeled as T_0 and T_6. T_0 is before deployment and T_6 is six months after the military returned home from deployment. Boxplots are incorporated within the violin plots to provide a more detailed overview.

Table F1

Statistical outcomes MASQ-GDA and MASQ-GDD

			BETTER		95% CI	
MASQ-GDA	*b*	*S.E.*	*t*	*p*	Lower	Upper
Val/Met	1.015	3.039	0.334	.739	-5.034	7.064
Time	-3.551	6.355	-0.559	.578	-16.200	9.097
ETI	0.104	0.216	0.484	.630	-0.325	0.534
Val/Met * Time	-1.455	4.271	-0.341	.734	-9.956	7.046
MASQ-GDD						
Val/Met	1.928	3.720	0.518	.606	-5.477	9.333
Time	2.845	7.779	0.366	.716	-12.638	18.328
ETI	0.304	0.264	1.151	.253	-0.222	0.829
Val/Met * Time	-4.148	5.228	-0.793	.430	-14.554	6.259

Note. Table F1 presents the statistical outcomes of the linear regression analysis for the Mood and Anxiety Symptom Questionnaire (MASQ) related to the Biological Effects of Traumatic Experiences, Treatment, and Recovery (BETTER) study. The statistical outcomes of the MASQ are subdivided in the General Distress Anxiety (MASQ-GDA) and the General Distress Depression (MASQ-GDD) subscales. *Val/Met* refers to the Val/Met genotype, *Time* indicates the time point of measurement, and the interaction term *Val/Met * Time* examines whether the effect of the Val/Met genotype on PTSD development changes over time. *DES* refers to Deployment Experiences Scale, and *ETI* refers to the Early Trauma Inventory. The values presented include regression coefficients (*b*), standard errors (*S.E.*), *t*-values, *p*-values, and 95% confidence intervals (Lower and Upper bounds).
Statistical significance is indicated by p-values less than .05. This analysis reveals no significant findings.