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No association between the serotonin transporter linked polymorphic region polymorphism and severity of posttraumatic stress disorder symptoms in combat veterans with or without comorbid depression

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ABSTRACT

Since both posttraumatic stress disorder (PTSD) and depression are associated with disturbances in the serotoninergic system, the aim of the study was to determine the association between severity of PTSD symptoms, serotonin transporter polymorphism (5-HTTLPR) and platelet serotonin (5-HT) concentration, in male combat veterans with PTSD ($n = 325$), who were subdivided according to presence of comorbid depression. The methodological approach included the psychiatric diagnostic interviews and rating scales (SCID for DSM-IV, HDRS, CAPS), polymerase chain reaction for 5-HTTLPR genotyping and spectrophotofluorometric method for measuring the platelet 5-HT concentration. PTSD veterans without depression had more severe PTSD symptoms, and less severe depressive symptoms, than PTSD veterans with depression. 5-HTTLPR genotype frequencies did not differ between veterans with mild, moderate and severe PTSD symptoms, and between depressed and non-depressed PTSD veterans. No significant association was found between the severity of PTSD symptoms and 5-HTTLPR genotype. Platelet 5-HT concentration was similar in PTSD veterans, with or without comorbid depression, and between two groups subdivided according to the severity of PTSD symptoms or 5-HTTLPR genotype. The study confirmed, on ethnically homogenous groups of veterans with matched combat experience, a lack of association between the PTSD symptoms severity and 5-HTTLPR or platelet 5-HT concentration.

1. Introduction

The neurobiological basis of posttraumatic stress disorder (PTSD) includes the abnormalities in different neurotransmitter and neuroendocrine systems (Michopoulos et al., 2015). The involvement of serotonin (5-hydroxytryptamine, 5-HT) in PTSD is suggested by its role in the regulation of mood, arousal and sleep (Jacobs, 1991), fear (Jovanovic and Ressler, 2010; Singewald et al., 2015) and by the clinical efficacy of antidepressant drugs in PTSD treatment (Kozaric-Kovacic, 2008). PTSD is associated with a very high rate of comorbidity, including depression (Kessler et al., 1995). PTSD develops as a consequence of the complex interplay between exposure to different types of traumatic event(s), gender, presence/absence of social support and early or prior stressful experience (Kilpatrick et al., 2007; Zoladz and Diamond, 2013). Regarding the severity of PTSD, combat veterans report more severe PTSD symptoms and more re-experiencing symptoms than non-combat subjects with PTSD (Brinker et al., 2007).

The activity and concentration of 5-HT in the synapse or in platelets is regulated by 5-HT transporter (5-HTT), encoded by the *5HTT* or *SLC6A4* gene (Lesch et al., 1996). The functional polymorphism of the *5-HTT* gene, a *5HTT* linked polymorphic region (5-HTTLPR), has been widely studied as a vulnerability and/or resiliency factor to different psychiatric disorders and traits (Lesch and Gutknecht, 2005), including PTSD (Goenjian et al., 2012; Koenen et al., 2009; Kolassa et al., 2010; Lee et al., 2005; Thakur et al., 2009; Wang et al., 2011; Xie et al., 2012), and depression (Karg et al., 2011; Munafo et al., 2009). This polymorphism affects the expression and function of the 5-HTT. The short variant reduces the transcriptional efficiency of the *5HTT* promoter, resulting in decreased 5-HTT expression (Lesch et al., 1996) leading to

Abbreviations: PTSD, posttraumatic stress disorder

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changes in 5-HT re-uptake and availability. The short (S) allele of the 5-HTTLPR has been reported to increase the risk for PTSD (Kolassa et al., 2010), to interact with environmental factors and contribute to PTSD (Kilpatrick et al., 2007; Koenen et al., 2009; Xie et al., 2009), or to represent a risk factor for PTSD after high trauma-exposure conditions (Gressier et al., 2013). However, a significant risk effect of the long (L) allele for PTSD was also found (Grabe et al., 2009), while another study (Thakur et al., 2009) and meta-analyses (Gressier et al., 2013; Navarro-Mateu et al., 2013) did not detect any significant association between PTSD and the 5-HTTLPR. Similar controversy exists for the association between the 5-HTTLPR variants and depression (Karg et al., 2011; Lopez-Leon et al., 2008; Munafo et al., 2009; Risch et al., 2009), though the presence of the S allele was associated with a higher load to develop depressive symptoms in subjects exposed to childhood abuse and adult traumatic events compared to carriers of the LL genotype (Grabe et al., 2012).

Blood platelets share similarities with the central 5-HT synaptosomes and are proposed to represent a limited peripheral model for the central serotonergic neurons (Camacho and Dimsdale, 2000; Stahl, 1985; Yubero-Lahoz et al., 2013). Platelet 5-HT concentration was found to be reduced in veterans with suicidal behavior (Kovacic et al., 2008), but was increased in veterans with psychotic features (Pivac et al., 2006) in combat related PTSD. In depression, platelet 5-HT concentration was either similar in depressed patients (Sagud et al., 2016), or higher (Pivac et al., 1997) in patients with psychotic depression, compared to control subjects.

The aim of the study was to determine the association between the 5-HTTLPR and platelet 5-HT concentration with severity of PTSD symptoms and comorbid depression in combat exposed veterans with PTSD. We hypothesized that in male veterans with combat related PTSD, more severe PTSD symptoms, as well as comorbid depression, will be associated with more frequent presence of the 5-HTTLPR S allele. Further hypotheses were that PTSD veterans with more and those with less severe PTSD symptoms, as well as those with or without comorbid depression, will differ in platelet 5-HT concentration. We expected higher platelet 5-HT concentration in veterans with more severe symptoms.

2. Methods

2.1. Subjects and clinical measures

Participants were male, medication-free combat veterans ($n=325$) with chronic and current combat related PTSD, from the Department of Psychiatry, University Hospital Dubrava. All subjects were recruited through the inpatient and outpatient unit of the Department of Psychiatry, University Hospital Dubrava in the period from 2001 to 2005. Participants were either drug-naive or drug free, and were not under psychological treatment, or were not treated with selective serotonin reuptake inhibitors (SSRI) 6 weeks prior to sampling. Participants were recruited consecutively after the admission. Those who met the exclusion criteria were excluded from the study. Veterans were all Caucasian subjects (Croatian origin), serving in the Croatian armed forces in the Homeland war, with comparable traumatic combat experience (3.0 ± 1.0 years) and comparable duration of time passed after traumatic experience (6.1 ± 2.7 years). The number of traumatic experiences could not be assessed during active military duty, since the war lasted 5 years, but all veterans participated in the same military operations. The diagnoses of PTSD and comorbid depression were done using the Structured Clinical Interview (SCID) for DSM-IV (First et al., 1995), the Hamilton Depression Rating Scale (HDRS) (Hamilton, 1960), and the Clinician Administered PTSD Scale (CAPS) (Blake et al., 1995). The CAPS scale assesses traumatic experience and incorporates 17 symptoms of PTSD in three symptom clusters: re-experiencing (5 symptoms), avoidance (7 symptoms), increased arousal (5 symptoms), duration of symptoms (at least one month: answers yes or no) and functional disturbances (3 symptoms). Items are rated as present or not present and require separate frequency and severity scores. The HDRS scale consists of 17 items scored from 0 to 4 points (0=symptom is not present, 4=symptom is markedly present) and is used for estimating the severity of depression (mild depression (score <18), moderate depression ($18 \leq$ score ≤ 25), severe depression (score >25)). Both scales are translated to Croatian and validated for use in the Croatian population (Arcel et al., 1998). Assessments were made by experienced psychiatrists in the field of psychotrauma who had been trained to apply particular scale and obtained licences to use it.

PTSD severity was evaluated according to CAPS scores and 92 (28.3%) subjects had mild (range 45–65) symptoms, 157 (48.3%) subjects had moderate (range 66–95) symptoms and 76 (23.4%) subjects had severe (range 96–136) PTSD symptoms. All PTSD subjects were subdivided according to the presence of comorbid depression into those without ($n=158$) and those with ($n=167$) comorbid depression.

Veterans completed a questionnaire, created by the psychiatrists at the Department of Psychiatry, University Hospital Dubrava, which included: basic demographic characteristics, smoking, alcohol consumption (not quantified), physical activity, presence of acute or chronic physical illness, and medication use. Exclusion criteria: any kind of pharmacological or psychological treatment, treatment with SSRI in the previous 6 weeks, a positive family history of psychiatric disorders, earlier history of acute psychosis, dementia, cognitive dysfunction, mental retardation, schizophrenia, mood disorders other than depression, personality disorders, substance abuse, past or current (within 3 months) alcohol or other substance abuse, cardiovascular, metabolic or neurological disorder, clinically significant abnormalities in electrocardiogram or laboratory findings, positive urine screen for illicit drugs and alcohol.

After complete description of the study protocol, written informed consent was obtained. All studies were approved by the local Ethics committee and conducted in accordance with the Helsinki Declaration as revised 1989.

2.2. Genotyping of the 5-HTTLPR and determination of platelet 5-HT concentration

Blood samples (8 ml) were collected into plastic syringes with 2 ml of acid citrate dextrose anticoagulant at 8.00 a.m. after fasting overnight. Genomic DNA was extracted from peripheral blood using a salting out method (Miller et al., 1988).

Genotyping was performed using polymerase chain reaction (PCR) as described previously (Noskova et al., 2008; Pivac et al., 2009). The primers used for amplification of 5-HTTLPR were 5′-GGCGTTGCCGCTCTGAATGC-3′ and 5′-GAGGGACTGAGCTGGACAACCAC-3′.

The concentration of 5-HT was determined spectrofluorimetrically in platelets isolated from platelet rich plasma (PRP), as described previously (Pivac et al., 2009). The measurement of the 5-HT fluorescence was performed on a Varian Spectrophotofluorimeter Cary Eclipse, on an exciting wavelength of 345 nm and emitted wavelength of 485 nm. The detection limit of the method was 10.0 ng/sample; with intra- and inter-assay coefficients of variation of 3.66% and 8.69%, respectively. Platelet protein concentrations were measured by the method according to Lowry et al. (1951). All laboratory procedures were performed blind to subject status.

2.3. Statistical evaluation

The results were expressed as numbers and percentages, median and 25th (Q1) and 75th (Q3) percentiles or mean ± standard error of the mean (SEM). Frequencies were evaluated using a χ^2-test with a Yates correction for continuity. Normality of the data was checked by Kolmogorov-Smirnov test. Two groups of demographic, clinical and biochemical data were evaluated with Mann-Whitney *U* test or Student's *t*-test, depending on the normality of the data. The interaction between 5-HTTLPR genotypes, severity of PTSD symptoms and platelet 5-HT concentration was evaluated with two-way ANOVA. Kruskal Wallis ANOVA was used to evaluate platelet 5-HT data between groups, since normality test failed. Standardized residuals (R) determined what category significantly contributed to rejecting the null hypothesis (Field et al., 2012). Results were evaluated using SigmaStat 2.0 (Jandell Scientific Corp. San Raphael, California, USA) and Microsoft Excel. G*Power 3 Software (Faul et al., 2007) was used for conducting power analyses, i.e. to determine *a priori* sample size and *post-hoc* power. Due to the multiple testing, *p* value was corrected to 0.025, avoiding the more conservative Bonferroni correction. For the analysis of the 5-HTTLPR genotype frequency with a χ^2-test (with $\alpha = 0.025$; power $(1-\beta)=0.800$; and with a small effect size ($\omega=0.2$; df=2), the required total sample size was 241 and actual total sample size was 325. *Post-hoc* computed achieved power $(1-\beta)$ was 0.842–0.861. For the ANOVA (with $\alpha=0.025$; power $(1-\beta)=0.800$; a small effect size ($\omega=0.20$; df=2, and number of groups=3), the required total sample size was 292, and actual total sample size was 325. *Post-hoc* computed achieved power $(1-\beta)$ was 0.813.

3. Results

Out of 325 veterans included in the study, 158 (48.6%) had PTSD and 167 (51.4%) had PTSD with comorbid depression. Veterans with PTSD without comorbid depression had significantly higher total CAPS scores compared to veterans with PTSD with comorbid depression (Table 1). Veterans with PTSD with comorbid depression had significantly higher HDRS total scores than veterans with PTSD without comorbid depression (Table 1). Besides total CAPS scores, the frequency of the PTSD symptoms severity differed significantly between veterans with PTSD with or without depression ($\chi^2=80.04$; df=2; $p<0.001$), since in the group without comorbid depression 30 (19.0%) subjects had mild, 57 (36.1%) subjects had moderate and 71 (44.9%) subjects had severe PTSD symptoms, while in the group with comorbid depression 62 (37.1%) subjects had mild, 100 (59.9%) subjects had moderate and 5 (3.0%) subjects had severe PTSD symptoms, respectively. The group of veterans with PTSD, without comorbid depression, with the most severe PTSD symptoms, was the major contributor to this significance (R=5.60).

Regarding the 5-HTTLPR genotype frequency, no deviation from the Hardy-Weinberg equilibrium was found in total sample ($\chi^2=0.19$; df=1; $p=0.666$), in veterans with PTSD without comorbid depression ($\chi^2=0.00$; df=1; $p=0.991$), and in veterans with PTSD with comorbid depression ($\chi^2=0.37$; df=1; $p=0.545$), respectively. The 5-HTTLPR genotype frequency was 39.3% for the LL, 46.8% for the LS and 13.9% for the SS genotype in veterans with PTSD without comorbid depression, and was similar ($\chi^2=0.71$; df=2; $p=0.702$) to the genotype frequency in veterans with PTSD with comorbid depression: LL (34.7%), LS (50.39%) and SS (15.0%).

Since the frequency of the homozygous SS genotype is around 14% in Croatian population (Noskova et al., 2008; Pivac et al., 2009), in our further analyses all subjects were grouped into the LL homozygotes and S carriers (the combined SS and SL genotypes). The frequency of the S carriers versus the homozygous LL genotype ($\chi^2=0.53$; df=1; $p=0.467$) did not differ between veterans with PTSD with or without comorbid depression.

To elucidate the possible association between 5-HTTLPR and severity of PTSD symptoms, the frequency of the S carriers and homozygous LL genotype was compared between veterans with PTSD, with or without comorbid depression, subdivided into those with mild, moderate and severe PTSD symptoms, and no significant ($p=0.051$; χ^2 test) difference was found (Table 2). To confirm this negative finding, we further evaluated the association of 5-HTTLPR genotype with PTSD symptom severity using Mann-Whitney *U* test with symptom severity as the outcome variable and 5-HTTLPR genotype as the grouping factor. No significant association was found between 5-HTTLPR genotype and total CAPS scores in veterans with PTSD ($U=2968.00$; $p=0.979$) or in veterans with PTSD with comorbid depression ($U=2555.50$; $p=0.042$).

Veterans with PTSD with comorbid depression had similar ($t=1.51$; df=323; $p=0.132$; Student *t*-test) platelet 5-HT concentration (0.95 ± 0.04) compared to veterans with PTSD without

Table 1
Demographic and clinical characteristic of patients with PTSD with or without comorbid depression.

	PTSD	PTSD with comorbid depression
Age (years)	41.0 (38.0–49.0)	43.5 (39.0–50.0) $U = 14{,}577.50$; $p=0.051$; Mann Whitney *U* test
CAPS total scores	88.0 (70.0–101.0)	69.0 (61.0–73.0) $U=6719.00$; $p<0.001$; Mann Whitney *U* test
CAPS-mild scores	58.5 (50.0–61.0)	56.5 (51.0–61.0) $U=902.00$; $p=0.818$; Mann Whitney *U* test
CAPS-moderate scores	73.0 (71.0–79.0)	72.0 (70.0–75.0) $U=2282.00$; $p=0.037$; Mann Whitney *U* test
CAPS-severe scores	1012.0 (98.0–110.0)	99.0 (97.0–101.3) $U=255.00$; $p=0.104$; Mann Whitney *U* test
HDRS total scores	21.0 (16.0–26.0)	26.0 (23.0–30.0) $U=18{,}077.00$; $p<0.001$; Mann Whitney *U* test

Data are expressed as median and 25th and 75th percentiles in parenthesis; HDRS=Hamilton Depression Rating Scale; CAPS=Clinician Administered PTSD Scale.

Table 2
The 5-HTTLPR genotypes and platelet 5-HT concentration in veterans with PTSD with or without comorbid depression subdivided into those with mild, moderate and severe PTSD symptoms (according to the CAPS scores).

CAPS scores PTSD symptoms	45–65 Mild	66–95 Moderate	96–136 Severe
PTSD			
LL homozygotes, *n* (%)	14 (46.7)	20 (35.1)	28 (39.4)
S carriers, *n* (%)	16 (53.3)	37 (64.9)	43 (60.6)
PTSD with comorbid depression			
LL homozygotes, *n* (%)	13 (21.0)	44 (44.0)	1 (20.0)
S carriers, *n* (%)	49 (79.0)	56 (56.0)	4 (80.0)
$\chi^2=11.04$, df=5, $p=0.051$; χ^2 test			
PTSD			
platelet 5-HT concentration, median (Q1-Q3)	0.87 (0.67–1.02)	0.99 (0.70–1.26)	1.07 (0.63–1.58)
PTSD with comorbid depression			
platelet 5-HT concentration, median (Q1-Q3)	0.93 (0.58–1.35)	0.94 (0.66–1.22)	0.67 (0.65–1.13)
$H=3.66$; df=5; $p=0.599$; Kruskal-Wallis ANOVA by ranks			

LL homozygotes=LL homozygous genotype; S carriers=the combined SL and SS genotype; CAPS=Clinician Administered PTSD Scale; 5-HTTLPR=5-HT transporter linked polymorphic region.

comorbid depression (1.03 ± 0.04). Based on their CAPS scores, veterans were subdivided into those with mild (0.92 (0.62–1.33)), moderate (0.95 (0.66–1.24)) and severe (1.06 (0.63–1.55)) PTSD symptoms. Platelet 5-HT concentration did not differ significantly between these three groups ($H=2.2$; df=2; $p=0.331$; Kruskal-Wallis ANOVA). When veterans with PTSD, with or without comorbid depression, were subdivided into veterans with mild, moderate and severe PTSD symptoms, there were no significant ($p=0.599$; Kruskal-Wallis ANOVA) differences in platelet 5-HT concentration among these groups (Table 2).

To evaluate the possible association between platelet 5-HT concentration and 5-HTTLPR genotypes, all veterans were subdivided into the S carriers (0.96 ± 0.03) and the LL homozygous genotype (1.04 ± 0.05). Student's t test ($t=1.41$; df=323; $p=0.161$) revealed that the S carriers and LL homozygotes had similar platelet 5-HT concentration.

To elucidate the possible combined effect of 5-HTTLPR genotypes and severity of PTSD symptoms on platelet 5-HT concentration, all veterans with PTSD were subdivided according to their 5-HTTLPR genotypes (into LL genotype carriers or S carriers) and based on CAPS scores into those with mild, moderate and severe PTSD (Table 3). Two-way ANOVA showed a slight ($p=0.027$) but non-significant (due to the p correction) effect of the CAPS severity scores, a lack of significant effect of the 5-HTTLPR genotype, and a lack of significant interactive effect of CAPS severity scores and 5-HTTLPR genotypes on platelet 5-HT concentration (Table 3). When veterans were subdivided according to presence of comorbid depression, CAPS scores and 5-HTTLPR genotypes, two-way ANOVA revealed no significant effects of the CAPS severity scores, a lack of significant effect of the 5-HTTLPR genotype, and a lack of significant combined effect of CAPS severity scores and 5-HTTLPR genotypes on platelet 5-HT concentration in veterans with PTSD or in those with PTSD and with comorbid depression, respectively (Table 3).

Table 3
Platelet 5-HT concentration in veterans with PTSD with or without comorbid depression subdivided according to the CAPS scores and 5-HTTLPR genotype.

CAPS scores PTSD symptoms	45–65 Mild	66–95 Moderate	96–136 Severe
All veterans	Platelet 5-HT concentration, mean ± SEM		
LL homozygotes	0.99 ± 0.10	0.98 ± 0.06	1.23 ± 0.10
S carriers	0.92 ± 0.06	0.94 ± 0.05	1.06 ± 0.07
$F=3.66$, $p=0.027^{*}$, CAPS severity scores			
$F=2.28$, $p=0.132$, 5-HTTLPR genotype			
$F=0.40$, $p=0.672$, CAPS severity scores × 5-HTTLPR genotypes			
PTSD	Platelet 5-HT concentration, mean ± SEM		
LL homozygotes	1.01 ± 0.15	0.91 ± 0.13	1.23 ± 0.11
S carriers	0.84 ± 0.14	0.99 ± 0.09	1.09 ± 0.09
$F=2.91$, $p=0.058$, CAPS severity scores			
$F=0.61$, $p=0.438$, 5-HTTLPR genotype			
$F=0.77$, $p=0.463$, CAPS severity scores × 5-HTTLPR genotypes			
PTSD with comorbid depression	Platelet 5-HT concentration, mean ± SEM		
LL homozygotes	0.97 ± 0.13	1.01 ± 0.07	1.22 ± 0.47
S carriers	0.95 ± 0.07	0.90 ± 0.06	0.76 ± 0.24
$F=0.01$, $p=0.993$, CAPS severity scores			
$F=1.17$, $p=0.282$, 5-HTTLPR genotype			
$F=0.39$, $p=0.679$, CAPS severity scores × 5-HTTLPR genotypes			

LL homozygotes=LL homozygous genotype; S carriers=the combined SL and SS genotype; CAPS=Clinician Administered PTSD Scale; 5-HTTLPR=5-HT transporter linked polymorphic region. Analysis was done using two-way ANOVA.

* not significant due to the p value correction.

4. Discussion

In contrast to our hypotheses, the main finding in the present study was a lack of significant association between the 5-HTTLPR variants and/or platelet 5-HT concentration with severity of PTSD symptoms in male war veterans with PTSD, with or without comorbid depression.

Central 5-HT activity is significantly affected by the 5-HTTLPR (Lesch et al., 1996). 5-HT depletion may contribute to hyperarousal symptoms characteristic for PTSD (Weiss, 2007). In the present study we found no significant association between the 5HTTLPR and PTSD symptoms severity (evaluated using the total CAPS scores, and additionally in veterans subdivided into those with mild, moderate and severe PTSD symptoms). Our data replicate findings showing no significant association of 5-HTTLPR with PTSD symptom clusters scores (Sayin et al., 2010), but disagree with a report suggesting that S allele is associated with more severe PTSD symptoms (Wang et al., 2011). However, this correlation ($r = 0.15$, $p = 0.03$) between the 5-HTTLPR and CAPS scores was significant (Wang et al., 2011) at the level which would not survive correction for multiple testing used in the present study. Other possible explanation might be the lack of data considering traumatic load (Kolassa et al., 2010) or the early traumatic life events (Kenna et al., 2012; Michopoulos et al., 2015). Significant interaction between the 5-HTTLPR genotype, childhood adversity and the risk for PTSD was detected in European-American carriers of one or two S alleles (Xie et al., 2009). Another data show either no significant differences in the number of traumatic experiences in Rwandan civil war refugees subdivided into 5-HTTLPR genotype groups (Kolassa et al., 2010), or the significant additive gene (5-HTTLPR triallelic L_A genotype) x environment (number of traumatic events) interaction effect on the manifestation of PTSD (Grabe et al., 2009).

Although the present study did not evaluate the association between the 5-HTTLPR and etiology of PTSD itself, our previous data (Kovacic, 2010) revealed that the LL (36.6%), LS (49.1%) or SS (14.3%) genotype frequency in 328 PTSD veterans did not differ significantly ($\chi^2=0.083$; df=2; $p=0.960$) from the LL (37.5%), LS (48.6%) or SS (13.9%) genotype frequency in 461 healthy control non-PTSD male subjects. This lack of association is affirmed by previous studies (Goenjian et al., 2012; Navarro-Mateu et al., 2013) that reported no significant relationship between 5-HTTLPR (evaluated as a biallelic or triallelic polymorphism) and PTSD in a large number of Caucasian PTSD subjects and controls (Navarro-Mateu et al., 2013).

In our study the distribution of the 5-HTTLPR genotypes was similar between veterans with PTSD, with or without comorbid depression. These results disagree with the significant association found between depressive symptoms and the S allele of 5-HTTPLR (Goenjian et al., 2012) in the earthquake victims in Armenia. Differences across studies might be explained by the differences in the evaluation of depression: self-rating Beck Depression Inventory for evaluating depressive symptoms (Goenjian et al., 2012) opposed to objective measures such as interviews using clinical scales (SCID and HDRS) applied in the present study, and in combat-exposed versus civilian subjects (Brinker et al., 2007). The association between depression and the 5-HTTLPR is inconsistent and complex (Kenna et al., 2012): no positive correlation (Munafo et al., 2009; Risch et al., 2009), or the association of S allele with a risk for depression, but with a small effect size (Karg et al., 2011; Lopez-Leon et al., 2008) were reported.

To the best of our knowledge, this is the first study to investigate in parallel the association of 5-HTTLPR genotypes and platelet 5-HT concentration, severity of PTSD symptoms and comorbid depression. Although an association between depressive disorders and altered platelet 5-HT concentration was reported (Pivac et al., 1997; Zahn et al., 2015), in our study comorbid

depression in PTSD had no effect on platelet 5-HT concentration. In line with data using different groups of Croatian combat veterans with less chronic form of PTSD (Muck-Seler et al., 2003; Pivac et al., 2002), in the present study, platelet 5-HT concentration was similar in veterans subdivided according to comorbid depression or severity of PTSD symptoms. Platelet 5-HT concentration was not affected by the 5-HTTLPR genotype in veterans with PTSD, confirming no significant association between platelet 5-HT concentration and 5-HTTLPR detected before in healthy Caucasian population (Pivac et al., 2009).

Limitations include the cross-sectional design, the assessment for biallelic and not triallelic 5-HTTLPR genotypes, the lack of data on the early traumatic experience or traumatic load, and inclusion of only Caucasian ethnicity and combat exposed veterans, thus the findings may not be generalizable. The A/G SNP (rs25531) affects the transcriptional activity of L allele (Lipsky et al., 2009; Navarro-Mateu et al., 2013). However, recent meta-analysis (Navarro-Mateu et al., 2013) elegantly showed that the triallelic approach, that included 627 patients with PTSD and 3524 controls, did not alter the results obtained with biallelic approach (1874 patients with PTSD and 7785 controls), either evaluated using the allele frequency, dominant or recessive model. Therefore, the differences between bi- and tri-allelic approaches presumably did not alter the obtained results. Regarding traumatic experience, no evidence that 5-HTLLPR moderates the effect of combat exposure on PTSD among combat veterans was reported (Liu et al., 2015). Our hypothesis-driven study provided some nominally/marginally significant associations which were lost after correction, suggesting that these negative results should be confirmed or rejected in the larger samples.

Strengths include the evaluation of the two measures of 5-HT system in ethnically homogenous Caucasian population with shared trauma, inclusion of medication-free male combat veterans with PTSD, with or without comorbid depression, the PTSD diagnosis and severity evaluation using SCID and CAPS, the sufficient sample size, statistical power, and corrected p value.

In conclusion, our results have shown similar distribution of the 5-HTTLPR genotypes and similar platelet 5-HT concentration in combat veterans with PTSD with or without comorbid depression, and in veterans subdivided according to the severity of PTSD symptoms. Additionally, platelet 5-HT concentration was not affected by the 5-HTTLPR genotype or PTSD symptom severity. Among numerous inconsistent data, our study, with all of its strengths and limitations, did not confirm that 5-HTTLPR or platelet 5-HT concentration might be used as peripheral markers of severity of PTSD symptoms in veterans with combat related PTSD with or without comorbid depression. This study does not support a direct main effect of the 5-HTTLPR polymorphism on the PTSD symptom severity, but it provides preliminary data from the ethnically homogenous population of combat veterans for the further meta-analyses. Our results might suggest that other symptoms and altered behaviors occurring in combat related PTSD could be associated with 5-HTTLPR and altered platelet 5-HT concentration.

Conflicts of interest

None.

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