WARNED

Risk factors for the development of PTSD

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Research in this dissertation was supported by the Dutch Ministry of Defence.

Printing of this dissertation was financially supported by the Dutch Ministry of Defence and the Brain Center Rudolf Magnus.

ISBN: 978-90-393-6914-2

Cover design and layout: Sander Pronk
Printed by: Off Page

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GEWAARSCHUWD

Risicofactoren voor het ontwikkelen van PTSS

(met een samenvatting in het Nederlands)

Proefschrift

ter verkrijging van de graad van doctor aan de Universiteit Utrecht op gezag van de rector magnificus, prof. dr. G.J. van der Zwaan, ingevolge het besluit van het college voor promoties in het openbaar te verdedigen op donderdag 18 januari 2018 des middags te 12.45 uur

door
Alieke Reijnen

geboren op 27 maart 1987
te Velp

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CHAPTER 01

General Introduction

Author: Alieke Reijnen

Introduction

The first time I was confronted with the impact of Posttraumatic Stress Disorder (PTSD), I was working as an intern at Foundation Centrum '45. It was sunny Friday morning and I joined a group of veterans for a coffee break. As always, they were talking about their deployments, the same stories I had heard before, but that was what they had in common. When I looked around, I noticed that one of the veterans was not participating in the conversation. He was staring out of the window, focusing on something. It was like he saw something outside but all I saw were trees moving in the wind. His breathing changed. All of a sudden, he started screaming that we should 'keep low' and that we had to give him his gun that stood behind the door. He was still starring out of the window and started shouting: 'They are coming. Look! Up there!'

In 2017, the total population of veterans in the Netherlands consists of 113.750 individuals that were deployed since World War II (Dutch Ministry of Defence, 2017). Over 26.000 veterans were deployed to Afghanistan as part of the International Security Assistance Force (ISAF; Dutch Ministry of Defence, 2017). During my years at the Military Mental Healthcare Research Centre, I interviewed about a hundred military men and women 10 years after their deployment to Afghanistan. For many of them the deployment was a very positive experience. They had finally performed the tasks they were trained for, they were able to help civilians by contributing to the rebuilding and security of Afghanistan, and they experienced the importance of comradeship.

Despite these positive memories, they all reported potentially stressful experiences; exposure to enemy fire, (the constant risk of) improvised explosive devices (IED's) and rocket attacks, and they witnessed colleagues and civilians who were injured or killed. Also, they described difficulties with the cultural differences and with being away from family and friends for a long time. For some, these experiences had a large influence on their adaptation to 'normal' life. Whereas most veterans reported to have adapted easily to life back in the Netherlands, some experienced difficulties after homecoming, such as nightmares, aggressive behavior and being overly alert. For most of them these difficulties disappeared within the first weeks, however, a small number of the participants kept having these symptoms for months or even years after their return. They had developed PTSD.

So, why does one person develop these mental health problems, whereas a colleague, who experienced the same situation, has no problem with adapting? How many develop symptoms of PTSD, when do they develop symptoms, and in what way do they differ from their resilient colleagues? The risk of developing mental health problems after deployment is a major concern to military personnel, their families and policy makers. Answering these

questions can contribute to better and timely treatment. More importantly, it can help identify military personnel at risk, might decrease the development of stress-related mental health problems and eventually improve the resilience of military personnel.

In this PhD thesis, the impact of deployment to Afghanistan on the development of mental health symptoms in Dutch military personnel is assessed. In addition, potential psychological and neuroendocrine risk factors for the development of posttraumatic stress symptoms are investigated. As an important quote of Desiderius Erasmus states: "Prevention is better than cure". Therefore, with this thesis we aim to assess if we can better predict who is at risk to develop PTSD symptoms after deployment, first and foremost to help military personnel but also those in their close environment. In other words, we aim to contribute to answering the question if military personnel can be *warned* for the development of stress-related mental health problems.

Prevalence and course of PTSD

Posttraumatic stress disorder is a debilitating disorder that can develop after experiencing a traumatic event. According to the fifth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5; American Psychiatric Association, 2013), PTSD is a trauma- and stressor-related disorder that is characterized by four clusters of symptoms. Individuals who suffer from PTSD often report persistently re-experiencing the traumatic event; acting or feeling as if the traumatic event is occurring, flashbacks, nightmares, and intense psychological distress and physiological reactions to cues that resemble an aspect of the traumatic event. In addition, they might persistently try to avoid situations, feelings or thoughts that remind them of the event. They report negative thoughts and feelings; e.g. overly negative thoughts or assumptions about oneself and the world, negative affect, difficulty experiencing positive affect, decreased interest, and estrangement from others. This is accompanied by symptoms of increased arousal; sleeping problems, outbursts of anger, hypervigilance, increased startle response and difficulty in concentrating. Since the DSM-5 (American Psychiatric Association, 2013) was only published in 2013 (after this project started), the current PhD thesis uses the diagnostic criteria as described in the DSM-IV-TR (American Psychiatric Association, 2000). Important changes in the DSM-5 criteria are that the A2 criterion (requiring that the traumatic response involved intense fear, hopelessness or horror) is removed, the avoidance and numbing cluster is separated and that three new symptoms are added; however, overall the symptoms are generally comparable.

In the Netherlands, about 80% of the general population experiences one or more traumatic events in their life. However, the lifetime prevalence of PTSD is only 7.4% (De Vries and Olff, 2009). Another study in the general population of the Netherlands reported that 52.2% described at least one traumatic event during their life and 3.8% fulfilled the criteria for current PTSD (Bronner et al., 2009). These rates are higher than the percentages found in other European countries. For instance, the European Study of the Epidemiology of

Mental Disorders reported a cross-European lifetime prevalence of PTSD of 1.9% (Alonso et al., 2004). Additionally, Darves-Bornoz et al. (2008) reported a 12-month prevalence in the Netherlands of about 2.6% compared to a cross-European rate of 0.9%, thereby showing that the apparent prevalence rates of PTSD differ between countries.

The prevalence estimates reported in military populations are also mixed. In military personnel from the United States (US), the prevalence rates for PTSD were reported to be between 4.7 and 16.6% after deployment to Iraq or Afghanistan (Hoge et al., 2004; Hoge et al., 2006; Milliken et al., 2007). In contrast, rates were found to be much lower in non-US countries. In military personnel from the United Kingdom (UK) and the Netherlands, the prevalence of PTSD was found to be around 5% after deployment to Iraq or Afghanistan (Hotopf et al., 2006; Rona et al., 2006; Engelhard et al., 2007; Iversen et al., 2009; Fear et al., 2010). This disparity might be explained by several deployment-related factors such as the location, duration and the year of deployment, the branch of service, and the differences in exposure to combat (Fear et al., 2010; Richardson et al., 2010; Hunt et al., 2014). In addition, there is a large heterogeneity in studies; differences in sampling strategies, measurement instruments, and sample sizes, which may contribute to the reported differences in the prevalence of PTSD (For review: Ramchand et al., 2010; Richardson et al., 2010). Also, the timing of the assessment might play an important role. Firstly, pre-deployment prevalence rates should be taken into account since assessment of the prevalence rates after return from deployment might lead to an overestimation of symptoms that are attributed to military deployment. For instance, Smith et al. (2008a) show that nearly half of the participants with symptoms prior to deployment, reported symptoms at follow-up, suggesting an overestimation in prevalence if assessed solely post-deployment. Moreover, pre-deployment rates of PTSD prevalence were found to be around 3% (Hotopf et al., 2006; Rona et al., 2006; Smith et al., 2008a; Fear et al., 2010). Secondly, performing only short-term follow-ups might underestimate the prevalence of mental health symptoms. Several studies have reported a two- to threefold increase in mental health problems several months after return compared to immediately after homecoming (Bliese et al., 2007; Milliken et al., 2007). Thirdly, the pre- to post-deployment development of PTSD symptoms in veterans shows heterogeneity. In general, four developmental trajectories are identified in the development of PTSD after military deployment (Bonanno et al., 2012): a low and stable trajectory (resilient), a moderate and improving trajectory (recovered), a worsening trajectory (delayed onset), and a high and stable trajectory (chronic). However, other trajectories might also be identified, for instance in a Danish study six trajectories were identified with three trajectories showing temporary benefits of deployment (Berntsen et al., 2012). This indicates that both pre-deployment and multiple (long-term) follow-up measurements are crucial to acquire a more reliable indication of the prevalence of PTSD, to better understand the complexity of PTSD development over time, and to timely identify individuals in need of treatment. Therefore, a prospective longitudinal cohort study, as used in the current PhD thesis, is necessary when examining mental health symptoms in Dutch military personnel after deployment to Afghanistan.

PRISMO

Starting in 2002, the Dutch Armed Forces participated in ISAF with the aim to provide security and to contribute to the rebuilding and development of Afghanistan. In 2004, Dutch military personnel were deployed to participate in the Provincial Reconstruction Teams (PRT), mainly in the province of Baghlan in the North of Afghanistan. In 2006, the Dutch government decided to participate in a mission in the province of Uruzgan, in the south of Afghanistan: Task Force Uruzgan (TFU). Their task was to create and provide stability and security in Uruzgan. During both the PRT and TFU, military personnel were exposed to combat-related stressors, such as enemy fire and rocket attacks, and civilians who were injured or killed. Also, between 2006 and 2010 almost 150 Dutch soldiers suffered mild to very serious injuries during combat actions or attacks and 25 military men died. In 2014, the Dutch participation to ISAF ended.

The size and the duration of the Dutch participation to ISAF in Afghanistan provided a unique opportunity to study the impact of deployment on Dutch military personnel and to identify potential risk or protective factors for the development of stress-related (mental) health problems. To assess this, a large prospective cohort study was started in 2005: Prospective Research In Stress-related Military Operations (PRISMO). This was the first study to assess psychological and biological aspects of the development of stress-related (mental) health problems using a prospective longitudinal design starting about one month before deployment and performing follow-ups at approximately 1 and 6 months, and 1, 2 and 5 years post-deployment (Figure 1). The measurements at 10 years post-deployment are currently being conducted. As displayed in Figure 1, during all the assessment participants were asked to fill out questionnaires. In addition, both blood and salivary samples were collected during the first three assessments to also be able to assess possible alterations after exposure to trauma. In the 10-years assessment psychiatric interviews were included in addition to the questionnaires. A total of 1032 military men and women signed up for participation in the study prior to deployment. Eventually, a total of 1007 were deployed between 2005 and 2008 for approximately 4 months. For an overview of the demographic characteristics in PRISMO, we refer to the infographic on the next page.

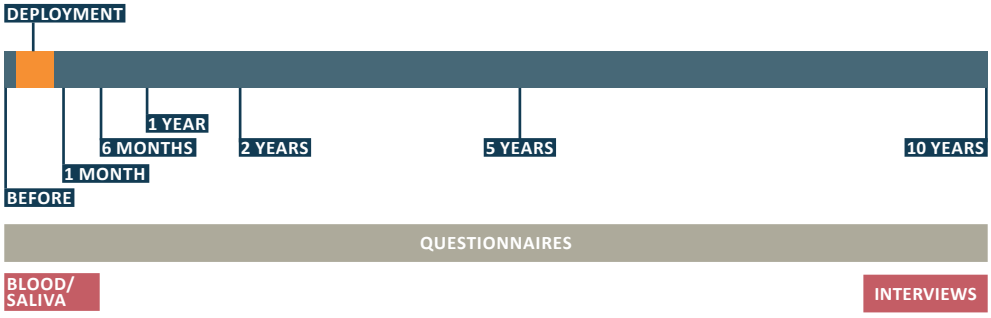
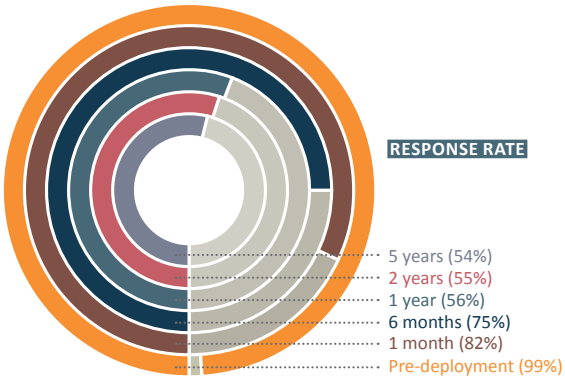
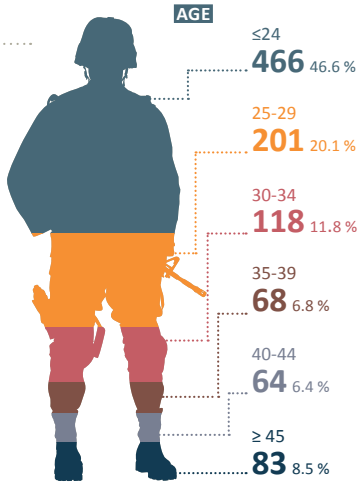
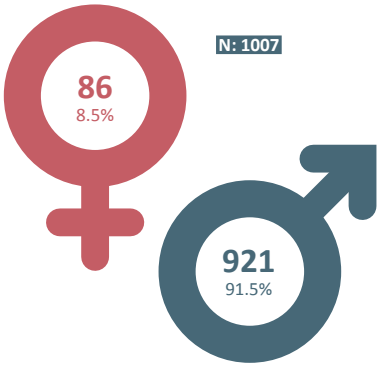


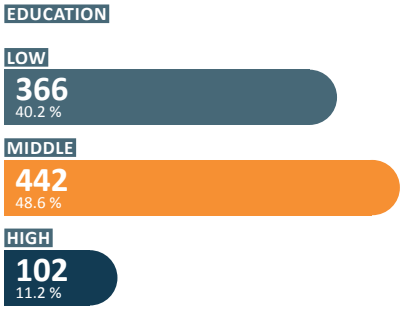
Figure 1. Design of the PRISMO study

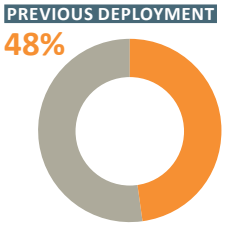
DEMOGRAPHIC CHARACTERISTICS PRISMO

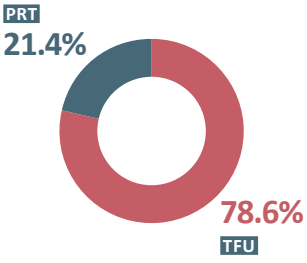


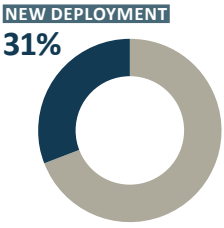


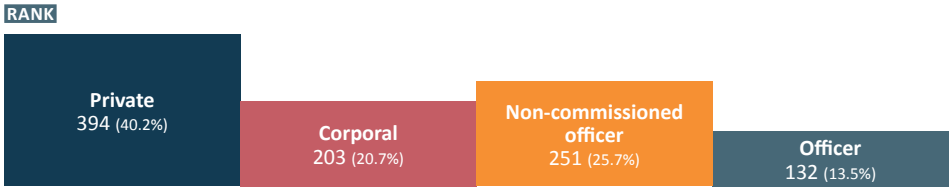












Risk factors for the development of PTSD

Since a large percentage of individuals exposed to trauma do not develop PTSD, this led to a growing interest in evaluating risk factors to determine who develops this disorder. PTSD is a very complex disorder and there are many behavioral and biological markers that have been assessed (For review: Baker et al., 2012; DiGangi et al., 2013; Zoladz and Diamond, 2013; Yehuda et al., 2015; Olff and van Zuiden, 2017). These factors can be divided in many different categories of risk factors; e.g. combat-related factors, demographic or individual characteristics, peri- and post-trauma factors. In addition to psychological risk factors, biological markers (biomarkers) can be used in order to predict the development of PTSD symptoms. A biomarker is any substance, structure or process that can be measured in the body or in its products that influences or predicts the incidence of outcome or disease (WHO International Programme on Chemical Safety, 2001). In this PhD thesis, the focus is on various potential psychological and biological risk or protective factors.

Combat-related characteristics

As previously described, increased estimates of PTSD are found after deployment to a combat zone. However, the findings regarding the role of deployment as a risk factor for PTSD are inconsistent (For review: Ramchand et al., 2010; Richardson et al., 2010; Kok et al., 2012). Whereas no overall effect of deployment on PTSD was found in several UK studies (Hotopf et al., 2006; Rona et al., 2009; Fear et al., 2010), it was associated with an increase in PTSD symptoms in US military personnel (Vasterling et al., 2010). A possible explanation for this is the difference in sampling strategy, the choice of comparison group, and the level of combat exposure (Richardson et al., 2010). Combat exposure or having a combat role, rather than deployment, is associated with the development of PTSD (Smith et al., 2008a; Rona et al., 2009; Fear et al., 2010; Jones et al., 2013). The frequency and intensity of exposure to combat is assumed to be an important predictor for the development of mental health problems among military personnel (Polusny et al., 2011). Other deployment-related factors that might be associated to the level of combat exposure, are the number of previous or new deployments, deployment year, and whether duties were inside or outside the military base. Also, higher rank might be associated with a decreased risk for PTSD (Iversen et al., 2008; LeardMann et al., 2009; Jones et al., 2013). Enlisted personnel were found to have a higher risk for PTSD than officers (Smith et al., 2008a). Importantly, although trauma exposure is a prerequisite for diagnosing PTSD, a large percentage of personnel exposed to combat do not develop PTSD, suggesting that the development of PTSD is not solely associated with combat exposure. Thus, in addition to the exposure, other potential risk and protective factors, such as individual characteristics and biological factors, should be taken into account to determine individual differences in the risk for PTSD.

Psychological risk factors

Individual characteristics

In general, and in military studies, the development of PTSD was found to be associated with younger age and lower education (Brewin et al., 2000; Riddle et al., 2007; Iversen et al., 2008; Seal et al., 2009; Richardson et al., 2010). Whereas female gender is associated with a higher risk for PTSD in the general population (Brewin et al., 2000; Ozer et al., 2003), in military personnel this gender difference is not consistently found. Whereas some state that female gender is a risk factor (Smith et al., 2008a; Luxton et al., 2010), other studies report that women are as resilient as men to combat-related stress and PTSD (Rona et al., 2007; Vogt et al., 2011; Street et al., 2013). Trauma and adversity during childhood is another important and generally known factor associated with PTSD, in the general population (Brewin et al., 2000; Ozer et al., 2003) and in military personnel (Iversen et al., 2007; Smith et al., 2008b; Polusny et al., 2011; Jones et al., 2012). The presence of PTSD symptoms prior to deployment is also found to be a relevant risk factor for PTSD (Rona et al., 2009). Post-deployment risk factors are, for instance, exposure to post-deployment life events (Polusny et al., 2011). In the current thesis, these factors were taken into account in the assessment of PTSD symptom development.

Personality traits

Personality traits are also potential risk or protective factors for the development of PTSD. The disorder was found to be positively related to traits such as neuroticism, harm avoidance, novelty-seeking, and self-transcendence and negatively related to extraversion, conscientiousness, and self-directedness (Jaksic et al., 2012). Furthermore, low self-directedness and cooperativeness and high self-transcendence and harm avoidance after trauma were related to developing PTSD after exposure to the Oklahoma City bombing (North et al., 2012). In prospective studies, pre-trauma high hostility and low self-efficacy were vulnerability markers for PTSD symptoms in firefighters after 2 years (Heinrichs et al., 2005). Also, in police officers trait anxiety was found to predict PTSD symptoms after one year (Meffert et al., 2008). In the PRISMO study, it was previously shown that high hostility and low self-directedness were significant predictors for PTSD symptoms six months after deployment (van Zuiden et al., 2011). Although these studies assessed personality as pre-trauma risk or protective factors, little research has been performed on the impact of traumatic experiences on the development of personality itself. So, it is unclear if personality is a pre-existing risk factor or if changes due to events, such as deployment, also influence the development or course of mental health problems.

Biological risk factors

In addition to psychological risk factors, biological measures might present more objective risk or protective factors for PTSD. There is an extensive amount of literature on potential

biological markers (biomarkers) for PTSD, especially in cross-section and retrospective study designs; particularly the hypothalamic-pituitary-adrenal (HPA) axis is often studied since it regulates and controls the responses to stress (For review: Baker et al., 2012; Schmidt et al., 2013; Zoladz and Diamond, 2013; Michopoulos et al., 2015). This research provides valuable information for the screening and diagnosis of PTSD and for examining the response to and effectiveness of treatment. However, the prospective longitudinal design used in PRISMO provides the opportunity to assess biomarkers as potential susceptibility markers for the development of PTSD. In this PhD thesis, several promising biological markers for the development of PTSD symptoms in military personnel will be discussed: testosterone, neuropeptide Y, oxytocin and vasopressin. The rationale to choose these markers is explained in the following sections.

Testosterone

Testosterone is an important product of the hypothalamic-pituitary-gonadal (HPG) axis, particularly in males. It is a steroid hormone secreted by the gonads that is involved in the reproduction process, secondary sex characteristics, and sexual functioning. In addition, higher levels of testosterone are associated with behavior: aggression (Popma et al., 2007), dominance, and competitiveness (Mehta and Josephs, 2006). Higher testosterone levels are also postulated to have an anxiolytic effect (Handa and Weiser, 2014). Furthermore, testosterone levels are thought to inhibit the functioning of the HPA-axis and cortisol has been shown to have an inhibiting effect on the HPG-axis (Viau, 2002). Although, as mentioned previously, gender might be a risk factor for the development of anxiety and stress-related disorders, the role of gonadal hormones is unclear.

In response to acute and prolonged stress, testosterone levels can be altered. For instance, serum testosterone levels were reduced after 12 hours of captivity during a US army survival course (Morgan et al., 2000). Moreover, after three weeks of military training and a 5-day combat course, decreased plasma testosterone levels were found (Gomez-Merino et al., 2005).

The studies examining testosterone levels in PTSD patients compared to healthy controls report mixed results. Whereas some studies reported higher levels of serum testosterone in PTSD patients (Mason et al., 1990; Karlović et al., 2012), others found no difference in morning plasma (Spivak et al., 2003) or lower levels in cerebrospinal fluid (CSF) concentration (Mulchahey et al., 2001). So, although stress is associated with a decrease in testosterone, it is unknown if testosterone levels are altered after military deployment and if either this alteration or the pre-deployment levels are associated with a high level of PTSD symptoms in the years after deployment.

Neuropeptide Y

Neuropeptide Y is an orexigenic peptide neurotransmitter that is abundantly expressed in the brain and in the peripheral nervous system. In the brain, high concentrations of NPY

are found in the hypothalamus, amygdala, brainstem and anterior pituitary (Hirsch and Zukowska, 2012). In the periphery, NPY is found in sympathetic nerves, where it often exists alongside norepinephrine, and in the adrenal medulla and platelets.

NPY can have a wide range of effects depending on the receptor type and location (Michel et al., 1998). In addition to many physiological functions such as food intake, energy homeostasis, the circadian rhythm and cognition (Eaton et al., 2007), NPY plays an important role in modulation of the stress response and in fear- and anxiety-related behavior (Wu et al., 2011; Hirsch and Zukowska, 2012). NPY can have a direct effect on the HPA-axis, thereby inhibiting excessive activation of the stress response (Kuo et al., 2007; Thorsell, 2010; Schmeltzer et al., 2016). Also, NPY can operate as a physiological brake to counteract and regulate the release and activity of norepinephrine (Schmeltzer et al., 2016).

Therefore, the NPY-system is suggested to have an important role in resilience to stress. In response to acute uncontrollable stress of interrogation during military survival training, significantly increased plasma NPY (pNPY) levels were found, which was related to superior performance and psychological resilience (Morgan et al., 2000; Morgan et al., 2002). In PTSD patients, lower levels of NPY in CSF were found compared to healthy non-traumatized controls (Sah et al., 2009), and trauma-exposed veterans (Sah et al., 2014). Furthermore, higher levels of plasma NPY were found in veterans without PTSD compared to PTSD patients and non-traumatized controls (Yehuda et al., 2006). In contrast, in veterans with PTSD peripherally measured NPY was found to be either reduced (Rasmusson et al., 2000) or no different from healthy controls (Morgan et al., 2003; Yehuda et al., 2006). Although these studies suggest that NPY might be a promising protective marker for PTSD, little is known about the predictive value of peripheral NPY as a risk or protective factor for the development of PTSD.

Oxytocin

Oxytocin (OT) is a nonapeptide, which is primarily produced in the paraventricular and supraoptic nuclei of the hypothalamus. Part of the OT is released in the brain where it works as a neurotransmitter in various brain regions, such as the hippocampus, amygdala, hypothalamus and striatum (Gimpl and Fahrenholz, 2001; Stoop, 2012). However, the majority of OT is released in the bloodstream via the posterior pituitary where it works as a hormone, for instance during parturition to induce uterine contraction and during lactation (Ludwig and Leng, 2006). In addition, OT is synthesized in various peripheral tissues and organs, such as the heart, ovary and testis (Nishimori et al., 2008). In addition to the known prosocial effects of OT (For review: Heinrichs et al., 2009), it might exert anxiolytic effects and reduce responses to stress (Neumann and Landgraf, 2012). OT has a bidirectional interaction with the HPA-axis. Stressors, such as the Trier Social Stress Test (TSST), can induce OT release (Pierrehumbert et al., 2009) and OT release can reduce the secretion of adrenocorticotropic hormone (ACTH) from the anterior pituitary in humans (Opacka-Juffry and Mohiyeddini, 2012). Furthermore, studies that used intranasal administration of OT in humans reported

decreased salivary cortisol during social stress (Linnen et al., 2012) suggesting that it can modulate the responsiveness of the HPA-axis.

Depending on previous negative life experiences OT levels can differ. For instance, lower levels of OT were found in CSF of women who were sexually abused in their childhood (Heim et al., 2009). Moreover, early life adverse experiences were related to lower plasma OT levels in physically healthy adult males (Opacka-Juffry and Mohiyeddini, 2012). However, when comparing the peripheral levels of OT between PTSD patients and healthy controls, the findings are inconsistent. Whereas some report no association of PTSD with plasma OT (Olff et al., 2013; Seng et al., 2013), others found lower salivary OT levels in policemen with PTSD after controlling for childhood trauma (Frijling et al., 2015). So, although it might be postulated that lower peripheral OT levels are associated with trauma exposure and/or PTSD, it is unclear if the peripheral OT levels can be used to predict individual differences in the development of PTSD symptoms over time.

Arginine Vasopressin

Arginine vasopressin (AVP), or antidiuretic hormone, is also a nonapeptide that is synthesized primarily in the paraventricular and supraoptic nuclei of the hypothalamus and secreted in the peripheral bloodstream by the posterior pituitary (Ludwig and Leng, 2006). AVP interacts with the HPA-axis, where it has a stimulatory effect on ACTH release through interaction with corticotropin-releasing hormone (CRH; De Kloet et al., 2005).

Like oxytocin, AVP serves as a neurotransmitter in various regions in the brain, such as the hippocampus, amygdala, and brain stem areas (Buijs, 1990). The centrally released AVP is associated with the regulation of brain functions, such as learning and memory and emotionality (Frank and Landgraf, 2008). In addition, AVP is secreted in the bloodstream as a neurohormone. The levels increase in response to a rise in plasma osmotic pressure or a fall in blood volume; it acts at the kidneys to promote water reabsorption and causes vasoconstriction (Ludwig and Leng, 2006). Vasopressin can have diverse effects in various tissues in the body since AVP acts via three receptor subtypes (V1a, V1b, V2) that all have different pharmacological properties (Greenberg and Verbalis, 2006)

Vasopressin is associated with anxiogenic and depressive actions (Landgraf and Neumann, 2004; Neumann and Landgraf, 2012) and with the regulation of aggression (Caldwell et al., 2008). In contrast with OT, intranasal administration of AVP was found to increase cortisol levels in response to a psychosocial stressor (Shalev et al., 2011). Furthermore, increased levels of AVP are reported in various mental health problems. For instance, higher plasma AVP levels were found in patients with major depressive disorder (Londen et al., 1997). In addition, a previous study in our group reported elevated plasma AVP levels in PTSD patients compared to both healthy non-exposed and trauma-exposed controls (De Kloet et al., 2008). However, a recent study by Frijling et al. (2015) found no differences in salivary AVP levels between police officers with and without PTSD. So, it might be postulated that higher peripheral AVP levels are associated with psychopathology; yet, it is unclear

whether this is secondary to the disorder or if differences in peripheral AVP levels prior to or shortly after deployment are predictive of the development of symptoms of PTSD.

Outline

This dissertation is divided in two parts. The first section (***Part 1***) focuses on the impact of deployment on Dutch military personnel. The prevalence of various mental health problems in the first 2 years after deployment is assessed and compared to pre-deployment (**Chapter 2**). Next, we assessed differences in the development of PTSD symptoms in the 5 years after deployment by identifying different symptom trajectories (**Chapter 3**). In addition, the relation between deployment and the character traits self-directedness and cooperativeness, and the association with the symptom trajectories of PTSD is examined (**Chapter 4**).

Part 2 focuses on peripherally measured neuroendocrine risk or protective markers for the development of PTSD in a large sample of military personnel. Whereas previous research often focused on these biomarkers as disease-related markers by comparing PTSD patients to healthy controls, in this thesis biological vulnerabilities prior to or evoked by experiences during deployment are assessed as candidate risk or protective factors for PTSD symptom development. In this section, we used different statistical techniques to assess the association between Testosterone (**Chapter 5**), Neuropeptide Y (**Chapter 6**) and Oxytocin and Vasopressin (**Chapter 7**) and the development of PTSD symptoms over time.

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PART 01

CHAPTER 02

Prevalence of mental health symptoms in Dutch military personnel returning from deployment to Afghanistan: a 2-year longitudinal analysis

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Published in: European Psychiatry 30 (2015) 341–346

Abstract

Recent studies in troops deployed to Iraq and Afghanistan have shown that combat exposure and exposure to deployment-related stressors increase the risk for the development of mental health symptoms. The aim of this study is to assess the prevalence of mental health symptoms in a cohort of Dutch military personnel prior to and at multiple time-points after deployment. Military personnel (N=994) completed various questionnaires at 5 time-points; starting prior to deployment and following the same cohort at 1 and 6 months and 1 and 2 years after their return from Afghanistan. The prevalence of symptoms of fatigue, PTSD, hostility, depression and anxiety was found to significantly increase after deployment compared with pre-deployment rates. As opposed to depressive symptoms and fatigue, the prevalence of PTSD was found to decrease after the 6-month assessment. The prevalence of sleeping problems and hostility remained relatively stable. The prevalence of mental health symptoms in military personnel increases after deployment, however, symptoms progression over time appears to be specific for various mental health symptoms. Comprehensive screening and monitoring for a wide range of mental health symptoms at multiple time-points after deployment is essential for early detection and to provide opportunities for intervention.

Introduction

Despite some controversy regarding the contribution of combat exposure (Jones et al., 2013), and the prevalence across nations, military personnel are at increased risk for the development of mental health symptoms and psychiatric morbidity following deployment. For instance, research demonstrates a two- to four-fold increase in lifetime prevalence of posttraumatic stress disorder (PTSD) among US combat veterans as compared to those obtained in the general US population (Richardson et al., 2010). Recent studies in troops deployed to Iraq and Afghanistan confirm that combat exposure and exposure to deployment-related stressors, including "minor" stressors like being away from family and friends, result in an increased risk for mental health symptoms (Hoge et al., 2004; Hoge et al., 2006; Milliken et al., 2007; Rona et al., 2009). Among soldiers and marines in the US, 19% screened positive for mental health concerns after deployment to Iraq compared with 11.3% after deployment to Afghanistan (Hoge et al., 2006). In UK military personnel, general psychiatric distress was found in 20.8% after deployment to Iraq and 17.3% after deployment to Afghanistan (Fear et al., 2010). Therefore, the impact of deployment on the mental health of military personnel and associated burden and cost to the healthcare system is of great public and academic interest (Harrison et al., 2010).

Following deployment to recent operations in Iraq and Afghanistan, prevalence rates in US military personnel for PTSD were 4.7-16.7%; for major depressive disorder (MDD) 4.7-10.3%; for anxiety disorders 6.6-7.9%, and for alcohol abuse 11.8% (Hoge et al., 2004; Hoge et al., 2006; Milliken et al., 2007). In UK military personnel, prevalence rates for PTSD were 1.3-4.8%; for MDD 3.7%; for anxiety disorders 4.5%, and for alcohol abuse 16.4-26% (Hotopf et al., 2006; Rona et al., 2006; Iversen et al., 2009; Fear et al., 2010). The prevalence of PTSD in Dutch military personnel deployed to Iraq was found to be less than 5% (Engelhard et al., 2007). Other studies in Dutch military personnel also demonstrated that forgetfulness, difficulty concentrating and fatigue were commonly endorsed symptoms (de Vries et al., 2000). So, although comparison is limited by differences in measurement instruments and the population studied, the rates show that functional impairments range beyond the scope of PTSD only and other manifestations of psychiatric morbidity should also be addressed.

Two issues appear to be of vital importance in determining prevalence rates of deployment-related mental health symptoms: assessment of pre-deployment functioning, and monitoring mental health symptoms at multiple intervals following deployment. Most studies assessed the prevalence of symptoms after return from the combat-zone only. However, this might lead to an overestimation of symptoms that are attributed to military deployment. In US military personnel, PTSD prevalence rates range from 2.4 to 7.6% prior to their deployment to Iraq (Hoge et al., 2004; Smith et al., 2008; Vasterling et al., 2010). Consequently, prospective studies that take pre-deployment mental health into account can be expected to yield lower deployment-related (corrected) prevalence rates for PTSD and other mental disorders. Indeed, a prospective study conducted in a Dutch sample of military personnel

deployed to Iraq found that prevalence rates of PTSD adjusted for pre-deployment PTSD were up to 3% lower than unadjusted prevalence rates (Engelhard et al., 2007). After adjustment for baseline characteristics, the prevalence rate of PTSD in UK military personnel did not differ from the pre-deployment rate (Rona et al., 2006), however, an increase in the rates for PTSD were found in US military personnel (Smith et al., 2008; Vasterling et al., 2010). In addition, the timing of post-deployment assessments may be of key importance. Several studies have shown that mental health symptoms are more likely to develop several months after returning from the military conflict compared to shortly following homecoming. The rates of psychological symptoms reported by a sample of U.S. military personnel were significantly higher at 120 days' post-deployment than immediately upon return (Bliese et al., 2007). Therefore, assessment shortly following deployment may underestimate the prevalence of mental health symptoms. A study by Milliken, Auchterlonie and Hoge (2007) showed increased mental health concerns several months after return in contrast to the rates reported directly after deployment (2007). Also, several studies in civilian (Sveen et al., 2011) and military samples (Orcutt et al., 2004; Dickstein et al., 2010; Berntsen et al., 2012; Bonanno et al., 2012) have demonstrated that the course of PTSD varies across individuals, and that in some individuals, PTSD symptoms may remain sub-threshold until months to years following deployment (see also, Bonanno, 2004). Thus, to assess the development of mental health symptoms after deployment, pre-deployment health status should be taken into account and participants should be followed for a prolonged period after their return to investigate possible changes over time.

The aim of the current study was to assess the prevalence of mental health symptoms in Dutch military personnel at multiple time-points before and after deployment. Specifically, this paper reports the self-reported prevalence of symptoms of PTSD, depression, anxiety, hostility and fatigue, somatic complaints, sleeping problems; starting prior to deployment and following the same cohort up to 2 years after return from Afghanistan.

Methods

Study population

This study was part of a prospective cohort study on deployment-related health problems in the Dutch armed forces. Participants were Dutch military personnel who were deployed to Afghanistan between 2005 and 2008 as part of the International Security Assistance Force (ISAF) of the NATO, either as part of a Provincial Reconstruction Team (PRT) or as part of Task Force Uruzgan (TFU). Military personnel were informed (oral and written) about this study at the army base. Those who were interested in the study could sign up for participation. There was financial compensation of €100, - for participation in three assessments. A total of 994 individuals volunteered to participate in the study. The study was approved by the Institutional Review Board of the University Medical Center Utrecht (Utrecht, the Netherlands).

Procedure

Individuals volunteered to participate prior to their deployment to Afghanistan. After a written and verbal description of the study, written informed consent was obtained. Approximately one to two months prior to deployment (T0) participants completed various paper-and-pencil questionnaires. After a 4-month deployment to Afghanistan, four assessments took place; at approximately one month (T1), six months (T2), one year (T3), and two years (T4) following homecoming. The first 3 assessments were completed at the army base. The T3 and T4 questionnaires were sent by mail. To maximize response rates and to try to reach non-responders or those who left the Armed Forces, participants were contacted a total of five times at each assessment through mail, e-mail and telephone.

Measures

Demographic measures included age, sex, education level, rank, marital status and the number of previous deployments. Exposure to potentially traumatic and combat-related stressors during the deployment was assessed with an 19-item checklist (see Table 1).

Mental health problems were assessed using the depression (16 items), anxiety (10 items), somatic complaints (12 items), sleeping problems (3 items), and hostility (6 items) subscales of the Dutch revised Symptom Checklist (SCL-90-R; Arrindell and Ettema, 2003). The SCL-90-R contains 90 items rated on a 5-point scale ranging from 1 (not at all) to 5 (very much).

Symptoms of PTSD were measured with the Dutch Self-Rating Inventory for PTSD (SRIP; Hovens et al., 2000; Hovens et al., 2002). The SRIP contains 22 items that correspond to symptoms in cluster B, C and D of DSM-IV (APA, 2000) diagnostic criteria for PTSD: re-experiencing (6 items), avoidance and numbing (9 items), and arousal (7 items). Symptom severity was rated using a 4-point Likert scale ranging from 1 (not at all) to 4 (very much). The SRIP has good internal consistency, discriminant validity and concurrent validity with other PTSD measures such as the Clinician Administered PTSD scale and the Mississippi scale for PTSD (Hovens et al., 1994; Hovens et al., 2002). The range of the cutoff (cutoff ≥38) provided the highest sensitivity and specificity for a PTSD diagnosis according to the DSM-IV (van Zelst et al., 2003).

Fatigue was assessed using the Checklist of Individual Strength (CIS; Ren et al., 1999; Vercoulen et al., 1999). This questionnaire consists of 20 statements that refer to aspects of fatigue experienced in the previous two weeks. Participants had to indicate to what extent the statements applied to them on a 7-point scale. Higher scores indicated more fatigue, more concentration problems and reduced motivation and activity level.

Table 1. Exposure to combat-related stressors (N=780).

Item	Item experienced	
	Count	%
Enemy fire	483	61.9
Witnessed people suffering	460	59.0
Witnessed wounded	367	47.1
Colleague injured or killed	350	44.9
Incoming fire	302	38.7
Witnessed dead	287	36.8
Rejected by locals	261	33.5
Personal danger	230	29.5
Witnessed others injured/ killed	156	20.0
Heard people screaming	153	19.6
Insufficient means to intervene	133	17.1
Mission experienced as useless	105	13.5
Insufficient control over situation	104	13.3
Memories of earlier deployments	94	12.1
Traffic accident	72	9.3
Held at gunpoint	37	4.7
Physical injuries	19	2.4
Colleague held hostage	3	0.4
Held hostage	0	0

Data Analysis

Analyses were performed with IBM SPSS 20 and Epi-info 7 (CDC, Atlanta, GA, USA). Characteristics of responders versus non-responders were compared using χ^2 tests and Spearman's rank correlation. Prevalence estimates, confidence intervals and odd's ratios were computed for clusters of mental health symptoms. Prevalence rates for all questionnaires were estimated based on 95th percentile scores as reported in the respective manuals or source publications. The prevalence rates at baseline and 1 month after deployment were compared with Fisher's exact tests. A χ^2 test for trends was used to assess possible trends in the prevalence rates across all post-deployment assessments, starting 1 month after homecoming.

Results

Descriptives and attrition

A total of 994 individuals participated in this study. Baseline assessment was completed by 863 participants (87%), 828 participants (83%) completed T1 assessment, 752 participants (76%) completed T2 assessment, 571 participants (57%) completed T3 assessment, and 565 participants (57%) completed T4 assessment. Non-response was found to be associated with being male ($\chi^2_{(1, N=994)} = 10.22$ $p<.01$), younger age ($\chi^2_{(5, N=992)} = 80.44$ $p<.001$), and lower rank ($\chi^2_{(3, N=985)} = 90.33$ $p<.001$). Furthermore, an association was found between reporting any mental health problem at one month after homecoming and non-response at

6 months, 1 and 2 years after deployment (Spearman's rank correlation or Spearman's rho (*rs*)=.081, N=994, p<.05; *rs*=.117, N=994, p<.001 and *rs*=.062, N=994, p=.052). Reporting symptoms at 6 months post-deployment was not related to non-response at 1 and 2 years (*rs*=.048, N=994, p>.05 and *rs*=.006, N=994, p>.05).

An overview of the characteristics of the total sample is shown in Table 2. Among the 994 participants who volunteered 903 (91%) were male. Mean age of the participants before deployment was 28.54 (SD 8.97). Furthermore, 426 participants (47.3%) had previously been deployed. Duties during deployment to Afghanistan included combat patrols, clearing or searching homes and buildings, participation in demining operations, and transportation across enemy territory. As shown in Table 1, military personnel experienced high-intensity warzone stressors such as exposure to enemy fire, armed combat, and seeing seriously injured fellow soldiers and civilians (including women and children). At the two-year assessment (T4) 20.1% (N=111) of the participants were deployed again.

Prevalence rates

The prevalence rates and odd's ratios (OR) of individuals scoring above cut-off (95th percentile) on the self-report questionnaires are presented in Table 3a and b. The prevalence of fatigue symptoms (cut-off ≥ 88) reported by military personnel was almost 3 to 4 times higher (7.1-9.9%; OR 2.90-3.92) after return from Afghanistan compared to the baseline rate (2.6%). The increase in the prevalence rates from baseline to one month after homecoming was significant (Fisher's exact test= .596, p<.001), and the increase over the various time-points after deployment was found to be trend significant (χ^2 test for trends=3.75, p=.053). The percentage of military personnel reporting symptoms of PTSD (cut-off ≥ 38) doubled from 4.3% before deployment to 8.6% (OR 2.12) at one month after homecoming (Fisher's exact test= 11.487, p<.001). PTSD symptoms were most prevalent (8.6-8.9%) up to 6 months after deployment and declined to 5.6% (OR 1.33) at two years after deployment. The gradually decreasing prevalence in PTSD symptoms over time after deployment was significant (χ^2 test for trends=5.58, p<.05).

Seven % (OR 1.82) of the participants report marked hostility (cut-off ≥ 11) at one month after deployment, as compared to 4% prior to deployment (Fisher's exact test= 7.40, p<.05). The prevalence rate increased to 7.8% (OR 2.05) in the first 6 months after homecoming and declined to 6.6% (OR 1.73) at 2 years post-deployment. The change in prevalence rates over the 2 years after deployment was not significant (χ^2 test for trends=0.23, p>.05). The prevalence of sleeping problems (cut-off ≥ 9) was around 4.3% (OR 1.70) at one month after deployment compared with 2.6% at baseline (Fisher's exact test= 3.75, p=.059). At T2, T3 and T4 the prevalence rates remained around 4% (OR 1.65-1.83). Odd's ratio's for sleeping problems were relatively stable across post-deployment assessments (χ^2 test for trends=0.01, p>.05).

Table 2. Pre-deployment characteristics of the total sample.

Variable	Frequencies	
	Count	%
Age (years) (N=992)[1]		
< 20	137	13.8
21-24	316	31.9
25-29	200	20.2
30-34	120	12.1
35-39	68	6.9
40-44	65	6.6
≥ 45	86	8.7
Sex (N=994)		
Male	904	90.9
Female	90	9.1
Education level[2] (N=919)[1]		
Low	33	3.6
Moderate	783	85.2
High	103	11.2
Marital status (N=903)[1]		
Single	333	36.9
Long-term relationship	176	19.5
Cohabiting	157	17.4
Married	228	25.3
Divorced/widowed	9	1.0
Rank (N=985)[1]		
Private	393	39.9
Corporal	202	20.5
Non-commissioned officer	257	26.1
(Staff) Officer	133	13.5
Number of prior missions (N=903)[1]		
0	477	52.8
1	232	25.7
2	107	11.8
≥ 3	87	9.6

[1] Count may not add up to 994 participants due to missing values.

[2] Education: Low = equivalent to some years of high school; Moderate = equivalent to finished high school; High = equivalent to some years of college or university education or more.

As shown in table 3a/b, the prevalence rates of depressive symptoms, anxiety and somatic complaints increased over the 2 years following deployment. The prevalence of depressive symptoms (cut-off ≥ 36) significantly increased from pre- to 1 month post-deployment (0.4%; Fisher's exact test= 18.862, p<.05). Furthermore, the prevalence rates increased significantly in the two year period after deployment (χ^2 test for trends=6.35, p<.05), with 2.2% (OR 6.32) of the military sample reporting symptoms at the T4 assessment. Compared to baseline, the prevalence rates of anxiety (cut-off ≥ 22), and somatic complaints (cut-off ≥ 27) showed no change from pre-deployment to 1 month post-deployment (Fisher's exact test= 0.00, p>.05; Fisher's exact test= 0.00, p>.05). However, the rates for both anxiety and somatic complaints increased significantly to 1.6% (OR 3.51 and 4.70 for anxiety and somatic symptoms respectively) at the T4 assessment (χ^2 test for trends=5.47, p<.05; χ^2 test for trends=8.00, p<.01).

To illustrate that military personnel might report a high level of symptoms on multiple symptom clusters, overlap between three clusters on three different questionnaires was assessed. Overlap between the symptom clusters depressive symptoms, PTSD symptoms and fatigue prior to and at 6 months post-deployment were examined. Prior to deployment 5.8% of the participants (N=40) scored above cut-off on one or more clusters. Of these individuals, 12.5% (N=5) scored above cut-off on two or more of the subscales. At 6 months post-deployment, 13.6% of the sample (N=98) scored above cut-off for symptoms of PTSD, and/or depression, and/or fatigue. Of these participants, 24.5% (N=24) scored above cut-off on two or more subscales; 5.1% (N=5) scored above cut-off on all three subscales, 17.4% (N=17) on PTSD symptoms and fatigue, and 2.0% (N=2) on depressive symptoms and fatigue. No participants scored above cut-off on both depressive and PTSD symptoms.

Table 3a. Prevalence rates, confidence intervals (CI) and odd's ratios (OR) for clusters of mental health symptoms.

	Pre-deployment				**1 month**					**6 months**				
	n/N	%	95% CI	OR[1]	n/N	%	95% CI	OR[1]	95% CI	n/N	%	95% CI	OR[1]	95% CI
Depressive symptoms	3/850	0.4	0.0–0.8	1.0	5/813	0.6	0.1–1.1	1.75	0.4–7.3	8/734	1.1	0.3–1.9	3.11	0.8–11.8
Anxiety symptoms	4/856	0.5	0.0–1.0	1.0	4/818	0.5	0.0–1.0	1.05	0.3–4.2	5/739	0.7	0.1–1.3	1.45	0.4–5.4
Somatic complaints	3/853	0.4	0.0–0.8	1.0	3/816	0.4	0.0–0.8	1.05	0.2–5.2	5/742	0.7	0.1–1.3	1.92	0.5–8.1
Sleeping problems	22/860	2.6	1.5–3.7	1.0	35/820	4.3	2.9–5.7	1.70	1.0–2.9	31/746	4.2	2.8–5.6	1.65	0.9–2.9
Hostility	34/860	4	2.7–5.3	1.0	57/819	7	5.3–8.7	1.82	1.2–2.8	58/746	7.8	5.9–9.7	2.05	1.3–3.2
PTSD-symptoms	30/704	4.3	2.8–5.8	1.0	66/764	8.6	6.6–10.6	2.12	1.4–3.3	66/745	8.9	6.9–10.9	2.18	1.4–3.4
Fatigue	22/862	2.6	1.5–3.7	1.0	58/822	7.1	5.3–8.9	2.90	1.8–4.8	55/745	7.4	5.5–9.3	3.04	1.8–5.0

Note: PTSD: posttraumatic stress disorder; N: the total number/sample of available data; n: the number of participants that score above cut-off.

[1] OR for 1 and 6 months compared to prevalence prior to deployment.

Table 3b. Prevalence rates, confidence intervals (CI) and odd's ratios (OR) for clusters of mental health symptoms.

	1 year					2 years				
	n/N	%	95% CI	OR1	95% CI	n/N	%	95% CI	OR1	95% CI
Depressive symptoms	8/559	1.4	0.4–2.4	4.10	1.1–15.5	12/548	2.2	1.0 –3.4	6.32	1.8–22.5
Anxiety symptoms	8/561	1.4	0.4–2.4	3.08	0.9–10.3	9/553	1.6	0.6–2.6	3.51	1.1–11.5
Somatic complaints	11/564	2	0.8–3.2	5.64	1.6–20.3	9/552	1.6	0.6–2.6	4.70	0.0–0.8
Sleeping problems	26/567	4.6	2.9–6.3	1.83	1.03–3.3	24/557	4.3	2.6–6.0	1.73	1.5–3.7
Hostility	37/567	6.5	4.5–8.5	1.70	1.1–2.7	37/557	6.6	4.5–8.7	1.73	2.7–5.3
PTSD-symptoms	38/566	6.7	4.8–9.0	1.62	1.0–2.6	30/536	5.6	3.7–7.5	1.33	2.8–5.8
Fatigue	56/568	9.9	7.4–12.4	4.18	2.5–6.9	52/558	9.3	6.9–11.7	3.92	1.5–3.7

Note: PTSD: posttraumatic stress disorder; N: the total number/sample of available data; n: the number of participants that score above cut-off.

[1] OR for 1 and 2 years compared to prevalence prior to deployment.

Discussion

In the current study, the prevalence of mental health symptoms in a cohort of Dutch military personnel was assessed prior to deployment and monitored up to 2 years after return from Afghanistan. The prevalence of symptoms of PTSD, depression, anxiety, somatic complaints, sleeping problems, and fatigue was found to increase after deployment compared with the rates found prior to deployment. This is in agreement with other studies showing that deployment to combat zones increases the risk for mental health symptoms (Hoge et al., 2004; Hotopf et al., 2006; Milliken et al., 2007; Smith et al., 2008; Rona et al., 2009; Fear et al., 2010; Vasterling et al., 2010). Nevertheless, these prevalence rates also show that a large percentage of deployed military personnel do not report a high level of mental health symptoms after deployment.

The highest post-deployment prevalence rates were observed for fatigue (7.1 - 9.9%), PTSD symptoms (5.6 - 8.9%), and hostility (6.5 - 7.8%). Roughly 4% of the deployed military personnel in the present study reported marked sleeping problems. Prevalence of post-deployment depressive, anxiety symptoms and somatic complaints ranged from 0.4 to 2.2%. Prevalence rates of sleeping problems and hostility remained relatively constant across all four post-deployment assessments whereas the prevalence rates of depressive and anxiety symptoms, somatic complaints and fatigue showed a gradual increase over time. The course of PTSD showed an increase in prevalence in the first 6 months after deployment after which the presence of symptoms gradually decreased. When interpreting the prevalence rates, however, it is important to realize that there is some overlap between various mental health symptoms.

Fatigue was the most prevalent health problem in the present study. The highest prevalence rate of fatigue symptoms was 9.9% at 1 year following deployment. These findings confirm previous findings that demonstrated that symptoms of fatigue are common among deployed troops (Johnson et al., 1999; de Vries et al., 2000; Dohrenwend, 2000), and that

deployment-related stress potentially composes a risk factor for chronic fatigue syndrome (Kang et al., 2003). Similarly, the present study underlines previous studies that found a high prevalence of hostility in deployed military personnel (Thomas et al., 2010; Elbogen et al., 2012).

In the current study, the highest prevalence of PTSD symptoms was found 6 months following deployment with 8.9% of the participants scoring above 95th percentile. In UK and US military personnel, the prevalence of PTSD was found to vary between 4.0 and 16.7% after deployment to Iraq or Afghanistan (Solomon and Mikulincer, 1990; Hotopf et al., 2006; Fear et al., 2010). Although the prevalence rate of PTSD in our as well as previous studies in military samples varies, the rates are considerably higher than the lifetime prevalence (1.9%) found in the general population of several European countries (Alonso et al., 2004).

Symptoms of depression and anxiety were less prevalent in the present study as compared to other studies. Depression rates ranged from 1.1 % at 6 months post-deployment to 2.2% at 2 years. Anxiety rates ranged from 0.5% at 1 month after deployment to 1.6% at 2 years. In US military personnel deployed to Afghanistan, the prevalence of MDD varied between 3.5 and 6.9% and the prevalence of anxiety varied between 6.6 and 7.9% (Hoge et al., 2004; Shen et al., 2012). The prevalence in UK military personnel was found to be respectively 3.7% and 4.5% after deployment to Iraq (Iversen et al., 2009). Although comparison is limited by differences in measurement instrument and the population studied, both the currently observed prevalence rates and those previously reported are considerably lower than the lifetime prevalence of MDD and anxiety for the general population of several European countries, which were 12.8% and 13.6% respectively (Alonso et al., 2004).

The increase in the prevalence rates of PTSD, depressive symptoms, anxiety, somatic complaints and fatigue from T1 to T4 assessments might be influenced by post-deployment factors. Mental health symptoms were previously found to be strongly related to adjustment problems after homecoming, lack of social support, reduced satisfaction with the military career, problems at home after the mission (e.g. financial issues and social functioning), and experiencing recent stressful life events (Polusny et al., 2011; Elbogen et al., 2012; Jones et al., 2013; Smid et al., 2013). Furthermore, in UK Armed Forces, personal factors unrelated to deployment, such as accidents, low rank and childhood adversity, were shown to be as important as combat exposure in the risk of developing PTSD (de Vries et al., 2000). This suggests that, in addition to factors prior to and during deployment, a broad range of factors after the mission might be involved in the development of mental health symptoms.

The current findings have various implications for military mental healthcare after deployment. In the assessment of deployment-related mental health symptoms the focus is often on PTSD, however, the current findings suggest that to meet the mental healthcare needs of military personnel after deployment (mental) health symptoms like fatigue, hostility and depression should also be addressed. Moreover, symptom progression over time, as depicted in changes in prevalence rates across time-points, appear to be specific for the

various mental health symptoms that were evaluated. Symptoms of PTSD and hostility, for example, seem to develop soon after homecoming whereas the prevalence of fatigue, depression and anxiety may continue to increase in the years following deployment. Furthermore, as opposed to the prevalence rates of depression and fatigue, the prevalence of PTSD was found to decrease after the 6-month assessment. The prevalence of hostility and sleeping problems was found to remain relatively stable over the two-year period after deployment.

The decrease in prevalence rates of PTSD symptoms after the first six months could be explained by an increased awareness for the development of PTSD following deployment. Although there was an overall reduction in the rate of PTSD, we did not examine whether different trajectories exist in the present sample, representing subgroups of soldiers differing in progression of PTSD symptomatology (e.g., recovered versus delayed-onset). Nevertheless, due to an increased awareness for PTSD (in military personnel and their personal network), we suspect that PTSD symptoms are more easily recognized by healthcare professionals, which will ensure appropriate treatment in an early stage. Also, reporting symptoms of PTSD and also fatigue after deployment might be more accepted among military personnel. In mental health support the stigma around mental health symptoms are still common barriers to care (Vermetten et al., 2014). These findings underline the importance of comprehensive post-deployment screening and monitoring of symptoms over time to be able to meet the mental health care needs of military personnel.

Although the current results provide a proper indication of the prevalence of mental health symptoms in the first two years after deployment, the study is limited by a number of factors. First, unfortunately we were able to maintain 57% of the original sample for the +1 and +2 year assessments only. Although attrition is inevitable in longitudinal cohort studies, it composes a continuing source of concern and a limitation on the generalizability of the results. Relatedly, the influence of non-response on the (change in) prevalence rates cannot be ruled out. In the present study reporting symptoms shortly after return from Afghanistan was found to be related to non-response in following assessments. Importantly however, no such association was found for the additional time-points. Secondly, it is unclear which factors attributed to changes in prevalence rates over time. We did not examine the development and progression of mental health symptoms in a non-deployed military cohort, and we did not systematically evaluate potentially relevant post-deployment factors that may have affected prevalence rates. Therefore, it remains unclear to what extent the present findings are attributable to the deployment to Afghanistan exclusively. Thirdly, a rather strict cut-off point was used possibly resulting in low prevalence rates for symptoms of depression, anxiety and sleep problems. However, since studies have shown that questionnaires might provide higher estimates of mental health symptoms than clinical assessments (e.g. Frueh et al., 2000; Engelhard et al., 2007) the prevalence rates reported in this paper are thought to present a better reflection of the actual prevalence of symptoms. Additionally, although there are doubts about the validity of the SCL-90-R regarding the multidimensionality (e.g. Schmitz et al., 2000) the SCL-90-R is extensively used as a diagnostic instrument to screen

for a broad range of psychiatric and psychosomatic symptoms (Hafkenscheid et al., 2007). Lastly, self-report questionnaires were used to obtain the prevalence rates whereas clinician-rated instruments would probably provide a more reliable estimate of the prevalence.

Conclusion

Although a large percentage of military personnel do not develop mental health symptoms, the current study confirms that the prevalence of mental health symptoms increases after a 4-month deployment to Afghanistan. Public and academic interest in deployment-related mental health symptoms is often confined to PTSD, however, the prevalence of other mental health symptoms was found to remain relatively stable or increased in the two years after deployment. This underlines the importance of investigating and paying attention to a broader range of (mental) health outcomes for a prolonged period after return.

To improve further research on the prevalence of deployment-related mental health symptoms it is important to take into consideration pre-deployment health status and timing of the assessments. In addition, it is important to include a non-deployed control group. To improve the mental health of military personnel after deployment and to meet the (changing) mental health care needs, comprehensive screening and monitoring for a wide range of mental health symptoms at various time-points after deployment is essential. The use of online screening methods and symptom-oriented interventions should be assessed in the future. For the individual participant, this ensures early detection, creates opportunities for self-monitoring, might increase self-reliance and can lower barriers to care.

Acknowledgement and disclosure

This work was financially supported by the Dutch Ministry of Defence.

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CHAPTER 03

Posttraumatic stress symptoms 5 years after military deployment to Afghanistan: an observational cohort study

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Published in: Lancet Psychiatry (2016) 3, 58-64

Abstract

Deployment can put soldiers at risk of developing posttraumatic stress symptoms. Despite several longitudinal studies, little is known about the timing of an increase in posttraumatic stress symptoms relative to pre-deployment. Longitudinal studies starting pre-deployment, in which participants are repeatedly measured over time, are warranted to assess the timing of an increase in symptoms to ultimately assess the timing of an increase in treatment demand after deployment. In this large observational cohort study, Dutch military personnel who were deployed to Afghanistan as part of the International Security Assistance Forces between March, 2005, and September, 2008, were assessed for posttraumatic stress symptoms with the Self-Rating Inventory for Posttraumatic Stress Disorder (SRIP) questionnaire. Participants were assessed 1 month before deployment and followed up at 1 month, 6 months, 12 months, 2 years, and 5 years after deployment, with changes in SRIP scores compared with pre-deployment using a mixed model analysis. The primary outcome was the total score of post-traumatic stress symptoms measured with SRIP at pre-deployment and the five follow-up assessments, with a score of 38 used as the cutoff to indicate substantial posttraumatic stress symptoms. Between March, 2005, and September, 2008, 1007 participants were recruited to this study. The results show two important effects of deployment on posttraumatic stress symptoms. A short-term symptom increase within the first 6 months after deployment (symptom increase coefficient for SRIP score vs pre-deployment (β)=0.99, 95% CI=0.50-1.48); and a long-term symptom increase at 5 years after deployment (β=1.67, 95% CI=1.14-2.20). This study underlines the importance of long-term monitoring of the psychological health of soldiers after deployment because early detection of symptoms is essential to early treatment, which is related to improved psychological health.

Introduction

Military populations are known to be at risk of developing posttraumatic stress disorder (PTSD) after military deployment (Smith et al., 2008; Vasterling et al., 2010; Kok et al., 2012; Reijnen et al., 2015). Accordingly, after deployment, the mental health status of military personnel is monitored and treatment is offered when necessary. To assess symptom development and new posttraumatic stress disorder prevalence after deployment, it is useful to examine how posttraumatic stress symptoms change over time. In longitudinal cohort studies, two important questions can be addressed: at what time(s) after a deployment are posttraumatic stress symptoms increased relative to the pre-deployment status? And what longitudinal developmental trajectories can be identified? Both these issues are important to assess the timing of an increase in treatment demand after deployment.

Changes in posttraumatic stress symptoms relative to the pre-deployment have been investigated in a longitudinal design with a pre-deployment measurement and one follow-up measurement within a year after the deployment (Hoge et al., 2004; Cabrera et al., 2007; Berntsen et al., 2012), which is a useful design to investigate the short-term effects. However, it limits the ability to look at possible long-term effects of deployment. Studies that did look at effects after more than 1 year, by means of a follow-up measurement at 2 or 3 years after deployment, did not look at symptom changes at several time-points (Smith et al., 2008; Rona et al., 2009; Reijnen et al., 2015). Other studies have investigated the heterogeneity in symptom development trajectories after deployment (Orcutt et al., 2004; Dickstein et al., 2010; Berntsen et al., 2012; Bonanno et al., 2012; Karstoft et al., 2013; Andersen et al., 2014; Karstoft et al., 2015). Mostly, four developmental trajectories are identified: a low and stable trajectory (resilient); a high and stable trajectory (chronic); a moderate improving trajectory (recovered); and a worsening trajectory (delayed onset; Bonanno et al., 2012; Karstoft et al., 2013, 2015). Some studies report additional trajectories for mild distress and low fluctuating symptom levels (Berntsen et al., 2012; Andersen et al., 2014). However, previous trajectory studies have either not addressed the change in posttraumatic stress symptoms relative to the pre-deployment or pre-trauma status of these symptoms (Orcutt et al., 2004; Karstoft et al., 2013), or had follow-up times of no longer than 3 years (Dickstein et al., 2010; Berntsen et al., 2012; Andersen et al., 2014). Moreover, these studies were only aimed at identification of posttraumatic stress trajectories (Schelling et al., 2006; Roberts et al., 2009; Shalev et al., 2012), whereas assessment of the timing of changes in levels of posttraumatic stress symptoms after deployment would also be interesting. This is especially important because an early detection of symptoms and subsequent early intervention is related to positive treatment outcome.

The direct effect of deployment on posttraumatic stress symptom levels and the developmental trajectories is probably moderated by several factors. In military studies, younger age, lower ranks, and a low education level are often identified as risk factors for PTSD (Brewin et al., 2000; Riddle et al., 2007; Iversen et al., 2008). Previous traumatic experiences might also play an important role. Several studies have suggested that although childhood trauma

is positively associated with PTSD (Yehuda et al., 1995), the presence of childhood trauma might result in lower reactivity to combat exposure (Cabrera et al., 2007). Furthermore, deployment characteristics, such as exposure to deployment stressors or active participation during combat versus a more supportive role (e.g., medical or logistic functions), have been shown to be related to the effect that deployment has on posttraumatic stress symptoms (Smith et al., 2008; Rona et al., 2009; Fear et al., 2010). Ascertaining factors that relate to changes in posttraumatic stress symptoms after deployment can help to identify military personnel that might be at increased risk of developing increased symptom levels.

We aimed to establish the time-points at which the effect of deployment on posttraumatic stress symptoms was increased or diminished. Furthermore, we aimed to identify trajectories of posttraumatic stress symptom development to distinguish groups of participants who respond differently to deployment. We also investigated the role of demographic characteristics, previous traumatic experiences, and the deployment characteristics on the development of posttraumatic stress symptoms. We postulated that these three factors have an important moderating effect on the relation between deployment and the development of posttraumatic stress symptoms over time.

Methods

Study design and participants

This study was part of a prospective cohort study on stress-factors related to deployment in military personnel. Participants were Dutch military personnel who were deployed to Afghanistan as part of the International Security Assistance Force (ISAF) between March, 2005 and September, 2008. The study was approved by the Institutional Review Board of the University Medical Center Utrecht, The Netherlands. Written informed consent was obtained from all participants after a written and verbal description of the study.

Procedures

The first measurement was done roughly 1 month before deployment. After a deployment period of four months, the follow-up assessments were done at roughly 1 month, 6 months, 12 months, 2 years, and 5 years after the soldiers returned home. The assessment before deployment and at the 1 and 6 months were done at the army-base (paper-and-pencil questionnaires). The 12 month and 2 years measurements were completed by mail (paper-and-pencil) and the 5 years follow-up was done by web-based questionnaire. To maximize the response rate, participants were contacted for a maximum of five times at each assessment by mail, email, and telephone.

We used the Self-Rating Inventory for Posttraumatic Stress Disorder (SRIP) questionnaire for all assessments (Hovens et al., 2002). The SRIP is a Dutch questionnaire used to assess posttraumatic stress symptoms in the past 4 weeks based on the DSM-IV criteria for PTSD and contains 22 questions with responses measured on a Likert scale ranging from 1

(never) to 4 (very frequent). The total score for posttraumatic stress was the sum of all 22 questions, where a higher score indicated more symptoms (range 22–88). A cutoff score of 38 was shown to achieve a good sensitivity and specificity when the SRIP was used as a screening instrument for PTSD (Hovens et al., 2002). The SRIP has good concurrent validity with other PTSD measures, such as the clinician-administered posttraumatic stress disorder scale (CAPS) and Mississippi scale for posttraumatic stress disorder (van Zelst et al., 2003).

The effect of age at the time of deployment was investigated as a continuous variable and also as a dichotomous variable separating the group younger than 21 years from the group that was 21 years or older. Educational level was assessed by categorically separating participants into three levels of education: low (i.e., some years of high school), medium (i.e., finished high school), and high (i.e., college or university education). Rank was separated in four categories: private, corporal, non-commissioned officer (NCO), and staff officer. Early traumatic experiences were assessed with the Early Trauma Inventory Self-Report-Short Form (ETISR-SF; Bremner et al., 2007), which measures potential traumatic experiences before the age of 18 years. The total sum of 27 items represents the total number of different traumatic events experienced. Furthermore, the number of previous deployments was assessed. The deployment characteristics were measured by several variables. At the 1 month after deployment assessment, exposure to potentially traumatic deployment-related stressors was measured with a 19-item deployment stressors checklist (DES; Reijnen et al., 2015). The indicated number of stressors was summed to obtain the total score for deployment stressors. Furthermore, the military function during deployment was assessed which indicated whether the deployment duties were inside the military base, outside the military base, or both. Subsequently, the deployment year was taken into account (2005, 2006, 2007, and 2008) and the number of new deployments after the deployment at study inclusion.

Outcomes

The primary outcome in this study was the total score of posttraumatic stress symptoms measured with SRIP at pre-deployment and at the five follow-up assessments (1 month, 6 months, 12 months, 2 years, and 5 years), with a score of 38 used as the cutoff to indicate substantial posttraumatic stress symptoms (van Zelst et al., 2003).

Statistical analysis

We predicted the outcome for the six time-points using dummy variables to assess the change in the outcome variable at each time-point relative to the pre-deployment score. The interaction of the potential moderating factors (i.e., demographic factors, previous traumatic experiences, or deployment characteristics) with the change in posttraumatic stress symptoms relative to pre-deployment was investigated at each time-point. The potential moderating factors were included one at a time. A random intercept was included to account for the variance between participants pre-deployment. The missing data in the outcome variable were not imputed, but handled by using the maximum likelihood estimation to obtain the model

variables. Missing data in the moderating factors were handled by multiple imputation before the analyses. Details about the missing value analyses and multiple imputation procedure are shown (supplementary material).

The trajectories of posttraumatic stress symptoms were examined in a latent growth mixture model with Mplus version 7.3 (Muthén and Muthén, 2012). A latent growth mixture model can be used to identify subpopulations, that have a different change over time after deployment. In this model, time was modelled as the actual time-points occurred (i.e., −1, 1, 6, 12, 24, 60), which was fixed between participants. The model included a linear, quadratic, and cubic slope; the slope variances were fixed at zero and the intercept variance was estimated. We investigated 1 to 6 class solutions and we used 10.000 iterations and 1000 start values to reach convergence. The models were compared on fit indices (e.g., the Bayesian Information Criterion), entropy, class sizes of no less than 1% of total count, and substantive interpretability. The effect of the moderating factors on the trajectory assignment was investigated in a three-step approach as described by Vermunt (2010). In the first step, a set of latent trajectories were extracted from the data. In the second step, each participant was assigned to the most likely trajectory. In the third step, the association between the assigned trajectories and moderating factors was investigated. Details about the trajectory analysis are shown (supplementary material).

Participants were included in the mixed models and trajectory analyses if they had an assessment at one or more time-points. We analyzed the data with mixed models using Stata version 12.1. A two-tailed p value of less than 0.05 was deemed statistically significant.

Results

Between March, 2005, and September, 2008, 1032 participants entered the study before their deployment. 25 participants were eventually not deployed, leaving 1007 participants in the study. Baseline characteristics are shown in Table 1. The average scores for posttraumatic stress symptoms and probable posttraumatic stress disorder rates are displayed at each time-point (Table 2). A full tabulation of the results for all analyses is shown (supplementary material). The basic mixed model analysis results with only the time-points included showed a significant increase of posttraumatic stress symptoms relative to pre-deployment at 1 month after deployment (symptom increase coefficient for SRIP score vs pre-deployment [β]=0.94, 95% CI= 0.46—1.43), at 6 months after deployment (β=0.99, 95% CI= 0.50—1.48), and at 5 years after deployment (β=1.67, 95% CI= 1.14—2.20). The latent growth mixture model analyses fit results of the models with 1 to 6 classes (supplementary material). The solution with three classes produced the best solution with respect to fit and theoretical interpretation; solutions with more than three classes had too small group sizes and encountered convergence issues. The model with three latent trajectories had one large group of 818 participants (85%) that had a low stable trajectory (i.e., resilient); a smaller group of 91 participants (9%) that had a trajectory with a moderate level of posttraumatic stress symptoms that increased heavily in the last time period (i.e., delayed onset); and another small group of 51 participants (5%)

that had increasing symptoms in the first year after deployment that then decreased after the first year (i.e., recovered). The estimated means for the three latent trajectories are displayed (Figure 1). The plots with the reported scores per estimated trajectory and the descriptive characteristics for the participants in the trajectories are presented (supplementary material).

Table 1. Baseline characteristics of participants who were deployed, separated for the participants included in the mixed model and latent trajectory analyses and participants with missing outcome values.

	Participants with a SRIP measurement at one or more time-points (N=960)[a] Count (%)	**Participants without a SRIP measurement** (N=47)[a] Count (%)	p-value
Gender			
Male	876 (91%)	45 (96%)	0.282
Female	84 (9%)	2 (4%)	
Age	*(N=958)*	*(N=42)*	
<21	130 (14%)	9 (21%)	0.150
≥21	828 (86%)	33 (79%)	
Education	*(N =883)*	*(N =27)*	
Low	33 (4%)	0 (0%)	0.590
Moderate	751 (85%)	24 (89%)	
High	99 (11%)	3 (11%)	
Rank	*(N =950)*	*(N =30)*	
Private	376 (40%)	18 (60%)	0.153
Corporal	199 (21%)	4 (13%)	
Non-commissioned officer	245 (26%)	6 (20%)	
Staff officer	130 (14%)	2 (7%)	
Previous deployment	*(N =875)*	*(N =27)*	
0	459 (52%)	20 (74%)	0.071
1	224 (26%)	5(19%)	
≥2	189 (22%)	2 (7%)	
Function during deployment	*(N =791)*	*(N =13)*	
Inside	244 (31%)	4 (31%)	0.501
Outside	474 (60%)	9 (69%)	
Both	73 (9%)	0 (0%)	
Deployment year	*(N =960)*	*(N =47)*	
2005/2006	234 (24%)	27 (57%)	<0.0001
2007/2008	726 (76%)	20 (43%)	
New deployments	*(N =579)*	*(N =0)*	
0	400 (69%)	-	
1	114 (20%)	-	
≥2	65 (11%)	-	
ETISR-SF total score	*(N =873)*	*(N =15)*	
Mean (SD)	3.44 (3.04)	6.13 (3.20)	0.0007
DES total score	*(N =705)*	*(N =2)*	
Mean (SD)	5.51 (3.22)	5.50 (4.95)	0.9963

Note: [a] Sample sizes might not add up to total due to missing data in the descriptive variables; SRIP : Self-Rating Inventory for Posttraumatic Stress Disorder; SD : standard deviation; ETISR-SR : Early Trauma Inventory self-report-short form; DES : Deployment stressors checklist; Differences on descriptive characteristics between participants with SRIP and participants without SRIP were tested with a *t*-test (continuous) or χ^2 -test (categorical).

Table 2. Dutch military personnel deployed to Afghanistan reporting posttraumatic stress symptoms at each time-point.

	N	Mean (SD)	Above cutoff (%)
Pre-deployment	680	26.76(5.03)	27(3.97%)
1 month	753	27.62(6.14)	62(8.23%)
6 months	737	27.73(7.07)	63(8.55%)
12 months	562	27.02(6.94)	38(6.76%)
2 years	528	26.64(5.90)	29(5.49%)
5 years	559	28.30(8.07)	72(12.88%)

Note: N: Total number of participants; SD: Standard Deviation; a SRIP score of 38 was used as cutoff value.

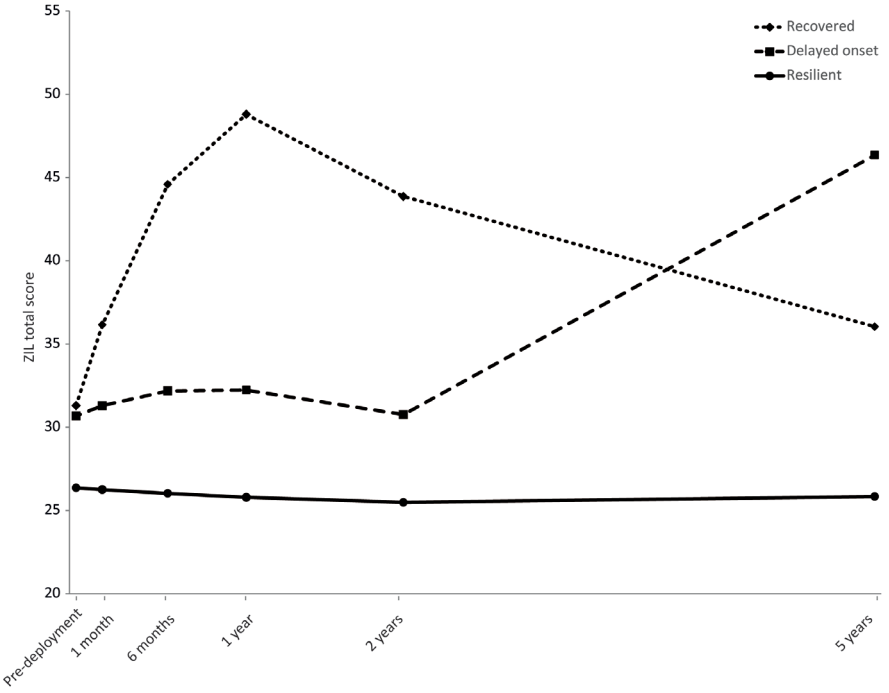

Figure 1. Latent developmental trajectories of posttraumatic stress symptoms (n=960). The solid line represents the resilient group (85.2%); the dashed line represents the delayed onset group (9.4%); the dotted line represents the recovered group (5.3%).

Age was significantly negatively related to the increase in posttraumatic stress symptoms at 5 years after deployment (effect of age at 5 years: β=−0.10, 95% CI=−0.16—−0.05), which suggests a higher increase in posttraumatic stress symptoms for younger soldiers. Moreover, soldiers younger than 21 years reported a greater increase in posttraumatic stress symptoms at 1 and 5 years after deployment than soldiers that were 21 years or older during deployment (<21 years at 12months: β=−2.63, 95% CI=−4.33— −0.93; <21 years at 5 years: β=−1.86, 95% CI=−3.53— −0.18) (Figure 2). The rank of the soldiers was strongly correlated

with age (r=0.73). Accordingly, the moderating effect of rank was strongest at the 5-year measurement, where the lower ranking soldiers (i.e., private and corporal) had more increased posttraumatic stress symptoms than the higher ranking soldiers (i.e., NCO and staff officer; higher rank at 5 years: β=-1.94, 95% CI=-3.01— -0.86). No rank differences were reported between the latent developmental trajectories. The educational level of the soldiers during their deployment did not affect the relation between deployment and the change in posttraumatic stress symptoms, nor did it differ between the subjects in the different latent developmental trajectories.

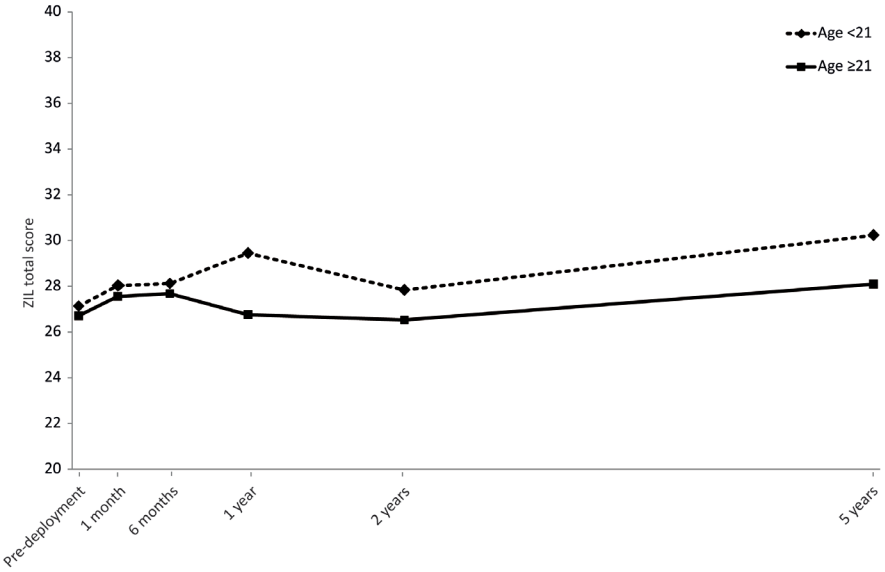

Figure 2. The average level of posttraumatic stress symptoms over time per age group. The soldiers younger than 21 years old are displayed by the dashed line (n=130) and soldiers of 21 years or older are displayed by the solid line (n=828).

Previous traumatic experiences only influenced the relation between deployment and the level of posttraumatic stress symptoms at 12 months after deployment (ETISR-SF at 12 months: β=-0.19, 95% CI=-0.38— -0.01). Additionally, the participants in the delayed onset trajectory had a slightly higher score on early trauma than the resilient group (β=0.13, 95% CI=0.01—0.25). Previous deployment, did not affect the relation between deployment and the change in posttraumatic stress symptoms, nor did it differ between the latent developmental trajectories. An increased level of deployment stressors was significantly related to a greater increase in

posttraumatic stress symptoms for all time-points apart from the 2-year assessment (Table 3). When the moderating effect for age was corrected for the moderating effect for deployment stressors, the effect of age at 5 years after deployment remained (age at 5 years: β=-0.08, 95% CI=-0.14— -0.02). The participants in the recovered trajectory and in the delayed onset trajectory had a significantly higher level of deployment stressors compared with the participants in the resilient trajectory (recovered: β=0.18, 95% CI=0.05—0.32; delayed onset: β=0.15, 95% CI=0.04—0.25). Furthermore, the group with combat duties outside the military base had significantly more increased posttraumatic stress symptoms at 12 months after deployment and at 5 years after deployment compared with the group that only resided inside the military base during deployment (outside the military base at 12 months: β=1.28, 95% CI=0.08— 2.47; outside the military base at 5 years: β=1.63, 95% CI=0.40— 2.86). Additionally, the units deployed in 2007 and 2008 had more increased posttraumatic stress symptoms at 5 years after deployment relative to the groups deployed in 2005 and 2006 (β=1.66, 95% CI=0.27—3.05). A new deployment after the main deployment did not have an effect on the change of posttraumatic complaints over time. The participants in the different latent developmental trajectories of posttraumatic stress symptoms did not differ in function during deployment, deployment year, or in the number of new deployments.

Table 3. Variable estimates for change in posttraumatic stress symptoms over time relative to pre-deployment status of Dutch military personnel deployed to Afghanistan (n=960).

	Time-effect		**Interaction with deployment stressors**	
	Coefficient (95% CI)	p-value	Coefficient (95% CI)	p-value
Intercept (pre-deployment)	25.81 (24.81 – 26.81)	<0.0001		
Δ 1 month[a]	-0.74 (-1.80 – 0.30)	0.160	0.29 (0.13 – 0.45)	<0.0001
Δ 6 months[a]	-0.86 (-1.94 – 0.22)	0.120	0.32 (0.15 – 0.48)	<0.0001
Δ 12 months[a]	-1.67 (-2.80 – -0.54)	0.004	0.37 (0.19 – 0.54)	<0.0001
Δ 2 years[a]	-0.93 (-2.17 – 0.31)	0.141	0.19 (-0.01 – 0.39)	0.058
Δ 5 years[a]	-0.29 (-1.49 – 0.92)	0.639	0.34 (0.15 – 0.53)	<0.0001

Note: [a] Δ indicates the difference relative to pre-deployment status; 95% CI : 95% Confidence Interval.

Discussion

The main objective of this study was to map the development of posttraumatic stress symptoms over time after deployment. As expected and previously identified (Hoge et al., 2004; Cabrera et al., 2007; Berntsen et al., 2012), during the first 6 months after deployment, the average level of posttraumatic stress symptoms increased compared with the pre-deployment score. This finding can be identified as the immediate effect of deployment on the development of posttraumatic stress symptoms. 1 year after deployment, the average level of posttraumatic stress symptoms was reported to drop back to the level at pre-deployment. This study identified a subsequent increase in posttraumatic stress symptoms 5 years after deployment, which was larger than at all previous time-points. This delayed effect was also identified in one of the developmental trajectories for posttraumatic stress symptoms, where this trajectory displayed moderate levels of posttraumatic stress symptoms in the first 2 years and an increased level at 5 years after deployment. Although the initial reaction to stress typically emerges in the short-term, in others, it might develop with a latency that can be described as delayed onset posttraumatic stress after military combat exposure (Horesh et al., 2013). Andreasen (2004) argues that the delayed onset of symptoms after traumatic experiences in military populations might occur because stress symptoms are not adaptive during combat. Moreover, belonging to a military group and feeling safety and understanding might support a resilient response to traumatic experiences. However, when the connection to a military group diminishes over time, resilience might wear off, contributing to a delayed stress response (Smid et al., 2009). Additionally, the longitudinal development of posttraumatic stress might be possible to explain with neurobiological mechanisms (van Zuiden et al., 2013; Vermetten et al., 2015). This has been a continuing effort in other studies in this cohort, and these clinical findings should be correlated with an endophenotype of the delayed response of posttraumatic stress disorder symptoms (i.e., the genetic characteristics that might explain posttraumatic stress symptoms; Geuze et al., 2012; van Zuiden et al., 2012). In view of this, early interventions by pharmacological approaches or trauma-focused psychotherapy might be beneficial to prevent late onset posttraumatic stress disorder (Schelling et al., 2006; Roberts et al., 2009; Shalev et al., 2012).

In this study, we identified three separate developmental trajectories of posttraumatic stress symptoms that are also found in previous studies. We did not uncover a fourth group with a chronic posttraumatic trajectory as mostly found in other studies (Bonanno et al., 2012; Karstoft et al., 2013; Karstoft et al., 2015). Previous studies use a pre-deployment measurement and only two follow-up measurements, whereas our study contained an increased number of measurements, which might be a reason for this difference. Our measurements in the first 2 years after deployment, showed an increased level of posttraumatic stress symptoms within the first year that subsequently decreased after 2 years, possibly because of successful treatment. This increase followed by a recovery process might have been missed if we used fewer follow-up measurements in the first 2 years after deployment.

Furthermore, as expected and previously identified, the effect of deployment on the increase of posttraumatic stress symptoms is positively moderated by combat stressors and having duties outside the military base (Rona et al., 2009). Moreover, the individuals with non-resilient developmental trajectories had experienced more combat stressors. Additionally, the groups deployed in 2007 and 2008 showed a larger increase in posttraumatic stress symptoms in the fifth year than those deployed in 2005 and 2006. This could also confirm the expectation that exposure to more severe combat stressors relate to increased posttraumatic stress symptoms because during the 2007 and 2008 deployments, the Dutch soldiers had more battlefield casualties and deaths compared with the deployments in 2005 and 2006 (Hoencamp et al., 2014). The moderating effect for age on the change in posttraumatic stress symptoms was partly explained by combat exposure. However, when the effect of age was corrected for the number of deployment stressors, the interaction at the 1 and 5-year timepoint was preserved. These findings implicate that younger soldiers need to be monitored more frequently after a deployment, because they might be at an increased risk of developing posttraumatic stress symptoms.

A limitation of this study is the underrepresentation of female soldiers, which might have affected the results, because men and women tend to respond differently to trauma (Nemeroff et al., 2006). However, this is a frequent limitation in military deployment studies due to the relatively low percentage of female soldiers in the army. For that reason, this sample is representative for generalization to an army population more than to the general population. Yet, our findings might extend to other populations, such as political prisoners and World Trade Center responders, where similar response patterns to traumatic events have been demonstrated (Maercker et al., 2013; Pietrzak et al., 2014). Moreover, we used self-report measurements to obtain the posttraumatic stress symptom levels, whereas a clinical interview might have resulted in more reliable scores. However, we expect that the change scores are not affected too much by the possible bias from self-report measurements. Other limitations are the absence of information on treatment, military employment status and comorbidity of other psychiatric conditions over time. For future studies, it may be useful to control for this time-varying information, as well as other covariates, such as deployment stressors before designing the study. The strength of this study is the large sample size that was included and the advanced handling of missing data in the sample that enabled the inclusion of a maximum level of information in the analyses. Moreover, this study has a large number of follow-up measurements during a long period of time, which is a great advantage compared with other studies that have either a long-term period with a small number of follow-up measurements, or a large number of follow-up measurements in a short-term period.

Overall, we identified two important effects of deployment on posttraumatic stress symptoms: a short-term symptom increase within the first six months after deployment; and a long-term symptom increase at 5 years after deployment. The findings implicate an increased demand for psychological care immediately after deployment and even 5 years

after a deployment has taken place. Healthcare providers need to be made aware of these potential treatment needs. Moreover, this study confirms that the experience of more deployment stressors increases posttraumatic stress reactions. Furthermore, younger military personnel respond more strongly to deployment with increased symptom levels. The results of this study underscore the importance of long-term monitoring of psychological health of deployed soldiers, because early detection of symptoms is essential to early treatment, which is related to positive effect on mental health.

Acknowledgements and disclosure

This study was financially supported by the Dutch Ministry of Defence.

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Supplementary material

Missing data analyses

The missing values in the posttraumatic stress symptom scores over time were investigated to assume the most plausible missing data mechanism. The probability of missing values was related to the posttraumatic stress symptom scores on the previous time point in order to study the correlation of study drop-out with symptom levels. Only the symptom level at six months after deployment were related to the probability of missing values at two years and five years after deployment. Additionally, we conducted Little's MCAR test to assess the differences of the observed mean and the estimated mean in each missing data pattern (Little et al., 1999). The missing data patterns are presented in Table 1. Little's MCAR test indicated that there were no differences, which relates to a missing completely at random mechanism. Based on the results from this missing value analysis, we assume a missing at random mechanism for the missing values in the posttraumatic stress symptom scores over time.

Table 1. Missing data patterns for the posttraumatic stress symptoms scores at each time-point.

Time-points						
pre-deployment	1 month	6 months	12 months	2 years	5 years	n[a]
						253
X						62
			X			18
	X					23
X	X					31
				X		28
			X	X		24
			X	X	X	76
				X	X	30
					X	23
			X		X	14
X					X	12
X				X	X	14
		X				12
		X	X	X	X	46
	X		X	X	X	16
	X	X	X	X	X	45
X			X	X	X	37
X		X	X	X	X	26
X	X	X	X	X	X	47

Note: X indicates a missing value for that time-point. n[a] indicates the number of participants that had the corresponding missing data pattern – patterns that occurred in less than 1% of the participants were omitted.

The missing values on the potential modifying factors were assumed to be missing at random as well, and were handled with multiple imputation prior to the analyses.

Multiple imputation procedure

The multiple imputation was performed in STATA with the "mi impute chained" command with predictive mean matching using 50 imputations (StataCorp, 2013; White et al., 2011). The imputation model included the subscales of the ETISR-SF and the SRIP and the baseline descriptive variables (i.e., age, gender, deployment year, function during deployment, rank, education, number of previous deployments and number of new deployments). The missing item variables in the deployment stressors checklist were imputed at the item level. The performance of the imputation procedure was checked by inspecting the iteration plots for each imputed variable. The total scores of the questionnaires were calculated with the "passive imputation" command by either summing the imputed subscales for the ETISR-SF or the imputed item scores for the deployment stressors checklist.

Latent trajectory growth mixture modeling results

The latent trajectories were extracted from the data via a latent growth mixture model in Mplus (Muthén and Muthén, 2012). In the latent growth mixture model, time was modeled as the actual time-points occurred (i.e., -1; 1; 6; 12; 24; 60), which was fixed between subjects. The model included a linear, quadratic and cubic slope, the slope variances were fixed at zero and the intercept variance was estimated. We investigated 1 to 6 class solutions and we used 10000 iterations and 1000 start values in order to reach convergence. The models were compared on fit indices (e.g. the Bayesian Information Criterion (BIC)), entropy, class sizes of no less than 1% of total count, and substantive interpretability (Table 2; Jung and Wickrama, 2008).

Table 2. Fit indices for one to six class solutions of the latent trajectory growth mixture models.

Fit indices	1 class	2 classes	3 classes	4 classes	5 classes	6 classes
AIC	23789.07	23421.08	23211.03	23033.00	22916.49	22786.31
BIC	23842.61	23498.95	23313.23	23159.54	23067.36	22961.52
Adj BIC[a]	23807.67	23448.13	23246.54	23076.97	22968.91	22847.19
Entropy		0.925	0.856	0.885	0.871	0.863
Proportion of		0.066	0.094	0.004	0.043	0.782
participants per		0.934	0.053	0.049	0.004	0.068
class[b]			0.852	0.828	0.800	0.020
				0.118	0.040	0.054
					0.112	0.003
						0.073

Note: [a] BIC adjusted for sample size; [b] Class proportions based on the estimated model.

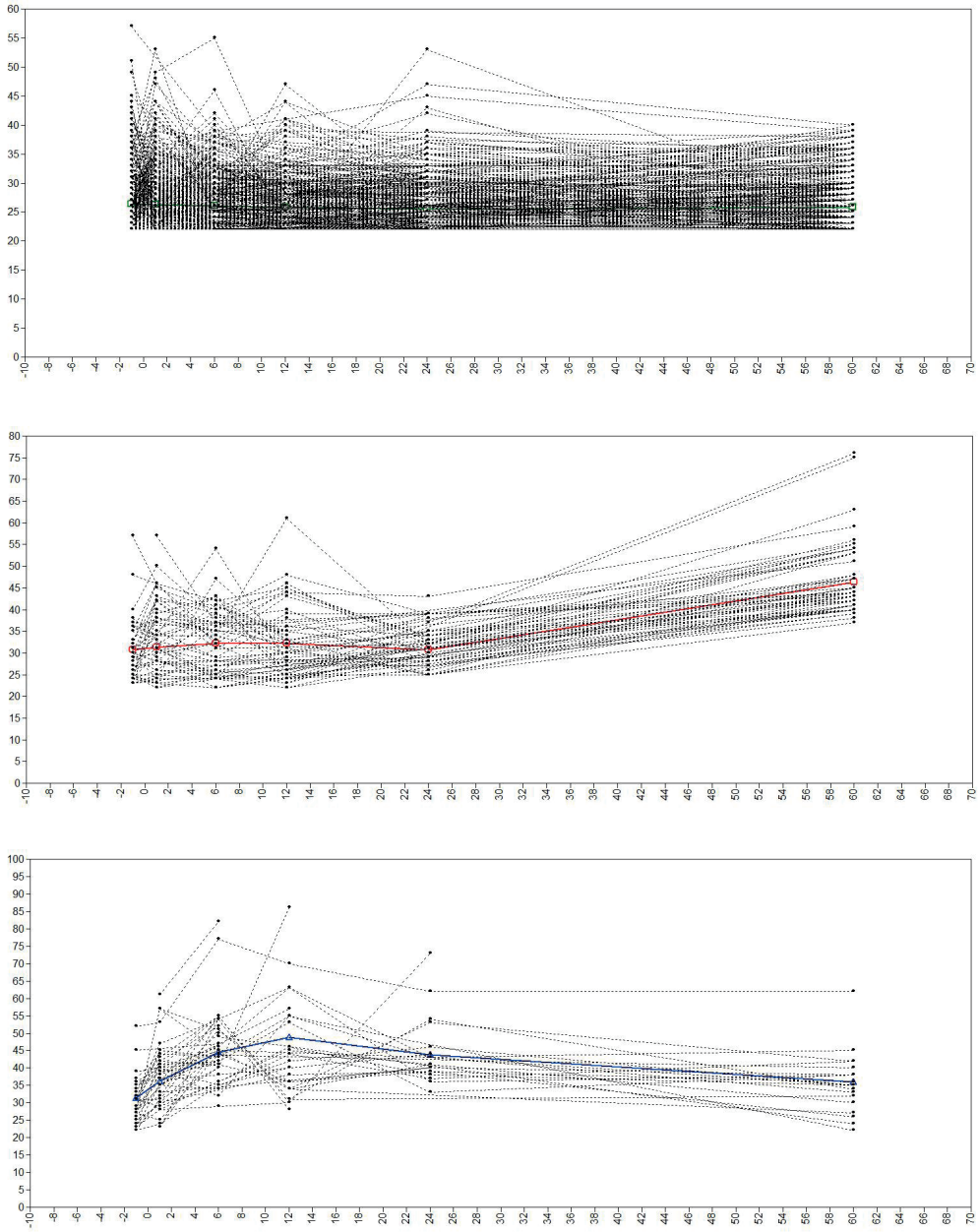

Figure 1. Estimated means and observed individual values for latent developmental trajectories.
Top panel presents the resilient trajectory, middle panel the delayed onset trajectory and the bottom panel the recovery trajectory.

Table 3. Descriptive characteristics estimated percentages from multiple imputed data for each trajectory in the three-class solution for the observed data.

Fit indices	Resilient trajectory (n=848)	Recovered trajectory (n=49)	Delayed onset trajectory (n=63)
Gender			
Male	91.2%	91.8%	92.1%
Female	8.8%	8.1%	7.9%
Age			
<21	12.9%	22.5%	15.9%
≥21	87.1%	77.5%	84.1%
Education			
Low	3.8%	8.6%	1.8%
Moderate	85.0%	82.9%	85.0%
High	11.2%	8.4%	13.2 %
Rank			
Private	39.1%	51.0%	39.7%
Corporal	20.7%	22.5%	23.8%
Non-commissioned officer	26.2%	20.4%	22.2%
Staff officer	14.0%	6.1%	14.3%
Previous deployment			
No	51.9%	70.0%	52.6%
Yes	48.1%	30.0%	47.4%
Function during deployment			
Inside	31.6%	20.6%	27.9%
Outside	59.2%	69.3%	59.9%
Both	9.2%	10.1%	12.2%
Deployment year			
2005/2006	25.2%	22.5%	14.3%
2007/2008	74.8%	77.5%	85.7%
New deployments			
No	68.4%	72.3%	75.1%
Yes	31.6%	27.7%	24.9%
ETISR-SF total score			
Mean(SD)	3.35(0.10)	4.48(0.59)	4.38(0.50)
DES total score			
Mean(SD)	5.36(0.12)	7.38(0.58)	6.58(0.47)

Note: ETISR-SR : Early Trauma Inventory self-report-short form; DES : Deployment stressors checklist; SD : Standard Deviation.

Analysis results
Mixed model analyses

Table 4. Parameter estimates for change in posttraumatic stress symptoms over time relative to pre-deployment status without interactions with potential moderators (n=960).

	Time-effect	
	Coefficient (95% CI)	p-values
Intercept (pre-deployment)	26.71 (26.25 – 27.18)	<0.0001
Δ 1 month[a]	0.94 (0.46 – 1.43)	<0.0001
Δ 6 months[a]	0.99 (0.50 – 1.48)	<0.0001
Δ 12 months[a]	0.41 (-0.12 – 0.94)	0.129
Δ 2 years[a]	0.20 (-0.34 – 0.74)	0.464
Δ 5 years[a]	1.67 (1.14 – 2.20)	<0.0001

Note: [a] Δ indicates the difference relative to pre-deployment status; 95% CI : 95% Confidence Interval.

Table 5. Parameter estimates for change in posttraumatic stress symptoms over time relative to pre-deployment status with age as moderator (n=960).

	Effect		Interaction time x age	
	Coefficient (95% CI)	p-values	Coefficient (95% CI)	p-values
Intercept (pre-deployment)	27.78 (26.21 – 29.35)	<0.0001		
Δ 1 month[a]	2.20 (0.54 – 3.85)	0.009	-0.04 (-0.10 – 0.01)	0.122
Δ 6 months[a]	2.20 (0.55 – 3.85)	0.009	-0.04 (-0.10 – 0.01)	0.135
Δ 12 months[a]	2.13 (0.34 – 3.91)	0.020	-0.06 (-0.12 – 0.00)	0.052
Δ 2 years[a]	0.40 (-1.45 – 2.25)	0.673	-0.01(-0.07 – 0.05)	0.775
Δ 5 years[a]	4.84 (3.03 – 6.65)	<0.0001	-0.10 (-0.16 – -0.05)	<0.0001
Age	-0.04 (-0.09 – 0.02)	0.174		

Note: [a] Δ indicates the difference relative to pre-deployment status; 95% CI : 95% Confidence Interval.

Table 6. Parameter estimates for change in posttraumatic stress symptoms over time relative to pre-deployment status with dichotomous age as moderator (n=960).

	Effect		Interaction time x age[21]	
	Coefficient (95% CI)	p-values	Coefficient (95% CI)	p-values
Intercept (pre-deployment)	27.12 (26.88 – 28.37)	<0.0001		
Δ 1 month[a]	1.15 (-0.15 – 2.46)	0.083	-0.24 (-1.65 – 1.16)	0.734
Δ 6 months[a]	0.96 (-0.39 – 2.31)	0.164	0.04 (-1.41 – 1.48)	0.961
Δ 12 months[a]	2.78 (1.18 – 4.39)	0.001	-2.63 (-4.33 – -0.93)	0.002
Δ 2 years[a]	0.80 (-0.88 – 2.48)	0.350	-0.67(-2.44 – 1.10)	0.457
Δ 5 years[a]	3.34 (1.76 – 4.92)	<0.0001	-1.86 (-3.53 – -0.18)	0.030
Age[21]	-0.47 (-0.15 – 2.46)	0.493		

Note: [a] Δ indicates the difference relative to pre-deployment status; Age[21] : dichotomous age variable that separates soldiers under 21 from soldiers of 21 or older; 95% CI : 95% Confidence Interval.

Table 7. Parameter estimates for change in posttraumatic stress symptoms over time relative to pre-deployment status with military rank as moderator (n=960).

	Effect		Interaction time x rank[b]	
	Coefficient (95% CI)	p-values	Coefficient (95% CI)	p-values
Intercept (pre-deployment)	26.86 (26.27 – 27.45)	<0.0001		
Δ 1 month[a]	1.14 (0.52 – 1.76)	<0.0001	-0.49 (-1.48 – 0.51)	0.338
Δ 6 months[a]	1.27 (0.63 – 1.91)	<0.0001	-0.67 (-1.66 – 0.33)	0.189
Δ 12 months[a]	0.65 (-0.07 – 1.37)	0.078	-0.55 (-1.62 – -0.52)	0.315
Δ 2 years[a]	0.20 (-0.56 – 0.96)	0.600	-0.10(-1.19 – 0.99)	0.855
Δ 5 years[a]	2.62 (1.88 – 3.35)	<0.0001	-1.94 (-3.01 – -0.86)	<0.0001
Rank[b]	-0.36 (-1.31 – 0.59)	0.455		

Note: [a] Δ indicates the difference relative to pre-deployment status; [b] the rank parameter indicates the difference between NCO & staff officer ranks versus soldier and corporal ranks (reference); 95% CI : 95% Confidence Interval.

Table 8. Parameter estimates for change in posttraumatic stress symptoms over time relative to pre-deployment status with the education level as moderator (n=960).

	Effect		Interaction time x education[medium b]		Interaction time x education[high b]	
	Coefficient (95% CI)	p-values	Coefficient (95% CI)	p-values	Coefficient (95% CI)	p-values
Intercept (pre-deployment)	30.0 (25.48 – 30.5)	<0.0001				
Δ 1 month[a]	1.13 (-1.48 – 3.74)	0.396	-0.16 (-2.83 – 2.51)	0.909	-0.61 (-3.64 – 2.41)	0.691
Δ 6 months[a]	1.41 (-1.24 – 4.05)	0.298	-0.34 (-3.04 – 2.37)	0.808	-1.15 (-4.21 – 1.91)	0.461
Δ 12 months[a]	0.99 (-3.29 – 5.28)	0.648	-0.51 (-4.89 – 3.88)	0.820	-1.20 (-5.75 – 3.35)	0.603
Δ 2 years[a]	-2.06 (-5.76 – 1.65)	0.276	2.34 (-1.42 – 6.10)	0.222	1.98 (-2.03 – 5.98)	0.334
Δ 5 years[a]	-0.19 (-4.05 – 3.66)	0.921	2.03 (-1.89 – 5.94)	0.310	1.14 (-3.03 – 5.30)	0.592
Education[medium b]	-1.26 (-3.83 – 1.31)	0.337				
Education[high b]	-1.76 (-4.69 – 1.18)	0.241				

Note: [a] Δ indicates the difference relative to pre-deployment status; [b]reference category is group with a low education level; 95% CI : 95% Confidence Interval; Education[medium] : medium level of education; Education[high] : high level of education.

Table 9. Parameter estimates for change in posttraumatic stress symptoms over time relative to pre-deployment status with the early trauma total score as moderator (n=960).

	Effect		Interaction time x ETISR-SF	
	Coefficient (95% CI)	p-values	Coefficient (95% CI)	p-values
Intercept (pre-deployment)	25.13 (24.44 – 25.83)	<0.0001		
Δ 1 month[a]	1.19 (0.45 – 1.93)	0.001	-0.08 (-0.25 – 0.09)	0.349
Δ 6 months[a]	1.06 (0.31 – 1.80)	0.006	-0.32 (-0.20 – 0.14)	0.714
Δ 12 months[a]	1.02 (0.21 – 1.82)	0.013	-0.19 (-0.38 – -0.01)	0.040
Δ 2 years[a]	0.26 (-0.56 – 1.08)	0.531	-0.03 (-0.22 – 0.15)	0.712
Δ 5 years[a]	1.52 (0.70 – 2.32)	<0.0001	0.03 (-0.15 – 0.21)	0.752
ETISR-SF total score	0.47 (0.31 – 0.63)	<0.0001		

Note: [a] Δ indicates the difference relative to pre-deployment status; ETISR-SF total score : Early trauma inventory self-report – short form; 95% CI : 95% Confidence Interval.

Table 10. Parameter estimates for change in posttraumatic stress symptoms over time relative to pre-deployment status with previous deployments as moderator (n=960).

	Effect		**Interaction time x previous deployments**	
	Coefficient (95% CI)	p-values	Coefficient (95% CI)	p-values
Intercept (pre-deployment)	26.90 (26.25 – 27.54)	<0.0001		
Δ 1 month[a]	1.02 (0.34 – 1.69)	0.003	-0.15 (-1.13 – 0.83)	0.769
Δ 6 months[a]	1.22 (0.53 – 1.91)	0.001	-0.45 (-1.46 – 0.55)	0.376
Δ 12 months[a]	0.79 (0.03 – 1.56)	xs0.042	-0.73 (-1.81 – -0.35)	0.186
Δ 2 years[a]	0.13 (-0.66 – 0.92)	0.739	0.11 (-0.99 – 1.21)	0.850
Δ 5 years[a]	2.02 (1.23 – 2.80)	<0.0001	-0.66 (-1.77 – 0.46)	0.249
Previous deployments	-0.38 (-1.34 – 0.57)	0.432		

Note: [a] Δ indicates the difference relative to pre-deployment status; 95% CI : 95% Confidence Interval.

Table 11. Parameter estimates for change in posttraumatic stress symptoms over time relative to pre-deployment status with the deployment stressors checklist total score as moderator (n=960).

	Effect		**Interaction time x DES**	
	Coefficient (95% CI)	p-values	Coefficient (95% CI)	p-values
Intercept (pre-deployment)	25.81 (24.81 – 26.81)	<0.0001		
Δ 1 month[a]	-0.74 (-1.80 – 0.30)	0.160	0.29 (0.13 – 0.45)	<0.0001
Δ 6 months[a]	-0.86 (-1.94 – 0.22)	0.120	0.32 (0.15 – 0.48)	<0.0001
Δ 12 months[a]	-1.67 (-2.80 – -0.54)	0.004	0.37 (0.19 – 0.54)	<0.0001
Δ 2 years[a]	-0.93 (-2.17 – 0.31)	0.141	0.19 (-0.01 – 0.39)	0.058
Δ 5 years[a]	-0.29 (-1.49 – 0.92)	0.639	0.34 (0.15 – 0.53)	<0.0001
DES total score	0.94 (-0.04 – 1.93)	0.059		

Note: [a] Δ indicates the difference relative to pre-deployment status; 95% CI : 95% Confidence Interval.

Table 12. Parameter estimates for change in posttraumatic stress symptoms over time relative to pre-deployment status with the military function as moderator (n=960).

	Effect		**Interaction time x function$^{outside\ b}$**		**Interaction time x function$^{both\ b}$**	
	Coefficient (95% CI)	p-values	Coefficient (95% CI)	p-values	Coefficient (95% CI)	p-values
Intercept (pre-deployment)	26.44 (25.58 – 27.3)	<0.0001				
Δ 1 month[a]	0.53 (-0.36 – 1.41)	0.224	0.59 (-0.52 – 1.69)	0.297	0.70 (-1.10 – 2.50)	0.446
Δ 6 months[a]	0.41 (-0.46 – 1.28)	0.356	0.87 (-0.22 – 1.97)	0.117	0.79 (-1.01 – 2.60)	0.388
Δ 12 months[a]	-0.27 (-1.19 – 0.64)	0.556	1.28 (0.08 – 2.47)	0.037	0.08 (-1.86 – 2.03)	0.934
Δ 2 years[a]	-0.10 (-0.99 – 0.80)	0.835	0.55 (-0.63 – 1.74)	0.360	-0.24 (-2.25 – 1.79)	0.819
Δ 5 years[a]	-0.71 (-0.22 – 1.63)	0.133	1.63 (0.40 – 2.86)	0.009	1.13 (-0.97 – 3.24)	0.291
Function$^{outside\ b}$	0.38 (-0.69 – 1.44)	0.487				
Function$^{both\ b}$	0.62 (-1.16 – 2.40)	0.496				

Note: [a] Δ indicates the difference relative to pre-deployment status; [b]reference category is group with function inside military base; 95% CI : 95% Confidence Interval; Function^{outside} : function outside military base; Function^{both} : function both inside and outside the military base.

Table 13. Parameter estimates for change in posttraumatic stress symptoms over time relative to pre-deployment status with the deployment year as moderator (n=960).

	Effect		**Interaction time x Year[2007/2008 b]**	
	Coefficient (95% CI)	p-values	Coefficient (95% CI)	p-values
Intercept (pre-deployment)	25.87 (24.81 – 27.12)	<0.0001		
Δ 1 month[a]	0.56 (-0.72 – 1.83)	0.392	0.47 (-0.91 – 1.85)	0.501
Δ 6 months	0.96 (-0.28 – 2.19)	0.129	0.06 (-1.28 – 1.41)	0.926
Δ 12 months[a]	1.21 (-0.09 – 2.52)	0.068	-1.01 (-2.44 – 0.42)	0.165
Δ 2 years[a]	0.63 (-0.62 – 1.89)	0.324	-0.57 (-1.97 – 0.83)	0.422
Δ 5 years[a]	0.47 (-0.79 – 1.72)	0.466	1.66 (0.27 – 3.05)	0.019
Year[2007/2008 b]	0.97 (-0.29 – 2.24)	0.059		

Note: [a] Δ indicates the difference relative to pre-deployment status; [b] reference category is the group deployed in 2005 and 2006; 95% CI : 95% Confidence Interval.

Table 14. Parameter estimates for change in posttraumatic stress symptoms over time relative to pre-deployment status with new deployments as moderator (n=960).

	Effect		**Interaction time x new deployments**	
	Coefficient (95% CI)	p-values	Coefficient (95% CI)	p-values
Intercept (pre-deployment)	26.86 (26.27 – 27.44)	<0.0001		
Δ 1 month[a]	1.09 (0.46 – 1.71)	0.001	-0.47 (-1.78 – 0.84)	0.478
Δ 6 months[a]	0.88 (0.24 – 1.51)	0.007	0.39 (-0.94 – 1.72)	0.564
Δ 12 months[a]	0.46 (-0.23 – 1.14)	0.190	-0.15 (-1.58 – 1.28)	0.838
Δ 2 years[a]	0.19 (-0.48 – 0.86)	0.576	0.05 (-1.32 – 1.42)	0.946
Δ 5 years[a]	1.96 (1.30 – 2.62)	<0.0001	-0.95 (-2.20 – 0.30)	0.138
new deployments	-0.45 (-1.34 – 0.57)	0.432		

Note: [a] Δ indicates the difference relative to pre-deployment status; 95% CI : 95% Confidence Interval.

Three-step analysis latent growth mixture model

Table 15. Multinomial logistic regression estimates from the 3-step procedure for the latent trajectories of the latent growth mixture model (n=960).

	Delayed onset versus resilient		**Recovered versus resilient**	
	Coefficient (SE)	p-values	Coefficient (SE)	p-values
Intercept	-5.174 (1.174)	0.004	-1.783 (1.512)	0.238
Year[a]	1.093 (0.688)	0.113	0.005 (0.399)	0.989
Age	-0.008 (0.037)	0.827	-0.078 (0.044)	0.079
Gender	0.289 (0.632)	0.647	0.256 (0.762)	0.737
Education^{medium}	0.979 (1.264)	0.439	-0.657 (0.698)	0.347
Education^{high}	1.462 (1.376)	0.288	-0.446 (0.993)	0.653
Rank[b]	-0.292 (0.593)	0.622	0.053 (0.560)	0.925
Function^{outside}	-0.367 (0.514)	0.475	-0.321 (0.710)	0.651
Function^{both}	0.234 (0.604)	0.698	0.382 (0.911)	0.675
Previous deployment	-1.607 (1.280)	0.210	-0.565 (1.403)	0.687
New deployment	0.740 (0.604)	0.221	0.197 (0.954)	0.836
DES total score	0.149 (0.065)	0.023	0.182 (0.070)	0.009
ETISR-SF	0.132 (0.061)	0.030	0.114 (0.066)	0.081

Note: [a] Year: year of deployment 2007/2008 versus 2005/2006; [b] Rank indicates higher ranks versus lower ranks; SE: Standard error; Education^{medium} : medium level education versus low level education; Education^{high} : high level education versus low level education; Function^{outside} : function outside military base versus functions inside the military base; Function^{both} : function both inside and outside the military base versus functions only inside the base; DES: Deployment stressors checklist; ETISR-SF:Early Trauma Inventory self-report – short form.

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CHAPTER 04

Development of self-directedness and cooperativeness in relation to PTSD symptom trajectories after deployment to a combat zone

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In submission

Abstract

Deployment to a combat zone increases the risk of experiencing traumatic events and developing stress-related mental health problems. To improve resilience and decrease the risk of psychopathology, it is important that personality traits mature with age, defined as high levels of self-directedness and cooperativeness. However, little is known about the impact of military deployment on the development of these character traits. Therefore, the aim of this study is to assess if military deployment is associated with changes in the traits cooperativeness and self-directedness. In a large cohort of military personnel these personality traits were assessed before, at 1 and 6 months, and 2 and 5 years after deployment to Afghanistan. Linear mixed effect models were used to assess the individual change in the traits over time, and to study the relation between potential predictors and the character scales. Cooperativeness was found to remain stable, whereas self-directedness was found to slightly decrease over time. This decrease was related to the development symptoms of posttraumatic stress disorder (PTSD) over time. Furthermore, low levels of the character traits were associated with the development of PTSD symptoms. It is clear from these findings that, although military personnel were exposed to potentially traumatic events, this did not directly influence the development of cooperativeness over time. The development of self-directedness over time was associated with the development of PTSD symptoms.

Introduction

Military deployment is a period of prolonged high intensity stress in which military personnel are exposed to deployment-related and potentially traumatic combat-related stressors, such as being in enemy fire, and seeing colleagues or civilians getting injured or killed. It is known that these stressors increase the risk of developing stress-related mental health problems, such as posttraumatic stress disorder (PTSD; Smith et al., 2008; Reijnen et al., 2015; Eekhout et al., 2016). Deployment to a combat zone might have an influence on the development of personality as well. Moreover, individual differences in personality traits are shown to be vulnerability- and also protective factors for PTSD symptoms (e.g. Jaksic et al., 2012). Nevertheless, to the author's knowledge, no study examined the influence of military deployment on the development of personality traits.

There has been an ongoing debate on the stability or changeability of personality and the factors that might influence this change (e.g. West and Graziano, 1989). Initially, personality traits were found to show little meaningful change past the age of 30 (McCrae et al., 2000; Terracciano et al., 2010). In this perspective, mean level changes in personality traits are attributed to genetic factors or intrinsic maturation and are not affected by environmental influences. In contrast, others have shown that personality continues to develop throughout adulthood and can also change as a result of life experiences (Helson et al., 2002; Roberts et al., 2006; Specht et al., 2011).

Although personality can change at any age, most change occurs in young adulthood (age 20-40; Roberts et al., 2006; Roberts and Mroczek, 2008). During young adulthood, individuals become more self-confident, conscientious, and emotionally stable (Roberts and Mroczek, 2008). In addition to intrinsic maturation and genetic effects, social demands and life experiences also account for changes (Lüdtke et al., 2011; Specht et al., 2011). For instance, the transition from school to university or work and a first relationship are found to have a positive influence on personality by decreasing neuroticism and increasing openness, agreeableness and conscientiousness (Bleidorn et al., 2016). Military training was associated with lower changes in agreeableness over time compared to civilian community service, which persisted 5 years after training (Jackson et al., 2012). Also, although the effects are small, experiencing extreme events is associated with increases in neuroticism, and decreases in openness to experience and agreeableness (Löckenhoff et al., 2009; Kandler et al., 2012). Therefore, it can be speculated that life experiences, such as military deployment, are associated with changes in personality.

The psychobiological model of temperament and character (Cloninger et al., 1993; Cloninger, 2008) postulates an interaction of genetics and environment. According to this model, temperament and character traits are interrelated domains that interact within the individual regulating the development of psychological functions. The model describes four temperament dimensions (novelty seeking, harm avoidance, reward dependence and persistence), which manifest early in life, and three character dimensions; self-directedness, cooperativeness, and self-transcendence. The dimensions are expected to change with age

towards psychological maturity due to increasing self-awareness and consequences of one's actions (Cloninger et al., 1993). Maturity is characterized by high self-directedness and high cooperativeness (Cloninger et al., 1993; Josefsson et al., 2011), which refers to the ability of individuals to regulate emotions and behavior to achieve their goals and values, and to be able to identify with and accept other people (Cloninger et al., 1993; Josefsson et al., 2013). This change towards maturity is in line with age-related increases in agreeableness and conscientiousness found in studies on the Big Five personality traits (McCrae et al., 2000; McAdams and Olson, 2010).

This psychological maturation of character has an important role in reducing the vulnerability for psychopathology, the development of resilience to environmental adversity, and in coping with challenges (Caspi et al., 2004; Josefsson et al., 2013). Moreover, the development of self-directedness and cooperativeness is positively related to well-being (Cloninger et al., 2010; Josefsson et al., 2011). Low levels of self-directedness and cooperativeness have been found to be associated with many psychiatric disorders, such as schizophrenia, mood and anxiety disorders (Cloninger et al., 2010) and also PTSD (Jaksic et al., 2012). Moreover, lower self-directedness prior to deployment predicted PTSD symptoms at 6 months after homecoming (van Zuiden et al., 2011). However, it is unclear to what extent personality reflects a pre-existing vulnerability factor or whether event-related changes in character also influence the development of mental health problems.

In sum, self-directedness and cooperativeness are thought to develop towards psychological maturity with age and especially young adulthood is an important time period for the development of character. This maturation is essential to improve resilience and reduce the vulnerability for psychopathology. Although military personnel have an increased risk of developing mental health problems, little is known about the impact of a prolonged period of high intensity stress on the character traits over time. Therefore, the aim of the current study was to investigate the relation between deployment to a combat zone and the development of self-directedness and cooperativeness over time and to assess the potential influence of demographic characteristics, life events, and developmental trajectories of PTSD.

Methods

Participants

Temperament and character were assessed as part of a large prospective cohort study named Prospective Research in Stress-related Military Operations (PRISMO). The aim of this study is to examine potential biological and psychological factors that are associated with the development of stress-related (mental) health problems. The participants are military personnel who were deployed to Afghanistan between 2005 and 2008 for a period of approximately four months. They were deployed as part of the International Security Assistance Force (ISAF) of NATO with either the Provincial Reconstructions Teams (PRT) or as the Task Force Uruzgan (TFU). At the first wave of the PRISMO study 1032 military men (N=939) and women (N=93)

volunteered to participate in the study. After excluding non-deployed individuals (N=25), the data of 1007 participants were eligible for further analyses.

Procedures

Military personnel were approached to volunteer and participate in the PRISMO study after receiving a written and verbal description of the study and informed consent was obtained. Participants completed the questionnaires used in this part of the study approximately 1 month prior to deployment (T0); at one (T1) and 6 (T2) months post-deployment; and at 2 (T3) and 5 (T4) years after return from Afghanistan. Paper-and-pencil questionnaires were filled out at the army base for T0 to T2. At T3 questionnaires were filled out at home and returned by mail and at T4 internet-based questionnaires were used. Participants received financial compensation for completing the assessments. This study was approved by the Institutional Review Board of the University Medical Center Utrecht, the Netherlands.

Measurements

Personality was assessed with the Dutch Short version of Temperament and Character Questionnaire (TCI-SF: Duijsens and Spinhoven, 2002). The TCI-SF is based on the psychobiological theory of personality (Cloninger et al., 1993; Cloninger, 2008). The questionnaire contains 105 dichotomous items (0: False; 1: True). In the current study, we focused on two character scales previously shown to be important in the development of PTSD symptoms; self-directedness and cooperativeness (Yoon et al., 2009; van Zuiden et al., 2011; Jaksic et al., 2012). Self-directedness refers to self-determination and willpower or the ability of an individual to control, regulate and adapt behavior to fit the situation in accord with individually chosen goals and values. Cooperativeness refers to identification with and acceptance of other people; being socially tolerant, empathic and compassionate. Both subscales consist of 15 dichotomous items.

Exposure to deployment and combat-related stressors after deployment was measured with the Deployment Experiences Scale (DES; Reijnen et al., 2015). This is a 19-item dichotomous checklist, which was specifically developed for this study. Potentially traumatic experiences during childhood (<18 years) were measured with the Early Trauma Inventory–Self Report short form (ETI-SR-SF; Bremner et al., 2007) prior to deployment.

The PTSD trajectories used in the current study were based on the level of symptoms of posttraumatic stress disorder, which was measured using the Dutch Self-Rating Inventory for PTSD (SRIP; van Zelst et al., 2003). The SRIP contains 22 items, corresponding to the three symptom clusters according to diagnostic criteria for PTSD of the DSM-IV (American Psychiatric Association, 2000). The scores on the items were summed for an indication of the severity. In this study, we used three trajectories of PTSD symptom development; namely a *resilient* trajectory (85%) reporting a low level of symptoms; a *recovered* trajectory (5%) reporting an increasing level of symptoms in the first year after deployment that then decreased; and a *delayed onset* (9%) trajectory reporting a moderate level of symptoms

that increased heavily at 5 years post-deployment. The identification of the trajectories was described in a previous study, for more information we refer to Eekhout et al. (2016).

Various demographic characteristics were measured prior to deployment; namely age, education, rank, relationship status, previous deployments. Age was categorized in five-year age groups using the lowest category (<21) as reference in the analyses. Life events, such as beginning and/or ending of a relationship, birth of children, marriage, moving in with partner, and new deployments were measured at 5 years post-deployment.

Statistical analysis

All data analyses were performed in R (RCoreTeam, 2014) using the packages lavaan (Rosseel, 2012), mice (van Buuren and Groothuis-Oudshoorn, 2011), and lme4 (Bates et al., 2015).

First, measurement invariance was assessed for the subscales self-directedness and cooperativeness. In models that study change in personality, it is important to assess if changes in the scores over time are due to real change in the construct, and not attributable to change in the relation between the indicators and the latent variables (Self-Directedness and Cooperativeness) over time. Measurement invariance of the subscales across time was assessed by means of confirmatory factor analyses (CFA) for ordinal variables (Hirschfeld and von Brachel, 2014). A series of nested models was examined, starting with the least restrictive model and comparing this to increasingly restrictive models. Model 1 includes no constraints for any of the parameter estimates. In model 2 factor loadings were constrained to be invariant (weak invariance) and in model 3 both factor loadings and intercepts (strong invariance) were constrained to be invariant over time. Little or no change in goodness of fit between the increasingly restrictive models, suggests invariance of the structure across time. In this study, the comparative fit index (CFI) and the root-mean-square error of approximation (RMSEA) were compared. According to Chen (2007), a change larger than -.010 in CFI, supplemented by a change larger than .015 in RMSEA would indicate a violation of invariance.

After measurement invariance was confirmed, missing item scores were imputed with multivariate imputation by chained equations using predictive mean matching (van Buuren and Groothuis-Oudshoorn, 2011). The missing values in the data were assumed to be missing at random and all the variables used in the analyses were included in the imputation model. For the outcome measures the missing and then deletion strategy of von Hippel (2007) was applied. So, the outcome variables were included in the imputation but deleted prior the data analyses, otherwise the variables were imputed as though they were not related to the outcome variable (Moons et al., 2006). A total of 30 imputed datasets were generated.

Next, a series of linear mixed effect models were fit to the data to assess the individual change in the subscale scores over time and the relationships between various predictors and the character scales. The outcome variables were the character scales self-directedness and cooperativeness. As fixed effects, the endorsement of traumatic experiences during childhood, and the experience of combat-related stressors were investigated. Additionally,

the added value of various demographic characteristics (age, gender, education, rank, relationship status prior to deployment, and previous deployments), potential life events in the period after deployment (beginning and/or ending of a relationship, birth of children, marriage, moving in with partner, new deployments), and trajectories of PTSD development (Eekhout et al., 2016) were assessed by adding one at the time. Lastly, relevant interactions between the variables were included in the models. Time was coded as time in years relative to deployment (-0.083, 0.083, 0.5, 2 and 5). A random intercept was used to account for the variance between participants where time = 0; also, a random slope for time was included in the model to account for the variance in slopes between participants. Visual inspection of the residual plots did not show deviations from homoscedasticity or normality. The results of the analyses were pooled according to Rubin's rules (Rubin, 1987). The most relevant predictors for each of the outcome measures were examined using the Wald test, which pools the p-values for comparing nested models using the method of Li et al. (1991). The p-values for the Wald tests were adjusted to correct for multiple testing with the correction as suggested by Benjamini and Hochberg (1995) using the "p.adjust" function in R (RCoreTeam, 2014).

Results

The data of 1007 military men and women were included in the analyses. An overview of the demographic information is presented in Table 1. The mean levels of self-directedness and cooperativeness at each time-point are displayed in Table 2.

Table 1. Demographic information and life events.

		N (Mean)	% (SD)
Age[1]	<21	139	13.9
	21-24	327	32.7
	25-29	201	20.1
	30-34	118	11.8
	35-39	68	6.8
	40-44	64	6.4
	≥45	83	8.3
Gender	Male	921	91.5
	Female	86	8.5
Education[1]	Low	366	40.2
	Middle	442	48.6
	High	102	11.2
Rank[1]	Private	394	40.2
	Corporal	203	20.7
	NCO	251	25.6
	Officer	132	13.5

Table 1. Demographic information and life events. (Continued)

		N (Mean)	% (SD)
Relationship status pre-deployment[1]	Married	224	25
	Cohabiting	156	17.4
	Long-term relation	172	19.2
	Single	335	37.4
	Divorced/Widowed	132	14.7
Early life trauma (N=943)[1]		(3.49)	(3.06)
Deployment-related stressors (N=707)[1]		(4.51)	(3.22)
Previous deployments[1]	Yes	424	47
	No	478	53
New deployments[1]	Yes	179	30.9
	No	401	69.1
PTSD trajectories[1]	Resilient	848	88.3
	Recovered	49	5.1
	Delayed	63	6.6
Life events after deployment			
Beginning a relation[1]	Yes	32	5.5
	No	548	94.5
Ending a relation[1]	Yes	71	12.2
	No	509	87.8
Marriage[1]	Yes	65	11.2
	No	515	88.8
Moving in together[1]	Yes	68	11.7
	No	512	88.3
Child(ren)[1]	Yes	113	19.5
	No	467	80.5

Note: SD: standard deviation; NCO: Non-commissioned officer; PTSD: Posttraumatic Stress Disorder. [1] Count may not add up to 1007 participants due to missing values.

Table 2. Descriptive statistics for cooperativeness and self-directedness over time.

	Self-Directedness			Cooperativeness		
	N[1]	Mean[2]	SD	N[1]	Mean[2]	SD
Pre-deployment	806	13.64	1.87	779	11.87	2.97
1 month	801	13.53	1.93	772	11.56	3.08
6 months	725	13.54	2.07	712	11.77	3.08
2 years	525	13.45	2.23	523	12.24	2.91
5 years	536	13.26	2.43	536	12.04	2.98

Note: N: Total sample; SD: Standard Deviation. [1] Count may not add up to 1007 participants due to missing values.
[2] The mean levels correspond to the "average range" according to the Dutch norm scores (Duijsens and Spinhoven, 2002)

The first step in the analyses was to establish measurement invariance for the character subscales over time. The results from the measurement invariance analysis are presented in the supplementary material, Table S1 and S2. The results indicate that strong invariance holds for both character subscales. In the model for cooperativeness one item (item 73) was

deleted from the analyses after inspection of the modification indices. As shown in Table S1 and S2, the resulting models all showed acceptable CFI and RMSEA fit and this suggests structural stability of the subscales self-directedness and cooperativeness across time.

The pooled results of the linear mixed effect models show that self-directedness decreases slightly but significantly over time (Table 3). The different age categories were positively related to the levels of self-directedness over time, indicating that for all age categories ≥21, participants showed higher self-directedness compared to participants with age <21. This effect was most pronounced for the age category 40-44 relative to age category <21. After correcting for the cooperativeness subscale in the model, the endorsement of early life trauma was negatively related to self-directedness whereas no significant association was found with deployment-related stressors. Including the PTSD trajectories (Eekhout et al, 2016) in the model revealed both a negative association of the delayed and recovered trajectory with self-directedness compared to the resilient trajectory. In addition, as displayed in Figure 1, a significant interaction effect was found between the PTSD trajectories and time, which indicates that the development of self-directedness over time was different for the participants with distinct trajectories of PTSD. No association was found with previous or new deployments, marital status prior to deployment, and life events (beginning and/or ending of a relationship, birth of children, marriage, moving in with partner) experienced in the period after deployment.

Table 3. Model estimates for self-directedness over time (N=972).

		Estimate (95%CI)	p
Intercept		11.02 (10.63 —11.41)	.000
Time in years		-0.04(-0.07— -0.002)	.039
Age[a]	21-24	0.34(0.04 —0.64)	.028
	25-29	0.57(0.24 —0.90)	.001
	30-34	0.58(0.20 —0.95)	.002
	35-39	0.55(0.12 —0.98)	.012
	40-44	0.80(0.36 —1.24)	.000
	≥45	0.44(0.03 —0.85)	.034
Early life trauma		-0.05(-0.08 — -0.02)	.001
Cooperativeness		0.21(0.18 —0.23)	.000
Trajectories PTSD[b]	Delayed	-1.18(-1.56 — -0.79)	.000
	Recovered	-0.99(-1.43 — -0.55)	.000
Time × Delayed[b]		-0.47(-0.58 — -0.36)	.000
Time × Recovered[b]		-0.20(-0.36 — -0.04)	.015

Note: 95%CI:95% Confidence interval. [a] Reference category is age <21. [b] Reference category is the resilient trajectory.

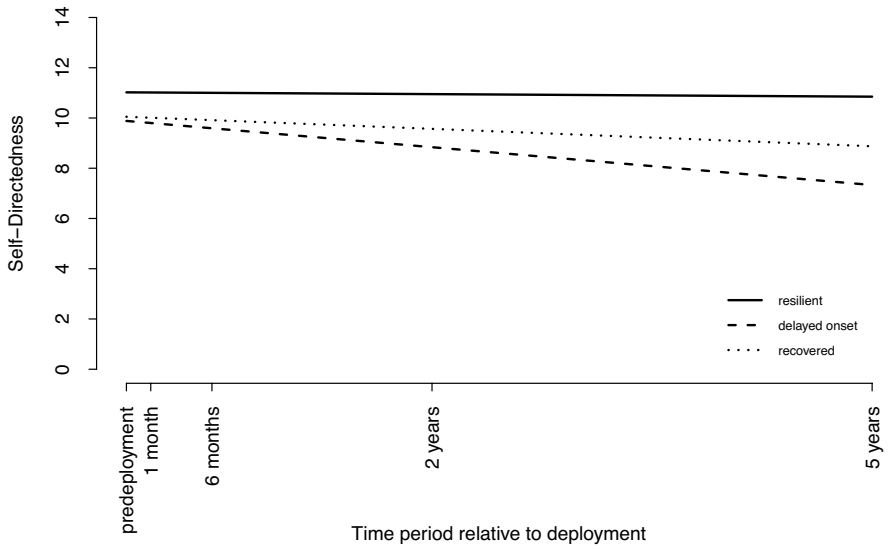

Figure 1. Development of self-directedness over time for the three PTSD trajectories.

In contrast to self-directedness, the subscale cooperativeness showed no significant association with time (Table 4). However, age and rank were positively related to the baseline level of cooperativeness. The results also show that gender is negatively related, with lower levels of cooperativeness in military men than in military women. After correcting for the subscale self-directedness, both the endorsement of early life trauma and the experience of deployment-related stressors were negatively related to cooperativeness. As displayed in Figure 2, the recovered trajectory of PTSD was negatively related to cooperativeness. However, there was no interaction effect between time and the PTSD trajectories. In agreement with findings of self-directedness, no association was found between cooperativeness and previous or new deployments, marital status prior to deployment, and the life events (beginning and/or ending of a relationship, birth of children, marriage, moving in with partner) experienced in the period after deployment.

Table 4. Model estimates for cooperativeness over time (N=963).

		Estimate (95%CI)	p
Intercept		7.82 (6.92 —8.72)	.000
Time in years		0.03 (-0.01 —0.07)	.173
Age[a]	21-24	0.03 (-0.46 —0.52)	.916
	25-29	0.36 (-0.24 —0.97)	.241
	30-34	0.99 (0.25 —1.73)	.009
	35-39	0.72 (-0.13 —1.58)	.097
	40-44	0.96 (0.08 —1.85)	.032
	≥45	1.29 (0.43 —2.15)	.003
Gender[b]	Male	-1.16 (-1.67 —-0.64)	.000
Rank[c]	Corporal	0.32 (-0.13 —0.76)	.165
	NCO	0.72 (0.14 —1.31)	.016
	Officer	1.21 (0.56 —1.85)	.000
Early life trauma		-0.09 (-0.14 —-0.04)	.000
Deployment-related stressors		-0.08 (-0.13 —-0.03)	.003
Self-directedness		0.36 (0.32 —0.40)	.000
Trajectories PTSD[d]	Delayed	-0.29 (-0.87 —0.30)	.335
	Recovered	-1.40 (-2.08 —-0.72)	.000

Note: 95%CI:95% Confidence interval; NCO: Non-commissioned officer; [a] Reference category is age <21.
[b] Reference category is female. [c] Reference category is private. [d]Reference category is the resilient trajectory.

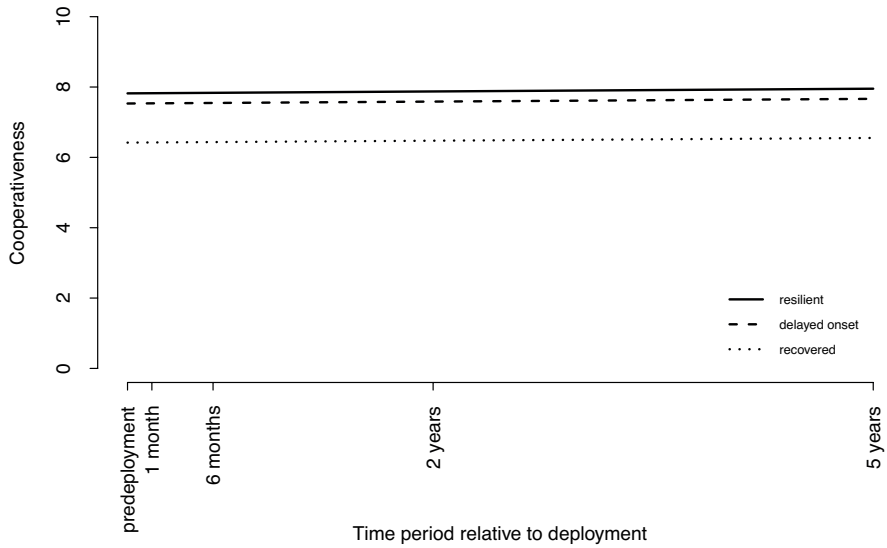

Figure 2. Development of cooperativeness over time for the three PTSD trajectories.

Discussion

Although serious adverse events are suggested to have an impact on the development of character, the current findings show that the character trait cooperativeness is relatively stable over time after deployment to a combat zone. However, a decrease in self-directedness over time was found, which was associated with the development of PTSD symptoms. In addition, the baseline levels of these character traits were associated with various demographic characteristics; age, gender, and rank. In agreement with the literature (Rademaker et al., 2008; de Carvalho et al., 2015), self-directedness and cooperativeness were negatively associated with the endorsement of early life trauma. Furthermore, as expected based on a previous study of our group (van Zuiden et al., 2011), lower self-directedness was related to the recovered and the delayed onset PTSD trajectories. Moreover, a stronger decline in the level of self-directedness over time was found in these trajectories compared to the resilient PTSD trajectory. In contrast, lower cooperativeness was only associated with the recovered PTSD trajectory.

Our findings show that, after controlling for age, the level of cooperativeness does not change as a result of military deployment. Both the level of cooperativeness and self-directedness over time were not associated with experiencing combat-related stressors. In line with our findings, a recent Swedish study reported that personal values also remained relatively stable after participating in a 6-month military deployment to Afghanistan (Sundberg, 2016). In contrast, previous studies have shown that experiencing serious adverse life events is associated with increases in neuroticism, and decreases in agreeableness and openness (Löckenhoff et al., 2009; Kandler et al., 2012; Riese et al., 2014). They suggest that the changes were not rooted in pre-exposure personality but emerged as a result of exposure to trauma. In addition, other life events, such as a first relationship and the transition to college or work, were shown to be associated with decreases in neuroticism and increases in openness and agreeableness (Edmonds et al., 2008; Bleidorn et al., 2016), yet the current findings show no association between the character traits and similar life events. Moreover, neither previous nor new deployments were related to the character traits. Thus, this might suggest that deployment to a combat zone is not directly related to changes in character traits over a 5-year time period.

An explanation for the stability of character after deployment might be that individuals with specific personality traits choose to join the military in the first place. As shown by Jackson et al. (2012), the decision to enter the military was predicted by lower levels of agreeableness, neuroticism and openness to experience. These so-called selection effects (Lodi-Smith and Roberts, 2007) suggest that joining the military but also some of the life events a person experiences are a result of personality. For instance, higher levels of neuroticism predict endorsing a wider range of negative events, higher extraversion predicts more positive life events, and openness to experience predicts more events in general (Lüdtke et al., 2011). In contrast, research by North et al. (2012) showed that low self-directedness and cooperativeness (and high self-transcendence and harm avoidance), were

associated with more PTSD after the Oklahoma City bombing. According to these authors, there was little to no link between personality and the likelihood of being at that location when the bomb was detonated. However, trauma exposure during military deployment is not necessarily a random occurrence, and certain traits might predispose to experiencing more trauma.

In addition to selection effects, personality change might be influenced by the pressure exerted on an individual to conform to meet the expectations of a group or society (Caspi et al., 2004; Lodi-Smith and Roberts, 2007). Jackson et al. (2012) reported an increase in agreeableness over time in military recruits; however, this change was smaller compared to individuals who chose civilian community service. The socialization effect (Lodi-Smith and Roberts, 2007) might explain the change in personality of military recruits. It is also possible that if an individual cannot conform to the expectations, it leads to dropping out of the military. However, in the current study character was measured prior to deployment and after an extensive period of training. So, it might be speculated that military lifestyle and training mediate the association between personality and combat-related experiences; however, further research starting with multiple assessments prior to deployment is necessary to truly assess this association over time.

In line with the literature on the maturity principle (Caspi et al., 2004; Edmonds et al., 2008; Lüdtke et al., 2011), the level of cooperativeness and self-directedness increased over the different age categories. As described in the introduction, this is important to improve resilience (Caspi et al., 2004), since lower cooperativeness and self-directedness are typically associated with different psychopathologies (Cloninger et al., 2010). Our findings confirm that lower levels of cooperativeness at baseline are associated with the development of PTSD symptoms in the first year after deployment. However, participants in the delayed PTSD trajectory showed similar levels of cooperativeness as the resilient PTSD trajectory. Higher cooperativeness refers to being socially tolerant, empathic, and compassionate and is related to greater perceived social support compared to low cooperativeness (Cloninger et al., 2010). Since post-deployment social support might reduce the severity of traumatic stress (Pietrzak et al., 2009), this support might initially enable these individuals to cope with their experiences, emotions and behavior. Further research should examine how the pre-existing personality profiles are associated with the course of PTSD symptom development.

For self-directedness, lower levels were observed for both the recovered and delayed trajectory compared to the resilient trajectory. However, whereas self-directedness was relatively stable over time in the resilient participants, an increase in PTSD symptoms over time was associated with a decline in self-directedness over time. Low self-directedness refers to difficulty in self-regulating emotions and adapting behavior to fit a situation, lack of long-term goals and determination, and low self-esteem (Cloninger et al., 1993), which and the development of PTSD symptoms is. However, from the current study we can conclude that self-directedness is more stable in participants that were resilient for the development of PTSD symptoms over time.

An important strength of the current study is that personality was assessed in a large sample over time starting prior to deployment to Afghanistan and included multiple follow-up assessments. Furthermore, measurement invariance was assessed for the subscales cooperativeness and self-directedness and strong invariance was given. However, there are some limitations that need to be taken into consideration. As previously discussed, individuals with specific personality traits enter the military and this might limit generalizability to populations outside the military. The generalizability is further limited due to the underrepresentation of female soldiers. However, this is a frequent limitation in military studies because of the low percentage of females in the military. In addition, in this study there is no non-deployed or civilian control group to assess differences in character trait development over time. To account for confounding influences of unobserved factors on personality change, it would be valuable to include an age-matched control group in future research.

In conclusion, although deployment to a combat zone increases the risk of experiencing potentially traumatic events and developing stress-related disorders, it is clear the character trait cooperativeness remained relatively stable after deployment whereas self-directedness decreased over time. Although the findings confirmed that low levels of the traits are related to the development of PTSD symptoms, it was also shown that there are differences in the relation between the traits and the course of PTSD symptoms over time. This underlines the importance of examining personality traits in an earlier phase to be able to assess stability or changes due to training and to identify personality profiles with an increased risk for PTSD. This might provide opportunities to assess and develop methods to promote the development of traits such as self-directedness and cooperativeness and increase the resilience of military personnel.

Acknowledgements and disclosure

The authors thank the Dutch commanders and troops and all members of the PRISMO team involved in data acquisition for their time and effort. Also, we thank Prof. dr. Joëls for her valuable comments and suggestions. This study was financially supported by the Dutch Ministry of Defence.

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Supplementary material

Table S1. Fit indices for measurement invariance tests for self-directedness over time.

Model*	X^2	df	CFI	RMSEA
1[a]	888.933	450	0.941	.038
2[b]	894.176	506	0.948	.034
3[c]	889.287	502	0.948	.034

Note: CFI=comparative fit index; RMSEA=root-mean-square error of approximation.
* Using robust estimation. [a] Unconstrained model. [b] Factor loadings invariant (weak invariance). [c] Factor loadings and intercept invariant (strong invariance).

Table S2. Fit indices for measurement invariance tests for cooperativeness over time.

Model	X^2	df	CFI	RMSEA
1[a]	862.835	385	.985	.043
2[b]	1.016.829	437	.981	.045
3[c]	948.784	433	.983	.042

Note: CFI=comparative fit index; RMSEA=root-mean-square error of approximation.
[a] Unconstrained model. [b] Factor loadings invariant (weak invariance). [c] Factor loadings and intercept invariant (strong invariance).

PART 02

CHAPTER 05

The effect of deployment to a combat zone on testosterone levels and the association with the development of posttraumatic stress symptoms; a longitudinal prospective Dutch military cohort study

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Published in: Psychoneuroendocrinlogy (2015) 51, 525–533

Abstract

There is limited evidence on the association of the activity of HPG-axis with stress and symptoms of stress-related disorders. The aim of the current study was to assess the effect of deployment to a combat-zone on plasma testosterone levels, and the possible association with the development of symptoms of posttraumatic stress disorder (PTSD). A total of 918 males were included in the study before deployment to a combat zone in Afghanistan. The effect of deployment on testosterone was longitudinally assessed; starting prior to deployment and follow-up assessments were performed at 1 and 6 months after return. Furthermore, the association with PTSD symptoms reported at 1 and 2 years post-deployment was assessed. Plasma testosterone levels were significantly increased after deployment compared with pre-deployment levels. Although no difference was found between individuals reporting high or low levels of PTSD symptoms, pre-deployment testosterone levels predicted the development of PTSD symptoms at 1 and 2 years post-deployment. This study provides evidence that not the alterations in testosterone levels shortly after deployment, but the pre-deployment testosterone levels are associated with PTSD symptoms, which is of value in the identification of biological vulnerability factors for the development of PTSD.

Introduction

Military deployment can be described as a period of prolonged stress in which military personnel are exposed to a variety of deployment and combat-related stressors, of which some may be potentially traumatic, such as enemy fire and seeing injuries. At the same time, it is a period of practicing professional skills as a soldier, executing specific military tasks in a highly motivated group. The stressors, the anticipation of going to a warzone and being there for months, but also the challenge to deliver a good performance might be associated with alterations in psychological and underlying biological mechanisms. Therefore, military deployment can provide a unique context to assess the relation between neuroendocrinology and psychological stress. In addition, the risk of developing stress-related mental health symptoms, such as symptoms of posttraumatic stress disorder (PTSD), was found to increase after deployment to a combat zone (Rona et al., 2006; Smith et al., 2008; Vasterling et al., 2010). In a previous study, we showed that the prevalence of PTSD symptoms significantly increased (5.6–8.9%) after deployment compared to pre-deployment rates (4.3%; Reijnen et al., 2015). Although the prevalence rates of PTSD vary, the rates are considerably higher than the lifetime prevalence (1.9%) found in the general population of several European countries (Alonso et al., 2004). In addition, military-related trauma is associated with more severe posttraumatic stress symptoms than criminal victimization (Naifeh et al., 2008). So, unraveling possible alterations in neuroendocrine systems in response to (traumatic) stress exposure might also contribute to the identification of biological vulnerability factors for the development of PTSD symptoms and the improvement of preventive intervention strategies.

Testosterone is an important product of the hypothalamic-pituitary-gonadal axis (HPG-axis) and is secreted by the adrenal gonads. It shows a diurnal rhythm with a near 50% decrease from morning to evening (Dabbs, 1990). The steroid hormone is involved in reproduction, male secondary characteristics, and sexual function. Furthermore, testosterone is related to various psychosocial and behavioral factors such as dominance, competitiveness and aggression (Mazur and Booth, 1998; Archer, 2006; Mehta and Josephs, 2006). Stress can alter testosterone levels, as has been shown by the inhibitory effects on human reproductive function and sex steroid release (Rivier and Rivest, 1991). Decreased testosterone levels have been found in response to acute stress. For instance, serum testosterone levels were significantly reduced during and after stress exposure in a US army survival course compared to baseline (Morgan et al., 2000). Alterations were also found after a prolonged period of stress exposure. Decreased plasma testosterone levels were found during and after 3 weeks of military training followed by a 5-day combat course (Gomez-Merino et al., 2005). In addition, testosterone was significantly decreased to clearly hypogonadal levels in young male soldiers after 8 weeks of extremely stressful training (Bernton et al., 1995). However, they demonstrated rapid recovery 72h after the training period. These findings illustrate that both acute and prolonged stress can alter testosterone levels.

In contrast to the decrease in response to acute stress, findings on testosterone levels in PTSD patients are contradictory. Higher serum testosterone levels were found in

PTSD patients (Mason et al., 1990) and in patients with combat-related PTSD without comorbid disorders (Karlović et al., 2012). In contrast, morning plasma testosterone levels were not found to differ (Spivak et al., 2003) and testosterone concentrations in cerebrospinal fluid concentrations were significantly lower in patients with PTSD (Mulchahey et al., 2001). Although these studies differ in measurement techniques, these studies emphasize that the association between PTSD and testosterone is not clear.

There is evidence that the relation between testosterone and stress-related disorders might be influenced by the interaction between neuroendocrine systems. Recent studies suggested that the hypothalamic-pituitary-adrenal axis (HPA-axis) might have a moderating role on the association between testosterone and aggressive-like behaviors, also known as the *dual hormone hypothesis* (Popma et al., 2007; Mehta and Josephs, 2010). In support of this hypothesis, testosterone was related to aggression (Dabbs et al., 1991; Popma et al., 2007) and dominance as measured in the domains leadership and competitiveness (Mehta and Josephs, 2010), only in individuals with low cortisol levels. This might be explained by the probable anxiolytic effect of high testosterone (Handa and Weiser, 2014), and the relationship of low cortisol with decreased stress and social approach (Dickerson and Kemeny, 2004). Although this hypothesis sounds promising, cortisol does not moderate the relation between testosterone and various indicators of antisocial behavior such as juvenile delinquency, alcohol, drugs and violence (Mazur and Booth, 2014). However, in contrast to the other studies, hormone and behavior measurement were not completed at the same time.

Research on neuroendocrine alterations in PTSD patients has often focused on the HPA-axis. Cortisol, an important product and regulator of the HPA-axis, is released in response to acute psychological stress (Dickerson and Kemeny, 2004) to reinstate homeostasis, and induce long-lasting adaptive changes (De Kloet et al., 2005). While cortisol levels are high in response to an acute stressor, lower cortisol levels were consistently demonstrated in patients with PTSD (i.e. Yehuda et al., 1995; Bremner et al., 2007a). Moreover, hypocortisolism might also represent a preexisting risk factor that promotes the manifestation of PTSD (Heim and Nemeroff, 2009). We reported that low levels of circulating cortisol measured shortly after trauma contributed as vulnerability factor for the development of PTSD symptoms (van Zuiden et al., 2013). Although PTSD research has focused on altered activity of the HPA-axis, less attention is paid to the interaction of the HPA-axis with other neuroendocrine systems such as the HPG-axis. There is thought to be a reciprocal relationship between the HPA-axis and the HPG-axis, both inhibiting the activity of the other axis on different levels (Viau, 2002). So, while the HPG-axis might influence the stress response system, the association of this axis with stress and stress-related disorders is inadequately examined.

In research, relatively little attention is paid to the influence of severe stress on the activity of the HPG-axis, and the association with the development of symptoms. As discussed, testosterone levels in military personnel decreased after exposure to acute stressors and after stressful training programs. Furthermore, findings on the association between HPG-axis and

PTSD are rather contradictory. In the current study, we examined the effect of deployment on testosterone levels in military men deployed to Afghanistan by measuring testosterone and cortisol levels pre-deployment, and at 1 and 6 months after homecoming. Also, the association of the testosterone levels with the development of posttraumatic stress symptoms in the 2-year period after return was assessed. Furthermore, since the HPG-axis and the HPA-axis are thought to interact, the possible moderating role of cortisol on the association between testosterone and the development of PTSD symptoms was addressed.

Methods

Participants

This study is part of a large prospective cohort study on the development and course of stress-related symptoms in Dutch military personnel in relation to a 4-month deployment to a combat zone. The participants were military men deployed to Afghanistan between 2005 and 2008 as part of the International Security Assistance Force (ISAF) of NATO, either as part of a Provincial Reconstruction Team (PRT) or as part of Task Force Uruzgan (TFU). The duties of the participants comprised the searching of houses and buildings, combat patrols, transportation across enemy territory, and demining operations. During deployment, they were exposed to typical warzone stressors such as exposure to enemy fire, armed combat, improvised explosive devices, and witnessing soldiers and citizens getting seriously injured. Prior to their deployment, military men could volunteer to participate after a verbal and written description of the study. Participants received financial compensation (a voucher in the value of € 100 for participation in 3 assessments). A total of 918 males volunteered to participate in the study. The study was approved by the Institutional Review Board of the University Medical Center Utrecht.

Measurement

Questionnaires

The Dutch Self-Rating Inventory for PTSD (SRIP) was used to measure symptoms of PTSD (Hovens et al., 2000; Hovens et al., 2002). The SRIP contains 22 items, which correspond to symptoms in the clusters B, C and D of DSM-IV (American Psychiatric Association, 2000) diagnostic criteria for PTSD, namely re-experiencing (6 items), avoidance and numbing (9 items), and arousal (7 items). Symptom severity was evaluated using a 4-point Likert scale ranging from not at all (score 1) to very much (score 4). The SRIP was demonstrated to have good concurrent validity with diagnostic clinical interviews for PTSD (Hovens et al., 2002). A cutoff of 38 was used to create two groups, since this range provided the highest sensitivity and specificity for a PTSD diagnosis according to the DSM-IV (van Zelst et al., 2003).

Exposure to combat and deployment-related stressors was assessed with a 19-item checklist developed for this study (Reijnen et al., 2015). The Dutch version of the short form self-report of the Early Trauma Inventory (ETI-SR-SF; Bremner et al., 2007b) was used to

assess potential exposure to traumatic experiences (physical, sexual and emotional abuse) before the age of 18 years. The questionnaire consists of 27 dichotomous items and the score indicates the composite severity of traumatic events experienced during childhood.

Plasma samples

Blood samples were collected approximately 2–4 weeks prior to deployment and at 1 and 6 months after deployment. For the plasma testosterone and cortisol measurements a venous blood sample was collected between 0800h and 1130h in EDTA vacutainers and immediately put on ice. The samples were centrifuged at 3500 rpm for 12 min at 4°C after which they were transported to the hospital and stored at −80°C until assayed.

Plasma testosterone levels were measured using an electrochemiluminescence immunoassay on the Modular E170 (Testosterone II, Roche Diagnostics GmbH, Mannheim, Germany). The lower limit of detection was 0.1 nmol/L and interassay variation was 5.4; 3.4 and 2.7% at 1.1; 6.6 and 33 nmol/L. Reference values for male participants were 7–31 nmol/L.

Plasma cortisol levels were measured using an electrochemiluminescence immunoassay on the Modular E170 (Roche Diagnostics GmbH, Mannheim, Germany). Lower limit detection was 3 nmol/L; interassay variation was <3%; and reference values were 170–540 nmol/L.

Plasma SHBG levels were measured using an electrochemiluminescence immunoassay on the Modular E170 (Roche Diagnostics GmbH, Mannheim, Germany). The lower limit of detection was 2 nmol/L; interassay variation was <4% in the range of 10–120 nmol/L. Reference values for male participants were 10–40 nmol/L (20–40 years) and 15–50 nmol/L (40–60 years).

Procedure

Military personnel were informed about the study at the army base prior to deployment. After receiving a verbal and written description of the study, military personnel could sign up for participation and informed consent was obtained. About one month prior to a 4-month deployment (T0) participants completed various paper-and-pencil questionnaires, and blood samples were collected. Follow-up assessments were done at approximately one month (T1), six months (T2) and one (T3) and two years (T4) after return. The first three assessments (T0–T2) were completed at the army base. At the one and two-year follow-up, questionnaires were completed at home and returned by mail. In order to minimize non-compliance and reduce dropout, all participants were contacted a total of five times, through e-mail, mail and/or telephone, to remind them to fill out the questionnaires.

Analysis

Analyses were performed with IBM SPSS 22. Descriptive statistics and the influence of non-response were examined using frequencies; independent samples *t*-tests and χ^2 -tests. Outliers were removed if the *z*-values of testosterone and/or cortisol were greater than ±3.29 (testosterone N=4; cortisol N=6). Bioavailable testosterone was calculated using the formula

based on the total testosterone and SHBG levels (Morris et al., 2004). Following this, linear mixed model analyses were used to assess the alterations in total testosterone and bioavailable testosterone over time and to assess possible differences in both testosterone levels between participants with high (cutoff SRIP ≥38) or low levels of PTSD symptoms one year post-deployment (T3). Mixed model analyses were used because listwise deletion in repeated measures ANOVA's would result in the loss of valuable data. Variables that differentiate between completers and dropouts were included as covariates when evaluating the effects of time and PTSD symptoms on the testosterone levels. Also, Spearman rank correlations were used to assess the association between the testosterone levels and the subscales of the SRIP (re-experiencing, avoidance, and hyperarousal) at one year post-deployment.

In addition, the predictive value of pre-deployment testosterone levels and the potentially moderating role of cortisol on the association between PTSD and testosterone were examined using hierarchical multiple regression analyses. First, if one testosterone and/or cortisol sample was missing, the values were imputed using multiple data imputation (N=779). Second, testosterone and cortisol scores were standardized and next these values were multiplied to calculate the testosterone-cortisol interaction term (see Mehta and Josephs, 2010). Third, three multiple regression analyses were conducted for the prediction of PTSD symptoms at T2, T3 and T4 with the covariates (age, ETI, exposure to traumatic stressors, PTSD score prior to deployment and the number of previous deployments) in step 1, the independent variables in step 2 and the interaction between testosterone and cortisol in step 3. Pairwise deletion was used and the pooled results of 5 imputations were reported. The variance inflation factors of all variables were below the tolerable limit of 10. Mahalonobis distance analysis (p< .001) was used to detect outliers and these were removed from the regression analysis (N=12).

Results

Participant characteristics and attrition

A total of 918 deployed male participants volunteered to take part in the study. Baseline assessments were completed by 908 participants (98.7%), 773 participants (84%) completed T1 assessment, 693 participants (75.3%) completed T2 assessment, 501 participants (54.5%) completed T3 assessment, and 474 participants (51.5%) completed T4 assessment (see also Table 1). The mean age of these participants was 28.60 (SD 9.06) prior to deployment (see Table 2). At 6 months after deployment 9.0% (N=60) of the males was found to report a high level of PTSD symptoms (cut-off SRIP≥38). The percentage of military personnel reporting symptoms at 1-year was 7.5% (N=37) and 5.7% (N=27) at 2 years post-deployment. The differences between the groups reporting high or low levels of symptoms at 1 year post-deployment are shown in Table 2.

Table 1. Flow of the participants (N) from the baseline to the two-year assessment.

	Testosterone			Cortisol			PTSD-questionnaire				
	T0	T1	T2	T0	T1	T2	T0	T1	T2	T3	T4
Completed	887	736	540	879	741	649	627	703	672	500	474
Non-response	31	133	224	39	128	201	164	135	178	308	322
Dropout	0	47	19	0	47	19	0	47	19	41	13
Not available for analysis	0	0	86	0	0	0	–	–	–	–	–
Ques. not administered	–	–	–	–	–	–	127	31	0	0	0
Deceased	0	2	0	0	2	0	0	2	0	1	0

Table 2. Participant characteristics of the complete sample and the difference between the subgroups with high or low levels of PTSD symptoms at 1 year after deployment.

	Complete sample		No PTSD symptoms at 1 year		PTSD symptoms at 1 year		
	N	M (SD)	N	M (SD)	N	M (SD)	*p*
Demographics[1]							
Age during deployment	909	28.60 (9.06)	461	31.57 (9.97)	37	27.22 (7.11)	.001
Previous deployments	818	0.88 (1.19)	427	1.06 (1.28)	31	.90 (1.35)	.528
Early Trauma Inventory	833	3.52 (3.07)	448	3.39 (2.94)	34	3.59 (3.66)	.714
Deployment stressors	644	4.52 (3.26)	364	4.13 (3.09)	29	6.07 (3.49)	<.001
Questionnaire[1]							
PTSD score T0	626	26.76 (4.95)	340	26.37 (4.37)	28	33.07 (9.29)	<.001
PTSD score T2	670	27.86 (7.16)	421	26.85 (6.12)	33	35.67 (9.25)	<.001
PTSD score T3	498	27.20 (7.09)	461	25.64 (3.69)	37	46.59 (10.13)	<.001
PTSD score T4	473	26.77 (5.74)	362	25.93 (4.95)	33	35.12 (7.58)	<.001
Hormones[1]							
Testosterone T0	885	17.94 (5.70)	453	17.17 (5.47)	37	16.08 (6.57)	.251
Cortisol T0	813	435.81 (131.48)	443	421.22 (126.55)	35	443.69 (140.80)	.316
Testosterone T1	734	18.55 (5.77)	409	17.85 (5.56)	32	16.66 (5.49)	.245
Cortisol T1	735	454.33 (135.55)	415	443.25 (132.38)	33	435.09 (143.66)	.735
Testosterone T2	538	18.11 (5.68)	326	17.55 (5.74)	30	17.82 (6.00)	.810
Cortisol T2	644	424.10 (128.70)	402	420.44 (127.98)	32	410.00 (107.55)	.654

[1] The number of participants varies due to missing values.

When comparing dropouts at the 6-month assessment with completers, dropouts were significantly younger prior to deployment ($t_{(489.31)}$=-11.39, p<.001), were potentially exposed to more traumatic stressors during childhood ($t_{(835)}$=-2.148, p<.05) and had less often been deployed prior to this deployment ($\chi^2_{(6,\ N=893)}$=17.44, p<.05). Nonetheless, both groups did not differ in pre-deployment PTSD symptoms ($t_{(627)}$=-.362, p=.717), cortisol levels before deployment ($t_{(879)}$=1.499, p=.134) and exposure to traumatic stressors during deployment ($t_{(646)}$=-.879, p=.380). A trend significant difference was found in testosterone levels before deployment with higher testosterone levels in dropouts ($t_{(887)}$=1.749, p<.081).

Testosterone and PTSD

To assess the effect of deployment, plasma testosterone levels were compared over time starting prior to deployment and at 1 and 6 months post-deployment. Linear mixed modeling revealed a significant increase of testosterone over time ($F_{(2,656.81)}$=4.93, p=.008; see Figure 1). This effect remained significant after controlling for age, childhood trauma, and the number of previous deployments ($F_{(2,578.73)}$=7.36, p>.01). There was no significant alteration of bioavailable testosterone over time ($F_{(2,674.91)}$=2.00, p=.136), however, a significant alteration was found after controlling for age, childhood trauma, and previous deployments ($F_{(2,602.44)}$=4.12, p=.017).

The association between testosterone and PTSD symptoms was examined by comparing the testosterone levels over time between the participants reporting high and low levels of PTSD symptoms at 1 year after deployment. There was a significant increase in testosterone levels after deployment ($F_{(2,490.89)}$=3.51, p=.031; see Figure 2). However, no significant interaction effect between time and group on testosterone levels was found ($F_{(2,490.89)}$=1.22, p=.297). Also, there were no differences between the groups ($F_{(1,403.65)}$=0.664, p=.416). These effect were comparable after controlling for the confounding factors (Time; $F_{(2,362.94)}$=5.52, p=.004, Interaction; $F_{(2,362.95)}$=2.18, p=.115, Group; $F_{(1,436.13)}$=2.41, p=.121). In addition, linear mixed modeling revealed no significant interaction between bioavailable testosterone and a high or low level of PTSD symptoms ($F_{(2,418.69)=.}$973, p=.379), even after controlling for the confounding factors ($F_{(2,379.26)}$=1.783, p=.170). Lastly, no significant associations were found between the subscales of the SRIP at one year post-deployment and the testosterone levels at the different time points.

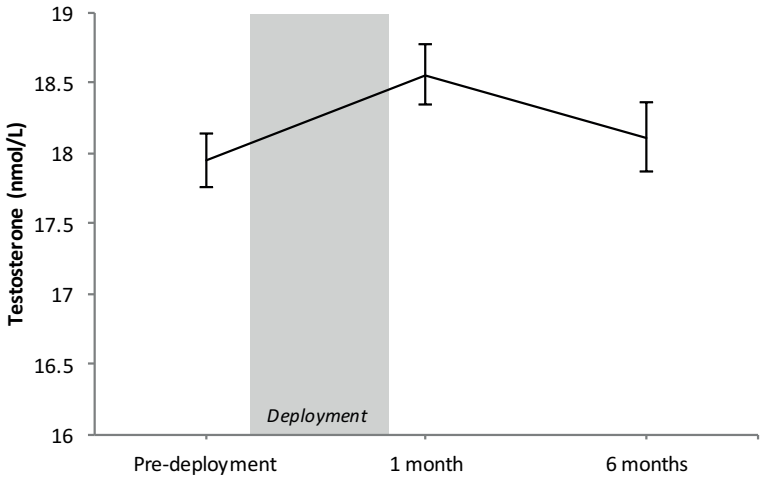

Figure 1. Testosterone levels (+SEM) over the three timepoints.

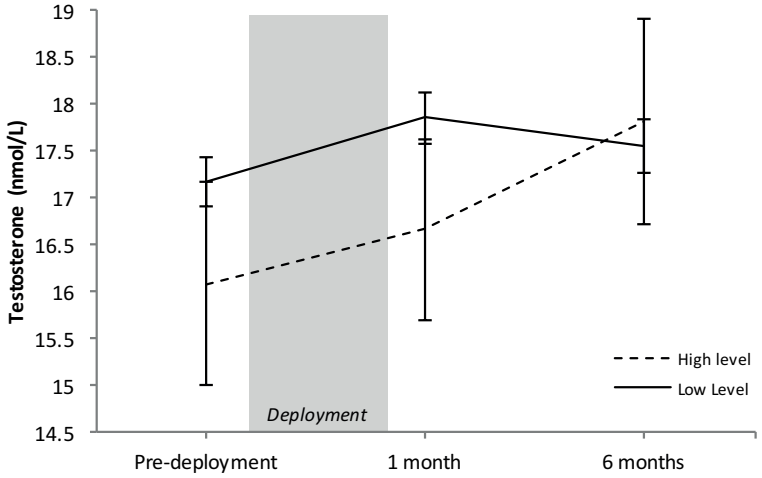

Figure 2. Testosterone levels (+SEM) over the three timepoints for military personnel with high (SRIP ≥38) or low levels of PTSD symptoms at one year post-deployment.

Testosterone, PTSD and cortisol

The predictive value of testosterone for the development of PTSD symptoms at various time-points post-deployment, and the possible moderating role of cortisol were examined using multiple regression analyses. The analyses revealed that pre-deployment testosterone levels significantly predicted a higher level of PTSD symptoms at 1 (B=-.991, p<.05) and 2 years post-deployment (B=-1.034, p<.001) after controlling for age, exposure to traumatic stressors during deployment, the number of previous deployments, pre-deployment PTSD symptoms, and potentially traumatic childhood experiences (see Table 3). The pre-deployment cortisol levels and the testosterone × cortisol interaction were not significant predictors in these models. The final models explained 21.4% and 20.2% of the variance in PTSD symptoms at, respectively, 1 and 2 years after deployment. Testosterone, cortisol and the testosterone × cortisol interaction did not predict the development of PTSD symptoms at 6 months after controlling for confounders.

Table 3. Predictive value of pre-deployment testosterone and cortisol levels for PTSD symptoms post-deployment.

	PTSD symptoms at 6 months		**PTSD symptoms at 1 year**		**PTSD symptoms at 2 years**	
	B	p	B	p	B	p
Age during deployment	-.051	.205	-.089	.050	-.060	.117
Previous deployments	-.121	.678	.051	.878	.035	.899
Early Trauma Inventory	.303	.003	.011	.922	.325	.001
Deployment stressors	.412	.000	.470	.000	.241	.010
PTSD score T0	.521	.000	.503	.000	.344	.000
Testosterone T0	-.358	.260	-.911	.012	-1.034	.001
Cortisol T0	.050	.868	-.487	.155	-.068	.815
Testosterone × Cortisol	.142	.667	.370	.323	.119	.708

Note: PTSD: Posttraumatic stress disorder

Discussion

In the current study, the effect of deployment to a combat zone on testosterone levels, and the association with self-reported PTSD symptoms was examined. Plasma testosterone levels were significantly increased after return compared to the levels at the pre-deployment assessment. The course of the testosterone levels did not differ between individuals that were reporting high or low levels of PTSD symptoms. Nevertheless, pre-deployment testosterone levels significantly predicted the development of symptoms at 1 and 2 years after deployment. Contrary to what was hypothesized, plasma cortisol levels were not found to have a moderating role in this association between testosterone and PTSD symptoms.

Relative to pre-deployment levels, testosterone levels were moderately increased after a 4-month deployment to a combat-zone. Although little is known about the effect of prolonged stress on plasma testosterone levels, medical evaluations of hostages (military and civilians) freed from captivity in Iran showed elevated levels of testosterone (Rahe et al., 1990). The increased levels are in line with the *challenge hypothesis*, which suggests that testosterone levels increase in preparation of a challenging encounter, thereby initiating approach motivation and reducing fear (Archer, 2006). In competitive situations, such as judo competitions and professional basketball games, testosterone levels were found to increase (Gonzalez-Bono et al., 2000; Serrano et al., 2000; Salvador, 2005). Interestingly, the response was not associated with the outcome, but rather with self-appraisal and the attribution of the outcome to personal effort. In addition, in cultures where honor is important testosterone levels increased in response to an insult (Cohen et al., 1996). These findings suggest that the increase in testosterone might be an adaptive biological mechanism to cope with the challenging situations encountered during exposure to threat and the challenge of combat.

Following this, the association between testosterone and the development of PTSD symptoms was assessed. The testosterone levels over time of participants reporting high or low levels of PTSD symptoms at 1 year post-deployment were comparable. This is in agreement with several studies that reported no differences in plasma testosterone levels between patients with combat-related PTSD and healthy controls (Mulchahey et al., 2001; Spivak et al., 2003). However, the pre-deployment levels were found to be predictive of the development of PTSD symptoms at 1 and 2 years after homecoming. Lower testosterone levels were also found to be a risk factor for the development of depressive symptoms (Shores et al., 2005; Hintikka et al., 2009) and anxiety symptoms (Berglund et al., 2011). In addition, high testosterone levels are thought to have an anxiolytic effect (Handa and Weiser, 2014). For instance, increased anxiety-like behavior was seen in men following chemical castration for prostate cancer treatment (Almeida et al., 2004). Also, the administration of testosterone has been shown to reduce subconscious fear (Van Honk et al., 2005). Therefore, it is tempting to postulate that low testosterone is a vulnerability factor for the development of mental health problems. However, it is also possible that other factors interact with the low testosterone levels, making individuals more prone to stress. For instance, higher testosterone levels are related to aggression (Popma et al., 2007), dominance and competitiveness (Sellers et al.,

2007; Mehta and Josephs, 2010), and novelty seeking (Gerra et al., 1999; Määttänen et al., 2013). On the other hand, higher levels of neuroticism and low levels of conscientiousness, extraversion, and agreeableness were linked to mental illnesses, such as anxiety disorders (Kotov et al., 2010). So, further research should investigate the role of intermediating factors, such as personality and temperament, in the association between testosterone and PTSD.

Support for the moderating role of cortisol in the association between testosterone and PTSD symptoms was also investigated. Testosterone is thought to inhibit HPA functioning, whereas cortisol has been shown to have an inhibitory effect on all levels of the HPG-axis (Viau, 2002). Handa and Weiser (2014) suggest that this interaction should be considered when exploring the normal and pathological responses to environmental stressors. For instance, aggression was found to be associated with high testosterone levels and low cortisol (Popma et al., 2007). Although aggression was found to increase after returning home from a combat zone (MacManus et al., 2013) and hostility symptoms were predictive of the development of PTSD symptoms (van Zuiden et al., 2011), the interaction between the HPA- and HPG-axis could not be confirmed in the current study. Paradoxically, low testosterone was predictive of the development of PTSD symptoms and cortisol had no moderating role. Since high levels of PTSD symptoms were not associated with a high level of depressive symptoms, possible depression-related elevations in cortisol could not have obscured the effects. This suggests that when assessing pre-deployment vulnerability factors, low testosterone can be considered as a predictor for PTSD symptoms, irrespective of the cortisol level measured prior to deployment.

Although the findings provide novel information on the effect of deployment to a combat zone on testosterone levels and PTSD symptoms, the interpretation of the findings of this study is limited by several factors. First, the generalizability of the results is limited due to non-response and attrition. This is inevitable in longitudinal cohort studies; however, by making use of mixed model analysis and data imputation this effect is reduced. Second, since participation in the study was voluntary, a selection bias cannot be ruled out. Third, no control group of non-deployed military personnel was added to the study, therefore, the effect cannot be attributed solely to deployment. Fourth, androgens show a diurnal rhythm with a steeper decline in the morning than in the afternoon (Dabbs, 1990). This may result in more variation, which may obscure underlying effects. However, research has indicated that the between-subject variation is larger than the within-subject variation (Dabbs, 1991). Lastly, self-report questionnaires were used to assess the presence of PTSD symptoms whereas clinician-rated instruments would probably provide a better and more reliable estimate of the severity of reported symptoms. Nevertheless, the SRIP was demonstrated to have good concurrent validity with diagnostic clinical interviews for PTSD (Hovens et al., 2002).

In conclusion, this study provides evidence that the activity of the HPG-axis in military personnel is altered after exposure to the high intensity stress of a combat zone. This alteration is not directly associated with the development of PTSD symptoms. However, low testosterone levels prior to deployment were found to be a vulnerability factor for the

development of PTSD symptoms 1 year after return home. So, in addition to the effect of deployment on the regulation of the HPA-axis, the results illustrate that not the alteration in testosterone levels shortly after deployment but the pre-deployment levels are associated with the development of PTSD symptoms. Future research should assess the testosterone levels in PTSD patients with different types of traumatic experiences. Research shows that the type, frequency, and intensity of traumatic events play a key role as potentiating factors for the development of PTSD (Perkonigg et al., 2000; Frans et al., 2005). Also, the context is associated with different symptom patterns (Naifeh et al., 2008), therefore, the underlying biological mechanisms might also differ. In addition, future research should assess the etiology of low testosterone and influence of intermediating factors, such as personality and temperament. This could contribute in the identification of biological vulnerability factors for the development of stress-related mental health symptoms.

Acknowledgements and disclosure

This work was financially supported by the Dutch Ministry of Defence.

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CHAPTER 06

Biological profiling of plasma neuropeptide Y in relation to posttraumatic stress symptoms in two combat cohorts

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In submission

Abstract

In order to decrease the risk of developing stress-related disorders after military deployment, biological vulnerability factors should be identified. Neuropeptide Y (NPY) is a peptide neurotransmitter that is associated with modulation of the stress response. Using the data of two longitudinal prospective cohort studies (N=892 and N=2427), plasma NPY (*p*NPY) was assessed as a possible susceptibility biomarker for the development of PTSD symptoms over time. Data collection started prior to deployment and follow-up assessments were completed up to two years after deployment. In *p*NPY levels, measured before and shortly after deployment, three distinct trajectories were identified. In both cohorts, these trajectories were not related to the level of reported PTSD symptoms over time and neither were pre-deployment *p*NPY levels. Whereas previous research suggested that high NPY levels might be a marker for resilience, the current findings suggest limited usefulness of peripherally measured NPY in predicting the development of PTSD.

Introduction

Although military personnel are highly trained to deal with stressful and traumatic events during deployment, high levels of combat exposure are associated with an increased risk of developing posttraumatic stress disorder (PTSD; Jones et al., 2013; Trautmann et al., 2017). In Dutch military personnel deployed to Afghanistan, 5.6-12.9% report symptoms of PTSD compared to 4.3% prior to deployment (Eekhout et al., 2016; Reijnen et al., 2015). In US combat veterans, new-onset PTSD symptoms were reported in 7.6-8.7% (Smith et al., 2008). Since only a subset of deployed military personnel develops PTSD, identification of biological susceptibility or resilience markers is important. This might provide a more objective measure to improve diagnosis of PTSD and, more importantly, to identify individuals at risk so as to be able to provide treatment in an early stage and to develop new treatments. Research on biomarkers for PTSD has often focused on the hypothalamic-pituitary-adrenal axis (HPA-axis) and the sympathetic nervous system, however, here we focus on neuropeptide Y (NPY) which is suggested to modulate both these systems (Heilig, 2004; Hirsch and Zukowska, 2012; Rasmusson et al., 2010).

NPY is a peptide neurotransmitter that is widely distributed in the central and peripheral nervous system (Hirsch and Zukowska, 2012; Thorsell, 2010). In the brain, NPY is abundantly expressed in numerous regions, including the amygdala, hippocampus and hypothalamus (Gehlert, 2004). In the periphery, it is primarily expressed in the sympathetic ganglia, where it is co-localized with noradrenaline, the adrenal medulla and platelets (Gehlert, 2004; Hirsch and Zukowska, 2012). NPY plays a role in a broad range of activities, such as the modulation of the stress response (Hirsch and Zukowska, 2012; Wu et al., 2011). NPY positively interacts with the HPA-axis, inhibiting excessive activation of the stress response, and can inhibit or regulate the release and activity of noradrenaline (Hirsch and Zukowska, 2012; Kuo et al., 2007; Schmeltzer et al., 2016; Thorsell, 2010). As animal data has shown, central administration of certain NPY receptor agonists decreases anxiety-like responses (Sajdyk, 2005) and promotes long-term resilience to stress (Sajdyk et al., 2008). Intranasal delivery of NPY to rats before or immediately after a single prolonged stress model of PTSD prevented the development of PTSD-like symptoms (Sabban et al., 2015; Serova et al., 2013) and even reduced symptoms in those rats that had already developed PTSD-like symptoms (Serova et al., 2014). In humans, significantly increased plasma NPY (*p*NPY) levels were found after acute, uncontrollable stress during survival training, with higher levels in Special Forces compared to non-Special Forces soldiers (Morgan et al., 2002; Morgan et al., 2000). The plasma NPY levels increased together with cortisol and noradrenalin (Morgan et al., 2001). This increase was associated with psychological resilience, less subjective distress and superior performance during military training (Morgan et al., 2002; Morgan et al., 2000). Moreover, higher plasma NPY levels were found in veterans without PTSD compared to PTSD patients and non-traumatized controls, and in PTSD patients with greater improvement in symptoms over time (Yehuda et al., 2006).

Decreased activity of the NPY system could result in reduced stress resiliency. In PTSD

patients, NPY levels in cerebrospinal fluid (CSF) were observed to be lower when compared to non-traumatized controls (Sah et al., 2009), and trauma-exposed veterans (Sah et al., 2014). However, plasma NPY levels in military men with PTSD were either reduced (Rasmusson et al., 2000) or did not differ from healthy controls (Morgan et al., 2003; Yehuda et al., 2006). Although there is some evidence that reduced *p*NPY is associated with PTSD, this is based on relatively small samples of (chronic) PTSD (in-) patients. Moreover, no significant differences in *p*NPY levels were found in other populations, for instance between female victims of intimate partner violence with and without PTSD and non-abused women (Seedat et al., 2003) and more recently between survivors of traffic accidents (Nishi et al., 2014).

This inconsistency might be associated with various other factors. First, trauma exposure rather than PTSD symptoms might be associated with reduced baseline NPY (Morgan et al., 2003). However, as shown in other research (Sah et al., 2014), trauma exposure in itself was not the primary contributor to low NPY in PTSD patients. Second, inter-individual differences in NPY might be present prior to trauma exposure and low levels might predispose individuals to develop stress-related mental health problems (Wu et al., 2011). Third, inter-individual variation in NPY might be due to environmental factors. A high level of early life adversity is related to psychopathology in adulthood (Weber et al., 2008) and is known to affect the individuals' stress response (Edwards et al., 2003). Moreover, higher levels of childhood trauma are shown to be significantly correlated with lower levels of plasma NPY after controlling for the degree of intimate partner violence (Seedat et al., 2003). Also, genetic variation in the NPY gene promotor and early life adversity affect HPA-axis responses to acute psychosocial stress (Witt et al., 2011) and might be a risk factor for expression of negative affect (Sommer et al., 2010).

Previous research on NPY focused on the difference between PTSD patients and healthy controls, thereby studying NPY as a marker for disease-related processes. However, in order to decrease the risk of developing PTSD after exposure to trauma, studies should focus on NPY as a susceptibility marker. Therefore, the aim of this study was to investigate if pre-deployment *p*NPY levels or changes in *p*NPY evoked by traumatic experiences are associated with PTSD symptoms over time in two large prospective longitudinal cohort studies; Prospective Research in Stress-related Military Operations (PRISMO) and Marine Resiliency Study (MRS). Furthermore, since there is an increasing interest in studying objective (biological) measures of psychopathology (Insel et al., 2010), we used a biological profiling perspective to assess if differences in pre- to post-deployment *p*NPY levels were related to the development of PTSD. Therefore, latent growth mixture modeling was used, which has previously been applied to address heterogeneity in PTSD symptom development by identifying distinct developmental trajectories of PTSD symptoms over time (e.g. Bonanno et al., 2012; Eekhout et al., 2016). In the current study, a similar approach was used, however, instead of identifying subpopulations based on the level of PTSD symptoms, distinct trajectories of *p*NPY over time were identified. Additionally, the moderating roles of early life trauma and deployment-related stressors on the relation between *p*NPY and the development of PTSD were assessed.

Experimental procedures

PRISMO

Participants

The first data sample used for this study is part of an ongoing large longitudinal cohort study, PRISMO, on the development and course of stress-related mental health symptoms in Dutch military personnel after deployment to Afghanistan. The military men were deployed for four months between 2005 and 2008 as part of the International Security Assistance Force (ISAF) of NATO (See also Reijnen et al., 2015). A total of 1032 individuals volunteered to participate prior to their deployment. After excluding women (N=93), non-deployed individuals (N=18), and those with no *p*NPY measurements (N=29), the data of 892 were available for the analyses.

Procedure

Military personnel could participate in the study after they received a complete verbal and written description of the study and informed consent was obtained. About one month prior to deployment (T0; baseline), and at approximately one (T1) and six months (T2) post-deployment participants completed various paper-and-pencil questionnaires, and blood samples were collected at the army base. At the one and two year follow-up (T3 and T4), only questionnaires were completed and returned by mail. For their participation, they received financial compensation (a voucher in the value of € 100 for participation in three assessments). The study was approved by the Institutional Review Board of the University Medical Center Utrecht.

Measurement

Questionnaires

The Dutch Self-Rating Inventory for PTSD (SRIP) was used to measure symptoms of PTSD (Hovens et al., 2002; Hovens et al., 2000). The SRIP contains 22 items, which corresponds to symptoms in the clusters re-experiencing (cluster B: 6 items), avoidance and numbing (cluster C: 9 items) and arousal (cluster D: 7 items) of the DSM-IV (American Psychiatric Association, 2000) diagnostic criteria for PTSD. Symptom severity (range 22-88) in the past month was evaluated using a 4-point Likert scale ranging from not at all (score 1) to very much (score 4). The SRIP was demonstrated to have good concurrent validity with diagnostic clinical interviews for PTSD, such as the Mississippi Scale for Combat-related PTSD (.82), and the MMPI PTSD subscale (.80) (Hovens et al., 2002). A SRIP cut-off score in the range of 39 showed good concurrent validity with the Clinician-Administered PTSD scale (sensitivity: 74%; specificity: 81%; van Zelst et al., 2003), therefore, this was used in the current study to diagnose a high level of PTSD symptoms.

The Dutch version of the Early Trauma Inventory self-report short form (ETISR-SF; Bremner et al., 2007) was used prior to deployment to assess potential exposure to traumatic experiences (general, physical, sexual and emotional abuse) before the age of

18 years. The questionnaire consists of 27 dichotomous items and the score indicates the composite severity of traumatic events experienced during childhood. Exposure to combat and deployment-related stressors was assessed at one month post-deployment with a 19-item dichotomous deployment stressor checklist (DES; Reijnen et al., 2015).

Plasma samples

For the plasma NPY measurements a venous blood sample was collected between 8AM and 1130AM in EDTA vacutainers and immediately put on ice. The samples were centrifuged at 3500 RPM for 12 minutes at 4°C after which they were transported to the hospital and stored at -80°C until assayed. Plasma neuropeptide Y levels were determined in a competitive enzyme immunoassay (Neuropeptide Y Enzyme Immunoassay (EIA) Kit, Ray Biotech Inc.). The sensitivity was 3 ng/mL and inter-assay variation was 15% at 22 ng/mL (n = 33).

MRS

Participants

The analyses were repeated in the Marine Resiliency Study (MRS). This is a large prospective longitudinal study on factors predictive of the development of combat-related PTSD (Baker et al., 2012). Approximately 2610 Marines, exclusively males, from four infantry battalions enrolled in the study. They were deployed for seven months to either Iraq (battalions 1 and 2, 2008-2009) or Afghanistan (battalions 3 and 4, 2009-2010). After excluding non-deployed individuals (N=15), and those with no plasma NPY samples (N=168), the *p*NPY data of 2427 were available for the analyses.

Procedure

Prior to a 7-month deployment, commanders of the infantry battalions were informed about the study goals and Marines in the battalions were invited to participate. Participation was voluntary and informed consent was obtained before enrolling in the study. Approximately one month prior to deployment (T0), and at three (T1) and six months (T2) post-deployment participants were interviewed and blood samples were collected on a Marine Corps base or at VA San Diego Medical Center. Participants received a 50-dollar gift card for blood draws at each study visit. The study was approved by the Institutional Review Board of the University of California, San Diego, VA San Diego Research Service, and Naval Health Research Center.

Measurements

Clinical interview

In this replication, the Clinician-Administered PTSD scale (CAPS) was used to assess the presence and severity of PTSD symptoms (Blake et al., 1995). This is a structured interview that corresponds to the DSM-IV criteria for PTSD (American Psychiatric Association, 2000). For more details, we refer to the supplementary material.

Questionnaires

Early life trauma was measured with the Childhood Trauma Questionnaire Short Form (CTQ; Agorastos et al., 2014; Bernstein and Fink, 1998). This 34-item questionnaire assessed emotional abuse, physical abuse, sexual abuse, emotional neglect, and physical neglect before the age of 18. To assess deployment-related trauma, a composite Deployment Risk and Resilience Inventory (DRRI) measure was used (King et al., 2006; Minassian et al., 2015). For more information, we refer to the supplementary material.

Plasma samples

For the plasma NPY measurements a venous blood sample was collected between 8AM and 5PM in EDTA vacutainers. The samples were centrifuged at 3000 RPM for 15 minutes at 4°C. Plasma neuropeptide Y levels were determined in a competitive radioimmunoassay using antiserum raised against synthetic NPY conjugated to bovine thyroglobulin (Catalog number 13-NPYHU-R100, ALPCO diagnostics, Salem, NH). The sensitivity was 3 pmol/L and inter-assay variations were 9.2% (n = 6, mean 42.4 pmol/L) and 7.6% (n = 6, mean 90.2 pmol/L).

Analysis

Analyses were performed with MPLUS version 7.3 (Muthén and Muthén, 2012), STATA (StataCorp, 2013) and R (RCoreTeam, 2014). Descriptive statistics were examined using frequencies; independent samples t-tests; and χ^2-tests. The variables were tested for normality and a square root or log transformation was performed where necessary.

First, the association between the pre-deployment *p*NPY and PTSD was assessed using logistic regression analysis with a high level of PTSD symptoms (SRIP cutoff ≥38 or PTSD diagnosis according to the DSM-IV) and a low level of symptoms (SRIP cutoff <38 or no PTSD diagnosis) as group variable.

Following this, latent growth mixture model was used to identify distinct classes of *p*NPY over time. A stepwise forward method was used to determine the optimal number of classes, starting with one class and adding one class at the time (1-6 classes). The time-points were modeled as the actual time-points; namely 0, 7 and 12 months. The model only included a linear slope; both the intercept and slope variances were estimated. Missing data were managed with the use of robust maximum likelihood estimation. The models were compared using various fit indices: the Bayesian Information Criterion (BIC); Akaike Information Criterion (AIC); Sample-size adjusted BIC (Adj. BIC); Vuong, Lo, Mendell and Rubin Likelihood ratio test (VLMR-LRT); Bootstrap likelihood ratio test (BLRT); posterior probabilities, entropy; class sizes no less than 1% of total count; and relevant differences between classes (Jung and Wickrama, 2008). The model with the optimal number of classes in the PRISMO study was also used in the data of the MRS.

Next, a series of linear mixed models were performed to assess the association between baseline *p*NPY levels and the *p*NPY trajectories and PTSD symptoms over time. Random intercepts were used to account for the variance between participants. Missing data

were handled using restricted maximum likelihood estimation. In the PRISMO cohort, the total level of PTSD symptoms at pre-deployment, one and six months and one and two years after deployment were used as the outcome variable. In MRS, the CAPS scores prior to deployment, and at three and six months were used. Either baseline *p*NPY levels or the *p*NPY trajectories were included as independent variables. Because of the highly skewed distribution of the CAPS scores in the MRS, zero-inflated negative binomial mixed models were used in this cohort. Additionally, because of the differences between the battalions in the MRS, the different battalions were controlled for in the analyses. The moderating role of early life trauma and deployment-related stressors on the relation between *p*NPY and the development of PTSD was examined by including the interaction terms in the models.

Results

PRISMO

The military men participating in this study (N=892) were between the ages of 18 and 60 with a mean age of 28.55 (SD 9.0) prior to deployment. Further demographic information is displayed in Table 1 and 2. Blood samples and questionnaires were collected at baseline for 878 participants (98.4%), at 1 month for 738 participants (82.7%) and at 6 months for 661 participants (74.1%). The questionnaires at one year were completed by 498 participants (55.8%) and at two years by 467 participants (52.4%).

Table 1. Demographics for the PRISMO sample (N=892).

Variable	Prevalence	
	Count	%
Age (years) (N=886)[1]		
< 21	125	14.1
21-24	287	32.4
25-29	172	19.4
30-34	101	11.4
35-39	63	7.1
40-44	61	6.9
≥ 45	77	8.7
Education level (N=807)[1]		
Low	30	3.7
Moderate	698	86.5
High	79	9.8
Marital status (N=797)[1]		
Single	297	37.3
Long-term relationship	153	19.2

Table 1. Demographics for the PRISMO sample (N=892). *(continued)*

Variable	Prevalence	
	Count	%
Cohabiting	132	16.6
Married	207	26.0
Divorced/widowed	8	1.1
Rank (N=867)[1]		
Private	355	40.9
Corporal	176	20.3
Non-commissioned officer	304	35.1
Officer	32	3.7
Number of prior missions (N=798)[1]		
0	409	51.3
1	206	25.8
2	100	12.5
≥ 3	83	10.4

[1] Count may not add up to 892 participants due to missing values.

Logistic regression analyses revealed that the pre-deployment pNPY levels were not associated with a high level of PTSD symptoms pre-deployment (b=-0.19, p=.29, n=21 cases), and at one month (b=0.01, p=.07, n=59 cases), 6 months (b=-0.01, p=.47, n=60 cases), at 1 year (b=0.02, p=.09, n=35 cases), and at 2 years (b=0.01, p=.33, n=27 cases) post-deployment.

Table 2. NPY levels and PTSD symptoms in the total sample and the three classes in Prismo.

	Sample (N=892)		**Low NPY** (N=837)		**Intermediate NPY** (N=39)		**High NPY** (N=16)		
	N1	Mean (SD)	N[1]	Mean (SD)	N[1]	Mean (SD)	N[1]	Mean (SD)	p
NPY (ng/ml)									
T0	878	41.06 (16.20)	824	38.2 (11.00)	39	77.08 (12.38)	15	104.41 (22.35)	<.001
T1	738	42.81 (17.39)	693	40.21 (12.55)	33	69.83 (18.44)	12	118.46 (22.28)	<.001
T2	661	40.66 (15.73)	625	38.37 (11.35)	27	68.59 (11.99)	9	115.83 (22.26)	<.001
PTSD Symptoms									
T0	620	26.7 (4.96)	583	26.73 (4.95)	24	28.38 (5.65)	13	26.54 (3.67)	.279
T1	695	27.72 (6.21)	652	27.66 (6.15)	32	28.91 (7.30)	11	28 (6.51)	.535
T2	668	27.9 (7.18)	630	27.83 (7.19)	28	29.5 (7.11)	10	28 (6.52)	.484
T3	498	27.23 (7.10)	464	27.15 (7.17)	27	28.85 (6.35)	7	26.43 (3.87)	.458
T4	467	26.8 (5.76)	435	26.73 (5.76)	25	27.96 (5.93)	7	28.14 (5.31)	.483
PTSD > 95th perc.		n > cutoff		n > cutoff		n > cutoff		n > cutoff	
T0	620	23	583	22	24	1	13	0	.762
T1	695	60	652	54	32	5	11	1	.299
T2	668	61	630	58	28	2	10	1	.929
T3	498	37	464	33	27	4	7	0	.254
T4	467	27	435	25	25	1	7	1	.452

Note: N: total sample; n: number of cases; SD: Standard Deviation: NPY: Neuropeptide Y; PTSD: Posttraumatic Stress Disorder. [1] N might not correspond to total sample size due to missing values.

Next, using a stepwise forward method, the three-class solution was found to be the best model for the PRISMO cohort (see Figure 1A). For an overview of the fit indices, we refer to table S1 in the supplementary material. The largest class (93.2%) showed a relatively low pattern of pNPY levels over time and was therefore named *low NPY class* (see Table 2). Relative to the pre-deployment pNPY level, this class showed a significant peak in NPY level at 1 month after deployment (b=1.75, p=.003, 95% CI= 1.60–2.90; 6 months; b=.11, p=.84, 95% CI= -.95–1.18). The second class (5.0%) showed a moderate and significantly decreasing level of pNPY over time compared to pre-deployment levels (1 month; b=-7.22, p=.06, 95% CI=-14.83–.38, 6 months; b=-8.52, p=.01, 95% CI=-14.78– -2.27), this class was named *intermediate NPY class*. The third class (1.8%) showed a high and slightly increasing level of plasma NPY and was therefore named *high NPY class* (1 month; b=15.17, p=.09, 95% CI=-2.81 – 33.16, 6 months; b=10.45, p=.36, 95% CI=-13.49 – 34.40). When comparing the three classes, a trend was found in age with younger participants in the high NPY class (F(2,883)=2.91, p=.055; see Table S2) and in the low class significantly more participants were smoking prior to deployment ($\chi(2)=6.32$, p=.04). The classes did not differ in the level of PTSD symptoms at the separate time-points, nor in the number of participants scoring above the 95th percentile (SRIP cutoff ≥38; see Table 2).

Baseline pNPY was not significantly associated with the development of PTSD symptoms over time (baseline NPY: b=0.01, p=.62, 95% CI=-0.02–0.02). Also, the intermediate and high NPY classes showed no significant difference in the development of PTSD over time compared with the participants in the low NPY class (intermediate NPY: b=1.14, p=.20, 95% CI=-0.60–2.88; high NPY: b=-0.52, p=.71, 95% CI=-3.30–2.26). The results are depicted in Figure 1B.

Following this, the number of deployment stressors did not influence the relation between the pNPY trajectories and PTSD symptoms over time (intermediate NPY×DES: b=-0.08, p=.80, 95% CI=-0.70–0.54; high NPY×DES: b=0.09, p=.86, 95% CI=-0.96–1.15); neither did the total level of early life trauma (intermediate NPY×ETI: b=-0.51, p=.08, 95% CI=-1.09–0.07; high NPY×ETI: b=-0.72, p=.14, 95% CI=-1.67–0.23).

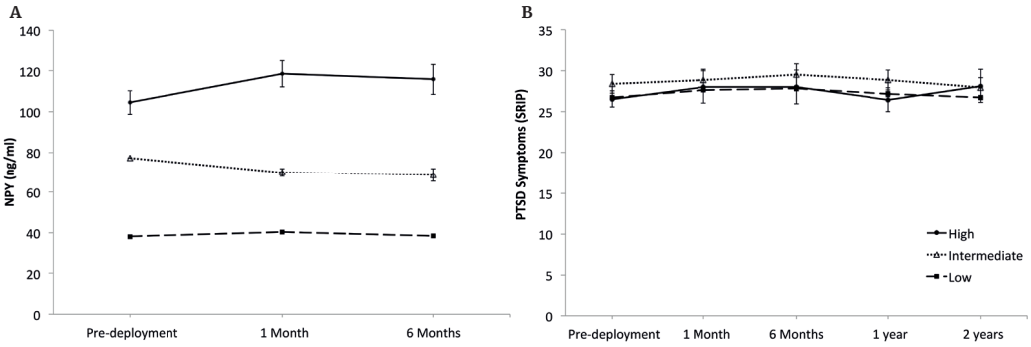

Figure 1. (A) Three NPY classes (+SEM) and (B) the level of PTSD symptoms for the three classes (+SEM) in **PRISMO**. The dashed line represents the low class (93.2%), the dotted line the intermediate class (5.0%) and the solid line the high class (1.8%).

MRS

The findings of the PRISMO study were replicated in the data of the MRS. In total, the data of 2427 military men were included in the analyses. Demographic information is displayed in Table 3 and 4. In agreement with the results of the PRISMO cohort, the logistic regression of pre-deployment *p*NPY levels showed no significant association with PTSD diagnosis at pre-deployment (b=0.00, *p*=.98, n=121 cases), 3 months post-deployment (b=0.06, *p*=.21, n=114 cases), and 6 months post deployment (b=0.05, *p*=.41, n=77 cases).

Table 3. Demographics for the MRS sample (N=2427).

Variable	**Prevalence**	
	Count	%
Age (years)(N=2394)[1]		
< 21	808	33.8
21 - 24	1151	48.1
25 - 29	327	13.7
30 - 35	72	3
35 - 39	28	1.2
40 -44	6	0.3
>= 45	2	0.1
Education level (N=2408)[1]		
Some high school	51	2.1
GED	51	2.1
High school	1525	63.3
Some College	631	26.2
Associates Degree	56	2.3
4 Year college	82	3.4
Master's	8	0.3
PhD	4	0.2
Marital Status (N=2388)[1]		
Never married	1464	61.3
Married	843	35.3
Divorced	51	2.1
Separated	30	1.3
Rank (N=2391)[1]		
E1-E3	1629	68.1
E4-E9	701	29.3
Officer	61	2.6
Number of prior deployments (N=2321)[1]		
0	1163	50.1
1	619	26.7
2	349	15.0
≥ 3	190	8.2

[1] Count may not add up to 2427 participants due to missing values.

Table 4. NPY levels and PTSD symptoms in the total sample and the three classes in MRS.

	Sample (N=2427)		Low NPY (N=2333)		Intermediate NPY (N=77)		High NPY (N=17)		
	N[1]	Mean (SD)	N[1]	Mean (SD)	N[1]	Mean (SD)	N[1]	Mean (SD)	p
NPY (pmol/L)									
T0	2401	91.1 (37.5)	2308	87.57 (31.08)	76	185.5 (34.71)	17	190.43 (107.12)	<.001
T1	1775	84.94 (30.46)	1703	82.59 (27.11)	60	131.06 (41.33)	12	187.99 (52.32)	<.001
T2	1549	86.86 (32.43)	1486	84.68 (28.82)	52	120.19 (45.77)	11	223.56 (41.78)	<.001
PTSD Symptoms									
T0	2417	15.19 (15.64)	2323	15.15 (15.5)	77	14.34 (15.81)	17	23.18 (28.44)	.10
T1	1800	17.46 (18.02)	1725	17.75 (17.95)	63	13.08 (19.08)	12	24.25 (19.92)	.19
T2	1579	15.27 (17.26)	1514	15.4 (17.28)	54	12.59 (17.49)	11	10.73 (10.96)	.50
PTSD (CAPS)		**n with**		**n with**		**n with**		**n with**	
T0	2420	121	2326	112	77	5	17	4	.04
T1	1804	114	1729	109	63	3	12	2	.39
T2	1580	78	1515	76	54	2	11	0	.55

Note: N: total sample; n: number of cases; SD: Standard Deviation: NPY: Neuropeptide Y; PTSD: Posttraumatic Stress Disorder. [1] N might not correspond to total sample size due to missing values.

Following this, the three-class solution was fitted onto the MRS data using latent growth mixture modeling (for fit indices see Table S3). As is demonstrated in Figure 2A, comparable trajectories were found in MRS. As in the PRISMO cohort, the largest class (96.1%) showed a relatively low pattern of *p*NPY levels over time, the *low NPY class* (3 months; b=-0.23, p<.001, 95% CI=-0.32 — -0.14, 6 months; b=-0.14, p=.007, 95% CI=-0.23 — 0.04). The second class (3.2%) showed a moderate and significantly decreasing level of *p*NPY over time (3 months; b=-2.20, p<.001, 95% CI=-2.86 — -1.70, 6 months; b=-2.67, p<.001 95% CI=-3.20 — -2.23), which is comparable to the *intermediate NPY class*. The third class (0.7%) was found to be small, and showed consistently high levels of plasma NPY comparable to the *high NPY class* (3 months; b=0.73, p=.59, 95% CI=-1.52 — 2.97, 6 months; b=1.38, p=.29, 95% CI=-1.15 — 3.91). For an overview of the demographic information for the different classes see Table S4 of the supplementary material.

In agreement with the results of the PRISMO cohort, baseline *p*NPY was not significantly associated with the development of PTSD symptoms over time (baseline NPY: b=0.01, *p*=.27, 95% CI=-0.01—0.03). As is depicted in Figure 2B, PTSD symptom levels in the high NPY class decrease at 6 months post-deployment, whereas the levels in the other classes are relatively stable. However, as compared to the low class, the intermediate and high NPY classes showed no significant difference in the development of PTSD over time (intermediate NPY: b=-0.11, p=.25, 95% CI=-0.30—0.08; high NPY: b=0.18, p=.36, 95% CI=-0.21—0.58).

Furthermore, the *p*NPY classes did not interact with the number of deployment stressors (intermediate NPY×DRRI: b=-0.09, p=.53, 95% CI=-0.38— 0.19; high NPY×DRRI: b=-0.35, p=.24, 95% CI=-0.94— 0.24) nor with the level of early life trauma in the association with PTSD symptoms according to the CAPS score (intermediate NPY×CTQ: b=0.01, p=.15, 95% CI=-0.004—0.02; high NPY×CTQ: b=0.01, p=.31, 95% CI=-0.01—0.03).

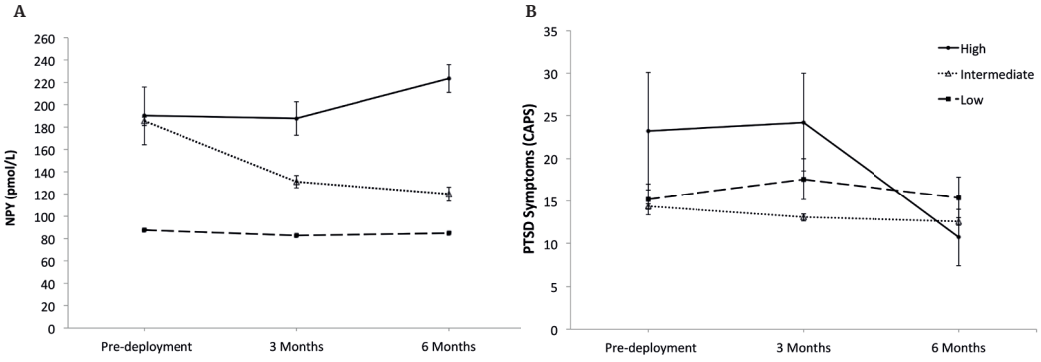

Figure 2. (A) Three NPY classes (+SEM) and (B) the level of PTSD symptoms for the three classes (+SEM) in **MRS**. The dashed line represents the low class (96.1%), the dotted line the intermediate class (3.2%) and the solid line the high class (0.7%).

Discussion

Despite the fact that NPY is often suggested to be associated with resilience, in this study increased basal plasma NPY levels were not found to be a protective factor for the development of PTSD symptoms in military personnel. Neither pre-deployment *p*NPY levels nor the difference from pre- to post-deployment *p*NPY levels were associated with the development of PTSD symptoms from pre-deployment up to two years after return. More importantly, these findings were replicated in an independent large military cohort followed up to 6 months post-deployment.

Pre-deployment *p*NPY levels were not significantly different between participants with (a high level of symptoms of) PTSD and a relatively resilient group. These findings are in agreement with some of the previous research in which plasma NPY levels in PTSD patients were similar to healthy (traumatized) controls (Morgan et al., 2003; Seedat et al., 2003; Yehuda et al., 2006). Comparable results were also found in survivors of traffic accidents, which showed no association between serum NPY and depression or PTSD at 1 month post-accident (Nishi et al., 2014). In contrast, another study reported reduced plasma NPY levels and blunted yohimbine-stimulated increases in patients with combat-related chronic PTSD, although in that study PTSD patients were compared to a small group of significantly younger controls (Rasmusson et al., 2000). More importantly, in all previous studies *p*NPY was investigated as a marker for disease by comparing PTSD patients with healthy controls. The current study longitudinally assessed *p*NPY as a *predisposition* for the possible development of PTSD symptoms over time. Additionally, instead of comparing participants based on the level of reported symptoms, this study used a biological profiling perspective to compare participants based on pre-deployment *p*NPY and pre- to post-deployment difference in *p*NPY. In sum, our findings suggest that the simple use of peripherally measured basal NPY levels as a susceptibility biomarker for the development of PTSD is limited.

In contrast, reduced NPY levels have been consistently reported in CSF of PTSD patients versus healthy (traumatized) controls (Sah et al., 2014; Sah et al., 2009). This might be explained by the difference between peripheral and central NPY levels. NPY levels from CSF and plasma show no cross-correlation (Baker et al., 2013). This indicates that plasma NPY levels may not accurately reflect the levels measured in CSF. Moreover, increased levels of NPY in the brain are thought to improve the ability to cope with stress in various brain regions important for emotional reactivity, such as the amygdala and locus coeruleus (Heilig, 2004; Sabban et al., 2015; Thorsell, 2010). However, NPY in the periphery is suggested to amplify the stress response, for instance, by regulation of the cardiovascular and immune systems (Dutton et al., 2006; Hirsch and Zukowska, 2012; Wang et al., 2009). Therefore, low NPY as predisposition for development of PTSD should be further assessed with NPY collected from CSF.

Furthermore, the role of NPY in resilience to stress is supported by studies on inter-individual variations in NPY-related genes. Genetics polymorphisms account for about 66% of the variance in the concentration of CSF NPY, and most likely also in plasma (Baker et al., 2013; Berrettini et al., 1988; Zhang et al., 2012). NPY genotypes associated with lower NPY levels might increase the risk to develop stress-related mental health problems by predisposing individuals to hyper-responsitivity to negative stimuli within various key brain structures that are associated with affective processing (Domschke et al., 2010; Mickey et al., 2011; Zhou et al., 2008). For instance, a NPY haplotype associated with low NPY predicted higher emotion-induced activation of the amygdala and a higher risk of developing stress-related disorders (Zhou et al., 2008). The inter-individual variability in NPY levels might be further complicated by the interaction between genotype and environment. The interaction between the variation in NPY gene promoter and early life adversity has been reported to influence the response of the HPA-axis to acute social stress in humans (Witt et al., 2011). However, in the current study, no interaction was found between early life stress and the reaction of the peripheral NPY system in the association with PTSD symptoms over time. Therefore, future research should further assess the role of NPY genotypes and the interaction with early life trauma, thereby taking into account the type and timing of the adversity, to better understand differences in the development of PTSD.

The strength of this study is that it assessed *p*NPY and PTSD symptoms in two large cohorts over a prolonged period of time before and after deployment. However, there are various limitations. First, *p*NPY levels were determined based on one venous blood sample at each time-point, which might show higher variability than multiple samples. Although *p*NPY was reported to undergo a circaoctohoran (8 hour) variation (Löckinger et al., 2004), more recent research reported no statistically significant NPY circadian rhythmicity in plasma (Baker et al., 2013). In future research, NPY levels should be measured repeatedly and/or contiguous to a stress challenge to assess if (subtle) changes in NPY activity are related to PTSD. Second, based on the fit indices, the three-class model would not be the ideal model for the MRS data since the high class is very small (See Table S3). However,

our findings underline that similar trends in *p*NPY over time are found in both studies with the largest number of military men showing a relatively low level of plasma NPY and a small group showing high levels of *p*NPY. Importantly, when predicting PTSD symptoms over time with the pre-deployment *p*NPY levels on a continuous scale similar results were found. Moreover, repeating the analysis with the two classes (Table S5) did not yield different results as is shown in the supplementary material. Third, although the influence of psychotropic drugs on plasma NPY levels is unclear, there are a small number of participants (PRISMO; N=6; MRS; N=30) that use anti-depressants. Deleting these participants from the samples did not influence our findings. Fourth, in the PRISMO study, self-report questionnaires were used to assess the level of PTSD symptoms. Clinical interviews, such as the CAPS used in the MRS cohort, might have resulted in more reliable scores. Lastly, previous studies suggested that *p*NPY levels were associated with trauma exposure rather than PTSD (Morgan et al., 2003; Yehuda et al., 2006). Since there was no control group of non-deployed military personnel with *p*NPY assay data, this could not be assessed. However, though this should be interpreted with caution, the three trajectories did not significantly differ in the level of reported deployment-related stressors and no simple correlation was found between the level of stressors and *p*NPY at different time-points. Also, no interaction was found between the level of stressors and the *p*NPY levels in relation to PTSD over time.

In conclusion, neither pre-deployment *p*NPY levels nor the *p*NPY trajectories were associated with the development of PTSD symptoms over time. While heightened NPY levels are proposed to be involved in the resiliency to stress, potentially decreasing the risk of developing stress-related disorders, our findings indicate that basal plasma NPY levels are not a simple susceptibility biomarker for the development of PTSD. While our findings cannot address the utility of plasma collected for NPY assay contiguous to a severe stress challenge, as in military training, the current findings show that, in contrast to findings in centrally measured NPY, plasma NPY was not associated with PTSD. Taken together, the use of a biological profiling perspective provided important novel information on the limited usefulness of peripheral NPY as a susceptibility biomarker for the development of PTSD over a longer, pre- to post-deployment timeframe.

Acknowledgments and disclosure

The authors thank the Dutch commanders and troops and all members of the PRISMO team involved in data acquisition for their time and effort. Also, we thank Prof. dr. Joëls and Dr. Kennis for their valuable suggestions to the manuscript.

The authors acknowledge all MRS co-investigators, as well as administrative core, of the MRS Team, including logistic coordinators, clinician-interviewers, and data collection staff listed in the Methods (Baker et al., 2012). We thank Daniel O'Connor and his laboratory for contributions to MRS. The authors also thank the U.S. Marine and Navy Corpsmen volunteers for military service and participation in this study.

PRISMO data collection and analysis was supported by the Dutch Ministry of Defence. MRS data collection and analysis was supported by VA Health Service Research and Development project no. SDR 09-0128; the Marine Corps; and the Navy Bureau of Medicine and Surgery. The sponsors had no role in the design of the study; in the collection, analysis, and interpretation of data; in the writing of the report; and in the decision to submit the paper for publication.

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Supplementary material

MRS-Clinical interview

The Clinician-Administered PTSD scale (CAPS) is considered a gold-standard structural interview (Blake et al., 1995). The 17-items correspond to the three clusters of the DSM-IV, namely re-experiencing (cluster B), avoidance and numbing (cluster C) and hyperarousal (cluster D). The symptoms are rated for frequency and intensity on a five-point scale (0-4) and summed to provide the severity ratings. The CAPS has good psychometric properties across a wide variety of clinical populations and research settings (Weathers et al., 2001).

MRS-Questionnaires

The Childhood Trauma Questionnaire Short Form (CTQ; Bernstein and Fink, 1998; Agorastos et al., 2014) is a 34-item questionnaire used to assess emotional abuse, physical abuse, sexual abuse, emotional neglect, and physical neglect before the age of 18. Items are rated on a five-point Likert-type scale (range 25-125) and summed to assess the severity of multiple forms of abuse and neglect (for more information on the used scoring and catogorization see Agorastos et al., 2014). The internal consistency coefficients of the original version ranged from .61 (physical neglect) to .95 (sexual abuse).

The subscales Combat Experiences, Post Battle Experience, Deployment Environment, and Deployment Concerns of the Deployment Risk and Resilience Inventory (DRRI) measure were used to assess deployment-related stressors (for more information on how to form a composite measure see King et al., 2006; Minassian et al., 2015). It was shown to demonstrate acceptable internal consistency for the subscales and convergent and discriminative validity (Vogt et al., 2008).

Table S1. Fit indices for the classes 1-4 in PRISMO.

Fit indices	1 class	2 classes	3 classes	4 classes
AIC	18683.61	18284.09	18181.86	18148.82
BIC	18721.96	18336.82	18246.97	18230.31
Adj. BIC	18696.55	18301.88	18204.51	18176.32
Entropy		.987	.965	.976
Prop. cases per class		.967	.044	.941
		.033	.938	.004
			.018	.044
				.011
VLMR p-value		.05	.018	.10
BLRT p-value		.00	.00	.00

Note: AIC: Akaike Information Criterion; BIC: Bayesian Information Criterion; Adj. BIC: Sample-size adjusted BIC; VLMR-LRT: Vuong, Lo, Mendell and Rubin Likelihood ratio test; BLRT: Bootstrap likelihood ratio test.

Table S2. Demographic information for the three classes in PRISMO.

Variable	Low NPY (N=837)		Intermediate NPY (N=39)		High NPY (N=16)	
	Count	%	Count	%	Count	%
Age (years)[1,2]	(N=831)		(N=39)		(N=16)	
< 21	115	13.8	5	12.8	5	31.3
21–24	269	32.4	12	30.8	6	37.5
25–29	161	19.4	7	17.9	4	25.0
30–34	94	11.3	6	15.4	1	6.3
35–39	59	7.1	4	10.3	–	–
40–44	60	7.2	1	2.6	–	–
≥ 45	73	8.8	4	10.3	–	–
Education level[1]	(N=747)		(N=36)		(N=14)	
Low	29	3.8	–	–	1	6.7
Moderate	652	86.4	32	86.5	14	93.3
High	74	9.8	5	13.5	–	–
Marital status[1]	(N=755)		(N=37)		(N=15)	
Single	278	37.2	12	33.3	7	50,0
Long-term relationship	141	18.9	9	25.0	3	21.4
Cohabiting	124	16.6	4	11.1	4	28.6
Married	196	26.2	11	30.6	–	–
Divorced/widowed	8	1.1	–	–	–	–
Rank[1]	(N=813)		(N=39)		(N=15)	
Private	333	41.0	15	38.5	7	46.7
Corporal	163	20.0	6	15.4	7	46.7
NCO	288	35.4	15	38.5	1	6.7
Officer	29	3.6	3	7.7	–	–
Number of prior missions[1]	(N=746)		(N=37)		(N=15)	
0	381	51.1	20	54.1	8	53.3
1	197	26.4	6	16.2	3	20.0
2	90	12.1	7	18.9	3	20.0
≥ 3	78	10.4	4	10.8	1	6.7
BMI[1]	N	M(SD)	N	M(SD)	N	M(SD)
Pre-deployment	755	24.84 (2,78)	37	25.03 (2.58)	15	24.05 (2.19)
1 month	334	24.88 (2.87)	16	25.28 (2.79)	6	24.04 (1.66)
6 months	322	25.17 (3)	16	25.45 (3.28)	5	24.13 (1.51)

Note: NCO: non commissioned officer; BMI: Body Mass Index; M(SD): Mean (Standard Deviation); N: Number of participants.
[1] Count may not add up to the total number of participants due to missing values. [2] $p<.05$.

Table S3. Fit indices for the three-class model per cohort in MRS.

Fit indices	Cohort 1	Cohort 2	Cohort 3	Cohort 4
AIC	7957.29	16313.15	14914.49	17368.22
BIC	8009.37	16376.86	14976.46	17434.77
Adj. BIC	7964.97	16332.41	14932.01	17390.31
Entropy	.990	.905	.917	.982
Prop. cases per class	.983	.945	.935	.984
	.013	.050	.012	.004
	.003	.004	.051	.013
VLMR p-value	.004	.45	.029	.17
BLRT p-value	.00	.00	.00	.00

Note: AIC: Akaike Information Criterion; BIC: Bayesian Information Criterion; Adj. BIC: Sample-size adjusted BIC; VLMR-LRT: Vuong, Lo, Mendell and Rubin Likelihood ratio test; BLRT: Bootstrap likelihood ratio test.

Table S4. Demographic information for the three classes in MRS.

Variable	Low NPY (N=2333)		Intermediate NPY (N=77)		High NPY (N=17)	
	Count	%	Count	%	Count	%
Age (years)[1]	(N=2301)		(N=76)		(N=17)	
< 21	774	3.6	27	35.5	7	41.2
21 – 24	1109	48.2	33	43.4	9	52.9
25 – 29	314	13.6	13	17.1	–	–
30 – 35	69	3.0	2	2.6	1	5.9
35 – 39	27	1.2	1	1.3	–	–
40 -44	6	0.3	–	–	–	–
>= 45	2	0.1	–	–	–	–
Education level[1]	(N=2315)		(N=76)		(N=17)	
Some high school	50	2.2	1	1.3	–	–
GED	47	2.0	4	5.3	–	–
High school	1466	63.3	45	59.2	14	82.4
Some College	612	26.4	16	21.1	3	17.6
Associates Degree	53	2.3	3	3.9	–	–
4 Year college	77	3.3	5	6.6	–	–
Master's	6	0.3	2	2.6	–	–
PhD	4	0.2	–	–	–	–
Marital Status[1]	(N=2295)		(N=76)		(N=17)	
Never married	1401	61.0	50	65.8	13	76.5
Married	815	35.5	24	31.6	4	23.5
Divorced	50	2.2	1	1.3	–	–
Separated	29	1.3	1	1.3	–	–
Rank[1,2]	(N=2298)		(N=76)		(N=17)	
E1–E3	1558	67.8	58	76.3	13	76.5
E4–E9	684	29.8	13	17.1	4	23.5
Officer	56	2.4	5	6.6	–	–
Number of prior deployments[1]	(N=2300)		(N=76)		(N=17)	
0	1110	48.3	45	59.2	8	47.1
1	666	29.0	21	27.6	4	23.5
2	337	14.7	8	10.5	4	23.5
≥ 3	187	8.1	2	2.6	1	5.9
BMI[1]	N	M(SD)	N	M(SD)	N	M(SD)
Pre-deployment	2298	25.68 (3.04)	76	25.35 (2.79)	17	25.44 (2.47)
3 months	1722	26.15 (3.06)	63	26.15 (2.79)	12	27 (2.06)
6 months	1514	26.5 (3.32)	54	26.51 (3.09)	11	26.39 (3.15)

Note: BMI: Body Mass Index; M(SD): Mean (Standard Deviation); N: Number of participants.

[1] Count may not add up to the total number of participants due to missing values. [2] $p<.05$.

Additional analyses

Table S5. Fit indices for the two-class model per cohort in MRS.

Fit indices	Cohort 1	Cohort 2	Cohort 3	Cohort 4
AIC	7992.463	16339.62	14943.09	17405.47
BIC	8033.387	16389.68	14991.79	17457.76
Adj. BIC	7998.5	16354.76	14956.86	17422.82
Entropy	.968	.998	.985	.963
Prop. cases per class	.972	.004	.019	.019
	.028	.996	.981	.981
VLMR p-value	.37	.00	.034	.01
BLRT p-value	.00	.00	.00	.00

Note: AIC: Akaike Information Criterion; BIC: Bayesian Information Criterion; Adj. BIC: Sample-size adjusted BIC; VLMR-LRT: Vuong, Lo, Mendell and Rubin Likelihood ratio test; BLRT: Bootstrap likelihood ratio test.

Mixed model analyses MRS with 2 NPY classes

The high NPY class showed no significant difference in the development of PTSD over time compared with the participants in the low NPY class (high NPY: b=0.11, p=.42, 95% CI=-0.16— 0.38). Furthermore, the number of deployment stressors did not influence the relation between the NPY trajectories and PTSD symptoms over time (high NPY×DRRI: b=-0.39, p=.05, 95% CI=-0.78— 0.00); neither did the total level of early life trauma (high NPY×LEC: b=0.01, p=.34, 95% CI=-0.01—0.02).

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CHAPTER 07

Individual variation in plasma oxytocin and vasopressin levels in relation to the development of combat-related PTSD in a large military cohort

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Published in: Journal of Psychiatric Research (2017) 94, 88–95

Abstract

In an attempt to decrease the risk of developing mental health problems after military deployment, it is important to find biological markers to identify those at risk. Oxytocin (OT) and arginine vasopressin (AVP) are potential biomarkers for the development of post-traumatic stress disorder (PTSD) because they are involved in the regulation of stress and anxiety. Therefore, the aim was to examine whether plasma OT (*p*OT) and AVP (*p*AVP) levels before and after deployment are biomarkers for the development of posttraumatic stress symptoms over time in addition to other known risk factors. This study is part of a large prospective cohort study on candidate markers for stress-related mental health symptoms and resiliency after deployment to a combat zone; Prospective Research in Stress-related Military Operations (PRISMO; N=907). Data was collected prior to deployment and follow-ups were performed at 1 and 6 months, and 1, 2, and 5 years post-deployment. Blood samples were collected in the first three assessments. The levels of *p*OT and *p*AVP were not significantly related to the development of PTSD symptoms over time. The results confirm that age, the experience of early life trauma, combat-related stressors and the presence of depressive symptoms are predictive for the development of PTSD symptoms over time. These findings showed that peripherally measured OT and AVP currently do not qualify as useful susceptibility biomarkers for the development of PTSD symptoms over time in military men after combat.

Introduction

Military personnel are at increased risk of developing mental health problems after deployment to a combat zone (Smith et al., 2008; Reijnen et al., 2015). In US combat veterans, a threefold increase in posttraumatic stress symptoms was found three years after deployment to Iraq or Afghanistan (Smith et al., 2008). In Dutch military personnel, a high level of posttraumatic stress symptoms was reported by 9% of the participants at six months and 13% at 5 years after deployment to Afghanistan compared to 4% prior to deployment (Eekhout et al., 2016b). Moreover, military personnel with mental health problems place an increased burden on consumption of (mental) health care (Eekhout et al., 2016a). Age, early life trauma and combat-related stressors were reported to be risk factors for the development of PTSD symptoms (Eekhout et al., 2016b). In addition to these factors, it is important to find biological susceptibility markers to identify those at risk for the development of combat-related posttraumatic stress disorder (PTSD).

Two potentially useful markers that have been proposed are oxytocin (OT) and arginine vasopressin (AVP; Kang et al., 2015). Both OT and AVP are nonapeptides, which are produced in the hypothalamus and secreted in the bloodstream by the posterior pituitary (Ludwig and Leng, 2006). They are potentially interesting because of their role in social behavior and in the regulation of stress and anxiety (Heinrichs et al., 2003; Ditzen et al., 2009; Neumann and Landgraf, 2012). Moreover, individual differences in the levels of these neuropeptides have been suggested to relate to symptoms of various psychiatric disorders (Meyer-Lindenberg et al., 2011).

Oxytocin is often reported to exert anxiolytic effects and to facilitate social interaction. For instance, intranasal administration of OT was found to decrease salivary cortisol levels during social stress (Linnen et al., 2012), decrease subjective ratings of anxiety in response to stress (Heinrichs et al., 2003), dampen amygdala activity to negative stimuli or fear-inducing visual stimuli (Kirsch et al., 2005; Domes et al., 2007) and increase emotion recognition and in-group trust (Van IJzendoorn and Bakermans-Kranenburg, 2012; MacDonald and Feifel, 2013). Therefore, OT is often studied as a promising treatment target for psychiatric disorders, such as schizophrenia and autism (Striepens et al., 2011; MacDonald and Feifel, 2013), and also for PTSD (Olff et al., 2010). Yet, cross-sectional studies on the peripheral OT levels in PTSD patients compared to healthy controls present inconsistent findings. For instance, in a group of pregnant women with PTSD, plasma OT (*p*OT) levels were not associated with PTSD (Seng et al., 2013). Also, no association was found between *p*OT levels and PTSD status in a group of traumatized individuals (Olff et al., 2013). In contrast, after controlling for childhood trauma, lower salivary OT levels were found in policemen with PTSD compared to trauma-exposed healthy controls, but not in women (Frijling et al., 2015).

Whereas central OT exerts anxiolytic and antidepressant effects, AVP is associated with anxiogenic and depressive actions (Neumann and Landgraf, 2012). For instance, increased cortisol responses to a psychosocial stressor were found after intranasal administration of AVP (Shalev et al., 2011). Furthermore, increased plasma AVP (*p*AVP) levels during the

processing of negative stimuli were found to be positively associated with amygdala activation in men (Motoki et al., 2016). Therefore, they suggested that *p*AVP might be a valid biological marker for the individual differences in anxiogenic effects in males. Higher *p*AVP levels are also reported to be associated with psychiatric disorders, such as major depressive disorder (Londen et al., 1997) and PTSD (de Kloet et al., 2008). In male veterans with PTSD, *p*AVP levels were elevated in comparison to both traumatized veterans and non-traumatized civilians (de Kloet et al., 2008). However, no difference in salivary AVP levels was found between police officers with and without PTSD (Frijling et al., 2015).

Although OT and AVP might be promising biological markers for PTSD, recent literature doubts whether peripherally measured OT and AVP levels reflect the level of central activity (Neumann and Landgraf, 2012; Grinevich et al., 2016). Recent studies found no correlation between the levels of *p*OT and those measured in cerebrospinal fluid (CSF; Jokinen et al., 2012; Kagerbauer et al., 2013) and similar doubts are present about AVP (Kagerbauer et al., 2013). Thus, it is questionable whether peripherally measured OT and AVP could be associated with behavioral changes. Another major concern is the validity of the methods used to measure OT and AVP levels, since values reported in the literature largely differ based on the method used (Szeto et al., 2011; McCullough et al., 2013; Leng and Ludwig, 2016). A third concern is that in exogenous administration studies, but also in studies on endogenous levels, sample sizes are relatively small and possibly underpowered, so reported findings might not represent true effects (Walum et al., 2016). Moreover, in a recent review and preliminary meta-analysis no evidence was found for altered *p*OT and *p*AVP levels in several psychiatric disorders (Rutigliano et al., 2016).

Although, recent literature regarding peripherally measured OT and AVP in relation to PTSD presents inconsistent findings, reliable comparison of studies is difficult because methods differ, sample sizes are relatively small and effects might be gender specific. In addition, to the authors' knowledge limited research is done on the predictive value of *peripheral* OT and AVP levels for the development of PTSD symptoms over a prolonged period. Therefore, we intended to contribute to the current discussion by assessing basal *p*OT and *p*AVP levels in a large cohort of military men before and after deployment and tested whether these hormones can serve as potential biomarkers for the development of posttraumatic stress symptoms over time in addition to other known risk factors.

Materials and Methods

Participants

The participants in the current study are military personnel who were deployed to Afghanistan between 2005 and 2008 for four months as part of the International Security Assistance Force (ISAF) of the NATO (For more information see Reijnen et al., 2015). A total of 1032 individuals volunteered to participate in the study. The data of 907 individuals was included in the present analyses (see Table 1); excluding women (N=93), non-deployed par-

ticipants (N=18), participants who were on antidepressant (N=6) or antihypertensive (N=7) medication on any of the blood draws.

Procedure

Plasma OT and AVP levels were determined as part of a large prospective cohort study on psychological and biological factors that might be associated with the development and course of stress-related mental health symptoms; Prospective Research in Stress-related Military Operations (PRISMO). After receiving a complete verbal and written description of the study, military personnel could volunteer to participate in the study and informed consent was obtained. Assessments were performed; approximately one month pre-deployment (T0), and one (T1) and six (T2) months and one (T3), two (T4) and five (T5) years post-deployment. In the first three assessments (T0, T1, T2) paper-and-pencil questionnaires were filled out and blood samples were collected at the army base. At T3 and T4 only questionnaires were filled out at home and returned by mail and at T5 internet-based questionnaires were filled out at home. Participants received financial compensation for completing all assessments. This study was approved by the Institutional Review Board of the University Medical Center Utrecht.

Measurements

Questionnaires

To measure the level of posttraumatic stress symptoms, the Dutch Self-Rating Inventory for PTSD (SRIP) was used (Hovens et al., 2000; Hovens et al., 2002). Symptom severity in the past month was assessed using a 4-point Likert scale (not at all – very much). This questionnaire contains 22 items, corresponding to the three symptom clusters according to diagnostic criteria for PTSD of the DSM-IV (American Psychiatric Association, 2000); re-experiencing (cluster B; 6 items), avoidance and numbing (cluster C; 9 items), and hyperarousal (cluster D; 7 items). The SRIP was demonstrated to have a good concurrent validity with diagnostic clinical interviews for PTSD (Hovens et al., 2002).

Exposure to combat-related stressors was assessed at one month post-deployment with the Deployment Experiences Scale (DES; Reijnen et al., 2015). This is a 19-item dichotomous checklist used to measure exposure to potentially traumatic deployment-related stressors. For an overview of the items, we refer to Reijnen et al. (2015). Potential traumatic events during childhood (<18 years) were assessed pre-deployment using Early Trauma Inventory Self Report short form (ETISR-SF; Bremner et al., 2007). The questionnaire consists of 27 dichotomous items. For both the ETISR-SF and the DES the composite score indicates the number of different traumatic events, during respectively childhood or military deployment. Depressive symptoms were measured with the SCL-90-R (Arrindell and Ettema, 2003) in the first 5 assessments (T0-T4) and with the Brief Symptoms Inventory (Derogatis and Melisaratos, 1983) at T5. Only the six corresponding items (5-point Likert scale) in both questionnaires were used to calculate the level of depressive symptoms present in the past week.

Plasma samples

The plasma samples were collected at T0, T1 and T2 using a venous blood sample collected between 8 AM and 1130 AM in EDTA vacutainers and immediately put on ice. The samples were centrifuged at 3500 rpm for 12 minutes at 4°C after they were transported to the hospital and stored at -80°C until assayed. Plasma OT and *p*AVP were measured after a C18 Sep-Pak column extraction procedure, with an elution solution of 95% acetonitrile/5% of TFA-H2O. The *p*OT levels in the extracted samples were determined in an ELISA (Oxytocin ELISA kit, ADI-900-153A, Enzo life Sciences). The sensitivity was 1 pg/mL and inter-assay variation ranged from 7.4% at 36 pg/mL to 5.7% at 127 pg/mL (n = 47). The *p*AVP levels in the extracted sample were measured by a competitive radioimmunoassay (Vasopressin-RIA, KIPERB319,DIAsource ImmunoAssays S.A, Belgium). The sensitivity was 20 pmol/L and inter-assay variation ranged from 5.5% at 4.8 pmol/L to 6.1% at 24 pmol/L (n = 43).

Statistical analyses

All data analyses and multiple data imputation were performed in IBM SPSS Statistics version 23 (IBMCorp, 2015). Data were screened and because a large percentage of the *p*AVP samples were below detection limit (a total of 487 over three time-points), an additional dichotomous variable was created (above/below detection level) to be able to also include estimates for the samples below 0.20 pg/ml. Variables were transformed when necessary, square-root transformation (*p*OT) or log transformation (*p*AVP) was used prior to imputation. Next, two outliers (values were > 5 SD from the mean) were removed in the oxytocin measurement on T2. In the vasopressin measurement two outliers on T0, one on T1, and two on T2 were removed (values were > 10 SD from the mean). Importantly, the deleted values were not related to the level of PTSD symptoms over time. After this, group-mean-centering was used to modulate *p*OT and *p*AVP levels over time. This was done to be able to separate the between-subjects and within-subject effects (Curran and Bauer, 2011; Wang and Maxwell, 2015), and both effects were included in the models. Grand mean centering was used to center the other continuous predictor variables to decrease the risk of multicollinearity and to calculate interaction variables prior to imputation (Hox, 2010).

Missing values in the predictor variables, demographic characteristics and covariates were imputed using multiple data imputation, which is an advanced method to handle missing data (Rubin, 1987; Sterne et al., 2009). The missing values were assumed to be missing at random. The imputation model included all the predictor variables and covariates that were used in the analyses. The outcome measure was also included in the imputation model: if not, the predictor variables were imputed as though they were not related to the outcome variable (Moons et al., 2006). However, the outcome was only used as a predictor variable because imputing outcome variables might add noise to the estimates (Kreft and de Leeuw, 1998). A total of 10 imputed datasets were generated.

A series of linear mixed model analyses were used to assess the predictive values of *p*OT and *p*AVP on the development of PTSD symptoms over time. Time was included as a covariate

in the models, because this leads to more accurate within- and between-subjects effects when looking into the relation between two time-varying variables (Wang and Maxwell, 2015). Time was coded as time in years (-0.083, 0.083, 0.5, 1, 2, 5). Random intercepts were used to account for the variance between participants. Also, a random slope for the time variable was included. Missing data in the outcome variable was handled using maximum likelihood estimation. The total level of PTSD symptoms from pre-deployment (T0) up to 5 years post-deployment (T5) was used as the outcome variable. The levels of *p*OT or *p*AVP, endorsement on early life trauma, and the number of combat-related stressors were included in the models as independent variables; additionally, demographic characteristics (age, education level, rank, relationship status) were added to the models. Next, potential interactions between relevant predictor variables (*p*OT and *p*AVP with time, age, early life trauma, and combat-related stressors) and cross-level interactions (*p*OT × *p*AVP) were added. To determine relevant predictor variables for the development of PTSD symptoms over time, we started with a basic model and assessed the change in -2 log-likelihood with each additional variable/interaction (Twisk, 2013). An unstructured covariance structure was applied to the models. After determining the final model, we also corrected for several covariates; smoking, alcohol use and Body Mass Index (BMI).

Results

The data of 907 male participants were used for the analyses. The participant characteristics of the original sample are displayed in Table 1. Repeatedly measured demographic information and questionnaires are presented separately for each time-point. For illustrative purposes, Figure 1 and 2 demonstrate the *p*OT and *p*AVP levels over time in the original sample.

Table 1. Demographic characteristics for the original sample.

		Pre-deployment	1 Month	6 Months	1 Year	2 Years	5 Years
Age[1]	M (SD)	28.38 (8.91)					
	N	900					
Education[1]	Low (%)	32 (3.9)					
(N=819)	Middle (%)	706 (86.2)					
	High (%)	81 (9.9)					
Rank[1]	Private (%)	364 (41.4)					
(N=880)	Corporal (%)	181 (20.6)					
	NCO (%)	302 (34.3)					
	Officer (%)	33 (3.7)					
Relation[1]	Yes (%)	492 (57.7)	215 (60.2)	220 (63.9)	240 (74.8)	355 (79.2)	425 (82)
	No (%)	316 (42.3)	142 (39.8)	124 (36.1)	81 (25.2)	93 (20.8)	93 (18)
Early life trauma[1]	M (SD)	3.53 (3.09)					
	N	824					
Combat-related stressors[1]	M (SD)		4.55 (3.28)				
	N		639				

Table 1. Demographic characteristics for the original sample. *(Continued)*

		Pre-deployment	1 Month	6 Months	1 Year	2 Years	5 Years
No. of previous deployments[1] (N=809)	0	422 (54.6)					
	1	205 (26.5)					
	2	100 (13)					
	>2	82 (5.9)					
Oxytocin (pg/ml)[1]	M (SD)	13.37 (5.79)	12.37 (5.48)	12.23 (5.09)			
	N	866	726	651			
Vasopressin below detection level	Yes (%)	112 (13.1)	110 (15.1)	130 (19.8)			
	No (%)	746 (86.9)	616 (84.9)	526 (80.2)			
Vasopressin (pg/ml)[1]	M (SD)	2.34 (6.39)	1.68 (3.66)	1.03 (1.35)			
	N	746	616	526			
PTSD symptoms[1]	M (SD)	26.57 (5.24)	27.66 (6.20)	27.80 (7.13)	27.11 (7.04)	26.72 (5.75)	28.33 (7.92)
	N	628	695	663	491	467	498
Depressive symptoms[1]	M (SD)	.44 (1.19)	.55 (1.30)	.68 (1.65)	.93 (2.27)	.94 (2.13)	2.58 (3.81)
	N	758	742	668	494	488	505
Smoking[1]	Yes (%)	371 (45.8)	158 (44.3)	144 (41.7)	118 (31.5)	134 (29.4)	136 (26.3)
	No (%)	440 (54.2)	199 (55.7)	201 (58.3)	257 (68.5)	312 (70.6)	382(73.7)
Alcohol use[1]	Yes (%)	441 (54.7)	205 (57.4)	183 (53.5)	155 (45.7)	180 (40.4)	231 (44.6)
	No (%)	366 (45.3)	152 (42.6)	159 (46.5)	184 (54.3)	266 (59.6)	287 (55.4)
BMI[1]	M (SD)	24.81 (2.66)	24.77 (2.67)	25.08 (2.85)	25.57 (3.05)	25.62 (2.91)	25.92 (3.10)
	N	818	349	336	313	386	507

Note: NCO: non-commissioned officer; BMI: Body Mass Index; M(SD): Mean (Standard Deviation); N: Number of participants; PTSD: posttraumatic stress disorder. [1] Count may not add up to the total number of participants due to missing values.

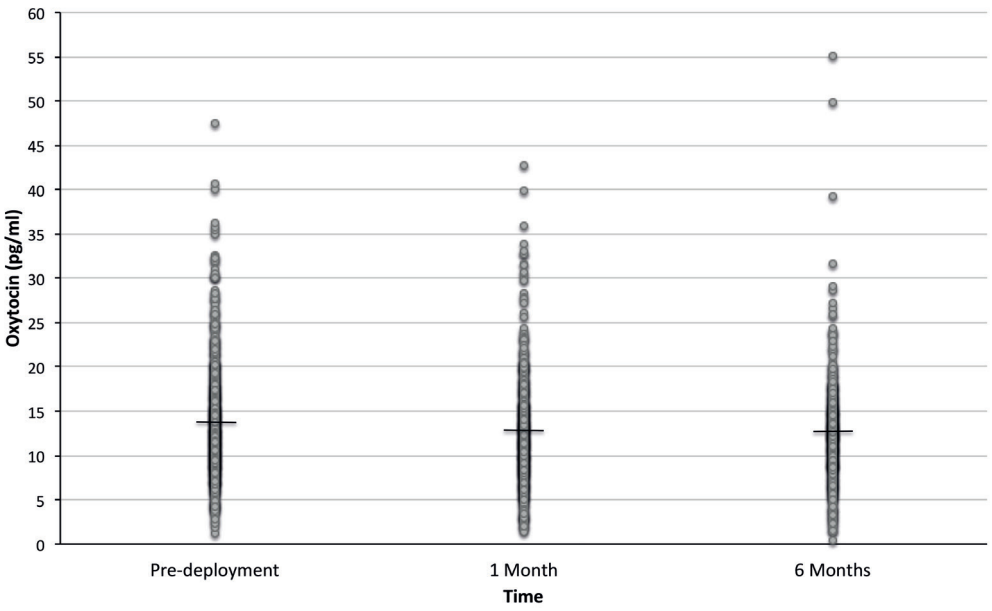

Figure 1. Plasma oxytocin levels in *original sample* over time.

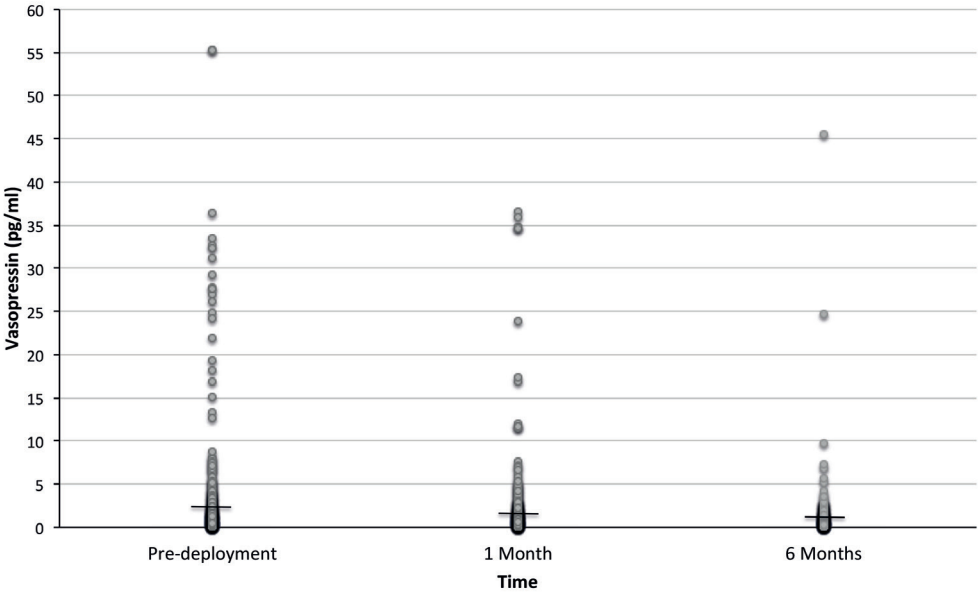

Figure 2. Plasma vasopressin levels in *original sample* over time.

Our pooled results showed that *p*OT levels were not significantly related to the development of PTSD symptoms over time. Both the differences between individuals (*b* = 0.36 (95% CI: -0.21 —0.94), p>.05) and within individuals (*b* = 0.24 (95% CI: -0.12 — 0.60), p>.05) were not predictive of the development of PTSD symptoms. After correction for the covariates, *p*OT levels were also not related to PTSD symptoms over time (*b* = 0.37 (95% CI: -0.21 —0.94), p>.05; *b* = 0.25 (95% CI: -0.11 —0.61), p>.05). The final models are displayed in Table 2. As shown, age was negatively related to the development of PTSD symptoms over time (*b* = -0.06 (95% CI: -0.10 —-0.02), p<.01). Early life trauma, combat-related stressors, and depressive symptoms were positively related predictors for the development of PTSD symptoms over time (*b* = 0.37 (95% CI: 0.25 —0.48), p<.01; *b* = 0.17 (95% CI: 0.07 —0.27), p<.01; *b* = 1.22 (95% CI: 1.03 —1.42), p<.01). The *p*OT levels did not significantly interact with early life trauma (Group-mean *p*OT × ETI: *b* = -0.03 (95% CI: -0.24 —0.17), p>.05; *p*OT × ETI: *b* = 0.07 (95% CI: -0.05 —0.19), p>.05) nor with combat-related stressors (Group-mean *p*OT × DES: *b* = 0.10 (95% CI: -0.07 —0.27), p>.05; *p*OT × DES: *b* = 0.03 (95% CI: -0.07 —0.14), p>.05).

Table 2. Model estimates for the predictive value of oxytocin for the development of PTSD symptoms with and without correction for the covariates.

	Uncorrected model**				Corrected model**			
			95% CI				95% CI	
	Estimate	**p**	Low	Up	**Estimate**	**p**	Low	Up
Intercept	−1.75	.087	−3.76	0.26	−1.60	.124	−3.65	0.44
Oxytocin–group mean	0.36	.213	−0.21	0.94	0.37	.212	−0.11	0.94
Oxytocin	0.24	.187	−0.12	0.60	0.25	.174	−0.21	0.61
Age	−0.06	.001*	−0.09	−0.02	−0.06	.001*	−0.10	−0.02
Time in years	−0.50	.243	−1.22	0.34	−0.51	.235	−1.35	0.33
Early life trauma	0.37	.000*	0.25	0.48	0.37	.000*	0.25	0.48
Combat-related stressors	0.17	.001*	0.07	0.26	0.17	.001*	0.07	0.27
Depression	1.23	.000*	1.03	1.42	1.22	.000*	1.03	1.42
Smoking					−0.07	.803	−0.60	0.47
Alcohol use					−0.24	.380	−0.79	0.30
Body Mass Index					−0.001	.993	−0.15	0.14

* p<.01 ** Uncorrected: −2LL = 12005.415, df = 12; Corrected: −2LL = 12004.270, df=15.

In our analyses *p*AVP levels were also not significantly related to the development of PTSD symptoms over time. Both the differences between individuals (b = −0.19 (95% CI: −1.12 — 0.74), p>.05) and within individuals were not predictive of the development of PTSD symptoms (b = −0.04 (95% CI: −0.74 —0.65), p>.05). The findings did not alter after correction for the covariates; (b = −0.19 (95% CI: −1.13 —0.75), p>.05; b = −0.03 (95% CI: —0.73 – 0.66), p>.05). There was no significant difference in PTSD symptoms over time between those with *p*AVP above and below detection limit, so this variable was not included in the final model. Again, age, early life trauma, combat-related stressors, and depressive symptoms were significant predictors for the development of PTSD symptoms over time (b = −0.06 (95% CI: −0.10 —−0.02), p<.01; b = 0.35 (95% CI: 0.24 —0.46), p<.01; b = 0.18 (95% CI: 0.08 —0.28), p<.01; b = 1.29 (95% CI: 1.07 —1.52), p<.01). The final models are displayed in Table 3. The *p*AVP also did not interact with early life trauma (Group-mean *p*AVP × ETI: b = −0.03 (95% CI: −0.41 —0.34), p>.05; *p*AVP × ETI: b = −0.17 (95% CI: −0.44 —0.09), p>.05) nor with combat-related stressors (Group-mean *p*AVP × DES: b = 0.17 (95% CI: −0.18 —0.52), p>.05; *p*AVP × DES: b = −0.06 (95% CI: −0.31 —0.19), p>.05).

When both *p*OT and *p*AVP levels were included in the model, the cross-level interactions were not significantly related to the development of PTSD symptoms. For an overview of this model we refer to Table S1 of the supplementary material. As displayed in Table S2 and S3, findings were similar to the previous models when the symptom clusters of PTSD, namely re-experiencing, avoidance and numbing, hyperarousal, were used as the outcome variable.

Table 3. Model estimates for the predictive value of vasopressin for the development of PTSD symptoms with and without correction for the covariates.

	Uncorrected model**				Corrected model**			
			95% CI				95% CI	
	Estimate	**p**	Low	Up	**Estimate**	**p**	Low	Up
Intercept	−0.54	.005*	−0.92	−0.16	−0.33	.196	−0.84	0.17
Vasopressin−group mean	−0.19	.686	−1.13	0.74	−0.19	.694	−1.12	0.75
Vasopressin	−0.04	.908	−0.74	0.65	−0.03	.924	−0.73	0.66
Age	−0.06	.001*	−0.09	−0.02	−0.06	.002*	−0.10	−0.02
Time in years	−0.57	.187	−1.42	0.28	−0.58	.183	−1.43	0.27
Early life trauma	0.35	.000*	0.24	0.46	0.35	.000*	0.24	0.46
Combat-related stressors	0.18	.000*	0.08	0.28	0.18	.000*	0.08	0.28
Depression	1.29	.000*	1.07	1.51	1.29	.000*	1.07	1.52
Smoking					−0.16	.541	−0.68	0.36
Alcohol use					−0.24	.366	−0.78	0.29
Body Mass Index					−0.01	.917	−0.15	0.13

* p<.01 ** Uncorrected: −2LL = 11983.302 df=12; Corrected: −2LL = 11979.817 df=15.

Discussion

The aim of the current study was to assess whether *p*OT and *p*AVP levels are susceptibility markers for the development of PTSD symptoms in addition to other known risk factors. The findings in this study show that age, early life trauma, combat-related stressors and the presence of depressive symptoms are significant predictors of the development of PTSD symptoms. Neither *p*OT nor *p*AVP levels were related to the level of posttraumatic stress symptoms over time. Also, no interaction was found with early life trauma and combat-related stressors.

Age, potentially traumatic experiences during deployment, and early life trauma were confirmed to be important risk factors for the development of PTSD symptoms over time. As mentioned, our findings are in agreement with previous studies reporting that younger age at trauma was predictive of developing (symptoms of) PTSD in military personnel (Brewin et al., 2000; Iversen et al., 2008). Also, rather than deployment in general, the exposure to combat experiences was associated with the onset of PTSD (Smith et al., 2008; Rona et al., 2009; Pietrzak et al., 2011). Furthermore, childhood trauma is known to increase the risk of developing mental health symptoms, such as PTSD (Cabrera et al., 2007). So, this study once more underlines the importance of these factors as risk factors for the development of PTSD symptoms over time.

Earlier studies investigating peripherally measured OT and AVP as biomarker for PTSD most often assessed OT and AVP as disease-related markers by comparing PTSD patients to healthy (traumatized) controls. In line with our findings, there are studies, mainly on female subjects, showing no difference in plasma OT levels between PTSD patients and healthy controls (Olff et al., 2013; Seng et al., 2013). However, in policemen with PTSD, lower salivary OT levels were reported compared to trauma-exposed controls (Frijling et al., 2015). Regarding the association with peripheral AVP levels, this study found no differences in salivary AVP levels of policemen with PTSD compared to trauma-exposed males. A previous study from

our group showed higher *p*AVP levels in PTSD patients compared to both combat-exposed veterans and healthy controls (de Kloet et al., 2008). In line with this study, the current study was designed to investigate *p*OT and *p*AVP as candidate susceptibility markers for the development of PTSD symptoms up to 5 years post-deployment. To our knowledge, only one recent study assessed the predictive value of serum OT levels and found no relation to PTSD, depression and anxiety symptoms at one month after a motor vehicle accident (Nishi et al., 2015). This suggests that plasma and serum OT and AVP levels might not be useful susceptibility biomarkers for the development of PTSD after trauma.

As mentioned previously, there is disagreement in the extent to which *p*OT and *p*AVP levels reflect the levels of OT and AVP in the brain (Neumann and Landgraf, 2012; Leng and Ludwig, 2016). There is evidence that *p*OT levels positively predict CSF OT levels and that both are negatively associated with trait anxiety in children (Carson et al., 2015). In contrast, others reported no cross-correlation between levels in plasma and CSF (Jokinen et al., 2012; Kagerbauer et al., 2013). They argue that the release patterns of central and peripheral OT and AVP might be independent under basal conditions. Moreover, the magnocellular neurons in the paraventricular and supraoptic nuclei of the hypothalamus secrete OT and AVP in the periphery through the posterior pituitary, whereas dendritic release of OT and AVP from these neurons in the extracellular space and parvocellular neurons of the hypothalamus contribute to release in the brain (Meyer-Lindenberg et al., 2011; Striepens et al., 2011; Beurel and Nemeroff, 2014). Also, because of the size of the molecules, they are not able to penetrate the Blood-Brain-Barrier (BBB) allowing independent regulation (Neumann and Landgraf, 2012; Kagerbauer et al., 2013). At the same time, there are suggestions for simultaneous or coordinated release of peripheral and central neuropeptides, for instance during birth, appetite-related and reproductive stimuli, and various forms of stress (Neumann and Landgraf, 2012; Leng and Ludwig, 2016).

Central and peripheral OT and AVP might also have functionally different effects (Neumann and Landgraf, 2012; Beurel and Nemeroff, 2014). In the body, OT and AVP are thought to mainly relate to physiological functions such as labor, lactation and antidiuresis, osmolality regulation and vasoconstriction respectively. Moreover, since AVP secretion is mainly driven by plasma osmolality there are doubts whether this can be overridden by socio-emotional stimuli (Kagerbauer et al., 2013). Thus, *central* neuropeptide levels might be more relevant in relation to socio-emotional behavior (Heinrichs et al., 2008). Multiple studies have shown that intranasal administration of OT has the ability to influence social behavior and amygdala activation in response to emotional stimuli (For review: Striepens et al., 2011). However, these effects might be influenced by both contextual and interindividual factors (Bartz et al., 2011; Olff et al., 2013). Also, to some, it is questionable how much of the intranasally administered OT reaches the brain and what the influence is of the increase in the periphery (Leng and Ludwig, 2016). A preliminary study by Motoki et al. (2016) showed that plasma AVP levels were positively associated with amygdala activation in men during the processing of negative emotions. The current findings once more underline the necessity

of studying the association between peripheral and central levels of OT and AVP serving the question whether the peripheral measurement is valuable in relation to psychiatric disorders.

In addition to the debate over peripheral versus central OT and AVP, it is not clear whether the levels in plasma, saliva and also urine are correlated. Some studies show that OT levels in plasma and saliva are closely related (Hoffman et al., 2012), however, others do not support that bioavailable levels of OT can be measured in saliva (Horvat-Gordon et al., 2005). Moreover, currently several authors suggest that the reliability of OT and AVP measurements in plasma but also in other bodily fluids is questionable, since limited research has shown whether measurements methods of bioactive OT and AVP are valid (Szeto et al., 2011; Kagerbauer et al., 2013; McCullough et al., 2013). Moreover, although extracted samples are used, non-OT molecules might be measured in addition to the OT levels (Szeto et al., 2011; McCullough et al., 2013). Thus, further research is necessary to assess more valid and reliable methods to determine peripheral levels of OT and AVP.

An important strength of this study is that *p*OT and *p*AVP levels were examined in a large homogeneous cohort of military men, using similarly extracted samples and robust statistics. Yet, we are aware that there are some limitations to the study. First, OT and AVP levels were determined based on one venous blood sample at each time-point and this might have resulted in higher variability of the samples over time, especially since oxytocin is released in a pulsatile fashion (Ludwig and Leng, 2006). Also, there was no information about the temperature during sample collection, food and water intake and the precise timing of this collection. Since variations might have occurred, caution needs to be emphasized with the interpretation of the findings. Second, in this study the development of PTSD symptoms was assessed on a continuous scale over a period of 5 years. Yet, PTSD is a very heterogeneous disorder and some features might not be specific to PTSD. It might be that biomarkers are more symptom-specific or specific for clusters of symptoms. However, as shown no association was found with the symptom clusters of PTSD. Also, although the homogenous cohort of deployed military men is an important strength in the current study, this might limit generalizability to other populations and other types of trauma. Further research should, for instance, assess plasma oxytocin levels in relation to the development of PTSD after traumatic experiences such as intimate partner violence or sexual abuse. Lastly, military cohorts have a strong social bond, which might have influenced the levels of *p*OT and *p*AVP and could have been a protective factor at least shortly after deployment.

In conclusion, to be able to identify military personnel at risk for developing stress-related disorders and to be able to provide and develop preventive treatment options, identification of reliable susceptibility biomarkers is crucial. This study shows that *p*OT and *p*AVP levels currently have limited usefulness as susceptibility biomarkers for the development of PTSD symptoms in a military cohort.

Acknowledgements and disclosure

The authors thank the Dutch commanders and troops and all members of the PRISMO team involved in data acquisition for their time and effort. Also, we thank Prof. dr. Joëls and Rosalie Gorter for their valuable comments and suggestions. PRISMO data collection and analysis was supported by the Dutch Ministry of Defence.

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Supplementary material

Table S1. Model estimates for the interaction between vasopressin and oxytocin in the development of PTSD symptoms with and without correction for the covariates.

	Uncorrected model**				Corrected model**			
			95% CI				95% CI	
	Estimate	**p**	Low	Up	**Estimate**	**p**	Low	Up
Intercept	−1.86	.064	−3.83	0.11	−1.69	.104	−3.73	0.35
Vasopressin–group mean	−0.17	.959	−6.66	6.32	−0.34	.919	−6.82	6.15
Vasopressin	−3.30	.298	−9.61	3.00	−3.28	.299	−9.55	2.99
Age	−0.06	.002*	−0.09	−0.02	−0.06	.004*	−0.10	−0.02
Time in years	−0.53	.226	−1.39	0.33	−0.54	.212	−1.40	0.31
Early life trauma	0.36	.000*	0.25	0.47	0.36	.000*	0.25	0.46
Deployment stressors	0.22	.000*	0.11	0.33	0.22	.000*	0.11	0.33
Depression	1.21	.000*	0.97	1.45	1.21	.000*	0.97	1.46
Oxytocin–group mean	0.38	.176	−0.17	0.94	0.38	.183	−0.18	0.95
Oxytocin	0.27	.144	−0.09	0.64	0.27	.137	−0.09	0.64
Vasopressin–group mean×Oxytocin–group mean	−0.01	.991	−1.80	1.79	0.04	.966	−1.76	1.83
Vasopressin×Oxytocin	−0.60	.567	−2.70	1.50	−0.60	.561	−2.67	1.47
Oxytocin–group mean×Vasopressin	0.89	.341	−0.97	2.74	0.88	.342	−0.96	2.73
Vasopressin–group mean×Oxytocin	0.86	.169	−0.36	2.07	0.87	.160	−0.35	2.09
Smoking					−0.06	.831	−0.61	0.49
Alcohol					−0.28	.303	−0.81	0.25
Body Mass Index					−0.01	.947	−0.20	0.18

* p<.01 ** Uncorrected: −2LL = 11996.208 df=18; Corrected: −2LL = 11990.313 df=21

Table S2. Model estimates for predictive value of oxytocin for the subscales of PTSD over time.

	SRIP–Re–experiencing		SRIP–Avoidance and numbing		SRIP–hyperarousal	
	Estimate (95% CI)	p	Estimate (95% CI)	p	Estimate (95% CI)	p
Intercept	6.28 (5.80–6.76)	.000*	10.21 (9.31–11.10)	.000*	8.95 (7.99–9.90)	.000*
Oxytocin–group mean	0.06 (−0.04–0.16)	.254	0.02 (−0.16–0.19)	.850	0.06 (−0.11–0.23)	.495
Oxytocin	0.07 (−0.07–0.20)	.322	0.20 (−0.06–0.45)	.125	0.06 (−0.21–0.33)	.677
Age	−0.02 (−0.03–−0.01)	.000*	−0.02 (−0.04–−0.01)	.005*	−0.02 (−0.04–−0.003)	.020**
Time in years	0.32(0.10–0.56)	.006*	0.24(−0.15–0.63)	.233	0.61(0.24–0.98)	.001*
Early life trauma	0.07 (0.05–0.10)	.000*	0.15 (0.10–0.20)	.000*	0.15 (0.09–0.20)	.000*
Deployment stressors	0.03 (0.003–0.05)	.028**	0.04 (0.01–0.09)	.035**	0.10 (0.05–0.15)	.001*
Depression	0.21 (0.17–0.26)	.000*	0.60 (0.51–0.69)	.000**	0.49 (0.40–0.57)	.000*

* p<.01 **p<.05

Table S3. Model estimates for predictive value of vasopressin for the subscales of PTSD over time.

	SRIP–Re–experiencing		SRIP–Avoidance and numbing		SRIP–hyperarousal	
	Estimate (95% CI)	p	Estimate (95% CI)	p	Estimate (95% CI)	p
Intercept	6.51 (6.42–6.60)	.000*	10.88 (10.71–11.1)	.000*	9.12 (8.94–9.30)	.000*
Vasopressin–group mean	0.09 (−0.14–0.32)	.438	−0.09 (−0.51–0.33)	.675	−0.17 (−0.61–0.28)	.465
Vasopressin	0.03 (−0.18–0.24)	.784	−0.09 (−0.44–0.27)	.622	−0.03 (−0.39–0.34)	.891
Age	−0.02 (−0.03–−0.01)	.000*	−0.02 (−0.04–−0.01)	.005*	−0.02 (−0.04–−0.003)	.018**
Time in years	0.32(0.09–0.56)	.007*	0.21(−0.19–0.60)	.308	0.58(0.19–0.96)	.003*
Early life trauma	0.07 (0.04–0.09)	.000*	0.14 (0.10–0.19)	.000*	0.14 (0.09–0.19)	.000*
Deployment stressors	0.03 (0.01–0.06)	.018**	0.05 (0.01–0.09)	.021**	0.10 (0.05–0.15)	.000*
Depression	0.22 (0.17–0.27)	.000*	0.63 (0.53–0.73)	.000*	0.51 (0.42–0.60)	.000*

* p<.01 **p<.05

CHAPTER 08

Summary and General Discussion

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Summary

This thesis has focused on results from a large prospective cohort study in Dutch military personnel (N=1007). The first data was collected in 2006 and the study was designed as a 10-year follow-up study. The goal was to investigate psychological and biological aspects of stress-related (mental) health problems to be able to better predict who is at risk for the development of symptoms after deployment to a combat zone.

The first aim of this thesis was to gain more insight in the impact of deployment of Dutch military personnel to a combat zone on the development of mental health problems, more specifically Posttraumatic Stress Disorder (PTSD), and personality traits. **Chapter 2** showed that despite the exposure to high intensity combat, the larger part of military personnel did not report mental health problems in the first two years after deployment to Afghanistan. However, compared to pre-deployment rates, the prevalence estimates of mental health problems (symptoms of fatigue, PTSD, hostility, depression, and anxiety) were found to increase after deployment. The course of this development appeared to be specific for various mental health problems. Whereas the prevalence of symptoms of PTSD increased shortly after deployment, the prevalence of other domains such as hostility and sleeping problems remained relatively stable over time. The prevalence of symptoms of depression and fatigue increased over a two-year period after deployment. In **Chapter 3**, in addition to the increase in PTSD symptom in the first 6 months, a delayed increase was found at 5 years post-deployment. Moreover, three different developmental trajectories for PTSD symptoms were identified. The largest part of the sample showed a trajectory typical of resilience to the development of PTSD symptoms (85%) over the five years post-deployment. A second trajectory (5%) showed an increase in PTSD symptoms in the first year after which it declined (recovered trajectory) and the third trajectory (9%) showed an increase in PTSD symptoms five years post-deployment (delayed onset trajectory). In **Chapter 4** the association between deployment and the character traits cooperativeness and self-directedness was assessed. The results show that whereas the level of cooperativeness was stable over time, self-directedness was found to decrease over 5 years post-deployment. This decline in self-directedness was found to be stronger in the recovered and delayed onset PTSD symptom trajectories. Overall, the results of ***Part 1*** indicate that a large percentage of Dutch military personnel does not develop mental health problems or show changes in character after deployment to Afghanistan. However, there is a group of individuals that report significant difficulties with adaptation to life in the Netherlands. Whereas in some these symptoms emerged immediately and decreased after a year, in others the symptoms of PTSD were only first reported as late as 5 years after deployment.

The second aim of this thesis was to look if potential stress-related biological risk or protective factors (so called biomarkers) correlated to the development of symptoms of PTSD over time in military men. To do so, testosterone, neuropeptide-Y (NPY), oxytocin (OT), and arginine vasopressin (AVP) levels were measured in plasma before and at 1 and 6 months

post-deployment. In **Chapter 5**, increased plasma testosterone levels were found after deployment to Afghanistan. However, this change in testosterone levels did not differ between participants with high versus low level of PTSD symptoms. Interestingly, low plasma testosterone levels pre-deployment were found to be a risk factor for PTSD symptoms at 1 and 2 years post-deployment. The predictive value of plasma NPY for the development of PTSD symptoms was assessed in **Chapter 6**. Neither the pre-deployment nor the pre- to post deployment difference in plasma NPY was related to the development of PTSD symptoms over time. These findings were replicated in a similar independent military cohort of US marines (**Chapter 6**). In addition, as shown in **Chapter 7**, plasma OT and AVP levels were not related to the development of PTSD symptoms. The findings in ***Part 2*** demonstrated that whereas low plasma testosterone levels prior to deployment might represent a risk factor, the peripherally measured neuropeptides NPY, OT and AVP currently do not qualify as useful susceptibility biomarkers for the development of PTSD symptoms in military men after combat.

General discussion

Prevalence of mental health symptoms

Military personnel that is deployed to a combat zone is often exposed to stressful and potentially traumatic experiences. This increases the risk of developing mental health problems after homecoming as has been shown in several international cohorts (Boulos and Zamorski, 2013; Banwell et al., 2016; Crum-Cianflone et al., 2016; Madsen et al., 2016). Since this is a major concern to individual veterans, military personnel as well as society in general, the aim was to examine the prevalence of mental health symptoms in Dutch military personnel after their deployment to Afghanistan.

Importantly, the larger part of military personnel included in the PRISMO study did not report any development of mental health problems after deployment (**Chapter 2**). Moreover, in agreement with previous studies (e.g. Dickstein et al., 2010; Berntsen et al., 2012; Bonanno et al., 2012), the largest percentage of participants (85%) reported a stable trajectory of low posttraumatic stress pre- to 5 years post-deployment (**Chapter 3**). This low level of symptoms might be expected since the sample included predominantly relatively young, well-trained military men, who are likely more resilient individuals. In line with this, feeling less prepared for deployment and the perception that work was above an individual's training and experience is strongly associated with PTSD (Iversen et al., 2008; Polusny et al., 2011). Military personnel that reported to be less prepared for deployment, reported high perceived threat even in low levels of combat (Renshaw, 2011). So, it is likely that appropriate preparation and training prior to deployment is partially responsible for the high levels of resilience in Dutch military personnel.

However, the prevalence of symptoms of fatigue, hostility, depression and anxiety was found to increase after deployment compared to pre-deployment rates (**Chapter 2**). Moreover, between 5.6 and 12.9% of deployed personnel reports a high level of PTSD symptoms in the 5 years post-deployment compared to 4.3% prior to deployment. Although the average rates might be comparable to the lifetime prevalence of 7.4% found in the Dutch population (De Vries and Olff, 2009), they are considerably higher than the 12-months' PTSD prevalence of 2.6% found in the Netherlands (Darves-Bornoz et al., 2008) and the cross-European lifetime PTSD prevalence of 1.9% (Alonso et al., 2004). As discussed in **chapter 1**, in military populations the prevalence estimates are difficult to compare because of the high heterogeneity in studies and deployment characteristics (Ramchand et al., 2010; Richardson et al., 2010). One other study in Dutch military personnel reported a PTSD prevalence of 21, 4 and 6% in three cohorts 5 months after deployment to Iraq based on questionnaires and rates <5% based on clinical interviews (Engelhard et al., 2007). So, the question remains whether Dutch military personnel have an increased risk of developing mental health problems after deployment.

Although a significant increase in symptoms was reported in our cohort, it is still difficult to determine if deployment increases the risk of mental health problems, since there

was no non-deployed and/or civilian control group included. Studies that compared military personnel to civilians found higher overall rates of mental health disorders (Kessler et al., 2014a; Goodwin et al., 2015). In contrast, a recent German study reported comparable 12-months' prevalence rates for various mental health disorders in civilians and military personnel, irrespective of deployment (Trautmann et al., 2017). However, they found elevated rates of PTSD and panic/agoraphobia in deployed personnel with high levels of combat experience. This might suggest that whereas deployment in itself might not be associated with elevated levels of psychiatric disorders, combat-related stressors during deployment might increase the risk of mental health problems. In UK armed forces personnel, deployment was not related to higher rates of PTSD than military service in general, however, holding a combat role was associated with PTSD in those deployed to Iraq or Afghanistan (Jones et al., 2013). Moreover, combat exposure or having a combat role has consistently been found as one of the strongest predictors of PTSD after deployment (Hoge et al., 2004; Hoge et al., 2006; Rona et al., 2006; Smith et al., 2008; Fear et al., 2010; Jones et al., 2013). Also, in the current thesis, it was confirmed that the experience of more combat-related stressors increased reporting of posttraumatic stress reactions (**Chapter 3**). So, the level of combat exposure, rather than deployment, might be related to the increased prevalence of mental health symptoms in Dutch military personnel after deployment.

Course of mental health symptoms

Another major finding in this thesis is that the progression of symptoms over time can differ, thereby showing that symptoms can develop over a prolonged period after return from deployment. Whereas the symptoms of fatigue and depression were found to increase over the two-year period after deployment, the prevalence of PTSD symptoms increased in the first 6 months, but declined at the 1 and 2-year assessment (**Chapter 2**). In contrast, the prevalence of more general distress symptoms, such as anger and hostility, showed a relatively stable course. Moreover, in PRISMO, largely stable developmental trajectories were found for hostility symptoms over time (Heesink et al., 2015). This might suggest that, in contrast to PTSD, these symptoms are not recognized as severe mental health problems in need of treatment (in an early stage). In sum, these findings suggest that symptom progression over time is specific for various types of mental health problems, however, further research is necessary.

Moreover, within a specific disorder, such as PTSD, the presentation and course of symptoms can vary between individuals. The classification method of the Diagnostic and Statistical Manual of Mental Disorders (DSM; American Psychiatric Association, 2013) is used to identify who has a disorder based on a specific set of symptoms. However, psychiatric disorders are very heterogeneous. Indeed, many clinicians will recognize that individuals with the diagnosis of PTSD can have very distinct symptom presentations. Galatzer-Levy and Bryant (2013) showed that the DSM-IV criteria for PTSD have a very high level of heterogeneity (79,794 combinations), which is even higher for the DSM-5 (636,120 combinations),

and they suggest that other methods are needed to address the heterogeneity and identify meaningful clinical outcomes. Therefore, latent growth mixture modeling (LGMM) was used in **chapter 3** to identify distinct subpopulations by latent symptom course over multiple occasions following deployment. In line with our findings in **chapter 2**, a large relatively resilient trajectory and a recovered trajectory, showing initially a high level of posttraumatic stress symptoms that declined over time, were identified. In addition, in line with Bonanno et al. (2012), a delayed onset trajectory was found with a moderate level of symptoms that increased significantly at 5 years post-deployment. So, in addition to the variations in the prevalence of different types of symptoms over time, there is heterogeneity in the course of posttraumatic stress symptoms from pre- to post-deployment.

Since a large group of participants showed a resilient trajectory, it is interesting to assess which factors are related to the heterogeneity in PTSD symptoms over the different time points. Our findings were in line with Dickstein et al. (2010) and Bonanno et al. (2012), who found that the development of a high level of PTSD symptoms was associated with potentially traumatic experiences during childhood and exposure to combat-related stressors (**Chapter 3**). In addition to this, soldiers younger than 21 showed a greater increase in post-traumatic stress symptoms at 1 and 5 years post-deployment. As expected based on van Zuiden et al. (2011), lower baseline and decreasing levels of self-directedness over time were associated with posttraumatic stress reactions, compared to the resilient trajectory (**Chapter 4**). Furthermore, comparable levels of cooperativeness were found for the resilient and the delayed-onset trajectory, whereas lower levels were found for the recovered trajectory. Since cooperativeness was related to perceived social support (Cloninger et al., 2010), it might be postulated that social support is an important factor in the development of PTSD symptoms in military personnel. Indeed, in Israeli soldiers adequate social resources were associated with longer delays in PTSD onset, thereby suggesting that social support helps military personnel to endure the effects of war trauma for a prolonged period after deployment (Horesh et al., 2013). In contrast, (Karstoft et al., 2013) reported that higher social support was associated with a decreased chance of belonging to a recovered or chronic group, but not a delayed PTSD group. So, whereas some factors might be associated with PTSD development in general, others might be related to specific developmental trajectories of PTSD.

The results in **Chapter 3** showed that military personnel could develop PTSD symptoms years after returning from deployment. Interestingly, the veterans in the delayed onset trajectory already reported a moderate level of symptoms early after deployment. A review of 39 prospective studies reported that the development of delayed-onset PTSD was often preceded by the presence of sub-threshold PTSD symptoms (Utzon-Frank et al., 2014). Sub-threshold or partial PTSD may develop into delayed onset PTSD (Smid et al., 2009) and is associated with functional impairment, elevated depression and suicidal ideation (Marshall et al., 2001; Cukor et al., 2010), and anger and aggression (Jakupcak et al., 2007). Although there are large differences in methodology between the studies, in US military personnel the rates of sub-threshold PTSD are suggested to be comparable to full PTSD (Bergman et al.,

2017). In addition, the proportion of delayed onset PTSD was reported to be twice as high in professional groups, such as military personnel and firefighters, compared to civilian groups (Utzon-Frank et al., 2014). Since treating sub-threshold PTSD might result in a larger and more rapid reduction of symptoms than full PTSD (Korte et al., 2016), treatment provided earlier in the developmental trajectory might decrease the risk of delayed onset PTSD.

In addition, as previously shown in the PRISMO cohort (Smid et al., 2013), the progression of posttraumatic stress symptoms following deployment might be related to increased responsiveness or sensitization to the effects of post-deployment stressors, such as divorce, accidents, and loss of a job, during the first year following return from Afghanistan. Also, participants often report that symptoms worsened after new stressful events, such as ending a relationship and leaving the military. Some evidence was found that veterans that leave the military are more likely to develop late-onset PTSD (Goodwin et al., 2012). However, a recent study found similar trajectories in active duty and separated military personnel despite differences between military and civilian life (Porter et al., 2017). In the current 10-year assessment of the PRISMO study, the efforts continue to explore differences in symptom development as well as collecting information on other potentially important factors, such as military employment status, post-deployment stressors, comorbid disorders, and treatment.

Biomarkers for the prevention of PTSD

Currently, clinical interviews and self-report questionnaires are used to determine if an individual meets the diagnostic criteria for PTSD. Biomarkers might provide more objective measure of PTSD, which might improve diagnosis and can also be used to identify individuals at risk or find markers of resilience (Lehrner and Yehuda, 2014). The prospective longitudinal design of the PRISMO study provides a unique opportunity to assess potential risk biomarkers before and after deployment. Therefore, the second aim of the current thesis was to assess pre-deployment and pre- to post-deployment alterations in stress-related biomarkers as potential susceptibility markers for the development of PTSD in military men.

Although extensive research focused on the hypothalamic-pituitary-adrenal (HPA) axis biomarkers (Olff and van Zuiden, 2017), in this thesis other potentially relevant neuroendocrine factors, as discussed in **chapter 1**, were examined in relation to PTSD symptoms. Moreover, to the authors' knowledge, limited research looked into the predictive role of these biomarkers. In **chapter 5**, lower pre-deployment testosterone levels were shown to be predictive of PTSD symptoms after deployment. In line with this, a recent study reported that first-time deployed US soldiers with blunted cortisol and blunted testosterone reactivity in response to a CO_2 challenge prior to deployment might have an increased risk for PTSD symptoms at higher levels of warzone stressor exposure (Josephs et al., 2017). So, reduced pre-trauma testosterone might play a role in PTSD susceptibility in military men.

In **chapter 6** and **7** peripherally measured neuropeptide Y (NPY), oxytocin (OT) and arginine vasopressin (AVP) were assessed as risk factors for the individual change in the reported

PTSD symptoms over time. The results show that neither plasma NPY (*p*NPY) (**Chapter 6**), nor plasma OT (*p*OT) and plasma AVP (*p*AVP) (**Chapter 7**) were related to the development of PTSD symptoms over time. Moreover, in **chapter 6** our findings were replicated in an independent cohort of US marines also showing no relation between plasma NPY levels before and after deployment and the reported PTSD symptoms over time. In line with this, in survivors of traffic accidents no association was found between serum NPY and OT on the one hand and PTSD, depression and anxiety symptoms one-month post-accident on the other hand (Nishi et al., 2014; Nishi et al., 2015). Based on these findings, it was concluded that peripherally measured neuropeptides currently have limited usefulness as susceptibility biomarkers for PTSD. However, as discussed in **chapter 6** and **7**, caution is emphasized with the interpretation of these findings because of the methodological difficulties in assessing plasma neuropeptides (Szeto et al., 2011; Baker et al., 2013; Kagerbauer et al., 2013; McCullough et al., 2013). In addition, peripheral effects of the neuropeptides might differ from central functions. For instance, central NPY appears to inhibit the sympathetic nervous system, whereas peripheral NPY shows the opposite effect (Schmeltzer et al., 2016). This discrepancy may be less pertinent in the case of testosterone, given the lipophilic character of this compound. Fluctuations in peripheral levels of other steroids, such as corticosterone, are known to be reflected in the brain too, at least in rodents (Droste et al., 2008).

Although many potential biomarkers have been identified (For review: Baker et al., 2012; DiGangi et al., 2013; Schmidt et al., 2013; Zoladz and Diamond, 2013; Michopoulos et al., 2015; Olff and van Zuiden, 2017), currently they do not qualify as reliable and specific biomarkers for PTSD. In the literature findings are contradictory and inconsistent (Zoladz and Diamond, 2013), which might be explained by the differences in symptom presentation, symptom development over time, and the comorbidity of PTSD with other psychiatric and medical conditions. A biomarker that is common to all different symptoms of PTSD is highly unlikely and it has been suggested that it is more promising to search for biomarkers for specific symptom clusters (Schmidt et al., 2013; Zoladz and Diamond, 2013). For instance, plasma AVP levels are correlated to avoidance symptoms in male veterans with PTSD (De Kloet et al., 2008). However, in **chapter 7**, the levels of *p*OT and *p*AVP were not related to the different symptom clusters of PTSD, supporting that the currently known potential biomarkers are still insufficiently robust.

The identification of specific biomarkers is further complicated by the interaction between different biological systems. For instance, the dual hormone hypothesis (Popma et al., 2007; Mehta and Josephs, 2010) assumes a cross talk between the HPA-axis and the hypothalamic-pituitary-gonadal (HPG) axis. Although in the current thesis no interaction was found between pre-deployment plasma cortisol and testosterone in relation to PTSD, Josephs et al. (2017) reported that pre-deployment testosterone reactivity might be protective for PTSD only in participants with blunted HPA-axis reactivity. In addition, many other biological factors, such as genetic, immunological, and neurobiological abnormalities, are related to PTSD (For review: Yehuda et al., 2015). This suggests a complex interplay of

biological systems underlying PTSD. So, to unravel the individual differences in PTSD development, it might be more valuable to look for different biomarker 'signatures' rather than searching for a specific biomarker for PTSD (Zoladz and Diamond, 2013).

Moreover, it seems unlikely that a single biomarker can be identified to measure the different stages of a disorder (Yehuda et al., 2013). Some biomarkers might be relevant at one time point and not at another (Schmidt et al., 2013). For instance, plasma testosterone might be a viable susceptibility biomarker for PTSD rather than a diagnostic biomarker. Thus, whereas low plasma testosterone was predictive of the development of PTSD (**Chapter 5**), studies that assessed plasma testosterone by comparing combat-related PTSD patients to healthy controls reported no difference (Mulchahey et al., 2001; Spivak et al., 2003). Also, although it is often assumed that markers of susceptibility and resilience represent opposites of the same dimension, some biomarkers might independently reflect resilience (Yehuda and Flory, 2007). For instance, although plasma NPY was not found to predict PTSD symptom development (**Chapter 6**), higher levels of plasma NPY were found in military personnel after survival training, which was associated with less distress and psychological resilience (Morgan et al., 2000; Morgan et al., 2002). Furthermore, the timing and the number of assessments of the biomarker might be important. In PRISMO, the neuroendocrine markers were determined with one sample collected pre- and post-deployment. However, some biomarkers might be more informative measured directly after the stressful or traumatic event. For instance, heightened NPY levels were found shortly after acute, uncontrollable stress (Morgan et al., 2000; Morgan et al., 2002). So, it might prove useful to study neuroendocrine markers contiguous to a stress challenge (e.g. Josephs et al., 2017) or in response to simulated trauma (Marshall et al., 2017).

Taken together, whereas NPY, OT and AVP might present potentially promising biomarkers, as discussed in **chapter 1**, **6**, and **7**, determining the levels in plasma was –in our hands– not found useful in relation to PTSD. Therefore, methods to investigate the central effects of these neuropeptides, for instance intranasal administration, in relation to psychiatric disorders should be further assessed (*but* see Leng and Ludwig, 2016, for a critical review). In addition, the current studies underline the necessity of large prospective longitudinal studies that focus on the complex interplay of biological systems to identify biomarkers, to differentiate between types of biomarkers, and to eventually be able to identify traumatized individuals at risk for (subtypes of) PTSD.

Can military personnel be **warned***?*

The reported prevalence rates of mental health problems in Dutch military personnel highlight the importance of screening tools and early interventions. However, as discussed, the interaction between different biological systems and the heterogeneity in PTSD thus far complicate the identification of reliable and specific biomarkers for PTSD. In addition to biological factors, pre-disposing psychological risk factors for PTSD were found in PRISMO, such as age during deployment, endorsement of early life trauma, personality characteristics

and the presence of PTSD symptoms prior to deployment (See Table 1). Moreover, in a review on pre-trauma risk factors for PTSD, six categories of predictors were reported; cognitive abilities, coping and response styles, personality, psychopathology, psychophysiological factors and social-ecological factors (DiGangi et al., 2013). The question remains, however, if pre-trauma factors can be reliably used to identify individuals at risk and to *warn* military personnel.

Efforts have been made to study the use of (psychological) screening tools to prevent the development of mental health problems. One study showed that pre-deployment mental health screening, as part of the standard medical screening, was associated with significant reductions in occupationally impairing mental health problems, medical evacuations from Iraq for mental health reasons, and suicidal ideation (Warner et al., 2011b). In contrast, Rona et al. (2006) did not find that screening on the basis of pre-deployment symptoms levels prior to deployment to Iraq was useful in predicting psychological morbidity 2 to 3 years after homecoming. A recent review on the usefulness of pre-employment and pre-duty screening in emergency service personnel showed that pre-duty screening protocols, with the assessment of personality and physiological and psychological coping strategies, may be able to identify only some individuals at increased risk for mental health problems (Marshall et al., 2017). The difficulty with screening for PTSD prior to trauma exposure is the lack of efficacy, the high false-positive rate, and low predictive value in settings in which prevalence of mental health problems is expected to be low (Rona et al., 2006; Terhakopian et al., 2008; Warner et al., 2011b).

Table 1. General overview of potential risk factors for PTSD symptoms in 5 years post-deployment reported in the current thesis.

		PTSD symptoms
Deployment-related characteristics	Exposure to combat-related stressors	+
	Previous deployment	≠
	New deployment	≠
	Rank	–
Individual characteristics	Age	–
	Educational level	≠
	Traumatic experiences in childhood	+
Character traits	Self-directedness	–
	Cooperativeness[1]	–
Biomarkers	Testosterone[2]	–
	*p*NPY[2]	≠
	*p*OT	≠
	*p*AVP	≠

Note: +/- found to be positively/negatively related; ≠ not found to be related.
[1.] In recovered trajectory [2.] PTSD symptoms up to 2 years post-deployment.

Moreover, pre-trauma risk factors might not be sufficient to predict mental health problems since PTSD develops in the context of trauma exposure. For instance, Rona et al. (2009) reported that pre-deployment screening is not effective because combat exposure is the strongest predictor of PTSD and unit support may have a protective effect on mental health outcome. However, although combat exposure is a strong predictor for PTSD (Hoge et al., 2004; Hoge et al., 2006; Rona et al., 2006; Smith et al., 2008; Fear et al., 2010; Jones et al., 2013), the intensity of the traumatic events might not be directly related to PTSD. As discussed, many military men and women who were exposed to high intensity trauma did not develop PTSD. Moreover, as shown in this thesis, previous and new deployments were not predictive of the development of PTSD. So, whereas trauma exposure is a prerequisite to develop PTSD, many other biological, environmental, behavioral and social factors are associated with the increased risk for PTSD. For instance, four single nucleotide polymorphisms (SNP's) in the FKBP5 gene, a gene that codes for glucocorticoid-related proteins, interact with the severity of childhood trauma to predict PTSD symptoms in adulthood (Binder et al., 2008).

Furthermore, deployment characteristics, peri-trauma and post-trauma, and social factors provide relevant and maybe necessary information to predict the development of PTSD. For instance, perceived life threat during trauma, peri-traumatic emotional responses and dissociation, and post-trauma social support are important factors (Brewin et al., 2000; Ozer et al., 2003; Xue et al., 2015). Ozer et al. (2003) even stated that peri-traumatic psychological processes are the strongest predictors of PTSD. Also, deployment-related characteristics, such as group cohesion and leadership, concerns about the family during deployment, and social support during and after deployment are related to mental health problems (Ramchand et al., 2015).

Although risk factors have been identified, it is not yet possible to identify individuals at risk for posttraumatic psychopathology prior to or after deployment. To find clinically useful and personalized predictors, many psychological and biological risk factors and the interaction between these factors have to be examined. Moreover, factors might be protective (e.g. social support; Ramchand et al., 2015), might be context-dependent (e.g. type of trauma), and might not be present in every individual (e.g. injuries). So, advanced statistical techniques are necessary to identify predictive sets of risk factors. Machine learning can integrate the psychological and biological factors to make risk predictions (e.g. Kessler et al., 2014b). This is a data-driven method that integrates large sets of information to identify models with optimal sensitivity and specificity to predict, for instance, post-deployment PTSD (Kessler et al., 2014b). Several equally predictive sets of early risk indicators were identified, including factors related to the traumatic event and admission to the emergency department (ED), to predict non-remitting PTSD in adults admitted to the ED (Galatzer-Levy et al., 2014). Moreover, in Danish military personnel, pre-deployment and early post-deployment risk indicators were identified with machine learning methods to predict individuals at risk for long-term PTSD after deployment to Afghanistan (Karstoft et al., 2015). So, machine learning is a valuable method that is increasingly used to develop risk prediction models.

In addition, as discussed, prediction is further complicated by heterogeneity in PTSD; the differences in symptom presentation, course of symptom development, and comorbidity with other psychiatric and medical conditions. However, alternative classification systems are proposed, such as Research Domain Criteria (RDOC; Insel et al., 2010; Schmidt and Vermetten, 2017). In this approach, a transdiagnostic approach is adopted and the focus is on common mechanisms that underlie disorders. Also, advanced statistical methods can be used to identify patterns in posttraumatic stress symptoms (Galatzer-Levy and Bryant, 2013). For instance, as shown in this PhD thesis, latent growth mixture modeling (LGMM) can be used to separate groups of individuals with different time courses of posttraumatic stress symptoms after trauma. Another example is latent class analysis (LCA), which uses the distribution of observed symptoms to identify subgroups in a population (e.g. Steenkamp et al., 2012). So, instead of relying on the DSM criteria, other approaches for classification can be used to address the heterogeneity in PTSD.

To conclude, currently pre-trauma risk prediction is not reliable and specific enough to identify individuals at risk for PTSD. Moreover, a screening tool to determine the individuals' risk for PTSD development will probably consist of a complex interplay between biological, psychological, and social factors measured prior to, during and for a prolonged period after deployment. Also, it is questionable if a single model can capture a heterogeneous disorder, such as PTSD. Therefore, the use of alternative classification systems, and complex and advanced statistical techniques in large prospective longitudinal studies, such as PRISMO, is essential in the development of risk prediction tools and personalized preventive interventions.

Should military personnel be **warned***?*

Although there are still many obstacles in the development of screening tools, the advances in statistical methods and the increase in large longitudinal studies give reason to be optimistic about the future possibility to predict the risk for PTSD. However, with this development, it is also important to consider the consequences of risk prediction. If it is possible to identify individuals at risk for developing PTSD after deployment, what are the ethical implications (see also Lehrner and Yehuda, 2014)?

At the first glance, there are many positive consequences. Individual risk prediction might protect individuals and their families for the development of a debilitating disorder for which trauma-focused therapy is only effective in roughly 50% of the cases (Bradley et al., 2005; Bisson et al., 2007; Haagen et al., 2015; Steenkamp et al., 2015). It can be used to develop resilience training or designate individuals for 'watchful waiting' to be able to provide treatment in an early stage. Early intervention may result in a shorter course of the disorder and fewer negative outcomes related to PTSD (Bryant et al., 2008; O'Donnell et al., 2008). Also, early treatment is related to a positive outcome and fewer symptoms are related to lower healthcare costs (Kearns et al., 2012). In an analysis of costs in the PRISMO study, healthcare costs were found to increase over time after deployment (Eekhout et al., 2016). So, preventive screening might decrease the burden and costs by intervening before

symptoms begin or by providing treatment in an early stage to prevent disorders from becoming chronic. However, although there are some preventive methods that show promise (For review: Kearns et al., 2012; Howlett and Stein, 2016; Qi et al., 2016), more research on effective preventive interventions is necessary.

On the other hand, if it is known that an individual has an increased risk, what does that mean for the individual, his/her unit, for employers and/or policy makers? A question often asked by participants of the PRISMO study is whether they will be excluded from deployment if they are screened as having an increased risk of developing mental health problems. Indeed, this is an important ethical question to consider. Should the individuals with high-risk profiles be excluded from deployment or should they be placed in jobs with a decreased risk of exposure (non-combat)? And how big should the risk be to exclude an individual from getting deployed? If someone is at increased risk this does not necessarily mean that they will develop the disorder. For instance, the BRCA1 gene does not identify the women that will develop breast cancer, only those at increased risk. Also, factors that put an individual at increased risk might also increase safety of the unit in theatre. For instance, high harm avoidance is a risk factor for mental health problems, but this might also make these individuals more alert and attentive to signs of danger.

In addition, if these individuals are deployed how does this affect the social relations within a unit? Is this interpreted as a sign of weakness? Does this increase the stigma around mental health problems? The stigma is already high in the military. Research showed that soldiers were significantly less willing to report mental health problems on a routine (non-anonymous) post-deployment health screening compared with an anonymous screening tool (Warner et al., 2011a). One can imagine that the knowledge of having increased risk will increase the individuals concerns about how they are perceived by their colleagues and their leaders, and more importantly how this might affect their military career. Also, the screening might have a negative effect on the individual's view of oneself, thereby decreasing self-esteem (Jones et al., 2003). Moreover, lower perception of group cohesion, relative to other members of the same unit, is associated with higher likelihood of PTSD (Breslau et al., 2016). So, there is a risk that with knowing that an individual is at increased risk for mental health problems, one actually achieves the opposite effect.

Some would even make the case that individuals at high-risk should not be allowed to enlist in the military. This raises concerns about potential discrimination. Also, it is questionable whether there are still enough military personnel to meet the requirements of a mission when this policy would be introduced. However, if someone with a high risk of mental health problems gets deployed and the risk of trauma exposure is high, who is responsible when the individual develops PTSD after deployment? Who is responsible for the costs associated with healthcare and compensation: the individual, the employer, or society? So, although the identification of susceptibility markers might have many beneficial aspects, it might also have social, ethical and legal implications, which should be considered before using them in practice.

Implications and future directions

In this PhD thesis, findings were presented from the first prospective longitudinal cohort study in soldiers measured before and at discrete moments post-deployment. Mental health problems were shown to develop for a prolonged period after deployment to Afghanistan. Currently, there are no reliable and specific screening tools to predict who is at increased risk of developing PTSD prior to deployment. However, an increasing number of biological and psychological susceptibility markers were identified. As discussed, further research on the individual variability in the response to trauma and the association with and between different markers is necessary to develop personalized preventive strategies. The use of 'Big' data and the development of advanced statistical methods, such as machine learning, increase the possibilities for finding clinically useful and personalized combinations of biological and psychological predictors. In addition, the use of different classification systems, such as RDOC (Insel et al., 2010; Schmidt and Vermetten, 2017), to address the heterogeneity of PTSD can prove useful to comprehend underlying mechanisms of stress-related mental health problems and to identify potential biomarkers. Also, it might support identifying which individuals will benefit from a particular intervention.

For now, the importance of monitoring a large range of symptoms even several years after deployment to provide treatment in an early stage is underlined. Younger age and a higher level of exposure were found to be important in the development of PTSD. So, it might be argued that individuals that were deployed under the age of 21 and were exposed to high levels of combat should be monitored more often. Also, the identification of risk factors could be used to study methods to increase resilience and develop secondary prevention strategies in military personnel. For instance, recovery and resilience might be enhanced by increasing interpersonal resources at home, work and in community and alleviating stressors, such as unemployment and family distress (Polusny et al., 2011; Horesh et al., 2013). Furthermore, healthy adaptive functioning might be promoted by methods increasing the level of self-directedness and cooperativeness. Training of these features prior to deployment might help to increase resiliency (Cloninger et al., 2010). Also, short-term interventions in individuals with sub-threshold PTSD might decrease the rate of delayed-onset PTSD (Korte et al., 2016).

Lastly, various pharmacological interventions to prevent stress-related disorders after trauma have been examined mainly in preclinical studies (For review: Howlett and Stein, 2016; Qi et al., 2016). For instance, preclinical research showed that intranasal delivery of NPY before and immediately after a prolonged stress model of PTSD prevented the development of PTSD-like symptoms by reducing anxiety-like behavior and normalizing HPA reactivity evoked by traumatic exposure (Serova et al., 2013; Sabban et al., 2015), making NPY attractive for PTSD prevention. Currently, no pharmacological intervention has enough evidence to justify clinical use (Howlett and Stein, 2016). However, lower PTSD symptoms at 3 months post-trauma were reported after receiving a 10-day course of low-dose hydrocortisone (Delahanty et al., 2013). Also, a recent study showed that intranasal oxytocin administration in emergency department patients initiated within 12 days post-trauma is a

promising preventive intervention for PTSD for individuals with high acute PTSD symptoms (van Zuiden et al., 2017). So, although peripherally measured neuropeptides were not related to PTSD symptom development in this thesis and more research is necessary, these preliminary studies show promise for the clinical use of pharmacological interventions to prevent the development of PTSD in military personnel.

Strengths and limitations

As discussed, the PRISMO study is the first study to assess both biological and psychological factors in relation to PTSD in a prospective longitudinal design, starting with the first measurement prior to deployment. Using this design, it was possible to assess risk factors for the development of PTSD and differentiate these from diagnostic markers. Moreover, advanced statistical methods were used to address the heterogeneity of symptoms of PTSD over time. Despite this, two important limitations have to be discussed.

First, self-report measures rather than clinical interviews were used to assess the presence of mental health problems. The Self-Report Inventory for PTSD has good concurrent validity with the Clinician-Administered PTSD scale (CAPS) and the Mississippi scale for PTSD (Hovens et al., 2002). However, questionnaires might provide higher prevalence estimates than interviews (Engelhard et al., 2007). Many might screen positive for PTSD on a questionnaire but may not have the disorder. An important explanation is that functional impairment is not included in questionnaires (Richardson et al., 2010). Therefore, in the 10-years assessment that is currently conducted, a clinical interview is added to the data collection to be able to differentiate between having symptoms and having a disorder. Based on the first observations, the high level of PTSD symptoms does not always correspond to the diagnosis of PTSD, but might also represent partial PTSD or another disorder.

A second limitation of this study is the limited generalizability of our findings to the general population. A strength of this study is that a cohort of military personnel (mainly men) were included, which were all exposed to combat-related stressors. However, the development of PTSD might be different for specific types of trauma. For instance, individuals are more likely to develop PTSD after combat exposure or sexual assault. In addition, only 5% of the veterans in the Netherlands are female (Dutch Ministry of Defence, 2017). So, although the low percentage of women in this study is representative to the military, there is an underrepresentation of women. Therefore, they were not included in the studies in ***Part 2*** of this thesis. However, as also shown in a report of the Veterans Institute (2016), it is important to study the impact of military deployment on women to be able to address their healthcare needs. Although many women in the PRISMO study fulfilled supportive roles during deployment, they reported many potentially traumatic events in the hospitals and outside of the base as part of the mission to win the 'hearts and minds' and to talk and connect to Afghan women. Also, women might be exposed to other types of stressors/trauma; sexual harassment, unit cohesion, or sexual trauma. So, if it is possible to develop a model

to identify individuals at risk, it is important to examine how this applies to other types of trauma, to women, and to other populations.

Conclusion

In conclusion, the increased level of various symptoms even years after military deployment underlines to importance of studying whether military personnel can be *warned*. However, although many biological and psychological risk factors are identified, it is currently not possible to reliably predict if an individual has an increased risk of developing PTSD after deployment. As discussed, there are many obstacles in identifying specific and reliable biomarkers for symptoms of PTSD. In this thesis plasma testosterone, but not peripherally measured NPY, OT and AVP, was associated with symptoms of PTSD. However, the advances in statistical methods to address the heterogeneity of PTSD and to identify multifactorial systems including both psychological and biological factors in large data sets are promising and should be further explored. Although identifying individuals at risk is valuable to develop personalized preventive interventions, the ethical implications of screening should be discussed before military personnel are *warned*.

The last time I was confronted with the impact of Posttraumatic Stress Disorder (PTSD), I was working as a PhD student at the Military Mental Healthcare Research Centre. It was a cloudy Friday afternoon and I had to interview a participant for the 10-year assessment of PRISMO. As always, I asked about his current situation, his work, his career, and how he looked back on his deployment to Afghanistan. He described his deployment as a positive experience and the comradeship was the most important reason for this. However, nobody had prepared him for the badly injured children he would be holding in his arms. Now, 10 years later, he is recovering from PTSD.

His motivation for participating in the 10-year assessment was to help find ways to prevent his colleagues from the struggle he went through. So, I asked him if he would have stayed in the Netherlands if we could have **warned** *him. He looked at me and gave me a firm answer: 'That is no option, I would not have missed it for the world!'*

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CHAPTER 09

Nederlandse samenvatting
Dankwoord
List of publications
About the author

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Nederlandse samenvatting

Veel militairen kijken positief terug op hun uitzending naar Afghanistan. Ze hebben de taken uitgevoerd waar ze voor getraind zijn, hebben kameraadschap ervaren en velen geven aan persoonlijk te zijn gegroeid. Desondanks zijn ze vaak blootgesteld aan mogelijke traumatische gebeurtenissen, zoals beschietingen, raketaanvallen en collega's of burgers die gewond zijn geraakt of zijn omgekomen. Toch kunnen veel militairen zich relatief eenvoudig aanpassen bij terugkomst in Nederland, maar er zijn ook militairen die last hebben van psychische klachten, zoals nachtmerries, agressief gedrag en overdreven alertheid. Bij de meeste militairen verdwijnen deze klachten binnen enkele weken na thuiskomst, maar bij anderen kan dit maanden of zelfs jaren aanhouden. Zij hebben een posttraumatische stressstoornis ofwel PTSS. Dit is een psychische stoornis die wordt gekenmerkt door het herbeleven van de traumatische gebeurtenis (bijv. nachtmerries, flashbacks), aanhoudende vermijding van prikkels die hen herinneren aan het trauma, negatieve gevoelens en gedachten en aanhoudende symptomen van verhoogde prikkelbaarheid (DSM-5; American Psychiatric Association, 2013). Maar waarom ontwikkelen sommige militairen deze klachten, terwijl anderen, die hetzelfde hebben meegemaakt, geen problemen ervaren? Hoeveel militairen ontwikkelen deze klachten, wanneer ontwikkelen zij deze klachten en op welke manier verschillen deze mensen van elkaar? Door het beantwoorden van deze vragen kan worden bijgedragen aan betere en tijdige behandeling van klachten. Daarnaast kunnen militairen met een verhoogd risico op klachten mogelijk worden geïdentificeerd om uiteindelijk de ontwikkeling van stress-gerelateerde klachten te verminderen en veerkracht te vergroten.

Het eerste doel van dit proefschrift was om meer zicht te krijgen op de ontwikkeling van psychische problematiek onder Nederlandse militairen over langere tijd na uitzending. Daarom is in het eerste deel van dit proefschrift gekeken naar de prevalentie en het beloop van stress-gerelateerde psychische klachten na uitzending naar Afghanistan. Ondanks dat het meemaken van een traumatische ervaring een vereiste is voor het diagnosticeren van PTSS (American Psychiatric Association, 2013), ontwikkelt slechts een klein deel van de uitgezonden militairen PTSS. Het tweede doel van dit proefschrift was om te onderzoeken of verschillende soorten risicofactoren kunnen worden gebruikt om te voorspellen wie posttraumatische stressklachten ontwikkelen na uitzending. Naast mogelijke psychologische en uitzendings-gerelateerde factoren, zoals demografische gegevens, persoonlijkheidstrekken en het meemaken van gevechtssituaties, zijn in het tweede deel van dit proefschrift verscheidene, in plasma gemeten, biologische risico- en beschermende factoren (testosteron, neuropeptide Y (NPY), oxytocine (OT) en arginine vasopressine (AVP)) onderzocht. Biologische factoren vormen een meer objectieve maat, die kan worden gebruikt bij het verbeteren van de diagnostiek naar PTSS en de identificatie van mensen met een verhoogd risico zodat behandeling eerder kan worden aangeboden en nieuwe therapieën kunnen worden ontwikkeld.

Het prospectieve longitudinale design, zoals gebruikt in dit proefschrift, gaf een unieke mogelijkheid om psychologische en biologische kwetsbaarheden te onderzoeken als

risicofactoren voor het ontwikkelen van PTSS-klachten. Wanneer we beter in staat zijn om te voorspellen wie een verhoogd risico heeft op het ontwikkelen van PTSS-klachten, kunnen militairen en hun omgeving beter worden geholpen. Met andere woorden, het doel van dit proefschrift was om een bijdrage te leveren aan het beantwoorden van de vraag of militairen kunnen worden *gewaarschuwd* voor het ontwikkelen van stress-gerelateerde psychische problematiek.

PRISMO

Om de invloed van uitzending op de psychische gezondheid van Nederlandse militairen en mogelijke risico- en protectieve factoren te onderzoeken is in 2005 een grote longitudinale studie gestart: Prospectie in Stress-gerelateerd Militair Onderzoek (PRISMO). Dit was de eerste studie die psychologische en biologische aspecten van het ontwikkelen van stress-gerelateerde psychische problematiek heeft onderzocht met een prospectief longitudinaal design. De eerste meting vond plaats vóór uitzending en vervolgens op 1 en 6 maanden en 1, 2 en 5 jaar na uitzending (*zie* figuur 1 in **hoofdstuk 1**). Momenteel wordt een meting 10 jaar na uitzending uitgevoerd. In totaal hebben 1032 militairen zich aangemeld voor deelname aan de studie, waarvan 1007 militairen tussen 2005 en 2008 zijn uitgezonden naar Afghanistan voor 4 maanden als onderdeel van de International Security Assistance Force (ISAF; voor meer informatie over de deelnemers *zie* infographic in **hoofdstuk 1**).

Resultaten

Deel 1

Om een indicatie te krijgen van de stress-gerelateerde psychische problematiek bij Nederlandse militairen na uitzending is in **hoofdstuk 2** gekeken naar de prevalentie vóór en op meerdere tijdstippen tot 2 jaar na de uitzending naar Afghanistan. Hieruit blijkt dat, ondanks het meemaken van potentieel traumatische gebeurtenissen, een groot deel van de militairen in de PRISMO-studie geen psychische klachten rapporteren na uitzending. Echter, vergeleken met de meting vóór uitzending, vonden we een stijging in de prevalentie van verschillende psychische klachten (vermoeidheid, PTSS, hostiliteit, depressie en angst). Het beloop van klachten over de tijd verschilt per klacht. We vonden dat de prevalentie van militairen met PTSS-klachten toenam kort na uitzending en daarna daalde, terwijl de prevalentie van depressieve- en vermoeidheidsklachten toenam over de twee jaar na uitzending. Hostiliteits- en slaapproblemen bleven relatief stabiel over de tijd na uitzending.

De bevindingen in **hoofdstuk 2** worden ondersteund door de bevindingen in **hoofdstuk 3**, waarbij is gekeken naar de ontwikkeling van PTSS-klachten over vijf jaar na uitzending. Hierin is te zien dat naast de toename in PTSS-klachten op 6 maanden na uitzending, ook een verlate toename was te zien op vijf jaar na uitzending. Daarop aansluitend, konden drie

verschillende groepen/trajecten worden onderscheiden op basis van de ontwikkeling van PTSS-klachten over de tijd. Zoals te zien is in figuur 1 van **hoofdstuk 3**, liet het grootste deel van de deelnemers een stabiel laag verloop van PTSS-klachten zien (veerkrachtig traject; 85%). Een tweede traject liet een toename van PTSS-klachten zien in het eerste jaar, waarna deze weer afnamen (hersteld traject; 5%) en het laatste traject liet een beloop zien met een toename in klachten op vijf jaar na uitzending (verlaat traject; 9%). In dit hoofdstuk zijn tevens verschillende risicofactoren voor de ontwikkeling van PTSS-klachten geïdentificeerd, zoals leeftijd, traumatische ervaringen in de jeugd en/of op uitzending.

Een eerdere studie in PRISMO heeft laten zien dat verschillende persoonlijkheidstrekken risicofactoren kunnen zijn voor PTSS bij militairen (van Zuiden et al., 2011). Het is echter onduidelijk of het gaat om kwetsbaarheidsfactoren of dat mogelijke veranderingen in persoonlijkheidstrekken door het meemaken van traumatische ervaringen ook de ontwikkeling van PTSS kunnen beïnvloeden. Daarom is in **hoofdstuk 4** de relatie tussen uitzending en de ontwikkeling van de karaktertrekken zelfsturendheid en coöperativiteit onderzocht van vóór tot 5 jaar na uitzending. Hieruit blijkt dat het niveau van coöperativiteit relatief stabiel was en de mate van zelfsturendheid afnam over de tijd. Deze afname bleek sterker in het herstelde en verlate traject van PTSS-klachten. Dit suggereert dat de ontwikkeling van PTSS-klachten is geassocieerd met een afname in de mate van zelfsturendheid. Daarnaast wordt in dit hoofdstuk aangetoond dat een lage mate van zelfsturendheid en coöperativiteit voor uitzending is gerelateerd aan het ontwikkelen van PTSS-klachten.

De resultaten van ***deel 1*** van dit proefschrift laten zien dat het grootste deel van de Nederlandse militairen na uitzending geen klachten ontwikkelen en ook geen verandering laten zien in karaktertrekken over tijd. Echter, er zijn militairen die problemen ervaren in de aanpassing aan het leven in Nederland. Sommigen ontwikkelen deze problemen kort na uitzending terwijl anderen pas vijf jaar na uitzending PTSS-klachten rapporteren.

Deel 2

In het tweede deel van dit proefschrift is onderzocht of er potentiële stress-gerelateerde biologische risico- of beschermende factoren zijn die samenhangen met de ontwikkeling van PTSS-klachten bij mannelijke militairen na uitzending. Eerdere onderzoeken hebben al vele mogelijke biologische factoren (biomarkers) geïdentificeerd, met name gericht op de hypothalamus-hypofyse-bijnier (HHB) as omdat deze van belang is in het reguleren van de stressrespons (Voor review: Baker et al., 2012; Schmidt et al., 2013; Zoladz en Diamond, 2013; Michopoulos et al., 2015). In ***deel 2*** worden de resultaten van andere mogelijke, aan de HHB-as gerelateerde, biologische factoren beschreven; namelijk testosteron, neuropeptide Y, oxytocine en vasopressine. De waarden werden bepaald in bloedplasma vóór, op 1 maand en op 6 maanden na uitzending.

In **hoofdstuk 5** werd gekeken of testosteronwaarden veranderen na uitzending en of deze verandering ofwel de waarden vóór uitzending gerelateerd zijn aan PTSS-klachten. We vonden dat testosteron levels toenamen na uitzending, maar dat deze verandering over

tijd niet verschillend was voor mensen met een hoge of lage mate van PTSS-klachten. Echter, lage testosteronwaarden vóór uitzending bleken wel voorspellend te zijn voor PTSS-klachten 1 en 2 jaar na uitzending.

Neuropeptide Y en oxytocine worden gezien als veelbelovende biologische factoren omdat het toedienen van deze peptiden (bijv. via neusspray) mogelijk als nieuwe therapie ingezet kunnen worden voor het verhogen van veerkracht bij militairen. Het is echter onduidelijk of het ook risicofactoren voor PTSS-klachten zijn, omdat eerdere onderzoeken keken naar deze stoffen als stoornis-gerelateerde marker door mensen met PTSS te vergelijken met gezonde controles. Daarom is in **hoofdstuk 6** de voorspellende waarde van plasma NPY voor het ontwikkelen van PTSS onderzocht. Hieruit blijkt dat zowel de NPY-levels vóór uitzending als de verandering in plasma NPY van vóór tot 6 maanden na uitzending niet gerelateerd is aan PTSS-klachten. Deze bevindingen zijn gerepliceerd in een onafhankelijk militair cohort van ruim 2400 mariniers uit de Verenigde Staten die zijn uitgezonden naar Irak of Afghanistan. Daarnaast zijn in **hoofdstuk 7** plasma oxytocine en vasopressine onderzocht als kwetsbaarheidsfactoren voor de ontwikkeling van PTSS-klachten. Hierin hebben wij laten zien dat, in tegenstelling tot bekende risicofactoren, zoals leeftijd, traumatische ervaringen in de jeugd en op uitzending, plasma OT en AVP niet gerelateerd zijn aan het ontwikkelen van PTSS-klachten tot 5 jaar na uitzending. Hierbij moet echter worden opgemerkt dat er methodologische obstakels zijn die zorgen dat de conclusies met enige voorzichtigheid moeten worden geïnterpreteerd.

De resultaten in ***deel 2*** van dit proefschrift laten zien dat lage testosteronwaarden vóór uitzending mogelijk een risicofactor vormen voor PTSS, maar dat de perifeer gemeten neuropeptiden, NPY, OT en AVP, op dit moment geen bruikbare kwetsbaarheidsfactoren zijn voor de ontwikkeling van PTSS in mannelijke militairen na uitzending.

Discussie

De prevalentie van psychische klachten

Een belangrijk resultaat is dat, zowel in **hoofdstuk 2** als **hoofdstuk 3**, het merendeel van de militairen in de PRISMO-studie geen klachten rapporteren na uitzending naar Afghanistan. Het is mogelijk dat de huidige voorbereiding en training voorafgaand aan de uitzending voor een deel zorgen voor deze verhoogde mate van veerkracht. Toch zien we ook dat de prevalentie van verschillende psychische klachten toeneemt in de jaren na uitzending. Het is echter de vraag of dit te wijten is aan de uitzending zelf. Op basis van literatuur en de huidige bevindingen, lijkt de stijging in de prevalentie van klachten eerder te wijten aan de blootstelling aan meer gevechtssituaties dan aan de uitzending zelf. Echter, om dit met meer zekerheid te kunnen stellen, zal in toekomstig onderzoek een niet-uitgezonden en burgercontrole groep moeten worden meegenomen.

Het beloop van psychische klachten

Een andere belangrijke bevinding is dat het soort klachten en het tijdstip waarop iemand klachten krijgt, kan verschillen tussen militairen. Het stabiele beloop van meer algemene klachten, zoals hostiliteit en slaapproblemen, doet vermoeden dat dergelijke klachten minder snel herkend en behandeld worden dan andere stress-gerelateerde klachten zoals PTSS. Daarnaast is er variatie in verschillende typen klachten over tijd en kunnen ook klachten binnen een stoornis variëren (bijv. PTSS). Zo is er heterogeniteit in de symptomen die mensen rapporteren en, zoals in **hoofdstuk 3** is beschreven, in de ontwikkeling van PTSS-klachten over de tijd na uitzending. Het is dan ook interessant om te onderzoeken welke factoren gerelateerd zijn aan de individuele verschillen in het ontwikkelen van PTSS-klachten over tijd. Zoals beschreven in **hoofdstuk 4**, is een lagere mate van zelfsturendheid gerelateerd aan het ontwikkelen van PTSS-klachten en is een lagere mate van coöperativiteit specifiek gerelateerd aan het herstelde traject. Dit suggereert dat waar sommige factoren gerelateerd zijn aan PTSS-ontwikkeling in het algemeen, andere factoren mogelijk gerelateerd zijn aan specifieke trajecten. Daarnaast blijkt dat individuen in het verlate traject al een verhoogde mate van klachten rapporteerden na uitzending, wat kan suggereren dat eerdere behandeling het risico op verlate PTSS mogelijk kan verlagen. In de vervolgmeting 10 jaar na uitzending zal het onderzoek naar individuele verschillen in het ontwikkelen van klachten moeten worden voortgezet en moet/kan de rol van mogelijke algemene en specifieke risicofactoren, zoals dienstverband en behandeling, verder worden onderzocht.

Biomarkers voor de preventie van PTSS

Op basis van ***deel 2*** van dit proefschrift, kan worden gesteld dat verlaagde testosteron vóór uitzending een mogelijke kwetsbaarheidsfactor vormt voor PTSS. Echter, ondanks dat NPY, OT en AVP mogelijk veelbelovende biomarkers zijn, bleek in onze studie dat de bruikbaarheid van deze perifeer gemeten neuropeptiden als voorspeller van PTSS op dit moment beperkt is. Daarom zal toekomstig onderzoek moeten kijken naar andere/betere metingen van neuropeptiden en moeten de centrale effecten van de neuropeptiden in relatie tot psychische problematiek worden onderzocht.

Ondanks dat vele potentiële biologische factoren zijn geïdentificeerd die gerelateerd zijn aan PTSS, zijn er momenteel geen biologische risico- of beschermende factoren die specifiek en betrouwbaar genoeg zijn om toe te passen in de praktijk. Dit kan worden verklaard doordat PTSS een zeer heterogene stoornis is met een hoge mate van co-morbiditeit met andere psychische en lichamelijke klachten. Daarnaast wordt het identificeren van specifieke biomarkers verder beperkt door de interactie met andere biologische systemen. Tevens lijkt het onwaarschijnlijk dat een enkele biologische factor kan worden geïdentificeerd om verschillende fasen van de stoornis te meten, dus het tijdstip waarop wordt gemeten en het aantal metingen is belangrijk. Dit benadrukt het belang van grote prospectieve longitudinale studies, zoals PRISMO, om de complexe samenhang tussen biologische systemen te onderzoeken en onderscheid te kunnen maken tussen verschillende

biomarkers om uiteindelijk mensen met een verhoogd risico op (subtypes van) PTSS te kunnen identificeren.

Kan een militair gewaarschuwd *worden?*

De aanwezigheid van psychische problematiek na uitzending geeft het belang aan van screeningsinstrumenten en vroegtijdige interventies. Ondanks dat veel onderzoek wordt gedaan naar mogelijke screeningsinstrumenten om PTSS te kunnen voorspellen vóór een traumatische gebeurtenis, zijn deze op dit moment niet specifiek en betrouwbaar genoeg om militairen met een verhoogd risico te kunnen identificeren. Een mogelijke verklaring hiervoor is dat risicofactoren voorafgaand aan trauma mogelijk niet voldoende zijn om PTSS te voorspellen omdat PTSS zich ontwikkelt in de context van een traumatische ervaring. Maar ondanks dat trauma een vereiste is voor het ontwikkelen van PTSS, kunnen ook vele andere psychologische, biologische, sociale en omgevingsfactoren tijdens of na trauma een rol spelen bij het ontwikkelen van PTSS. Daarnaast kunnen factoren beschermend zijn, context-afhankelijk en zijn deze niet bij ieder individu aanwezig. In de ontwikkeling van een screeningsinstrument zal moeten worden gekeken naar een complexe samenhang tussen biologische, psychologische en sociale factoren gemeten vóór, tijdens en na uitzending en daarvoor zijn geavanceerde statistische technieken, zoals 'machine learning', nodig. De vraag is echter of één specifiek model kan worden toegepast voor een heterogene stoornis, zoals PTSS. Daarom zal in toekomstig onderzoek, in plaats van PTSS volgens de DSM-5 (American Psychiaric Association, 2013), gebruik moeten worden gemaakt van andere classificatiesystemen (bijv. RDOC; Insel et al., 2010) of van complexe statistische technieken in grote prospectieve longitudinale studies, zoals PRISMO, om predictiemodellen en persoonsgerichte behandelingen te kunnen ontwikkelen.

Zou een militair gewaarschuwd *moeten worden?*

Ondanks de vele obstakels in het ontwikkelen van screeningsinstrumenten, zorgt de ontwikkeling van statistische technieken en de toename in grote longitudinale studies ervoor dat er reden is om optimistisch te zijn over de toekomstige mogelijkheid om het risico op PTSS te voorspellen. Maar het is belangrijk om ook na te denken over de consequenties van een dergelijke ontwikkeling. Als het mogelijk is om mensen te waarschuwen, wat zijn dan de ethische implicaties?

Op het eerste gezicht vallen met name positieve consequenties op. Zo kan eerder behandeling worden aangeboden, waardoor de stoornis bij minder mensen chronisch wordt en uiteindelijk de kosten voor de zorg worden verminderd. Echter, daarvoor moet meer onderzoek gedaan worden naar effectieve behandelingen van PTSS. Aan de andere kant is het de vraag, of als bekend is dat mensen een verhoogd risico hebben op PTSS, zij moeten worden uitgesloten voor uitzending? Of moeten zij een functie krijgen met een lager risico op het meemaken van trauma? Wat doet dit met sociale relaties en het zelfbeeld van de militair? En stel dat iemand wel wordt uitgezonden, wie is dan verantwoordelijk? In onderzoek naar

screeningsinstrumenten moet dan ook worden nagedacht over de ethische implicaties bij het gebruiken van een dergelijk instrument.

Implicaties en toekomstig onderzoek

De bevindingen in dit proefschrift laten zien dat het belangrijk het is om militairen na een uitzending langdurig (zelfs jaren) te monitoren op het ontwikkelen van een scala aan psychische klachten om zo klachten tijdig te kunnen detecteren en behandelen. Aangezien een hogere mate van blootstelling aan traumatische gebeurtenissen op uitzending en een jongere leeftijd belangrijke factoren bleken in relatie tot PTSS, is het mogelijk van belang deze groep vaker te monitoren. Daarnaast kan het identificeren van risicofactoren bijdragen aan het ontwikkelen van preventietechnieken en het verhogen van veerkracht, bijvoorbeeld door methoden te ontwikkelen die de mate van zelfsturendheid en coöperativiteit verhogen. Tot slot zijn er eerste aanwijzingen voor het gebruik van farmacologische interventies, zoals de intranasale toediening van oxytocine (van Zuiden et al., 2017), om het ontwikkelen van PTSS te voorkomen.

Op dit moment is het nog niet mogelijk om individuen te screenen op verhoogd risico, maar het gebruik van grote datasets ('Big data') en de ontwikkeling van geavanceerde statistische technieken verhogen de kans op het vinden van gepersonaliseerde en klinische bruikbare combinaties van risico- en beschermende factoren. Daarnaast kan het gebruik van alternatieve classificatiesystemen nuttig zijn om onderliggende mechanismen van PTSS te begrijpen en potentiele biomarkers te identificeren.

Conclusie

De gevonden toename in stress-gerelateerde psychische problematiek na uitzending benadrukt het belang van onderzoek naar methoden om militairen te kunnen *waarschuwen*. Ondanks dat vele mogelijke psychologische en biologische risicofactoren zijn geïdentificeerd, is het op dit moment niet mogelijk om op betrouwbare wijze te voorspellen of een individu een verhoogd risico heeft op het ontwikkelen van PTSS. Zoals besproken zijn er vele obstakels in het vinden van specifieke en betrouwbare biomarkers. In dit proefschrift is gevonden dat plasma testosteron vóór uitzending, in tegenstelling tot NPY, OT en AVP-levels in plasma, was geassocieerd met PTSS-klachten na uitzending. Echter, de geavanceerde statistische methoden om een multifactorieel systeem te identificeren met zowel psychologische als biologische factoren in grote datasets zijn veelbelovend. Ondanks dat het waardevol is om individuen met een verhoogd risico te identificeren met het oog op het ontwikkelen van persoonlijke preventieve behandelingen, moeten de ethische consequenties hiervan worden besproken voordat militairen worden *gewaarschuwd.*

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Dankwoord

Al vroeg in mijn studie klinische psychologie raakte ik geïnteresseerd in de gevolgen van traumatische ervaringen en het feit dat sommige mensen hierdoor klachten ontwikkelen en anderen niet. Maar ik had nooit gedacht dat ik ooit zou promoveren op dit onderwerp. In de afgelopen jaren heb ik zowel opgezien als uitgekeken naar het moment waarop ik het dankwoord kon gaan schrijven, want zonder het vertrouwen, de mentale steun en de praktische hulp van heel veel mensen was mij dit niet gelukt. Hieronder wil ik het woord richten aan een aantal mensen in het bijzonder.

Beste PRISMO-deelnemers, jullie zijn altijd mijn belangrijkste motivatie geweest voor het volbrengen van dit project. Zonder jullie was en is er geen PRISMO. In de afgelopen jaren heb ik velen van jullie zelf mogen spreken en ik heb er veel van geleerd. Ik vond het fantastisch met jullie te werken en ik ben onder de indruk van de openheid en de bereidheid waarmee jullie je hebben ingezet voor dit onderzoek en, belangrijker nog, voor het welzijn en de gezondheid van jullie collega's. Uiteraard hoop ik dat ik daar met dit proefschrift ook iets aan heb kunnen bijdragen. Ik wil jullie bedanken voor de interesse, de betrokkenheid en de bereidheid om ('telkens weer') mee te doen. Ontzettend bedankt!

Beste Professor Vermetten, beste Eric, ik wil je bedanken voor het vertrouwen en de kans die je mij hebt gegeven om dit onderzoek te doen. Bedankt voor de vrijheid die je me hebt gegeven in de uitvoering van het onderzoek, de vele gesprekken, de mogelijkheden, de interesse en betrokkenheid en voor het plaatsen van kritische vragen bij mijn artikelen.

Beste Professor Joëls, beste Marian, bedankt voor de mogelijkheden en de steun u mij hebt geboden in dit promotie traject. U daagde mij uit om uit mijn comfort zone te treden en dat heb ik zeer gewaardeerd, dank daarvoor.

Beste dr. Geuze, beste Elbert, in 2011 ben ik komen werken bij het onderzoekscentrum als stagiair om vervolgens verder te gaan als onderzoeksassistent. Maar promoveren 'dat was toch niks voor mij'. Jij gaf mij het vertrouwen dat ik het kon en steunde en motiveerde mij tijdens dit gehele project. We hebben verschillende leuke projecten samengedaan, veel met elkaar gesproken en ik heb dan ook veel van je mogen leren. Ik wens je alle goeds en hoop dat je een hele mooie tijd tegemoet gaat in het 'nieuwe' onderzoekscentrum.

Geachte beoordelingscommissie, Professor Kenemans, Professor Meijer, Professor Penninx, Professor van Os en Professor Kleber, hartelijk bedankt voor het lezen en beoordelen van mijn proefschrift. Beste Professor Kleber, beste Rolf, bedankt voor je inspiratie en interesse tijdens mijn studie en daarna.

Beste collega's van het onderzoekscentrum,
Joke, mijn vaste steun en toeverlaat tijdens al mijn jaren bij het onderzoekscentrum. We konden eigenlijk altijd over alles praten en ik zal dit gaan missen. Bedankt voor je interesse, je betrokkenheid, je hulp en steun.
Fenne, bedankt voor je enthousiasme, je eerlijkheid, je betrokkenheid en natuurlijk je hulp bij de PRISMO-interviews. Ik wens je heel veel succes met je eigen promotietraject.
Milou, bedankt voor onze gesprekken, de interesse en het meedenken. Heel veel succes met jouw proefschrift en vergeet niet af en toe gewoon 'even weg' te gaan.
Tim, bedankt voor je humor en je interesse en ook jij heel veel succes met je proefschrift!
Rosalie, bedankt voor het meedenken met mijn artikelen, voor het leren van 'R' en voor het vertrouwen in mijn kunnen. Ik hoop dat je snel je eigen promotie kan afronden!
Eva, bedankt voor de interesse en de gezellige gesprekken. Ik vind het heel stoer dat je promoveren combineert met het werken in de praktijk en ik wens je veel geluk met jullie kleine wonder.
Onderzoeksassistenten: Hester, jij kwam mij ondersteunen in het laatste half jaar en wat ben ik blij met jou geweest. Je pakte alles aan en nam verantwoordelijkheid. **Iris,** de afgelopen maanden kwam ook jij mij ondersteunen en je hebt je met jouw enthousiasme gelijk helemaal ingezet voor het project. Ik wil jullie ontzettend bedanken. Doordat ik PRISMO aan jullie kon toevertrouwen waren de laatste maanden een stuk makkelijker. Ik wens jullie veel succes en jullie gaan ongetwijfeld leuke banen tegemoet!
Sanne, jij gaat verder met PRISMO en wat ben ik jaloers ;) Ik heb er vertrouwen in dat jij het met succes gaat doen. Ik hoop dat je het net zo leuk gaat vinden als ik en ik wens je een hele mooie tijd en natuurlijk heel veel succes!

Beste oud-collega's, ook jullie wil ik bedanken voor de leuke en gezellige tijd en de samenwerking: **Loes, Mirjam, Arthur, Thomas, Rachel, Charlotte, Claire, Daniëlle. Sanne,** van het eerste promotietraject waar ik ooit direct getuige was, heb ik veel geleerd. Ik heb nog vaak aan je gedacht in de afgelopen maanden en ik heb nu nog meer respect voor hoe jij het hebt volbracht. **Iris,** jouw bijdrage aan mijn proefschrift was groot. Bedankt voor de hulp bij de analyses, voor het meedenken en beantwoorden van mijn vragen, zelfs ver na je vertrek bij het OC. **Lieke,** we waren kamergenoten in de start van onze projecten en hebben een hele leuke tijd gehad (met een beetje hulp van MJ en Robbie Williams) met vele gezellige borrels, uitstapjes en congressen. Bedankt voor de gezelligheid en de hulp en ik hoop dat je het naar je zin hebt in je nieuwe baan.

Beste stagiaires van het onderzoekscentrum, in het bijzonder mijn eigen stagiaires**: Bernice, Lisa, Ian, Annelot** en **Sascha,** ik heb ontzettend veel geleerd van het coördineren en begeleiden van jullie stages. **Annelot** en **Sascha,** ontzettend bedankt voor jullie hulp bij het uitvoeren van de PRISMO 10-jaarsmeting.

Beste collega's van de MGGZ, in het bijzonder kolonel IJzerman, kolonel-vliegerarts van der Meulen en kolonel Bergman, bedankt voor de belangstelling en voor het mogelijk maken van dit onderzoek. Ria, Pauline en Harry, bedankt voor het regelen van de vergoedingen voor onze deelnemers.

Beste collega's van de stressmeeting, bedankt voor jullie interesse en enthousiasme en voor de scherpe vragen tijdens mijn presentaties waardoor ik werd uitgedaagd altijd op kritische wijze naar mijn eigen onderzoek te blijven kijken.

Dear Dewleen Baker, Caroline Nievergelt and Adam Maihofer, thank you for the opportunity to combine our work in one paper. Although we were tested on our persistence, I think our collaboration resulted in a valuable manuscript. Thank you!

Lieve vrienden uit 'het Oosten', in het bijzonder Robert, Manon, Marcel, Marleen, Paul en Joyce, bedankt voor jullie interesse, de gezellige (fiets)weekendjes weg en de vele gezellige borrels. **Marcel,** bedankt dat jij er voor Sander bent. **Sanne en Aad,** bedankt voor de appjes en wat fijn dat we samen borrelen!

Lieve William, Nicol en Pascal, bedankt voor alle gesprekken, de moed en het vertrouwen dat jullie mij hebben gegeven en natuurlijk de afleiding met de vele gezellige borrels, etentjes en natuurlijk de Zwarte Cross.

Lieve Sjors, Sil, Wessel, Taike en Maurice; het Moortgat, de Move, Tivoli, het Hart, de Max pub, Lowlands, Pinkpop, de Zwarte Cross, Pukkelpop, met andere woorden: we houden wel van een feestje/concertje. Bedankt voor de mooie tijden! **Maurice,** wat fijn dat wij onze defensieverhalen konden delen en jij soms enige toelichting kon geven bij de vele afkortingen.

Lieve paranimfen, wie anders dan jullie wil ik op mijn verdediging naast me hebben.
Jessie, terwijl ik met koffie in mijn hand stond om mij aan je voor te stellen, vroeg jij of ik misschien koffie wou en dat was de start van alles. Heel veel (halve) bakjes koffie volgden en deze werden later aangevuld met wijntjes, biertjes, bitterballen en kaasstengels. De avonturen op weg naar deelnemers, asymmetrische gewaden, Kapitein Smulders, rolstoelraces, verhuizingen/huizen kopen, de start van relaties en jouw huwelijk met Jeroen. We hebben vele mooie avonturen beleefd, maar ook op mindere momenten konden we samen praten. Ik ben je dankbaar voor alle gezelligheid, de support en het feit dat jij me altijd een beetje in de gaten houdt. Lieve Jessie, ik wil jou, samen met Jeroen, een hele gelukkige maar vooral gezonde toekomst wensen.
Mitz, we hebben lange tijd samengewerkt bij het onderzoekscentrum en delen de interesse voor onze deelnemers en de problematiek. We hadden in die tijd hele rituelen opgebouwd waarbij standaard gestart werd met koffie (of thee in mijn geval). We hebben altijd gepraat over onze onderzoeken, elkaar geholpen, ideeën uitgewisseld en elkaars werk gelezen. Ik heb

dat ontzettend gewaardeerd, want ik kon altijd op je terugvallen en jij gaf mij het vertrouwen dat ik het kon. Samen naar congressen in San Diego, Toronto, Wenen, waar we het altijd erg naar ons zin hebben gehad. Maar ook in ons leven naast het werk hebben we veel gedeeld en de wekelijkse borrels (inmiddels iets minder ;)) waren ons moment. Bedankt voor de super samenwerking, de support en natuurlijk ook voor alle gezelligheid! Lieve Mitzy, ik wens jou en Matthijs heel veel geluk samen in jullie nieuwe (eigen) huis!
Jeroen en Matthijs, bedankt dat jullie er zijn voor deze meiden, ze een beetje in de gaten houden en ons vergezellen op onze eetdates.

Lieve schoonfamilie, Annie, Wim, Mariël, Manfred, Lucas, Dennis en **Carola,** vanaf het begin hebben jullie ervoor gezorgd dat ik me op mijn gemak heb gevoeld bij jullie. Bedankt voor jullie steun, hulp en natuurlijk voor de gezelligheid.

Lieve familie,
Koen en **Sam,** mijn grote kleine broers. Wij zijn de drie musketiers en we zijn nooit lang van elkaar gescheiden. Jullie zijn er altijd in goede en in minder goede tijden. Samen in de kroeg of om de tafel zijn we op ons best, maar op de latten of in een festivaltent komt het ook altijd goed. **Koen,** we kunnen altijd praten en ik vind het fijn dat wij onze interesses delen. Ik heb veel bewondering voor jouw betrokkenheid en liefde voor de mensen die minder kansen hebben gehad dan wij. **Sammie,** met jouw ongekende energie en vrolijkheid weet je altijd een lach op mijn gezicht te toveren (hoe irritant ook). Je bent nooit te beroerd om een handje in de lucht te steken (?!). Bedankt jongens, voor alles!
Anais, thank you for being there for Koen and for all your enthousiasm and support during these past years. You really helped me to 'don't stop believing'! Merci infiniment.
Egbert en Riky, papa en mama, het moeilijkste stuk van dit hele dankwoord en de reden dat ik ertegenop heb gezien om dit te schrijven. Ik ben jullie namelijk ontzettend dankbaar; voor jullie vertrouwen, jullie liefde, jullie steun (op soms hele moeilijke momenten) en voor de vele adviezen en (soms strenge) motiverende woorden. Jullie hebben ons geleerd je altijd in te zetten voor de medemens en om nooit op te geven en een resultaat daarvan hebben jullie nu in handen. Zonder jullie had ik dit nooit gekund (al zullen jullie zeggen van wel). Bedankt dat jullie er altijd zijn voor ons.

Lieve Sander, jij vond het niet nodig dat ik je zou bedanken in mijn proefschrift en dat is precies zoals jij bent. Maar zonder jou had ik dit nooit gekund (al is het maar vanwege de vormgeving ;)). San, jij hebt mij hier doorheen geholpen op alle mogelijke manieren en dankzij jou ligt dit 'boekje' hier. Naast het promoveren hebben we vele mooie momenten en uitdagingen gehad in de afgelopen jaren, zoals een huis kopen, reizen en samen fietsen, maar ook in de moeilijke periodes hebben wij geleerd hoe sterk wij samen kunnen zijn. Ik ben je dankbaar voor je bizarre hoeveelheid geduld, je rust, je liefde, je humor (hoe flauw ook) en je steun. Jij maakt mij ontzettend gelukkig!

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In submission

Reijnen, A., Geuze, E., Eekhout, I., Maihofer, A.X., Nievergelt, C.M., Baker, D.G., Vermetten, E., Biological profiling of plasma neuropeptide Y in relation to posttraumatic stress symptoms in two combat cohorts. In submission.

Reijnen, A., Geuze, E., Gorter, R., Vermetten, E. Development of self-directedness and cooperativeness in relation to PTSD symptom trajectories after deployment to a combat zone. In submission.

Curriculum Vitae

Alieke Reijnen was born on March 27, 1987 in Velp, the Netherlands. In 2005, she started her bachelor in Clinical and Health Psychology at Utrecht University, where after she enrolled in the master in Clinical and Health Psychology. She fulfilled both her clinical internship and her master thesis at Foundation Centrum '45, working with veterans and refugees and studying daily stressors and the quality of life of refugees in multidisciplinary treatment. In 2010, she obtained her Clinical and Health Psychology master's degree and also finished a bachelor in Neuropsychology, after which she started her master in Neuropsychology. During this year, she wrote a thesis on physiological responses to subliminal and supraliminal signals of fear under the supervision of dr. Baas and dr. Heitland. In addition, because of her interest in trauma-related mental health problems, she conducted a research internship at the research centre of the Military Mental Healthcare under supervision of Dr. Geuze and Dr. Rademaker. In 2011, she obtained her second master's degree cum laude. After this, she started working at the research centre as a research assistant on various projects and continued as a PhD student in the ongoing study: 'Prospective Research on Stress-related Military Operations' under supervision of Prof. dr. Joëls, Prof. dr. Vermetten, and Dr. Geuze. She studied potential risk factors for the development of posttraumatic stress disorder in Dutch military personnel after deployment to Afghanistan. Her work resulted in this dissertation.