The neurobiological background of posttraumatic stress symptomatology throughout the lifespan

Hilberdink, C.E.

Publication date
2023

Document Version
Final published version

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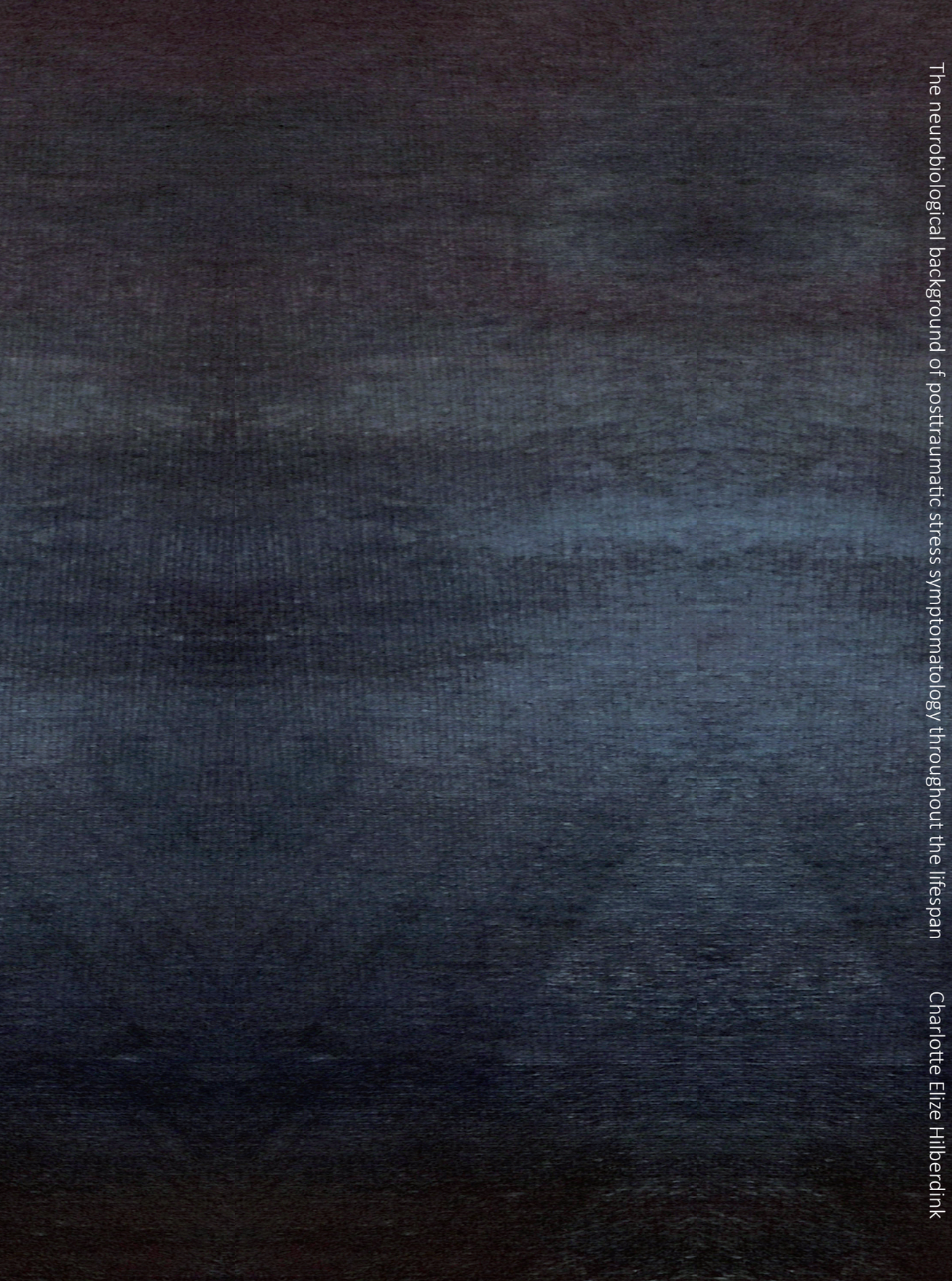

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COLOFON

The research for this doctoral thesis received financial assistance from research grants from The European Commission Horizon 2020 [#874739]; a Brain and Behavior Research Foundation [#24928]; The Netherlands Organisation for Health Research and Development, ZonMw [Veni #91617037]; The Amsterdam Brain and Cognition Centre (ABC, University of Amsterdam), and Nutricia Research Foundation.

Author: Charlotte Elize Hilberdink
Cover art, painted by: Dr. Mandy Spaan
Printed by: Print Service Ede

ISBN: 978-90-832951-6-9

Printing of this thesis was financially supported by Amsterdam UMC, location Amsterdam Medical Center (AMC).

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ACADEMISCH PROEFSCHRIFT

ter verkrijging van de graad van doctor
aan de Universiteit van Amsterdam
op gezag van de Rector Magnificus
prof. dr. ir. P.P.C.C. Verbeek

ten overstaan van een door het College voor Promoties ingestelde commissie,
in het openbaar te verdedigen in de Agnietenkapel
op vrijdag 24 februari 2023, te 16.00 uur

door Charlotte Elize Hilberdink
geboren te Nunspeet

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Faculteit der Geneeskunde

In Flanders Fields by Major John McCrae – 1915 – Boezinge

Written on the battlefield, where he worked as a medical doctor and lost his life to the war

In Flanders Fields the poppies blow
Between the crosses, row on row,
That mark our place; and in the sky
The larks, still bravely singing, fly
Scarce heard amid the guns below.
We are the Dead. Short days ago
We lived, felt dawn, saw sunset glow,
Loved, and were loved, and now we lie
In Flanders Fields.
Take up our quarrel with the foe:
To you from failing hands we throw
The torch; be yours to hold it high.
If you break faith with us who die
We shall not sleep, though poppies grow
In Flanders Fields

CONTENTS

CHAPTER 1

GENERAL INTRODUCTION

GENERAL INTRODUCTION

Trauma and PTSD

The majority of people experience at least one potentially traumatic event throughout their lives (Benjet et al., 2016; de Vries & Olff, 2009). A potential traumatic experience is a stressful event that poses threat or serious injury, such as a major car accident, physical or sexual assault, or threatened death, that can be directly experienced or indirectly witnessed by an individual (The American Psychiatric Association, 2013). It is common that individuals initially experience negative responses to such an event in its acute aftermath. Feelings of distress or anxiety when thinking about the event are considered to be normal but will usually fade away within the first few days or weeks. Yet, in a minority of individuals these responses do not disappear and can result in psychopathology, such as depression, anxiety and posttraumatic stress disorder (PTSD; e.g. Atwoli et al., 2015; Goldstein et al., 2016; McLaughlin et al., 2012, 2013). Approximately 10% of individuals develops PTSD after experiencing a potentially traumatic event with an estimated lifetime worldwide prevalence of 5.6% in an adult population (Koenen et al., 2017) and an overall PTSD prevalence rate ranging from 4.7-25% in children and adolescents (Alisic et al., 2014; Copeland et al., 2007; McLaughlin et al., 2013).

Characteristic symptoms of PTSD are re-experiencing the traumatic event, avoiding stimuli that are related to the traumatic event, exhibiting negative alterations in cognition and mood, and hyperarousal and –reactivity (American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5), The American Psychiatric Association, 2013). These symptoms are associated with substantial distress, impaired functioning on multiple psychosocial domains such as self-care, social interactions and relationships, and mobility (Jellestad et al., 2021; National Institute for Health and Clinical Excellence, 2005). Additionally, PTSD is highly comorbid with other psychological disorders, such as personality disorders, depression, anxiety, and substance abuse (Debell et al., 2014; Grant et al., 2008; Kessler, 1995; Koenen et al., 2008b; Lewis et al., 2019; Morris et al., 2012; Perkonigg et al., 2000; Rytwinski et al., 2013; Snoek et al., 2021). As a result, PTSD is a considerable burden for the individual's wellbeing as well as for the larger society.

Several theories exist on the core behavioural disturbances that underlie PTSD's hallmark symptoms (Pitman et al., 2012). These theories most prominently focus on impaired fear inhibition and extinction, and impaired memory processing in PTSD. A theory describing the behavioural disturbances related to hyperarousal and hyperreactivity symptoms suggests that experiencing intense arousal and fear in response to specific reminders of a traumatic event, such as smells, sounds and sights, would generally diminish in the first few hours or days after the traumatic event. However, in individuals with PTSD these fear reactions do not diminish and remain present, even in the absence of a real threatening situation, and are induced in response

to triggers that may even be unrelated to the traumatic event (Buckley, 2000; Chemtob et al., 1988; Foa et al., 1989; Lissek & van Meurs, 2015; Rothbaum & Davis, 2003). This overgeneralization of fear may ultimately result in attentional bias towards perceived threatening information, either related or unrelated to the trauma (Aupperle et al., 2012; Fani et al., 2012; Green et al., 2017; Lis et al., 2020; Litz et al., 2000; Milad et al., 2006, 2009; Thome et al., 2018). Another cognitive model describes behavioural disturbances involved in re-experiencing memories of the traumatic event and suggests that poor contextualization during memory encoding in the aftermath of trauma is affected in individuals with PTSD, resulting in increased automatic and spontaneous retrieval and triggering of emotional intrusive memories related to the traumatic event (Brewin, 2009, 2014, 2015; Ehlers et al., 2004; Ehlers & Clark, 2000; Meyer et al., 2015; Michael et al., 2005).

Neurobiological correlates of PTSD

Dysfunctioning of fear inhibition and extinction and affected memory processing in PTSD are potentially associated with disruptions in neurobiological processes. Over the past decades, several studies found evidence for the link between cognitive models of behavioural disturbances and their potential underlying disrupted neurobiological processes and correlates (for review, see e.g. (Akiki et al., 2017; Hayes et al., 2012a; Hayes et al., 2012b; Liberzon & Abelson, 2016; Quinones et al., 2020). Especially disruptions in processes of the stress systems emerged as neurobiological correlates that play a critical role in PTSD (Jovanovic & Norrholm, 2011; Liberzon & Abelson, 2016; Rauch et al., 2006; Samuelson, 2011; Shalev & Bremner, 2016), although much remains unknown about the neurobiological correlates of PTSD.

Neural structure and functioning

Various meta-analytic studies have yielded convergent evidence and demonstrated associations between PTSD and neural functioning using neuroimaging methods such as structural and functional magnetic resonance imaging (MRI, fMRI), positron emission tomography (PET) and single-photon emission computed tomography (SPECT). Predominantly, a dysfunctional neurocircuitry consisting of fronto-limbic brain regions was identified in individuals with PTSD symptoms during a state of rest (Koch et al., 2016; Wang et al., 2016) and in response to a variety of stressors (Hayes et al., 2012a; Patel et al., 2012; Sartory et al., 2013). Key neural regions within this neurocircuitry are the amygdala, hippocampus, anterior cingulate cortex and medial prefrontal cortex (mPFC) (Bremner et al., 2008; Liberzon & Sripada, 2007; Shin, 2006). For example, the most common impairments that have been found concerning the amygdala are increased volume and increased activation in response to emotional or threatening stimuli (e.g. Etkin & Wager, 2007; McLaughlin et al., 2014; Pitman et al., 2012; Rauch et al., 2000), whereas the hippocampus and mPFC show smaller volumes (Kitayama et al., 2005; Pitman et al., 2012) and reduced activity to similar stress-related stimuli (Etkin & Wager, 2007; McLaughlin et al., 2014; Pitman et al., 2012). In addition, impaired communication between the amygdala and mPFC in response to threatening stimuli may underlie the emotional and fear-related problems in

individuals with PTSD (Gilboa et al., 2004; St. Jacques et al., 2011; Stevens et al., 2013; Wolf & Herringa, 2016). These neural alterations seem to covary with the severity of PTSD symptoms (Etkin & Wager, 2007; Pitman et al., 2012; Thome et al., 2020; Wolf & Herringa, 2016) and some changes in fronto-limbic regions appear to be enduring with long-term alterations still present after trauma exposure, which suggests persistent adaptation of these neural regions (Bremner, 2003; Bremner et al., 2008; Dannlowski et al., 2012; Dickie et al., 2011; George et al., 2022; Milad et al., 2009). Even after recovery of PTSD some of their alterations remain to exist (Yoon et al., 2017) and appear to be critical for remission, probably due to their inherency with fear extinction and extinction retention processes in PTSD (George et al., 2022). The alterations in this neurocircuitry are mostly based on research in adults but seems to be largely overlapping between children, adolescents and adults, although studies show some inconsistencies between affected regions (Karl et al., 2006; Kribakaran et al., 2020).

Hypothalamic pituitary adrenal axis and autonomic stress system functioning

Converging evidence also links PTSD to abnormal regulation and functioning of the hypothalamic pituitary adrenal (HPA) axis and autonomic nervous system (ANS; for overview see Figure 1). Both the HPA axis and ANS are central regulators of physiological responses to and recovery from a stressor. The HPA axis is the main coordinator of neuroendocrine stress responses and after activation by a stressor a hormonal cascade will commence that eventually leads to secretion of its end product; the stress hormone cortisol (Herman et al., 2016). Thereafter, cortisol binds to glucocorticoid receptors (GR) to activate a negative feedback loop that consequently inhibits cortisol release and restores the steady state of homeostasis. Activation of the ANS by a stressor will mainly control involuntary bodily responses to a stressor in order for an individual to survive under stressful circumstances, such as increasing heart rate and breathing to flee from or fight a danger (McCorry, 2007). The ANS consists of the sympathetic (SNS) branch that predominates during unexpected stressful situations, and the parasympathetic (PNS) branch that predominantly activates during rest and digestive processes, which interact and alternate their activity for the maintenance of homeostasis and survival. Several cross-sectional and prospective studies identified core features of HPA and ANS functioning in individuals who suffer from PTSD symptoms. Concerning HPA axis functioning in PTSD, associations have been found between impairments in HPA axis and GR functioning and (pre)clinical symptoms and diagnosis of PTSD (de Kloet et al., 2006; Dunlop & Wong, 2019; Heim & Nemeroff, 2009; Klaassens et al., 2012; Pan et al., 2018; Schumacher et al., 2019; Sherin & Nemeroff, 2011; Speer et al., 2019). Several studies investigating the predictive value of HPA and GR functioning as a risk factor for PTSD symptom development identified low cortisol levels in the first few hours after trauma to predict PTSD (e.g. Mouthaan et al., 2014; Olff & van Zuiden, 2017). These low levels may be related to increased sensitivity and frequency of GRs after trauma exposure and subsequent high negative feedback on cortisol output shutting down the stress response too quickly (Rohleder et al., 2010; van Zuiden et al., 2013, 2012a; Yehuda et al., 2004). This fits with findings that dysregulated GR sensitivity and specific genetic factors involved in HPA and glucocorticoid pathway functioning are present

before exposure to trauma (van Zuiden et al., 2013). Accordingly, specific single nucleotide polymorphisms of the GR gene related to alterations in HPA and GR sensitivity have been identified as genetic vulnerabilities in individuals with PTSD (Banerjee et al., 2017; Boks et al., 2015; Castro-Vale et al., 2016; Koenen et al., 2009; Logue et al., 2015; Navarro-Mateu et al., 2013; Sheerin et al., 2020; Steudte-Schmiedgen et al., 2015). Yet, results on HPA functioning in patients with PTSD are still somewhat unequivocal and require further investigation. For example, adolescents with (partial) PTSD showed a blunted cortisol response to trauma-related stimuli (Zantvoord et al., 2019), whereas in adults both increased and blunted responses were identified (de Kloet et al., 2006; Elzinga et al., 2003; Zorn et al., 2017). Additionally, long-term alterations in HPA axis functioning were demonstrated in adult women who were exposed to trauma during their lives, although there are indications that effects may only be persistent after exposure to a traumatic event during childhood rather than during adulthood (Klaassens, 2010). Findings concerning functioning of the other major stress system, the ANS, in relation to PTSD mostly comes from research that examined sleep patterns, cardiovascular functioning or fear conditioning. Specifically, studies found activation markers of the SNS early after trauma exposure that appeared to predict subsequent PTSD. For example, elevated heart rate responses have been demonstrated in adults with PTSD during rest, in response to stress-inducing, trauma-related stimuli (Pole, 2007), and soon after exposure to a potential traumatic event (Morris et al., 2016).

Developmental timing of life adversity

The effects of potential traumatic events seem to depend on its timing throughout the lifespan (Dunn et al., 2017; Ogle et al., 2013; Shrira et al., 2012; Straussner & Calnan, 2014; Thornberry et al., 2001). Particularly during early development, the body and brain appear to be more sensitive to traumatic events, and adverse experiences during critical periods of development may have a strong impact on later physical and mental health (Dunn et al., 2017; Herringa, 2017; Heyn et al., 2019; Lupien et al., 2009; Ogle et al., 2013; Weems, 2019). For example, maternal stress during the early prenatal period and traumatic maltreatment during childhood subsequently increase the risk for psychopathological outcomes (Glover et al., 2018; Teicher & Samson, 2013). However, exposures to adversity such as traumatic life events during later life periods beyond childhood and adolescence, may also trigger psychopathology development (de Vries & Olff, 2009; Klaassens et al., 2012) with the potential to have a chronic course (Hiskey et al., 2008). Although adversity affects the risk for development of psychopathology throughout the entire lifespan, the exact resulting psychopathological vulnerabilities may differ depending on its timing (Zlotnick et al., 2008). As there are indications that the vulnerability and involvement of neurobiological mechanisms may differ across distinct life periods (Herringa, 2017; Heyn et al., 2019; Lupien et al., 2009; Weems, 2019) the impact of adversity on the risk to develop PTSD symptomatology could therefore be differential based on the time of exposure to adversity across the lifespan. Yet, the involved stress-related neural, endocrine and cardiac mechanisms are still largely unknown and this lack of knowledge currently hampers us to improve our understanding of who is at risk for development of PTSD symptoms. For example, information about HPA and ANS functioning

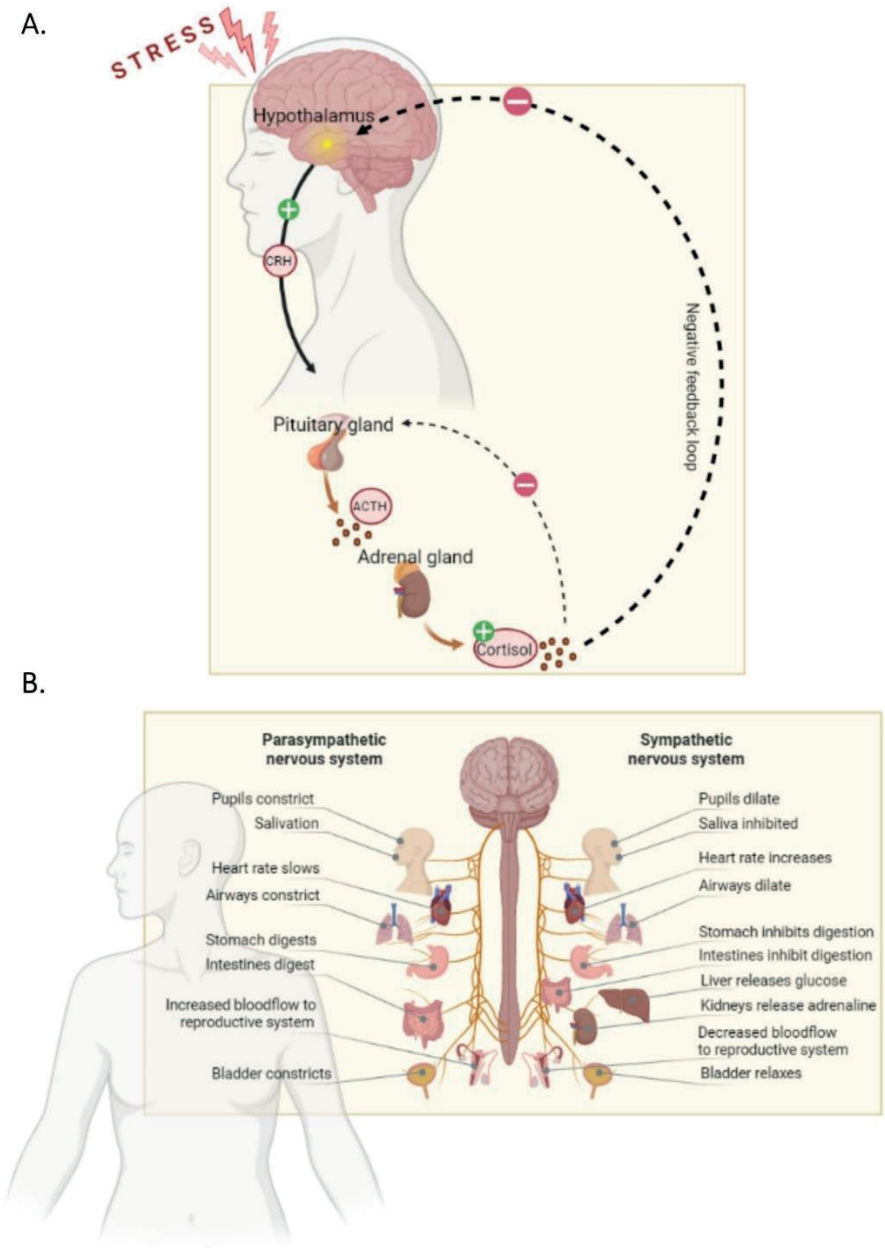

Figure 1. Overview of **A.** The hypothalamic pituitary adrenal (HPA) axis and **B.** The sympathetic and parasympathetic branch of the autonomic nervous system (ANS).

around the time of a potential trauma in relation to subsequent PTSD symptoms in children and adolescents is scarce and inconsistent (Jones-Alexander et al., 2005; MacMillan et al., 2009; Siciliano et al., 2022). The underlying vulnerability of involved neurobiological mechanisms may not be directly generalizable to all populations, across children, adolescents and adults, and warrants further elucidation to improve and identify individual risk to ultimately promote early preventive interventions.

Outline of this thesis

This thesis addresses the following aims:

In **chapter 2**, we aim to offer insight in how timing of adversity across the lifespan impacts the presence and type of trauma-related symptomatology during late adulthood within men and women participating in the Dutch famine birth cohort study. Potential differential impact of exposure to adversity across the lifespan is considered during the prenatal period, childhood, and mid-to-late adulthood.

In **chapter 3**, we aim to improve and broaden our understanding of the effects of timing of adversity on PTSD vulnerability by exploring the impact of multiple trauma exposures across these three life periods on PTSD symptom severity during late adulthood. We additionally intend to investigate whether the impact of adversity is modulated by genetic variation related to dysfunctional biological correlates of PTSD. As in **chapter 2**, this study is performed in members of the Dutch famine birth cohort study.

In **chapter 4**, we specifically focus on adolescents to gain insight in neurobiological stress-related mechanisms at the level of neural, endocrine and cardiac functioning in response to a psychosocial stressor that are associated with high levels of PTSD symptoms after trauma exposure. We present findings from a neuroimaging study within a subset of participants from the Amsterdam Born Children and their Development birth cohort.

In **chapter 5**, we present findings regarding the individual variation in the neurobiological and cognitive stress mechanisms that underlie intrusive memory development in a healthy adult male population. To increase the translational value and create a more real-life trauma setting, we applied an adjusted trauma film paradigm in this cross-sectional randomized-controlled trial.

In the final **chapter 6**, we provide a summary of our main findings and describe their implications in a general discussion, and offer suggestions for future research.


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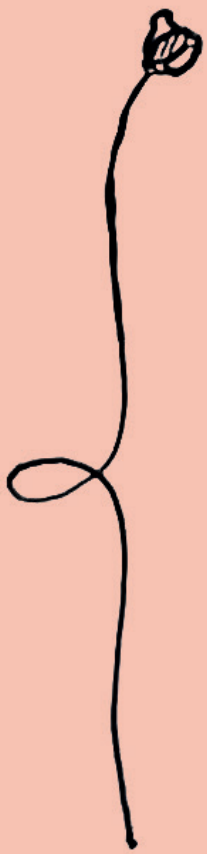


CHAPTER 2

The impact of adversities across the lifespan on psychological symptom profiles in late adulthood: a latent profile analysis

Charlotte E. Hilberdink, Mirjam van Zuiden, Miranda Olff, Tessa J. Roseboom & Susanne R. de Rooij

Submitted for publication, Preprint: OSF (2022)*, 10.31219/osf.io/c6fgd*

Keywords: prenatal undernutrition, childhood adversity, trauma, mood and anxiety, psychosis

Abstract

People commonly face adverse circumstances throughout life, which increases risk for psychiatric disorders, such as anxiety, depression, psychosis and posttraumatic stress disorder (PTSD). Adversities may occur during different periods in life. Especially adversity during early periods has been suggested to put individuals at risk for adverse mental health outcomes. Here, we investigated whether timing of adversity during the prenatal period, childhood, or mid-to-late adulthood differentially impacted classification into late adulthood symptom profiles.

We performed sex-stratified Latent Profile Analysis to identify latent profiles regarding anxious, depressive, psychotic and PTSD symptoms in *n*=568 Dutch famine birth cohort members (*n*=294 women, *n*=274 men, mean age (SD)=72.9(0.8)). Cross-sectional late adulthood symptomatology, childhood traumatic maltreatment and adulthood trauma were based on self-report questionnaires. Prenatal adversity was considered present when individuals were prenatally exposed to the 1944-45 Dutch famine.

In both men and women, we identified one anxious/depressive profile and three profiles with approximately equal severity of all symptom types within each profile, yet differentiating in overall severity (low, mild, high) between profiles. We additionally found a PTSD symptom profile in women. In men, logistic regression models showed significant associations between prenatal, childhood and adulthood adversity and profile classification, with differential effects depending on timing and most profound effects of child maltreatment. In women, childhood and adulthood adversity significantly increased classification probability into almost all profiles, with no significant effect of prenatal adversity.

These findings support a time-dependent and sex-specific impact of adversity during different periods across the lifespan on psychological health, with consequences into late adulthood.

1. Introduction

People commonly face a variety of adverse circumstances throughout their lives, which may include physical or psychosocial environmental threats, e.g. poor nutrition, maltreatment, and traumatic accidents (Emerson, 2013). Although many psychological and biological factors are involved in determining an individual's mental health outcome upon exposure to adversity (Berens et al., 2017; Jacob, 2013), exposure to adversities per se is well known to increase the risk for a broad range of psychological problems later in life (Hughes et al., 2017; Scott et al., 2010). These psychological problems may go well beyond the psychiatric disorders formally recognized in the DSM-5 as being stressor- or trauma-related, such as posttraumatic stress disorder (PTSD), as exposure to adversity is associated with increased risk for subsequent mood-, anxiety-, schizophrenia spectrum and other psychosis-related disorders (Beards et al., 2013; Betts et al., 2015; El-Khoury et al., 2021; Mandelli et al., 2015; van den Bergh et al., 2020). Also, the risk for adverse mental health outcomes is commonly found to increase dose-dependently with increasing adversity severity and frequency (Dohrenwend, 2006).

Adverse circumstances may occur during various periods in life. Adversities during early life periods are considered to particularly put exposed individuals at risk for developing unfavourable mental health outcomes, as the brain undergoes critical changes during these developmental periods which may render it particularly vulnerable to adverse circumstances (Hemady et al., 2022; Nelson & Gabard-Durnam, 2020; Opendak & Sullivan, 2016). These critical periods include the prenatal (*in utero*) period and childhood extending into adolescence (Rice & Barone, 2000).

Regarding prenatal exposure, maternal exposure to both psychological and physical stressors as well as maternal psychological distress during pregnancy have been associated with an increased risk for mood and anxiety disorders, schizophrenia spectrum disorders (van den Bergh et al., 2020), and PTSD (Betts et al., 2015) in the offspring during early adulthood. Additionally, famine exposure during the prenatal period has been associated with an increased risk for a multitude of mental disorders in adulthood, including depression, schizophrenia, and psychosis (Brown et al., 2000; Hoek, 1996; Lumey et al., 2011; van den Broek & Fleischmann, 2019). Findings from the Dutch famine birth cohort have demonstrated that specific outcomes may depend on timing of exposure during pregnancy and sex. Exposure to undernutrition during early gestation was associated with increased anxious and depressive symptoms in men in mid-adulthood, while no associations were found in men exposed during mid or late gestation, nor in women (de Rooij et al., 2011).

Childhood adversities have also been demonstrated to have strong associations with adverse mental health outcomes, including all classes of mood and anxiety disorders (Green et al., 2010; Kessler et al., 2010; Mandelli et al., 2015), as well as PTSD (Brewin et al., 2000; El-Khoury et al., 2021; Messman-Moore & Bhuptani, 2017) and psychosis (Trotta et al., 2015; Varese et al., 2012), with persistent risk from childhood throughout mid adulthood. Of note, specific subtypes of

adversity during childhood have been shown to particularly increase the risk for mental health problems. Varied methodological approaches, such as the use of different categorizations and measuring methods (Hales et al., 2022; Kessler et al., 2010; Negriff, 2020), may have led to inconsistent findings between studies. However, Sayyah et al. (2022) found differential effects and observed strong associations between childhood maltreatment and adult depressive, anxious and PTSD symptoms, whereas adversities related to maladaptive family function less broadly predicted adult PTSD symptoms and not depressive and anxious symptoms.

Although not defined as a vulnerable developmental period, exposure to adversity during adulthood is also associated with increased risk for a broad range of subsequent psychological problems. For example, exposure to traumatic events in adulthood is associated with increased risk of subsequent onset or worsening of depressive symptoms (Aldinger & Schulze, 2017), presence of both anxious and depressive symptomatology (van Veen et al., 2013), PTSD prevalence (Frans et al., 2005), onset and presence of psychotic symptoms (Beards et al., 2013) and psychotic relapse (Martland et al., 2020).

There is thus strong evidence that exposure to adversity during prenatal life, childhood and adulthood increases the risk for a wide range of psychological symptoms and disorders in general, and particularly for mood-, anxiety-, schizophrenia spectrum and other psychosis-related disorders as well as the trauma-related disorder PTSD. However, few studies have investigated the specific impact of adversity during multiple distinctively different periods in life on mental health within the same study population. The ones that did, measured cumulative exposure to adversity within and across different life periods (Copeland et al., 2018), and did not study exposure within different periods across the lifespan separately. In addition, most studies investigating the effects of adversity on mental health, irrespective of the life period of exposure, focused either on specific psychological disorders and their respective symptoms without taking the absence or presence of other comorbid disorders and their respective symptoms into account (Brewin et al., 2000; Mandelli et al., 2015), or only investigated comorbidity between maximally two disorders at the same time (Betts et al., 2015). Investigating comorbidity among a broad range of psychological disorders is relevant, as it may influence and exacerbate the course within disorders and outcome of symptoms (Breslau, 2001). Furthermore, comorbidity may interfere with diagnostic and treatment efficacy which consequently reduces symptom improvement and recovery (Tang et al., 2018). It thus remains largely unknown whether exposure to adversity across different periods in life increases risk for specific or comorbid psychological disorders.

Latent Profile Analysis (LPA) is a statistical approach that can be used to identify groups of individuals with similar symptom patterns based on their occurrence and severity levels (McCutcheon, 1987) and to study specificity and/or comorbidity of a broad range of psychological symptoms in a comprehensive manner. In the present study, we used LPA to identify latent profiles of anxious, depressive, psychotic and PTSD symptoms and subsequently investigated

whether exposure to adversity at different periods across the lifespan impacted classification into the observed symptom profiles measured in late adulthood. This was done in the Dutch famine birth cohort, a historical birth cohort of men and women born around the time of the 1944–1945 Dutch famine in Amsterdam, the Netherlands, aiming to investigate effects of prenatal famine exposure on adult health (Bleker et al., 2021). This cohort provides the unique opportunity to measure impact of adversities during three different periods across the lifespan, specifically during the prenatal period, early childhood, or mid-to-late adulthood, on a wide range of psychological symptoms and their comorbidity within the same population. Of note, we specifically focused on profiles of anxious, depressive, PTSD- and psychotic symptoms as their occurrence in adulthood has frequently been associated with exposure to adversity during these three life periods and they are often comorbid (Dernovšek & Šprah, 2009; Qassem et al., 2021; Spinhoven et al., 2014). Furthermore, we performed our analyses in men and women separately, as previous studies showed differences in the impact of specific adversities on risk for subsequent psychological problems between men and women, including in the cohort currently investigated (de Rooij et al., 2011). Also, there is evidence that that classification of participants into these latent profiles is biased if within-pattern sex differences were not accounted for (van Zuiden et al., 2022).

2. Methods

2.1 Participants

Participants were members of the Dutch famine birth cohort consisting of men and women born as term singletons in the Wilhelmina Gasthuis in Amsterdam around the time of the Dutch famine (1 Nov 1943-28 Feb 1947). At the start of the study, a total of N=2414 eligible participants were included (complete overview of cohort establishment and following data collection waves (Bleker et al., 2021; Ravelli et al., 1998)). Data for the current study were collected in follow-up wave-V, starting in 2018. Eligible cohort members (N=1207) were invited by mail to participate in a paper-and-pencil survey. A total of N=595 (49.3%) cohort members provided written informed consent to participate (flowchart in Supplementary Figure S1), of which N=568 (47.1%, *n*=294 women, *n*=274 men) completed the relevant questionnaires for this study. Descriptive overview of participant's demographics, characteristics, exposure to adversities and late adulthood psychological symptoms are displayed in Table 1. The Medical Ethics Committee of the Academic Medical Center, Amsterdam, Netherlands, concluded that a full review and official approval of this study wave was not required according to Dutch law for medical research.

2.2 Measures

2.2.1 Adversity during different periods in life

Prenatal undernutrition

Prenatal undernutrition was considered present if average daily maternal rations during any 13-week period of gestation were below 1000 calories (Burger et al., 1948; Ravelli et al., 1998).

Periods of 16 weeks were delineated to differentiate between those who were mainly exposed during late gestation (born between 7 Jan and 28 Apr 1945), mid gestation (born between 29 Apr and 18 Aug 1945), and early gestation (born between 19 Aug and 8 Dec 1945; for overview, see Supplementary Figure S2)). People born before 7 Jan 1945 and conceived after 8 Dec 1945 were considered unexposed to famine in utero and acted as control group.

Childhood traumatic maltreatment

We used the Dutch self-report Childhood Trauma Questionnaire (CTQ, 27 items, range 1-5 'Never true' to 'Very often true') (Bernstein et al., 2003; Thombs et al., 2009; van Schie et al., 2017) to measure traumatic maltreatment experiences during childhood and adolescence, which includes five subscales: Emotional; Sexual; and Physical Abuse; Emotional; and Physical Neglect. One item (item 24) was excluded from analyses because of previous invalid translation (Thombs et al., 2009). We calculated sum scores to measure total reported childhood traumatic maltreatment and subscale scores to measure reported childhood maltreatment subtypes. The Minimization-Denial subscale (MD; range 0-3) was calculated to determine response bias for possible underreporting childhood maltreatment by recoding its three items; 1 till 4=0 and 5=1.

Adulthood trauma exposure

We measured mid-to-late adulthood trauma exposure using the Dutch self-report Life Events Checklist (LEC-5) (Boeschoten et al., 2014a) including 17 traumatic event types, either directly experienced, witnessed, encountered in the line of work or occurring to close family members/friends. We specifically inquired on events in the past 15 years to be able to investigate parallel changes in anxiety and depression symptom severity (planned future research), measured over this 15-year period. We calculated sum scores (range 0-17) to measure total number of types experienced.

2.2.2 Late adulthood psychological symptoms

We measured depression and anxiety symptoms in the past month using the Dutch Hospital Anxiety and Depression Survey (HADS, 14 items, range 0-4, higher scores indicating more symptoms) (Spinhoven et al., 1997) that contains Anxiety and Depression subscales (both seven items). We assessed PTSD symptoms in the past month using the Dutch PTSD Checklist for DSM-5 (PCL-5, 20 items range 0-4, higher scores indicating more symptoms) (Boeschoten et al., 2014b) measuring four DSM-5 diagnostic symptom clusters: Cluster B-*Intrusions;* Cluster C-*Avoidance;* Cluster D-*Negative Cognitions and Mood;* Cluster E-*Arousal and Reactivity alterations.* We assessed (sub)clinical psychotic symptoms using the shortened Dutch Prodromal Questionnaire (PQ-16, 16 items, 0=Disagree 1=Agree) (Ising et al., 2012) measuring perceptual abnormalities/hallucination (9 items); unusual thought content/delusional ideas/paranoia (5 items) and negative symptoms (2 items).

2.3 Statistical analysis

All analyses were performed in women and men separately. A maximum of one missing item per questionnaire was allowed (2 for LEC-5), in which case the missing item was imputed by the participant's mean score on the other items (cases imputed: *n*=1 for HADS Depression and PCL-5 Cluster E; *n*=3 for HADS Anxiety, PCL-5 Cluster B and C; *n*=2 PCL-5 Cluster D; *n*= 19 for PQ-16; *n*=63 for LEC-5). Participants were excluded from all subsequent analyses due to suspected unreliable answers combined with psychotropic medication use and extremely high scores on HADS and PQ-16 questionnaires (*n*=1); multivariate outliers on psychological symptom scores (Mahalanobis Distances; men *n*=20, women *n*=11); or in case of missing Mahalanobis Distances if additionally >25% of items to calculate sub- or total scores were missing and these scores were univariate outliers (Z≥3.29; men *n*=4, women *n*=6).

In the remaining N=526 participants (88.1%, *n*=277 women, *n*=249 men) we performed latent profile analyses (LPA) in Mplus (v8.6 (Muthén & Muthén, 2012)) to identify psychological symptom profiles based on cross-sectional continuous HADS, PQ-16, and PCL-5 total and subscale scores. We followed a recommended 3-step procedure (Asparouhov & Muthén, 2014; Stride et al., 2015; Vermunt, 2010). Model evaluation was based on pre-identified indicators of best-fitting model by testing fit of a 1-profile model and subsequently increase profile number by 1, until addition of a profile was no longer optimal or improved (Nylund et al., 2007). Indicators were Bayesian Information Criterion (BIC), Akaike Information Criterion (AIC) and adjusted-AIC (lower value: better fit), entropy (>.80: indicates adequate profile division) (Celeux & Soromenho, 1996), Lo-Mendell-Rubin-adjusted likelihood ratio test (LMR-A) and bootstrap likelihood ratio test (BLRT, for both tests p<.05 indicates the complex model is relatively better-fitted than a simpler model (Lo et al., 2001; Yang, 2006). Models were estimated with multiple initial random starts and final stage optimizations to reduce risk of reaching local maximum that introduces bias in each bootstrap sample (Asparouhov & Muthén, 2014). For BLRT, we requested 500 starting value sets in the first and 200 in the second step of optimization to avoid local likelihood maxima. Robust maximum likelihood estimator was used as this allowed for inclusion of participants with missing data with robustness against non-normality and non-independence of observations.

To assess associations between adversity during different life periods and symptom profile assignment, we subsequently performed multinomial logistic regression modelling on symptom profile assignment. We ran separate models with differential effects dependent on timing of prenatal undernutrition (dummy-coded; early, mid, and late gestation versus controls), childhood traumatic maltreatment (continuous, CTQ total scores), childhood maltreatment subtypes (continuous, CTQ subscale scores), and adulthood trauma (continuous, LEC total score) as predictors. Several continuous and dummy-coded covariates extracted from questions about demographics and aging-related sensory impairments within the survey were included: educational level (continuous), marital status (2 dummies; widow(er) versus long-term relationship, single versus long-term relationship), aging sensory impairments (dichotomous), and

receiving help filling out the questionnaire (dichotomous). We additionally included covariates for perceived social economic status during childhood (SES; continuous) and MD scores (continuous) in the models with childhood traumatic maltreatment as predictor. Parameters were fixed in case of empty cells in the joint distribution of predictors, covariates and latent profile variables (Djelantik et al., 2021). Results are given as log odds indicating the probability likelihood for classification into assignment in the target profile versus the reference low-symptom severity profile (Table 3 and 4). A False Discovery Rate threshold (5%) was applied to correct the alpha value for significance for multiple comparisons (Benjamini & Hochberg, 1995).

3. Results

3.1 Latent symptom profile labels

Results for latent profile model estimation are described in Supplementary Materials S3. For men, the best-fitting model consisted of 4-profiles and for women 5-profiles (Figure 1).

Descriptive profile labels were based on means of sub- and total scores that determined profile membership (Table 2). In men, we interpreted the resulting profiles as (Figure 1A); “low symptoms” (73.5%) with lowest levels for all four psychological symptom types relative to the other profiles; “anxious/depressive symptoms” (6.0%) with relatively highest levels for anxious and depressive symptoms relative to lower levels for PTSD and psychotic symptoms within-profile and compared to the low and mild symptoms profiles; “mild symptoms” (16.9%) with moderate levels for all four symptom types compared to the other profiles; “high symptoms” (3.6%) with relatively highest levels for all four symptom types compared to the other profiles. In women, similar profiles occurred (Figure 1B, “low symptoms” (58.5%), “anxious/depressive symptoms” (4.7%), “mild symptoms” (20.2%), “high symptoms” (4.7%)), with one additional profile; “PTSD symptoms” (6.9%) with relatively highest levels for PTSD symptoms compared to the other symptom scores within-profile and compared to the low, mild and anxious/depressive symptom profiles.

3.2 Associations between life adversity and profile assignment

3.2.1 Prenatal undernutrition

We found a significant association between undernutrition in early gestation and profile assignment in men (Table 3). Findings suggested that in men exposed to famine during early gestation, probability was higher for classification into the mild- than low-symptom profile compared to unexposed men.

3.2.2 Childhood traumatic maltreatment

We found significant associations between childhood traumatic maltreatment and profile assignment in men (Table 3) and women (Table 4). Findings suggested that men who reported to have experienced more childhood maltreatment were associated with higher probability for classification into the anxious/depressive and high- than the low-symptom profile. In case they

reported more emotional abuse, the probability was higher for classification into the mild-symptom, but lower for classification into the anxious/depressive than low-symptom profile. In case they reported more physical neglect, the probability was higher for classification into the high- than low-symptom profile. In women, reports of more childhood traumatic maltreatment was associated with higher probability for classification into the mild-, PTSD, and high- than low-symptom profile. Reports of more emotional abuse was associated with higher probability for classification into the mild-symptom profile, but when women reported more sexual abuse, the probability was higher for classification into the high- than low-symptom profile.

3.2.3 Mid-to-late adulthood trauma

We found significant associations between adulthood trauma and profile assignment in men (Table 3) and women (Table 4). In men, reports of experiencing more adulthood trauma was associated with higher probability for classification into the high- than low-symptom profile. In women, reports of more adulthood trauma was associated with higher probability for classification into all other symptom profiles compared to the low-symptom profile.

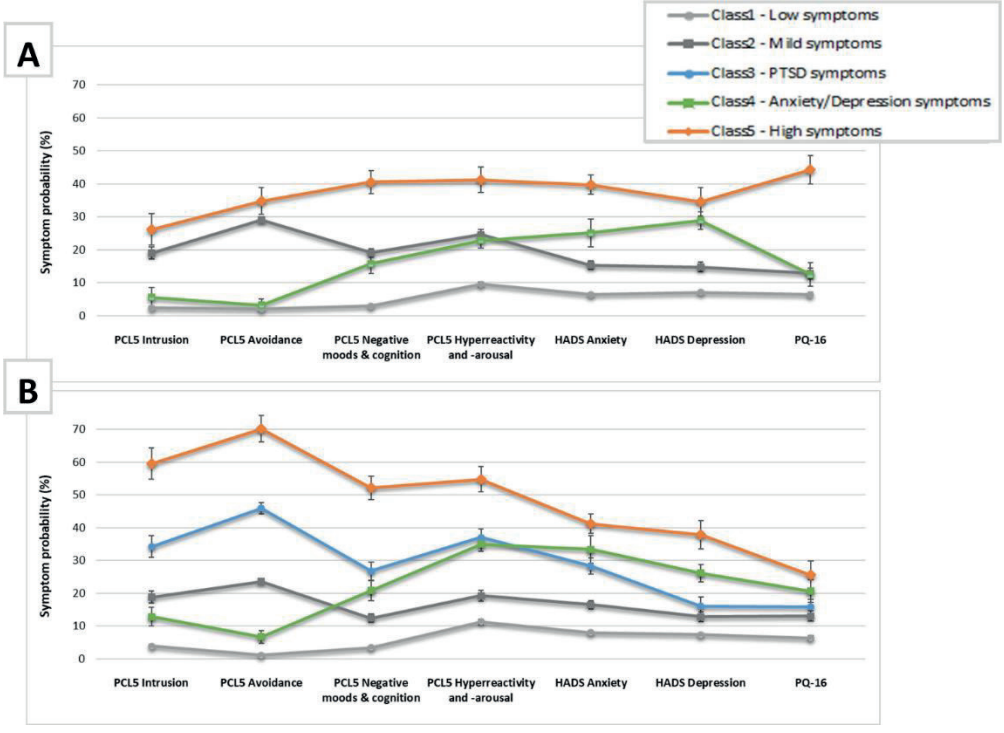


Figure 1. Latent symptom profiles based on psychiatric symptom levels in men **(A)** and women **(B)**. Symptom probability is based on the probability (in %) of the relative symptom score within-profile based on questionnaire subscores of the PCL-5 for PTSD–related DSM5 symptoms, HADS Anxiety and Depression subscores for anxious and depressive symptoms, and total score of PQ-16 for psychotic symptoms. Error bars indicate standard errors.

4. Discussie

In the Dutch famine birth cohort, we observed that in men exposure to undernutrition during early gestation, traumatic maltreatment in childhood and trauma in mid-to-late adulthood were all associated with symptom profile classification. In women, classification was impacted by childhood maltreatment and adulthood trauma, while no effect of prenatal undernutrition was observed. Lastly, we found distinct associations between specific subtypes of childhood adversities and symptom profile classification in both men and women.

Observed psychological symptom profiles

We observed four different symptom profiles in men and five symptom profiles in women. In both men and women, we observed three profiles that included all symptom types of approximately equal severity within each profile, yet differentiated between profiles in terms of their overall severity into low, mild and high severity. We also observed profiles that showed a clear prominence of specific symptom types. Firstly, there was a distinct profile for anxious/depressive symptoms in both men and women in the presence of low PTSD and psychotic symptoms. Within this distinct profile, the severity of anxious and depressive symptom levels was descriptively somewhat lower than within the high-symptom profile, and higher than within the low- and mild-symptom profiles. This particular profile was not unexpected as increased levels of anxious and depressive symptoms have consistently been shown to be comorbid, more so than with other psychiatric disorders (Angold et al., 1999), and both symptoms increase the risk of subsequently developing the other disorder, regardless of variation in study methodology (Saha et al., 2021). Secondly, a distinct PTSD symptom profile was identified in women only, implying that there was a subset of women specifically experiencing PTSD symptoms without comorbidity of the other investigated symptom types. Momartin et al. (2004), who identified a pure PTSD profile next to a comorbid PTSD/depression and pure depression profile using diagnostic grouping in a community sample of Bosnian refugees and war survivors, discussed that this may depend on the character of the experienced trauma. For example, a life-threatening character of an experienced traumatic event solely predicted pure PTSD compared to three other dimensions describing specified traumatic events that were previously extracted from interviews within this sample. As females appear to report more perceived life threat than males after experiencing a similar trauma (Irish et al., 2011), and it seems unclear how the likelihood of having pure PTSD or with any PTSD comorbidity is influenced by gender (Hourani et al., 2016; Kline et al., 2013; Maguen et al., 2012; Walter et al., 2022), an explanation for identifying this profile only in females could be related to gender differences in trauma appraisal. Psychotic symptom levels were overall low across profiles, although levels covaried with severity across the three profiles consisting of all symptom types. The low levels suggest that cohort members experienced few psychotic symptoms which limited variance in PQ-16 scores for LPA, but is in line with what is expected given their low prevalence in a general population (Perälä et al., 2007).

Adversity and psychological symptom profiles in women

Within women, childhood and adulthood adversity both generally increased the probability for classification into all symptom profiles other than the low symptom profile in late adulthood. This corresponds with previous studies demonstrating strong predictive effects of childhood and adulthood traumatic adversity for several DSM-IV disorders in adult men and women, including our symptoms of interest, with little apparent specificity across disorders (Carr et al., 2013; Do et al., 2019; Kessler et al., 2010; Kuzminskaite et al., 2022). Yet, we observed that specific forms of childhood adversity in women had differential impact on symptom severity, as women who reported more emotional abuse had higher risk for classification into specifically the mild- compared to low-symptom profile, while risk was higher for the high-symptom profile after experiencing more sexual abuse. In line with our findings, several subtypes of childhood maltreatment were previously found to generically predict many types of psychological symptoms, although some types more than the other (Kessler et al., 2010; Rameckers et al., 2021; Sayyah et al., 2022; Wood & Bhatnagar, 2015). Krause et al. (2003) hypothesized that chronic inhibition of experiencing and expressing emotions as coping strategy can be functional during childhood to deal with maltreatment, but mediates the association between childhood maltreatment and a range of adult psychological disorders. Accordingly, it is possible that specific coping strategies in response to adversity may determine psychopathology severity, however, we did not assess this in our study.

Adversity and psychological symptom profiles in men

In men, child maltreatment seemingly had the most profound effect as it increased probability for classification into all other profiles than low symptoms, whereas exposure to famine in early gestation aspecifically and exclusively increased probability for mild rather than low symptoms and adulthood trauma increased probability for high compared to low symptoms. The association with exposure to undernutrition during early gestation was only observed amongst men and no associations were found in prenatally exposed women. This implies a time-dependent impact of adversity within the prenatal gestational period in addition to a sex-specific vulnerability to prenatal adversity in general. This latter observation conforms to existing literature on the increased vulnerability of males to prenatal adversity, probably due to faster *in utero* fetus growth (Bale, 2016). It is essential to note that sample sizes of our prenatally exposed groups were limited, which could explain the absence of further significant associations. Yet, early gestation has been repeatedly demonstrated to be sensitive to famine exposure in relation to psychological health risk in later life (Betts et al., 2015), also within our study cohort (de Rooij et al., 2011; Wiegersma et al., 2022). Our observations in men further support a time-dependent effect of adversity on psychological health, extending from the prenatal period through childhood into late adulthood, thereby adding to a growing literature (Atzl et al., 2019; Daskalakis et al., 2013; Dunn et al., 2018; Narayan et al., 2013).

Notably and similar to what we found in women, specific subtypes of childhood adversity had differential impact in men, and not only affected overall symptom severity but also symptom type. For example, emotional abuse in men increased probability for mild rather than low symptoms, but lowered risk for anxious/depressive symptoms. In case of more physical neglect, probability was higher for high symptoms. As mentioned previously, specific stressor types experienced during childhood and adolescence distinctly predict specific adult psychopathology (Carr et al., 2013) and severity (Martins et al., 2014). Although it is still unclear what underlies the differential impact of childhood trauma subtypes on long-term symptom specificity, a hypothetical pathway has been put forward by Sayyah et al. (2022). They suggested that specific subtypes of childhood maltreatment interfere with the development of specific age-salient socio-emotional concepts that are formed during childhood and portend certain symptoms when disrupted. For example, interfering with the consolidation of attachment and emotion regulation could manifest in adult maladjustment of internalizing behaviour (related to anxious and depressive symptoms) or traumatic stress (related to PTSD). Accordingly, childhood trauma subtypes differentially affect personality traits (Cohen et al., 2014) and brain regions underlying specific affected cognitive-behavioural processes (Cassiers et al., 2018).

The different associations we found in men and women with respect to adversity impact during the prenatal period, early childhood and mid-to-late adulthood, may be regulated by differential underlying neurobiological (epigenetic) mechanisms regarding the development and susceptibility of psychological symptoms. For example, the faster rate of growth in male fetuses (Eriksson et al., 2010), protective characteristics of the female placenta during brain development (Bale, 2016), and disparate brain maturation and aging (Douet et al., 2014) have previously been identified to be sex-dysmorphic vulnerability and protective factors for health outcomes. Although these sex-differential effects need further investigation and could be influenced by other factors, such as trauma accumulation or trauma type, our findings stress the importance of the use of the Sex and Gender Equity in Research (SAGER) (Heidari et al., 2016) guidelines to study men and women separately in future research on psychological health.

Study implications

A major implication of our findings is that exposure to adversity across the lifespan still has measurable impact on psychological health into late adulthood, even when the adversity happened approximately 75 years ago as was observed in men exposed to undernutrition during early gestation. These findings seem to fit with the concept of developmental programming of mental health contending that adverse events in early life persistently impact risk for long-term psychopathology through disruption of neurobiological developmental processes that take place during these critical life periods (Coffman, 2020; Heim et al., 2019; Mustard, 1999; Nelson et al., 2020; Smith & Pollak, 2020; van den Bergh, 2011). Importantly, our findings highlight that long-term risk may also apply when adversity occurs outside of critical neurodevelopmental periods, during mid-to-late adulthood, independent of whether early life adversity was experienced. Yet,

other additional factors such as genetic predisposition (McIlwrick et al., 2016; Shadrina et al., 2018) may also be related to specificity of the type, complexity and severity of late adult symptoms (Berthelot et al., 2022; Koenen et al., 2008a).

Strengths and limitations

A major strength of this study is that we assessed adversities during different periods across the lifespan, including the prenatal period, and their associations with a wide range of psychological symptoms, making it possible to study whether effects of adversity exposure on patterns of psychological symptoms depend on timing of exposure. Another strength is our multidimensional statistical approach of using LPA. This person-centered method utilizes multivariate continuous data and full ranges of symptoms of interest and their severity, as well as their interdependence instead of relying on dichotomous categorical diagnoses, allowing for comprehensive consideration of symptom specificity and comorbidity (Lanza & Cooper, 2016; McCutcheon, 1987). Furthermore, we stratified our analyses for men and women as recommended in the SAGER guidelines (Heidari et al., 2016) and given previous findings on sex specificity of latent symptom profiles and impact of adversity thereon (de Rooij et al., 2011; van Zuiden et al., 2022). We indeed observed sex-specific effects, which may likely have gone undetected without performing this stratification.

Our study also has some limitations. First, although our cohort study is longitudinal in itself, psychological symptoms were assessed cross-sectionally. Consequently, we could not study potential differential effects regarding symptom chronicity and course. Future studies should adopt a longitudinal perspective to be able to investigate this. Another methodological limitation was that childhood and adulthood adversity were retrospectively assessed, possibly introducing memory bias with representation of inaccurate perceptions, interpretations and recollections (Baldwin et al., 2019). False positive memories in childhood adversity reports are rare (Hardt & Rutter, 2004), but underreporting is more prevalent (Maughan & Rutter, 1997). To account for potential underreporting, we included Minimization-Denial (MD) scores as covariate in our models regarding exposure to childhood adversity. Furthermore, women prenatally exposed to famine have overall higher mortality risk than unexposed women and exposed men (van Abeelen et al., 2012). This may have resulted in selective survival and participation of more healthy (female) cohort members. Additionally, our aging cohort members may have become physically and/or mentally ill, which could have increased loss to follow up of adults with poorer health. As those exposed to famine in early gestation have previously been shown to have increased risk for several adverse cardio-metabolic disease outcomes as well as other adverse mental and physical health outcomes, selective participation of this group of participants is likely and may have led to underestimation of effects in this study. Lastly, we did not take potential effects of accumulation of or interactions between several adversity types across the lifespan into account. Although it would have been valuable to assess the impact of multiple hits by adverse events across the

lifespan on psychological symptoms, our modest sample size only allowed for reliable investigation of main effects for distinct exposure periods.

Conclusion

We observed specific impact of adversity during different periods across the lifespan on psychological symptom profiles later in life, which appeared to be time-dependent as well as sex-specific. Effects of exposure to adversity during early gestation or during childhood were still visible in late adulthood suggesting an ongoing lifelong impact of adverse events that happened over 7 decades ago.

Table 1. Participant characteristics.

	Men (*n*=274)	**Women** (*n*=294)	Statistics
Age *(years)*	72.8 (0.9)[11]	72.9 (0.8)	*U*=41,585.00, *p*=.456
PSYCHOLOGICAL SYMPTOMS			
HADS Depression subscale[1]	3.3 (3.4)	3.5 (3.3)	*U*=41,343.50, *p*=.582
Probable depression (score ≥8)	26 (9.5%)	33 (11.2%)	X^2(1)= 0.46, *p*=.498
HADS Anxiety subscale[1]	3.1 (3.3)	4.2 (3.8)[6]	*U*=46,951.00, *p*<.001
Probable anxiety (score ≥8)	24 (8.8%)	53 (18.0%)	X^2(1)= 10.40, *p*=.001
PCL-5 PTSD symptoms[2]			
Cluster B - Intrusions	1.5 (2.5)[7]	2.6 (3.6)[8]	*U*=46,856.50, *p*<.001
Cluster C - Avoidance	0.7 (1.3)[7]	1.0 (1.6)[10]	*U*=43,446.00, *p*=.012
Cluster D – Negative cognitions and mood	2.7 (3.9)[8]	3.1 (4.2)[10]	*U*=42,582.50, *p*=.066
Cluster E – Arousal and reactivity alterations	3.7 (3.2)[6]	4.7 (3.8)[6]	*U*=46,021.50, *p*=.002
Probable PTSD (total score ≥23)	25 (9.1%)	41 (13.9%)	X^2(1)= 3.21, *p*=.073
PQ-16 Psychotic symptoms[3]			
Total score	1.7 (2.5)[6]	1.7 (1.9)[10]	*U*=41,626.50, *p*=.212
Probable psychosis (score ≥6)	15 (5.5%)	16 (5.4%)	X^2(1)<0.01, *p*=.986
LIFE ADVERSITIES			
Prenatal undernutrition			
Early gestation	24 (8.8%)	25 (8.5%)	
Mid gestation	26 (9.5%)	48 (16.3%)	
Late gestation	48 (17.5%)	46 (15.6%)	
Unexposed controls	176 (64.2%)	175 (59.5%)	
Childhood traumatic maltreatment[4]			
Emotional Abuse	6.7 (3.1)	7.5 (4.1)[6]	*U*=42,719.50, *p*=.144
Physical Abuse	5.8 (2.3)	5.8 (2.6)[6]	*U*=38,672.00, *p*=.264
Sexual Abuse	4.5 (2.0)	5.0 (2.8)[6]	*U*=43,634.50, *p*=.004
Emotional Neglect	11.5 (5.0)	12.1 (6.0)[9]	*U*=40,634.50, *p*=.639
Physical Neglect	7.3 (2.7)[6]	8.0 (3.1)[8]	*U*=43,570.00, *p*=.042
Total score	35.9 (11.4)[6]	38.1 (13.8)[9]	*U*=41,996.50, *p*=.211
Minimization/Denial	0.7 (1.0)	0.7 (1.0)[8]	*U*=38,424.00, *p*=.390
Mid-to-late adulthood trauma[5]			
Number of traumatic events (<15 years)	1.0 (1.6)	1.2 (1.5)	*U*=43,684.50, *p*=.065
Other health problems (*n(%)*)			
Neurological	3 (1.1%)	6 (2.0%)	*p*=.507
Hearing	21 (7.7%)	24 (8.2%)	X^2(1)= 0.05, *p*=.826
Vision	11 (4.0%)	18 (6.1%)	X^2(1)= 1.30, *p*=.254
Dizziness with falling	3 (1.1%)	5 (1.7%)	*p*=.726

Educational level (*n(%)*)			
Less than 6 primary school classes	1 (0.4%)	3 (1.0%)	*p*<.001
6 primary school classes	10 (3.6%)	19 (6.5%)	
More than primary school/primary school with uncompleted further education	15 (5.5%)	56 (19.0%)	
Practical training	47 (17.2%)	22 (7.5%)	
Secondary vocational education	126 (46.0%)	131 (44.6%)	
Pre-university education	17 (6.2%)	22 (7.5%)	
University/higher professional education	56 (20.4%)[7]	39 (13.3%)[7]	
Marital status (*n(%)*)			
Single *(Divorced/unmarried)*	29 (10.6%)	51 (17.4%)	*p*<.001
Long-term relationship *(Married/living together-unmarried)*	224 (81.8%)[7]	174 (58.1%)[8]	
Widow(er)/partner passed away	19 (6.9%)	66 (22.4%)	
Perceived SES during childhood			
Low	93 (34.0%)	95 (32.3%)	*p*=.108
Medium	84 (30.7%)	76 (25.9%)	
High	97 (35.4%)	121 (41.1%)[7]	
Received help with questionnaire (*n(%)*)	13 (4.7%)[6]	19 (6.5%)[6]	$X^2(1)= 0.79$, *p*=.375

Scores are displayed as mean (SD) for continuous variables or *n* (%) for categorical variables. Age was calculated based on date of birth and date of filling out the questionnaires (in case this was missing (*n*=7 men, *n*=18 women), date of signing Informed Consent was used). [1]Measured with HADS: Hospital Anxiety and Depression Scale; [2]Measured with PCL-5: PTSD Checklist for DSM-5; [3]Measured with PQ-16: Prodromal Questionnaire; [4]Measured with CTQ: Childhood Trauma Questionnaire; [5]Measured with LEC-5: Life Events Checklist, number of experienced traumatic event types in the past 15 years when experienced personally, witnessed it, learned about it happening to close family members or friends, or if it happened at work; SES: social economic status, *p*<0.05; [6]*n*=1 missing; [7]*n*=2 missing; [8]*n*=3 missing; [9]*n*=4 missing; [10]*n*=5 missing; [11]*n*=1 missing.

Table 2. Estimated mean total- and subscores for anxiety, depression, PTSD and psychotic symptom profiles of best-fitting models in men and women.

Men	**Low symptoms** (*n*=183)	**Anxiety/Depression symptoms** (*n*=15)	**Mild symptoms** (*n*=42)	**High symptoms** (*n*=9)	
Depressive symptoms[1]	2.0 (0.2)	8.1 (7.1)	4.1 (0.5)	9.7 (1.2)	
Anxious symptoms[1]	1.8 (0.3)	7.0 (2.9)	4.3 (0.6)	11.1 (0.7)	
PTSD symptoms[2]					
Cluster B-*Intrusions*	0.5 (0.1)	1.1 (2.5)	3.8 (0.4)	5.2 (0.7)	
Cluster C-*Avoidance*	0.2 (0.0)	0.3 (0.2)	2.3 (0.8)	2.8 (0.5)	
Cluster D-*Negative Cognitions and Mood*	0.8 (0.1)	4.4 (2.9)	5.3 (1.1)	11.4 (1.2)	
Cluster E-*Arousal and Reactivity*	2.3 (0.3)	5.5 (2.3)	5.9 (0.9)	9.9 (0.5)	
Psychotic symptoms3	1.0 (0.1)	2.0 (0.6)	2.1 (0.3)	7.1 (1.2)	
Women	**Low symptoms** (*n*=162)	**Anxiety/Depression symptoms** (*n*=27)	**Mild symptoms** (*n*=56)	**High symptoms** (*n*=13)	**PTSD symptoms** (*n*=19)
Depressive symptoms[1]	2.1 (0.2)	7.3 (0.7)	3.6 (0.4)	10.6 (1.2)	4.5 (0.8)
Anxious symptoms[1]	2.2 (0.2)	9.4 (1.2)	4.6 (0.4)	11.5 (0.8)	7.9 (0.7)
PTSD symptoms[2]					
Cluster B-*Intrusions*	0.8 (0.1)	2.6 (0.6)	3.8 (0.4)	11.9 (1.0)	6.9 (0.7)
Cluster C-*Avoidance*	0.1 (0.0)	0.5 (0.2)	1.9 (0.1)	5.6 (0.3)	3.7 (0.2)
Cluster D-*Negative Cognitions and Mood*	0.9 (0.1)	5.8 (0.8)	3.5 (0.4)	14.6 (1.0)	7.5 (0.8)
Cluster E-*Arousal and Reactivity*	2.7 (0.2)	8.4 (0.5)	4.6 (0.4)	13.2 (0.9)	8.9 (0.6)
Psychotic symptoms[3]	1.0 (0.1)	3.3 (0.6)	2.1 (0.3)	4.1 (0.7)	2.5 (0.4)

Scores are displayed as mean (SE). [1]HADS: Hospital Anxiety and Depression Scale, both range 0-28; [2]PCL-5: PTSD Checklist for DSM-5, ranges Cluster B-*Intrusions* 0-20, Cluster C-*Avoidance* 0-8, Cluster D-*Negative Cognitions and Mood* 0-28, Cluster E-*Arousal and Reactivity* 0-24; [3]PQ-16: Prodromal Questionnaire, range 0-16.

Table 3. Multinomial regression analysis for associations between life adversities and probability of profile assignment in men.

	Anxiety/Depression symptoms				Mild symptoms				High symptoms			
Men (N=249)	Versus low symptoms				Versus low symptoms				Versus low symptoms			
	Odds	SE Odds	95% CI Odds	*p*	Odds	SE Odds	95% CI Odds	*p*	Odds	SE Odds	95% CI Odds	*p*
Model 1 (N=246) Prenatal undernutrition due to famine exposure												
Early gestation	3.0	2.9	0.5-19.8	.256	3.8	2.4	1.1-13.3	.039*	3.0	3.7	0.3-34.0	.371
Mid gestation	0.6	0.6	0.1-3.8	.575	0.9	0.7	0.2-3.9	.875	1.4	1.6	0.1-14.0	.777
Late gestation	0.3	0.4	<0.1-2.8	.310	0.9	0.5	0.3-2.5	.882	0.8	0.9	0.1-7.5	.843
Educational level	0.8	0.2	0.5-1.4	.454	1.4	0.2	1.0-1.8	.031*	1.2	0.3	0.7-1.4	.606
Marital status												
Single vs long-term relation	0.9	0.8	0.2-5.1	.928	2.3	1.3	0.8-6.7	.125	***	***	***	<.001*
Widower vs long-term relation	1.7	1.2	0.5-6.5	.423	0.4	0.3	0.1-1.8	.262	265.4	<0.1	265.4-265.4	1.000
Other health problems	0.5	0.7	<0.1-7.6	.648	1.3	0.7	0.4-3.9	.662	1.2	1.4	0.1-11.5	.898
Received help with questionnaire	1.5	1.9	0.1-19.0	.761	1.3	1.6	0.1-15.9	.862	4.5	5.6	0.4-52.1	.232
Model 2 (N=245) Childhood traumatic maltreatment												
Childhood maltreatment[1]	1.1	<0.1	1.0-1.1	.010*	1.1	<0.1	1.0-1.1	.051	1.2	<0.1	1.0-1.2	.032*
Educational level	0.8	0.3	0.5-1.5	.554	1.4	0.2	1.1-1.9	.016*	1.3	0.3	0.7-1.9	.486
Marital status												
Single vs long-term relation	0.8	0.8	0.1-5.3	.843	4.5	2.7	1.4-14.6	.013*	***	***	***	<.001*
Widower vs long-term relation	1.6	1.2	0.4-6.6	.530	0.3	0.2	0.1-1.0	.054	157.2	<0.1	157.2-157.2	1.000

MD score - *Response bias CTQ*	1.1	0.5	0.5-2.5	.880	0.6	0.2	0.3-1.3	.187	0.5	0.3	0.2-1.6	.251
Perceived SES during childhood	1.2	0.4	0.7-2.2	.468	0.7	0.1	0.5-1.0	.061	1.1	0.3	0.7-1.8	.612
Other health problems	0.8	0.9	0.1-8.3	.827	1.4	0.8	0.5-4.3	.579	1.5	1.7	0.2-14.4	.730
Received help with questionnaire	1.8	2.5	0.1-17.1	.676	1.4	1.8	0.1-17.1	.814	4.9	7.1	0.3-83.6	.276
Model 3 (N=245) Childhood traumatic maltreatment subtypes												
Emotional abuse[1]	0.8	0.1	0.7-2.0	.036*	1.2	0.1	1.0-1.4	.032*	1.3	0.2	1.0-1.8	.080
Physical abuse[1]	1.2	0.2	0.9-1.6	.158	0.9	0.1	0.7-1.1	.275	0.6	0.2	0.4-1.1	.123
Sexual abuse[1]	1.1	0.2	0.8-1.4	.757	0.9	0.2	0.7-1.2	.513	1.1	0.4	0.5-2.2	.879
Emotional neglect[1]	1.1	0.1	0.9-1.3	.280	1.0	0.1	0.9-1.2	.754	0.9	0.1	0.7-1.2	.507
Physical neglect[1]	1.3	0.2	1.0-1.7	.103	1.1	0.1	0.9-1.4	.398	1.4	0.2	1.1-1.9	.008*
Educational level	<1.0	0.3	0.5-2.0	.951	1.4	0.2	1.1-2.0	.023*	1.3	0.4	0.8-2.3	.344
Marital status												
Single vs long-term relation	<1.0	1.2	0.1-9.9	.985	3.9	2.1	1.3-11.4	.015*	***	***	***	<.001*
Widower vs long-term relation	1.4	1.1	0.3-6.2	.699	0.3	0.2	0.1-1.0	.055	***	***	***	1.000
MD score - *Response bias CTQ*	1.0	0.5	0.4-2.5	.941	0.7	0.3	0.3-1.4	.276	0.3	0.2	0.1-1.1	.075
Perceived SES during childhood	1.3	0.4	0.7-2.4	.481	0.7	0.1	0.5-1.1	.087	1.4	0.5	0.8-2.6	.254
Other health problems	0.7	0.9	0.1-7.0	.794	1.3	0.8	0.4-4.3	.635	2.3	2.7	0.2-23.7	.491
Received help with questionnaire	2.8	3.4	0.3-30.0	.396	1.1	2.1	<0.1-45.3	.953	5.0	10.3	0.1-273.4	.428
Model 4 (N=246) Adulthood trauma												
Adulthood trauma[2]	0.6	0.2	0.3-1.2	.170	1.2	0.2	0.9-1.5	.277	2.0	0.4	1.4-2.9	<.001*
Educational level	0.8	0.2	0.4-1.4	.337	1.3	0.2	1.0-1.7	.054	1.1	0.3	0.7-1.9	.626

Marital status												
Single vs long-term relation	<1.0	0.8	0.2-5.0	.980	1.8	0.9	0.7-4.8	.218	***	***	***	<.001*
Widower vs long-term relation	1.6	1.1	0.4-6.0	.493	0.5	0.3	0.3-1.7	.285	332.3	<0.1	332.3-332.3	1.000
Other health problems	0.8	1.0	0.1-8.6	.839	1.3	0.7	0.4-4.0	.641	<1.0	1.3	0.1-14.0	.983
Received help with questionnaire	1.8	2.3	0.2-21.5	.646	0.9	1.4	0.2-16.9	.960	4.2	6.1	0.3-71.0	.317

[1]Childhood traumatic maltreatment subtypes was measured using the Childhood Trauma Questionnaire (CTQ); [2]Adulthood trauma was measured with LEC-5: Life Events Checklist, number of experienced traumatic event types in the past 15 years when experienced personally, witnessed it, learned about it happening to close family members or friends, or if it happened at work; Odds indicates the B value corresponding to the log odds, with Odds > 1 representing higher odds – higher probability for assignment into the target profile versus the low-symptom severity profile, and Odds < 1 lower odds – lower probability for assignment into the target profile versus the low-symptom severity profile; SES: Social economic status, SE: Standard error, CI: Confidence interval of B log odds. *** missing values.

Table 4. Multinomial regression analysis of estimates and odds for associations between life adversities and probability of profile assignment in women.

	Mild-symptoms				PTSD symptoms				Anxiety/Depression symptoms				High symptoms			
Women (N=277)	Versus low symptoms				Versus low symptoms				Versus low symptoms				Versus low symptoms			
	Odds	SE Odds	95% CI Odds	*p*	Odds	SE Odds	95% CI Odds	*p*	Odds	SE Odds	95% CI Odds	*p*	Odds	SE Odds	95% CI Odds	*p*
Model 1 (N=273) Prenatal undernutrition due to famine exposure																
Early gestation	0.6	0.5	0.1-2.7	.508	0.6	0.7	0.1-5.1	.646	1.5	1.0	0.4-5.8	.556	0.9	0.9	0.1-7.2	.889
Mid gestation	1.3	0.6	0.6-3.1	.523	2.1	1.3	0.6-6.9	.220	0.6	0.4	0.1-2.3	.426	0.5	0.6	0.1-4.4	.536
Late gestation	0.8	0.4	0.3-2.2	.711	0.3	0.3	<0.1-2.8	.285	0.3	0.3	0.1-1.7	.175	0.6	0.4	0.1-2.2	.396
Educational level	1.1	0.1	0.9-1.4	.327	1.2	0.3	0.8-1.8	.496	1.0	0.2	0.7-1.4	.995	1.3	0.3	0.9-1.9	.244
Marital status																
Single vs long-term relation	0.9	0.2	0.5-1.5	.639	1.4	0.5	0.7-3.0	.339	0.8	0.3	0.4-1.7	.542	1.3	0.6	0.5-3.0	.612
Widower vs long-term relation	1.5	0.4	0.9-2.6	.129	0.7	0.4	0.3-1.9	.517	1.1	0.5	0.4-2.7	.863	1.4	0.7	0.6-3.5	.478
Other health problems	0.7	0.4	0.2-2.2	.506	1.0	0.9	0.2-6.1	.972	1.7	1.2	0.5-6.7	.422	3.0	1.8	0.9-9.6	.067
Received help with questionnaire	0.2	0.3	<0.1-2.3	.216	2.0	0.3	0.3-12.3	.460	***	***	***	<.001*	1.2	1.5	0.1-12.9	.869
Model 2 (N=268) Childhood traumatic maltreatment																
Childhood maltreatment[1]	1.1	<0.1	1.0-1.1	.002*	1.1	<0.1	1.0-1.1	0.030*	1.0	<0.1	0.9-1.1	.916	1.1	<0.1	1.0-1.1	.013*
Educational level	1.2	0.2	0.9-1.5	.233	1.2	0.3	0.8-1.8	0.525	<1.0	0.1	0.7-1.3	.739	1.3	0.2	0.9-1.8	.207

Marital status																
Single vs long-term relation	0.9	0.3	0.5-1.5	.680	1.3	0.5	0.6-2.9	0.455	0.8	0.3	0.3-1.8	.583	1.2	0.5	0.5-2.8	.693
Widower vs long-term relation	1.5	0.5	0.8-2.7	.201	0.7	0.4	0.5-2.2	0.591	1.1	0.5	0.5-2.7	.795	1.5	0.8	0.5-4.1	.435
MD score - *Response bias CTQ*	0.9	0.2	0.6-1.3	.436	0.5	0.2	0.2-1.3	0.126	0.9	0.2	0.6-1.4	.545	0.7	0.3	0.3-1.7	.396
Perceived SES during childhood	1.3	0.2	1.0-1.8	.048*	1.1	0.2	0.7-1.7	0.691	<1.0	0.2	0.7-1.4	.858	1.1	0.3	0.6-1.9	.806
Other health problems	0.6	0.4	0.2-2.0	.377	0.7	0.7	0.1-4.5	0.711	1.5	0.9	0.4-5.1	.527	2.4	1.7	0.6-9.5	.220
Received help with questionnaire	0.3	0.4	<1.0-3.3	.354	2.0	2.1	0.3-15.3	0.510	***	***	***	<.001*	1.6	1.8	0.2-15.4	.697
Model 3 (N=266) Childhood traumatic maltreatment subtypes																
Emotional abuse[1]	1.1	0.1	1.0-1.3	.036*	1.1	0.1	0.9-1.3	.627	1.2	0.1	<1.0-1.4	.151	1.1	0.1	<1.0-1.3	.155
Physical abuse[1]	0.9	0.1	0.8-1.2	.520	1.0	0.1	0.8-1.3	.987	<1.0	0.1	0.7-1.3	.705	0.8	0.1	0.5-1.1	.153
Sexual abuse[1]	1.1	0.1	<1.0-1.3	.171	1.1	0.1	<1.0-1.3	.080	0.9	0.2	0.6-1.4	.642	1.3	0.1	1.0-1.6	.027*
Emotional neglect[1]	<1.0	0.1	0.9-1.1	.872	1.1	0.1	<1.0-1.2	.238	1.0	0.1	0.8-1.2	.932	1.1	0.1	<1.0-1.3	.121
Physical neglect[1]	1.1	0.1	0.9-1.3	.443	1.0	0.1	0.8-1.2	.722	0.8	0.2	0.6-1.2	.313	<1.0	0.1	0.7-1.2	.666
Educational level	1.1	0.2	0.9-1.5	.405	1.2	0.3	0.8-1.8	.502	0.9	0.1	0.7-1.1	.292	1.3	0.3	0.8-2.0	.240
Marital status																
Single vs long-term relation	0.9	0.3	0.5-1.5	.603	1.3	0.5	0.6-2.9	.456	0.7	0.4	0.3-1.9	.517	1.2	0.5	0.5-2.9	.676
Widower vs long-term relation	1.6	0.5	0.8-3.0	.183	0.7	0.4	0.2-2.3	.596	1.3	0.6	0.5-3.2	.638	1.4	0.8	0.5-4.2	.575
MD score - *Response bias CTQ*	0.8	0.2	0.5-1.2	.289	0.5	0.3	0.2-1.4	.161	1.0	0.2	0.6-1.4	.641	0.8	0.4	0.3-2.1	.592

Perceived SES during childhood	1.4	0.2	<1.0-1.8	.057	1.1	0.3	0.7-1.7	.775	0.9	0.2	0.6-1.4	.617	1.0	0.3	0.6-2.0	.908
Other health problems	0.6	0.4	0.2-2.1	.404	0.8	0.7	0.1-5.1	.774	1.3	0.9	0.3-4.8	.728	2.7	2.1	0.6-12.4	.207
Received help with questionnaire	0.3	0.4	<0.1-3.7	.368	2.0	2.1	0.3-15.9	.509	***	***	***	<.001	1.8	2.2	0.2-19.1	.623
Model 4 (N=273) Adulthood trauma																
Adulthood trauma[2]	1.4	0.2	1.1-1.8	.021*	1.5	0.2	1.1-2.0	.015*	1.4	0.2	1.1-1.8	.015*	1.6	0.3	1.0-2.4	.037*
Educational level	1.1	0.1	0.8-1.4	.736	1.0	0.2	0.6-1.6	.946	0.9	0.1	0.7-1.2	.387	1.1	0.2	0.7-1.7	.709
Marital status																
Single vs long-term relation	0.8	0.2	0.5-1.4	.400	1.3	0.5	0.6-2.9	.553	0.7	0.3	0.3-1.6	.419	1.1	0.4	0.5-2.3	.838
Widower vs long-term relation	1.7	0.5	<1.0-3.0	.064	0.8	0.4	0.3-2.2	.667	1.3	0.6	0.5-3.0	.591	1.8	0.8	0.7-4.5	.217
Other health problems	0.6	0.4	0.2-1.9	.367	0.7	0.6	0.1-3.5	.646	1.4	0.9	0.4-4.9	.626	2.4	1.6	0.7-8.8	.185
Received help with questionnaire	0.2	0.3	<0.1-2.5	.218	1.6	1.5	0.3-9.4	.583	***	***	***	<.001*	<1.0	1.1	0.1-8.1	.974

[1]Childhood traumatic maltreatment subtypes was measured using the Childhood Trauma Questionnaire (CTQ); [2]Adulthood trauma was measured with LEC-5: Life Events Checklist, number of experienced traumatic event types in the past 15 years when experienced personally, witnessed it, learned about it happening to close family members or friends, or if it happened at work; Odds indicates the B value corresponding to the log odds, with Odds > 1 representing higher odds – higher probability for assignment into the target profile versus the low-symptom severity profile, and Odds < 1 lower odds – lower probability for assignment into the target profile versus the low-symptom severity profile; SE: Standard error, CI: Confidence interval of log odds. *** missing values.

Financial Support

This work was supported by the European Commission Horizon 2020, project LongITools [Grant number #874739]; a Brain and Behavior Research Foundation NARSAD Young Investigator Grant awarded to dr. M. van Zuiden [#24928]; and Veni grant from The Netherlands Organisation for Health Research and Development ZonMw awarded to dr. M. van Zuiden [Grant number #91617037].

Conflict of Interest

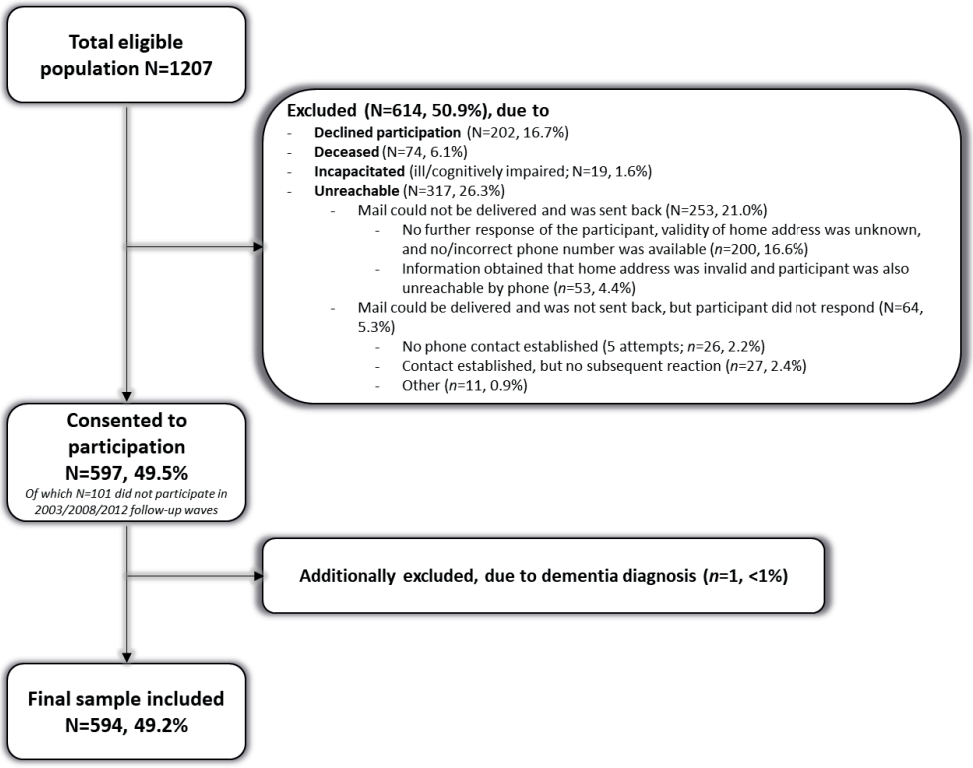
None.

Ethical standards

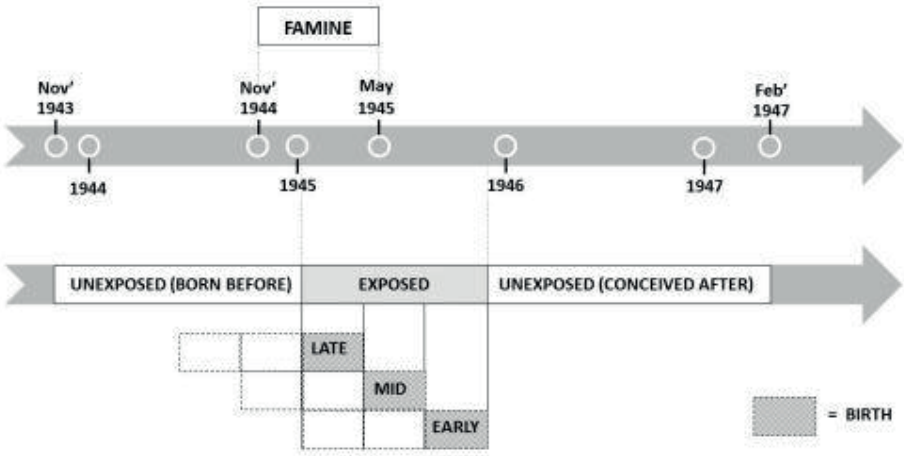
The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national guidelines on human experimentation (SAGER guidelines) and with the Helsinki Declaration of 1975, as revised in 2008, and the Medical Ethics Committee of the Academic Medical Center, Amsterdam, Netherlands, concluded that a full review and official approval of this study wave was not required according to Dutch law for medical research.

5. Supplementary Materials

S1. Flowchart of participant inclusion of the Dutch Famine birth cohort follow-up study and schematic presentation of gestational famine exposure. Schematic presentation of gestational famine exposure was copied from Bleker et al. (2021).



S2. Overview of exposure to prenatal undernutrition during gestation.



S3.1 Results of Latent Profile Analyses (LPA) on best-fitted classification model in men and women using pre-identified criteria and selection indicators.

	1-class model	2-class model	3-class model	4-class model	5-class model
Men (*n*=249)					
AIC	7819.071	7188.439	6995.689	6877.281	
BIC	7868.315	8265.823	7101.213	7010.945	
Adjusted-AIC	7823.934	7196.082	7006.111	6890.483	
Entropy	-	0.965	0.961	0.967	
LMR-A					
Value	-	632.307	204.125	131.430	
p-value	-	.0069	.1481	.7597	
BLRT					
-2LL difference	-	646.632	208.749	134.408	
p-value	-	<.001	<.001	<.001	
Women (*n*=277)					
AIC	9649.041	8824.544	8428.428	8335.714	8230.576
BIC	9699.777	8904.272	8537.149	8473.427	8397.280
Adjusted-AIC	9655.385	8834.513	8442.023	8352.934	8251.421
Entropy	-	0.950	0.962	0.936	0.972
LMR-A					
Value	-	822.223	403.155	106.350	118.505
p-value	-	.1323	.0014	.5293	.2301
BLRT					
-2LL difference	-	840.497	412.115	108.714	121.139
p-value	-	<.001	<.001	<.001	<.001

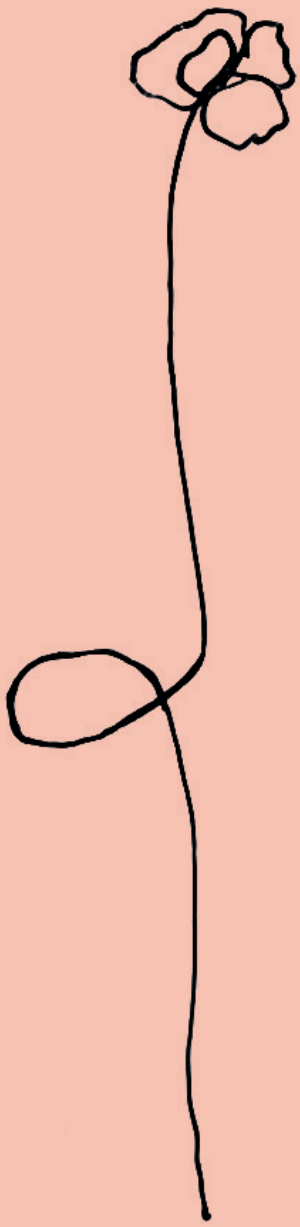
AIC: Akaike Information Criterion; BIC: Bayesian Information Criterion; LMR-A: Lo-Mendell-Rubin-adjusted likelihood ratio test; BLRT: bootstrap likelihood ratio test; -2LL: -2 times log-likelihood difference between an NK class solution and K–1 class solution.

S3.2 Final model selection and latent symptom profiles

In men, model indicators were compared across 1-, 2-, 3-, 4-, and 5-class models. However, increasing the number of classes to a 5-class model resulted in an unreliable model due to non-convergence and non-replication of the best log likelihood values and diminished gains. With the inclusion of 2-, 3- and 4-classed the model improved as increasing the number of latent classes was accompanied by significance of BLRT (p<.001) and decreasing values for BIC, AIC and adjusted-BIC, with lowest values in the 4-class model (Nylund et al., 2007; Yang, 2006). LMR-A significantly improved only with inclusion of 2 classes (p=.007) and not with inclusion of 3 or 4 classes. Classification quality according to entropy statistics was considered adequate for all classes (p>0.961), indicating marginal and substantive information gain by increasing the number of latent classes. A negligible 0.4% decrease in entropy took place with inclusion of a 3- over a 2-class model and 0.6% increase in a 4- over 3-class model. In consideration of all indicators – particularly AIC, BIC, adjusted-BIC, BLRT and entropy comparisons, the 4-class model was likely the most meaningful and acceptable fit with acceptable interpretability of average assignment class-probabilities and high precision (class-1 0.955; class-2 0.934; class-3 0.995; class-4 0.996).

In women, increasing the number of latent classes in a 1- till 6-class model were compared. Increasing the number of classes to a 6-class model resulted in an unreliable model due to non-convergence and non-replication of the best log likelihood values. Similarly, as in men, increasing the number of classes was accompanied by decreasing values for BIC, AIC and adjusted-BIC and significance of BLRT (p<.001; Table 2). Entropy statistics was considered adequate for all classes (p>.936), with lowest values in the 5-class model. LMR-A significantly improved only with inclusion of 3 classes (p=.001) and not with inclusion of 2, 4 or 5 classes. A negligible 1.3% increase in entropy took place with inclusion of a 3- over a 2-class model, 2.7% decrease in a 4- over 3-class model, and 3.9% increase in a 5- over 4-class model. Considering particularly AIC, BIC, adjusted-BIC, BLRT and entropy comparisons, the 5-class model was likely the most meaningful and acceptable fit with acceptable interpretability of average assignment class-probabilities and high precision (class-1 0.994; class-2 0.97; class-3 0.997; class-4 0.925; class-5 0.999).


2



CHAPTER 3

Effects of prenatal exposure to the 1944-45 Dutch famine and glucocorticoid receptor polymorphisms on later life PTSD susceptibility

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Submitted for publication

Keywords: PTSD, prenatal adversity, glucocorticoid receptor, NR3C1, HPA axis

Abstract

Background: Exposure to adversity *in utero* is thought to increase susceptibility to develop posttraumatic stress disorder (PTSD) following later life trauma, due to neurobiological programming effects during critical developmental periods. It remains unknown whether effects of prenatal adversity on PTSD susceptibility are modulated by genetic variations in neurobiological pathways implicated in PTSD susceptibility.

Objective: We investigated whether genetic variation in GR modulated effects of prenatal famine exposure on late adulthood PTSD symptom severity after trauma exposure in childhood and mid-to-late adulthood.

Method: We included term-born singleton N= 439 adults (mean age: 72 years, 54.2% women) from the Dutch Famine Birth Cohort, born around the time of the Dutch Famine of 1944/1945, divided into exposure and control groups based on timing of the famine during gestation. Participants filled out self-report questionnaires on childhood (Childhood Trauma Questionnaire) and mid-to-late adulthood (Life Events Checklist for DSM-5) trauma, and current PTSD symptom severity (PTSD Checklist for DSM-5). GR haplotypes were determined from four functional GR single nucleotide polymorphisms (ER22/23EK, N363S, *Bcl*I and exon 9β) in previously collected DNA. Linear regression analyses were performed to investigate associations of GR haplotype and prenatal famine exposure in conjunction with later life trauma on PTSD symptom severity.

Results: We observed a significant three-way interaction between the GR B*cl*I haplotype, famine exposure during early gestation, and adulthood trauma exposure on PTSD symptom severity in late adulthood. Only participants exposed to famine during early gestation without the GR B*cl*I haplotype showed a significantly stronger positive association between adulthood trauma and PTSD symptom severity than non-exposed participants, indicating increased PTSD susceptibility.

Conclusions: Our results illustrate the importance of integrated approaches considering genetics and environmental contexts throughout various life periods, including the rarely investigated prenatal environment, to elucidate how PTSD susceptibility evolves throughout life.

1. Introduction

Posttraumatic stress disorder (PTSD) is a common adverse consequence of traumatic events, with a worldwide conditional risk of 4% following traumatic events (Kessler et al., 2017). The fact that most trauma-exposed individuals do not develop PTSD indicates individual differences in susceptibility to PTSD development. Early life trauma is considered an important risk factor for PTSD development upon later trauma exposure during adulthood (Nishith et al., 2000; Schumm et al., 2006). This is thought to be influenced by permanent changes in neurobiological stress systems in response to early life trauma that promote survival of harsh circumstances in the near future, but thereby also result in increased susceptibility to (mental) health problems later in life (Boyce, 2016). The effects of early life adversity that occurs before birth, such as *in utero* exposure to undernutrition, maternal stress and maternal psychological problems, addictive substances or toxins, are potentially even larger because it coincides with critical periods of organogenesis and brain formation (Boyce, 2016). Because of the large and long lasting effects of prenatal adversity as well as its high prevalence throughout the world (Slopen et al., 2015), it is essential to investigate the extent of its adverse consequences, also in the context of PTSD.

Prenatal adversity is associated with increased risk for a broad range of negative long-term consequences in the offspring, including physical and mental health problems (Lumey et al., 2011; van den Bergh et al., 2020). It is of potential relevance for PTSD susceptibility following traumatic events that prenatal adversity has been associated with long-term changes in glucocorticoid (GC) levels and in the signaling of glucocorticoid effects throughout the body (Harris & Seckl, 2011; Reynolds et al., 2013; Seckl & Meaney, 2006). Glucocorticoids (cortisol in humans) are the end product of the hypothalamic-pituitary-adrenal (HPA) axis, which is activated upon (traumatic) stress, and play a pivotal role in stress reactivity and recovery. Increasing evidence from prospective studies in trauma-exposed adults indicates that individual variability in GC signaling during and acutely following trauma is associated with PTSD susceptibility (Steudte-Schmiedgen et al., 2016; van Zuiden et al., 2011, 2012a). However, prenatal adversity has only been scarcely studied in relation to PTSD susceptibility. One previous study showed that self-reported major symptoms of prenatal maternal genital infection were associated with increased risk for PTSD in early adulthood, and these effects were more apparent when only analysing trauma-exposed individuals (Betts et al., 2015). This suggests that prenatal adversity increases PTSD susceptibility after trauma exposure in later life.

In the Dutch famine birth cohort, also investigated in the current study, we recently observed that men exposed to famine during early gestation more commonly reported a broad range of mild psychological symptoms, including PTSD-, psychotic, depression and anxiety symptoms, in late adulthood compared to unexposed men (Hilberdink et al., 2022, preprint). It was, however, not investigated whether prenatal exposure to famine influenced the effect of trauma exposure in later life on PTSD susceptibility.

Accumulating evidence indicates that differences in susceptibility to adverse mental health outcomes after childhood adversity are dependent on genotypic variation. For example, it has been observed that the effects of childhood adversity on trauma-induced mental health problems throughout adulthood are moderated by single nucleotide polymorphisms (SNPs) in genes regulating GC signaling throughout the body (Binder et al., 2008). The effects of GCs are signalled via two types of receptors, of which the glucocorticoid receptor (GR) is primarily activated under stressful conditions when GC levels are high, initiating subsequent genomic and non-genomic stress responses (de Kloet et al., 2005; Oakley & Cidlowski, 2013). Additionally, a negative GC feedback loop via GRs in the hypothalamus and anterior pituitary results in HPA axis inhibition and thereby recovery from stress (Oakley & Cidlowski, 2013). Several common functional SNPs in the GR gene *NR3C1* have been identified, which directly influence GR function, in the direction of either hyposensitivity (i.e. SNPs GR9β (Kumsta et al., 2009) and ER22/23 EK (Manenschijn et al., 2009; Quax et al., 2013)) or hypersensitivity (i.e. SNPs *Bcl*I (DeRijk, 2009; van Rossum et al., 2003) and N363S (Manenschijn et al., 2009) to the effects of GCs. Several studies have observed that these SNPs are associated with PTSD susceptibility following childhood as well as adulthood trauma, with repeated but not fully consistent findings of increased PTSD susceptibility in carriers of the *Bcl*I SNP (Castro-Vale et al., 2021; Hauer et al., 2011; Lian et al., 2014). This fits with findings that pre-trauma high peripheral GR numbers and higher signalling of GC effects by GRs predicted increased susceptibility to develop PTSD symptoms following trauma exposure in adulthood (van Zuiden et al., 2011, 2012a, 2012b). Moreover, pre-trauma GR numbers were increased in adult carriers of a *Bcl*I SNP haplotype who were previously exposed to childhood trauma compared to non-carriers or non-childhood trauma exposed participants (van Zuiden et al., 2012a). As of yet, it remains unknown whether the effects of prenatal adversity on PTSD susceptibility are also conditionally dependent on genotypic variation in GR function.

In the current study we investigated whether associations between childhood trauma or mid-to-late adulthood trauma and PTSD symptom severity in late adulthood were moderated by prenatal exposure to famine. Furthermore, we investigated whether variation in the GR gene moderated the association between prenatal exposure to famine, childhood or adulthood trauma and PTSD symptom severity in late adulthood. We investigated these associations in members of the Dutch famine birth cohort, a historical birth cohort of individuals born around the time of the Dutch famine of 1944-1945, who have been followed over the past decades with the aim to investigate long-term effects of prenatal famine exposure on adult health (Bleker et al., 2021).

2. Methods

Participants and procedures

Participants were members of the Dutch famine birth cohort. This cohort consists of N=2414 individuals who were born alive as term singletons in the Wilhelmina Gasthuis (WG) hospital in Amsterdam between 1 November 1943 and 28 February 1947. The selection procedures and

follow-up of the cohort have been described in detail elsewhere. Six waves of data collection occurred (Bleker et al., 2021). In the current study, we used DNA collected from fasting blood samples in wave II (2002-2004), approximately 60 years after the famine. We additionally used questionnaire data collected in wave V (2018-2019), approximately 75 years after the famine. During wave V, eligible cohort members (N=1207) were invited by mail to participate in a paper-and-pencil survey, which also included questionnaires on childhood trauma, adulthood trauma and PTSD symptom severity further described below. Additionally, participants provided demographic information including marital status and highest completed education. Eventually N=595 (49.3%) cohort members provided written informed consent to participate (Hilberdink et al., 2022, preprint). In the current study, we ultimately included N=439 (73.8%) participants, for whom DNA samples and questionnaire data on PTSD symptom severity were available. The Medical Ethics Committee of the Academic Medical Center, Amsterdam, the Netherlands, approved the study for wave II and concluded that a full review and official approval for wave V was not required according to Dutch law for medical research. The study was carried out in accordance with the Declaration of Helsinki. Participants provided written informed consent for each data collection wave.

Measures

Exposure to famine

Exposure to famine during gestation was defined as an average maternal daily calorie intake of less than 1000 calories during any 13-week period of gestation, as based on the official daily rations of the general population (Ravelli et al., 1998). As such, children born in Amsterdam between 7 January 1945 and 8 December 1945, were considered to be exposed to famine during prenatal life. In line with all previous publications on the cohort, three 16-week periods were distinguished; children who were mainly exposed during late gestation (born between 7 January and 28 April 1945), mid gestation (born between 29 April and 18 August 1945) or early gestation (born between 19 August and 8 December 1945). A sample of the individuals born within 1year before the famine or conceived up to 1year after the end of the famine were considered unexposed to famine and were eligible as controls for comparisons (born before the famine between 1 November 1943 and 7 January 1945 or conceived after the famine and born between 9 December 1945 and 28 February 1947).

Childhood trauma

Childhood trauma (CT) was assessed with the short form of the Dutch Childhood Trauma Questionnaire (CTQ-SF) (Bernstein et al., 2003; van Schie et al., 2017). This self-report questionnaire assesses traumatic maltreatment in childhood across five dimensions: physical, sexual and emotional abuse as well as physical and emotional neglect. It additionally contains the Minimization-Denial (MD) subscale which assesses reporter bias (Thombs et al., 2009). Participants rated each item on a scale ranging from 1-5, “Never True” to “Very Often True”. Two items from physical and five from emotional neglect were reverse coded before total and subscale

scores were calculated. Due to translation inconsistencies, one item of the original questionnaire relating to sexual molestation was removed from the Dutch CTQ, resulting in a total of 27 items (4 for sexual abuse, 3 for MD and 5 for all other subscales) (van Schie et al., 2017). The total CTQ score (range 24-120) was determined by summing all item scores, except for the items of the MD subscale. Higher total CTQ scores indicated more severe exposure to trauma during childhood. The MD subscale score was obtained by recoding the scores on the 3 corresponding CTQ items (1 to 4=0 and 5=1), resulting in a value ranging from 0-3, with higher scores indicating higher probability of under-reporting CT.

Adulthood trauma

Exposure to potential traumatic events (PTEs) in mid-to-late adulthood was assessed with a self-report version of the Dutch Life Events Checklist (LEC-5) for the Diagnostic and Statistical Manual of Mental Disorders, fifth edition (DSM-5) (Boeschoten et al., 2018). This questionnaire enquires about 17 different PTEs that could be either directly experienced, witnessed, encountered during work or happened to a close friend or family member. Participants were specifically asked about events in the past 15 years, since the completion of wave IV. A total score (range 0-17) was determined by summing the types of PTEs endorsed. Participants with more than 2 missing items were removed from the analyses (n=5).

PTSD symptom severity

The Dutch version of the PTSD checklist for the DSM-5 (PCL-5) was used to assess PTSD symptom severity in the past month (Bovin et al., 2016; van Praag et al., 2020). This self-report questionnaire consists of 20 items, each measuring the severity of one of the DSM-5 symptom clusters of PTSD (intrusions, avoidance, negative alterations in cognition and mood, and alterations in arousal and reactivity) on a scale from 0-4, ranging from “Not at all’ to “Extremely”. A total score (range 0-80) was determined by adding up all item scores, with higher scores indicating higher overall PTSD symptom severity. Missing data for maximally one item per participant (N=6) was imputed with the participant’s grand mean when calculating the PCL-5 total score, participants with more than one missing item were removed from the analyses (n=11).

Genotyping and haplotype identification

Using genomic DNA extracted from fasting blood samples (de Rooij et al., 2006), genotyping of four GR SNPs (ER22/23EK (rs6189/rs6190), N363S (rs6195), *Bcl*I (rs41423247) and exon 9β (rs6198)) was conducted with TaqMan allelic discrimination assays. Applied Biosystems (Foster City, CA) designed and optimised these assays. Cluster separation was tested by running assays on 90 blood bank samples. Two ng of genomic DNA was used in the assay, which was run on the Taqman Prism 7900HT platform. Independent end-point readings from this platform were used to analyse genotyping results which were confirmed by repeating analyses on a randomly selected 5% of samples (Fang et al., 2005).

We investigated the effects of GR haplotypes instead of individual SNPs, which increases the power to detect associations between genetic variants and complex diseases (Morris & Kaplan, 2002), such as PTSD. Haplotypes are sets of polymorphisms that are inherited together and thus capture the combined effects of the present SNPs within a specific gene within an individual. The R-package Haplo.stats 1.8.7 (Sinnwell et al., 2022) was used to determine the haplotypes present in the current study sample. This package uses an expectation-maximization algorithm to assign each participant to a haplotype pair and to obtain maximum likelihood estimations of haplotype frequencies across both alleles per participant. Posterior probabilities of haplotype pairs for every participant with complete information for at least one SNP were also calculated. For N=30 participants (6.8%) at least one SNP was missing and thus had a haplotype pair posterior probability <1. The lowest posterior probability for a haplotype pair was 0.321 and this was the case for n=4 (0.9%) participants. These participants were retained in the analyses as they were robust to sensitivity analyses removing them to explore potential influence of these low posterior probabilities on the results. Haploview (Barrett et al., 2005) was used to test potential deviation from Hardy-Weinberg equilibrium (HWE) to avoid drawing false associations between genotype and phenotype.

Statistical analyses

All analyses were performed in R (v4.0.5) (R core team, 2021). Potential differences between participants in the four exposure groups (unexposed, exposed in early, mid or late gestation) were tested using Kruskal-Wallis tests for continuous variables and Fisher's exact tests for categorical variables. PCL-5 total scores and CTQ total scores were log-transformed due to heteroscedasticity. We performed linear regression analyses to investigate the associations of GR haplotype and prenatal famine in conjunction with later life trauma on PTSD symptom severity (PCL-5 total score). Each model included three main effects for prenatal famine (dichotomous dummy variables for early, mid and late exposure groups resulting in the unexposed control group as the reference), a main effect for GR SNP haplotype carrier status (dichotomous dummy variable, coded as carriers (homozygous (two copies of the investigated haplotype present within a participants' haplotype pair) or heterozygous (one copy present within the haplotype pair) versus non-carriers (no copies present within the haplotype pair)) and a main effect for later life trauma (containing either childhood trauma: continuous CTQ total score; or adulthood trauma: continuous LEC total score). Additionally, the regression analyses included 2-way interactions for prenatal famine*later life trauma; prenatal famine*haplotype; and later life trauma*haplotype; and a three-way interaction for prenatal famine*later life trauma*haplotype. Separate models were applied for each haplotype as well as for childhood and adulthood trauma. Sex and marital status were included as covariates in all models. Marital status was dummy coded with single or widower compared to long-term relationship as reference group, with N=4 (0.9%) participants excluded due to missing data. MD scores were included as an additional covariate in the childhood trauma regression models to correct for potential minimization of childhood trauma. Participants

with missing data on either CTQ total score (n=6 (1.4%), CTQ MD score (n=4, 0.9%) or LEC-5 score (n=5, 1.1%) were removed from the respective analyses in a pairwise manner.

As previous studies observed sex-specificity in both the effects of prenatal adversity (Betts et al., 2015; de Rooij et al., 2011) and genetic variation in GR (Lindholm et al., 2020; Sarubin et al., 2017) on psychological symptom severity in adulthood, we additionally explored sex-specificity of the observed effects by performing exploratory analyses for men and women separately for the models with significant haplotype interaction effects. For each model, robust bootstrapped linear regression models with bias corrected and accelerated (BCA) confidence intervals, based on 5000 samples were used. A Bonferroni-corrected confidence interval of 97.5% was used to correct for multiple comparisons with regard to later life trauma type (adulthood and childhood trauma) in the primary analyses. This confidence interval was also used in the exploratory analyses. As the haplotypes consisted of SNPs within the same gene and are therefore not independent, no multiple testing correction was applied to account for the separate haplotype models (Castro-Vale et al., 2021).

3. Results

Participant characteristics

All participants were in their late adulthood (range 71-74) when filling out the questionnaires on trauma exposure and PTSD symptom severity. No significant differences in sex, educational level, marital status, nor in reported childhood and adulthood trauma exposure were observed between participants exposed to famine in early, mid or late gestation and non-exposed control groups (Table 1). Group differences in PCL-5 total scores were not tested as these were included as outcome measure in our regression models.

Identified GR haplotypes

All SNPs conformed to HWE (all p-values > 0.6). Five haplotypes were found, with the most frequent haplotype (41.9%) consisting of the common major alleles of the four SNPs as reported in the general population (Figure 1). Other identified haplotypes were: the B*cl*I haplotype, containing the minor allele of the B*cl*I SNP and the major alleles for all other three SNPs (frequency: 35.9%); the exon 9β haplotype, containing the minor allele of the exon 9B SNP and the major alleles for all other three SNPs (frequency: 15.2%), the N363S haplotype, containing the minor allele of the N363S SNP and the major alleles for all other three SNPs (frequency: 3.8%), and finally the ER22/23K+exon 9β haplotype, containing the minor alleles for these two SNPs and the major alleles for the two remaining SNPs (frequency: 3.2%). Haplotype frequencies for these five haplotypes did not differ significantly between those exposed to famine in early, mid or late gestation and those unexposed (Fisher's exact test, p = .873, Figure 2). Subsequent analyses on the associations between haplotype carrier status and PTSD symptom severity could not be conducted for the two least frequent haplotypes, N363S and ER22/23K+exon 9β, as the number

of participants who were haplotype carriers within the exposure groups was too small to yield reliable results (Figure 2). Thus, we performed linear regression models for the most common haplotype, the B*cl*I and the exon 9β haplotypes only.

Effects of GR haplotype, prenatal famine and childhood trauma on PTSD symptom severity

We investigated the effects of haplotype carrier status, prenatal famine exposure and childhood trauma, and their interactions on PTSD symptom severity for each of the 3 haplotypes (most common haplotype; B*cl*I; and exon 9β) separately (see Table 2). In the model regarding the B*cl*I haplotype, we found a significant main effect of CTQ total score on PCL-5 total score (B = 1.21, SE = 0.32, 97.5% CI [0.49 – 1.93]). A similar main effect for CTQ total score was found in the model regarding the exon 9β haplotype (B = 1.18, SE = 0.25, 97.5% CI [0.62 – 1.73]). We did not find a significant main effect of CTQ total score in the model regarding the most common haplotype. No significant main effects of prenatal famine exposure or haplotype carrier status, nor any significant interaction effects were observed within any of the haplotype models.

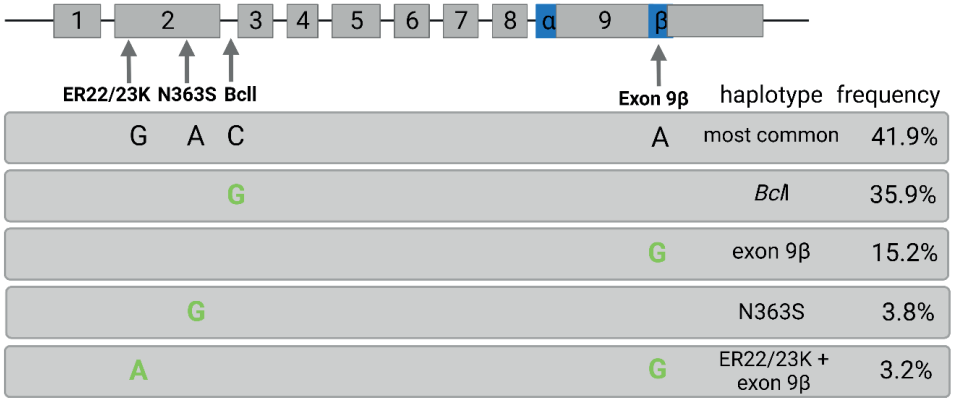

Figure 1. Overview of the five identified glucocorticoid receptor haplotypes based on the four investigated common single nucleotide polymorphisms (SNPs) within the *NR3C1* gene, and their respective frequencies within the cohort (based on two estimated haplotypes per participant).

Effects of GR haplotype, prenatal famine and adulthood trauma on PTSD symptom severity

We investigated the effects of haplotype carrier status, prenatal famine exposure and adulthood trauma, and their interactions on PTSD symptom severity for each of the 3 haplotypes (most common haplotype; B*cl*I; and exon 9β) separately (see Table 3). For the model regarding the *Bcl*I haplotype, we found a significant three-way interaction between LEC total score, exposure to famine in early gestation and *Bcl*I haplotype carrier status (B = -1.01, SE = 0.28, 97.5% CI [-1.77 - -0.18]; Figure 3), a significant two-way interaction between LEC total score and exposure to famine in early gestation (B = 0.58, SE = 0.20, 97.5% CI [0.17-1.01]), and an additional significant two-way interaction between *Bcl*I haplotype carrier status and exposure to famine in early gestation (B = 1.48, SE = 0.51, 97.5% CI [0.05-2.72]). We also found a significant main effect of LEC total score in

all haplotype models (most common haplotype; B = 0.22, SE = 0.08, 97.5% CI [0.02 – 0.44], B*cl*I; B = 0.16, SE = 0.07, 97.5% CI [0.01 – 0.30] and exon 9β; B = 0.19, SE = 0.06, 97.5% CI [0.06 – 0.33]). The association between LEC score, i.e. the amount of adulthood trauma, and PCL-5 total score, i.e. PTSD symptom severity, was positive for participants exposed to famine in early gestation without the B*cl*I haplotype, just as it was for the other exposure and control groups irrespective of their haplotype carrier status. However, this positive association was more pronounced in participants exposed to famine in early gestation than in participants who were both non-carrier of the B*cl*I haplotype and not exposed to famine during gestation (Figure 3, left panel). In contrast, participants carrying the *Bcl*I haplotype and exposed to famine in early gestation showed a negative association between LEC total score and PCL-5 total score (Figure 3, right panel). Regarding the most common and exon 9β haplotype models, there were no significant main or interaction effects of haplotype carrier status nor of prenatal famine exposure on PTSD symptom severity.

Potential sex-specific effects

Exploratory analyses were performed with separate models for men and women to investigate potential sex-specific effects for the model containing the significant three 5-way interaction between B*cl*I haplotype carrier status, prenatal exposure in early gestation, and adulthood trauma on PTSD symptom severity. In both the models for men and women, the directionality of the effects was the same as in the model including men and women combined. Although the 97.5% confidence intervals overlapped between men and women, the three-way interaction between B*cl*I haplotype carrier status, exposure in early gestation, and LEC total score was only significant in men (B = -1.44, SE = 0.44, CI [-2.60 - -0.01]) and not in women (B = -0.64, SE = 0.41, CI [-1.92 – 0.39]; Supplementary Table 1). None of the other effects concerning haplotype carrier status, prenatal famine exposure, and LEC total scores were significant.

4. Discussion

In the current study we investigated whether prenatal famine exposure and genetic variation in the GR moderated the associations between childhood trauma or mid-to-late adulthood trauma and PTSD symptom severity in late adulthood. We observed that famine exposure in early gestation and the GR B*cl*I haplotype together moderated the association between adulthood trauma and PTSD symptom severity, but not the association between childhood trauma and PTSD symptom severity. Participants exposed to famine during early gestation, but only those not carrying the GR B*cl*I haplotype, showed a significantly stronger association between adulthood trauma and PTSD symptom severity compared to non-exposed controls, indicating increased PTSD susceptibility following later life trauma. This increased susceptibility was not observed for participants who were exposed to famine during mid or late gestation. It was previously observed that prenatal exposure to maternal major infection increased PTSD susceptibility upon lifetime trauma exposure in 21 year old adults (Betts et al., 2015). Just as for this previous study, our

exploratory analyses tentatively indicate that the observed effects were more pronounced in men exposed to prenatal adversity than in women exposed to prenatal adversity.

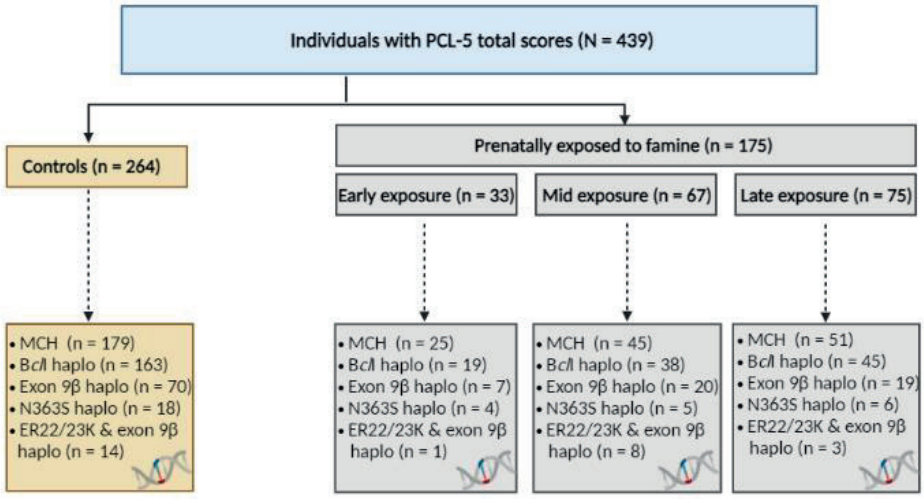

Figure 2. Number of haplotype (hap) carriers (either homozygous with two copies per haplotype pair, or heterozygous with one copy per haplotype pair) in controls and in each of the three exposure groups divided according to timing of exposure to famine during gestation. No significant differences in haplotype frequencies between exposure groups were found (Fisher's exact test, p = .873). MCH: most common haplotype.

Our findings also extend the previous findings on the impact of prenatal adversity on PTSD susceptibility in three ways. First, we investigated potential differential effects based on gestational timing of exposure within pregnancy, and observed that the effects were specific to exposure during early gestation. Secondly, our findings indicate that the influence of prenatal adversity on PTSD susceptibility following later life trauma extends well beyond the timeframe of early adulthood in the previous study by Betts et al. (2015), as we inquired on adulthood trauma exposure occurring within mid-to-late adulthood. Thirdly, we observed that the effect of prenatal adversity on PTSD susceptibility after later life trauma was dependent on common genetic variation. Significant moderation effects were observed for the GR B*cl*I haplotype, containing the minor allele of the functional B*cl*I SNP, a C to G nucleotide substitution, 646 base pairs downstream from exon 2 on the *NR3C1* gene, associated with GR hypersensitivity to GCs (van Rossum et al., 2003). Several previous studies in adults demonstrated that the presence of the B*cl*I SNP was associated with increased PTSD symptom development following adulthood trauma (Hauer et al., 2011; Lian et al., 2014). Furthermore, both the B*cl*I SNP and haplotype have been associated with increased presence of GR-related phenotypes of PTSD susceptibility in adults (e.g. higher peripheral GR number, lower acute post-trauma cortisol), either as main effect or in interaction with childhood trauma (Bachmann et al., 2005; Hauer et al., 2011; van Zuiden et al., 2012a). However, our findings contrasted these previous findings, which indicated increased PTSD

susceptibly in adulthood for B*cl*I carriers. Instead, we observed that participants exposed to famine during early gestation with the GR B*cl*I haplotype seemed to be protected from the adverse effects on PTSD susceptibility following adulthood trauma. Previously, it was observed that the GR B*cl*I SNP moderated the effect of prenatal maternal psychological symptoms measured during week 20 of pregnancy on emotional and behavioural problems in their three year old children in a similar manner as in our study: children of mothers with psychological problems during pregnancy carrying the G allele of the B*cl*I SNP had fewer emotional and behavioural problems than children of mothers with psychological problems during pregnancy without the SNP (Velders et al., 2012). Additionally, within the Dutch Famine Birth Cohort, previous studies have shown gene-early environment interactions suggesting that the direction of protective or harmful effects of various SNP alleles on physical health conditions turned around depending on prenatal famine exposure (Botden et al., 2012; de Rooij et al., 2006; van Hoek et al., 2009). For instance, minor alleles of two *SIRT1* SNPs, which in the general population increase the risk for type 2 diabetes, were associated with a lower prevalence of diabetes in individuals who had been exposed to famine prenatally (Botden et al., 2012).

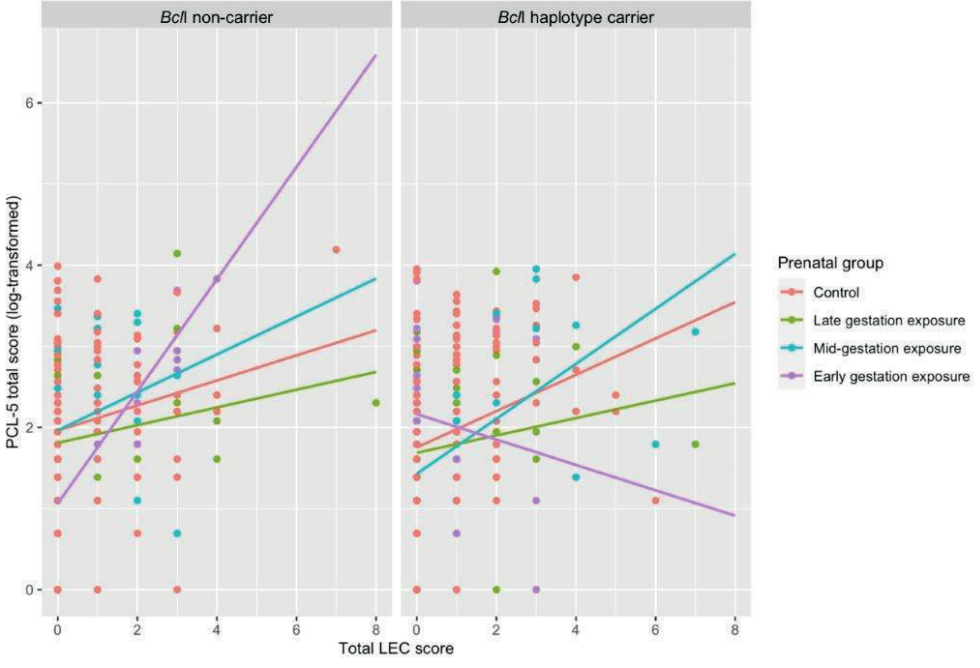

Figure 3. Visualization of PTSD symptom severity scores as a function of *Bcl*I haplotype carrier status, prenatal famine exposure and adulthood trauma. The association between the number of trauma types experienced during adulthood (LEC total score) and PTSD symptom severity (log-transformed PCL-5 total scores) is depicted for each prenatal exposure group and controls separately, divided into non-carriers (left panel) and *Bcl*I haplotype carriers (homozygous or heterozygous, right panel).

In the absence of reported mid-to-late adulthood trauma, participants exposed during early gestation with the B*cl*I haplotype had higher mean PTSD symptoms than those exposed during early gestation without the B*cl*I haplotype. Although we did not observe significant moderation effects of prenatal famine exposure nor of the B*cl*I haplotype on the association between childhood trauma and PTSD symptom severity, we cannot exclude that these higher PTSD symptoms resulted from exposure to traumatic events occurring between the time periods of childhood and mid-to-late adulthood investigated within this study. Additionally, we cannot exclude that our observed higher PTSD symptoms result from higher scores on those questionnaire items associated with other psychological problems, such as depression and anxiety, especially as the B*cl*I SNP was previously found to be associated with higher state anxiety (Lindholm et al., 2020) and increased risk for depression (van Rossum et al., 2006) in adulthood.

Our observed three-way interaction between GR haplotype, prenatal famine exposure and adulthood trauma on PTSD symptom severity seem to fit with the previously proposed three-hit model of vulnerability and resilience for stress-related mental health disorders following early life adversity (Daskalakis et al., 2013). This model posits that the interaction between genetic predisposition (Hit 1) and early life environmental factors (Hit 2) gives rise to particular programmed neurobiological phenotypes. These programmed phenotypes may promote either vulnerability or resilience to psychological problems in later life depending on the circumstances in the environment during later life (Hit 3).

Although the exact neurobiological mechanisms underlying our observed interactions between the GR B*cl*I haplotype, famine exposure during early gestation and adulthood trauma exposure cannot be inferred from the current study, our findings tentatively imply that the B*cl*I haplotype may have influenced famine exposure-related *in utero* alterations in HPA axis function and GC signalling by GRs. The Dutch famine undoubtedly induced physical as well as psychological stress in the exposed pregnant women, likely resulting in extensive prolonged HPA axis activation and high GC levels. Placental 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) partially protects the foetus from high maternal GC levels although this protection is not complete, and might not be present during the earliest phases of pregnancy when the placenta is still being formed. After the placenta starts to function, placental 11β-HSD2 expression increases, before it peaks in mid-pregnancy, after which it decreases again through late gestation (Howland et al., 2017). Thus, it is conceivable that during early pregnancy, famine exposure particularly resulted in high *in utero* GC levels compared to later gestation, resulting in compensatory alterations in the developing foetal HPA axis and central GRs modulated by functional GR SNPs. Such prenatal adversity-induced effects on HPA axis function and GC signalling by GRs in offspring were previously found to persist well into adulthood (Harris & Seckl, 2011; Howland et al., 2017; Reynolds et al., 2013; Seckl & Meaney, 2006), and may be further influenced by the presumed persistent functional effects of the carried GR haplotypes on GC signalling throughout life.

The Dutch famine birth cohort is unique in terms of its exposure to prenatal adversity confined to specific gestational periods. Together with the long-term follow up of individuals now more than seven decades following their prenatal exposure, this provides a rare opportunity to investigate the current research questions. However, the sample size was small, especially for the genetic analyses and even more so for the exploratory sex-specific analyses. Another limitation resulting from our small sample size was the combined analysis of homozygote and heterozygote haplotype carriers, as differences in associations between the investigated GR SNPs and susceptibility to develop mental health problems were previously observed between individuals with one versus two copies of the investigated haplotypes (Bachmann et al., 2005; Bet et al., 2009; Velders et al., 2012). Furthermore, we specifically investigated selected common SNPs with functional consequences within a probable neurobiological pathway underlying PTSD susceptibility. Although our study provides first evidence that such SNPs may interact with environmental factors throughout multiple periods in life to contribute to PTSD susceptibility, there are inherent limitations and pitfalls to using SNP based analyses to predict complex traits and disorders, such as PTSD (Wray et al., 2013). Therefore, the current results should be interpreted with caution and should ideally be validated in a larger population, although this will likely be difficult to achieve as populations with equal long-term follow-up as our study remain scarce. Furthermore, childhood trauma and adulthood trauma were retrospectively assessed. Although our childhood trauma-related analyses were corrected for potential underreporting (Thombs et al., 2009), we cannot exclude that other types of reporting bias may have occurred, especially when considering the sample consisted of aging individuals. Furthermore, we only investigated linear (interaction) effects of childhood or adulthood trauma exposure on PTSD symptom severity, while the described three-hit model also poses potential non-linear dose-dependent effects of the amount of trauma exposure on susceptibility to (traumatic) stress-related mental health problems (Daskalakis et al., 2013).

In conclusion, we observed that the association between famine exposure in early gestation and adulthood PTSD symptom severity was only detected when considered in interaction with genetic variation in a potential neurobiological pathway underlying PTSD susceptibility upon adulthood trauma exposure. Although our results remain to be validated, they illustrate the necessity of an integrated approach that considers genetics and the environmental context throughout various life periods, including the rarely investigated prenatal environment, when aiming to elucidate how PTSD susceptibility evolves throughout life.

Table 1. Characteristics of participant groups according to timing of exposure to prenatal famine.

	Controls		Prenatal famine exposure			
	Born before famine (n=139)	Conceived after famine (n=125)	Late gestation (n=75)	Mid gestation (n=67)	Early gestation (n=33)	
Age (years)	73.3 (1.0)	71.5 (0.5)	72.8 (0.4)	72.0 (0.2)	72.0 (0)	
PCL-5 total score[a]	10.5 (10.9)	10.9 (11.3)	8.3 (9.9)	11.2 (11.2)	12.9 (12.9)	
						p-value
CTQ total score[b]	36.8 (10.6)	37.5 (15.5)	36.9 (11.3)	37.8 (12.4)	39.1 (14.8)	.929
LEC total score[c]	1.04 (1.33)	0.92 (1.17)	1.24 (1.53)	1.27 (1.26)	1.27 (1.28)	.233
Sex (%)						
Women	73 (52.5)	65 (52.0)	37 (49.3)	44 (65.7)	19 (57.6)	.185
Man	66 (47.5)	60 (48.0)	38 (50.7)	23 (34.3)	14 (42.4)	
CTQ MD score[d]	0.76 (1.04)	0.71 (0.98)	0.72 (1.05)	0.49 (0.93)	0.61 (0.90)	.166
Marital status[e] **(%)**						
Long-term relationship	87 (62.6)	92 (73.6)	49 (66.2)	46 (68.7)	25 (75.8)	.732
Single	23 (16.5)	15 (12.0)	14 (18.9)	8 (11.9)	2 (6.1)	
Widower	28 (20.1)	16 (12.8)	11 (14.9)	13 (19.4)	6 (18.2)	
Education level	4.8 (1.4)	4.7 (1.5)	4.9 (1.5)	4.8 (1.2)	4.9 (1.1)	.743

Data are displayed as means (SD) or frequencies (%). [a]PTSD checklist for the DSM-5. [b]Childhood trauma questionnaire. [c]Life events checklist for the DSM-5. [d]Minimization denial score of the childhood trauma questionnaire. [e]Missing data for n=4 participants (n=3 controls, n=1 late gestation).

Table 2. Results of linear regression analyses including the effects of GR haplotype carrier status, prenatal famine exposure and childhood trauma on PTSD symptom severity in late adulthood, with separate models for the most common haplotype, the B*cl*I haplotype and the Exon 9β haplotype.

	Most common haplotype			BclI SNP haplotype			Exon 9β SNP haplotype		
Predictors	*B*	*SE*	*BCA CI (97.5%)*	*B*	*SE*	*BCA CI (97.5%)*	*B*	*SE*	*BCA CI (97.5%)*
Intercept	-0.65	1.41	-3.81 to 2.51	-2.27	1.16	-4.87 to 0.33	-2.32	0.90	**-4.35 to -0.29**
CTQ total score[a]	0.70	0.39	-0.18 to 1.57	1.21	0.32	**0.49 to 1.93**	1.18	0.25	**0.62 to 1.73**
Sex (female)[b]	0.14	0.09	-0.08 to 0.35	0.15	0.09	-0.06 to 0.36	0.17	0.09	-0.04 to 0.38
CTQ MD score[c]	-0.07	0.05	-0.19 to 0.05	-0.08	0.05	-0.20 to 0.04	-0.08	0.05	-0.20 to 0.04
Marital status (single)[b]	0.21	0.13	-0.10 to 0.51	0.18	0.13	-0.11 to 0.48	0.20	0.13	-0.10 to 0.50
Marital status (widower)[b]	0.19	0.13	-0.10 to 0.48	0.20	0.13	-0.09 to 0.49	0.20	0.13	-0.09 to 0.49
Late exposure[b]	0.80	2.72	-5.33 to 6.92	-0.23	2.61	-6.11 to 5.65	3.24	1.91	-1.06 to 7.54
Mid exposure[b]	-1.46	2.79	-7.75 to 4.82	0.23	2.41	-5.20 to 5.65	2.62	1.91	-1.68 to 6.92
Early exposure[b]	1.84	5.13	-9.71 to 13.39	-3.66	3.22	-10.90 to 3.57	-1.05	2.07	-5.71 to 3.60
Haplotype carrier status (≥1 copies)[d]	-1.88	1.59	-5.47 to 1.70	0.49	1.43	-2.72 to 3.70	1.47	1.65	-2.24 to 5.18
CTQ*Late exposure	-0.20	0.75	-1.89 to 1.48	-0.01	0.72	-1.62 to 1.60	-0.97	0.53	-2.17 to 0.22
CTQ*Mid exposure	0.39	0.77	-1.33 to 2.11	-0.08	0.67	-1.58 to 1.42	-0.76	0.53	-1.95 to 0.44
CTQ*Early exposure	-0.44	1.44	-3.68 to 2.80	1.04	0.89	-0.96 to 3.03	0.26	0.57	-1.01 to 1.54
CTQ*Haplotype	0.56	0.44	-0.43 to 1.55	-0.19	0.40	-1.08 to 0.70	-0.38	0.46	-1.41 to 0.66
Late exposure*Haplotype	3.28	3.39	-4.35 to 10.90	5.26	3.33	-2.23 to 12.75	-0.71	3.59	-8.79 to 7.38
Mid exposure*Haplotype	3.09	3.41	-4.59 to 10.77	0.83	3.19	-6.33 to 8.00	-5.72	3.48	-13.55 to 2.12
Early exposure*Haplotype	-2.71	5.54	-15.19 to 9.76	4.39	4.01	-4.63 to 13.41	3.39	5.74	-9.53 to 16.31
CTQ*Late exposure*Haplotype	-1.01	0.94	-3.12 to 1.09	-1.43	0.92	-3.50 to 0.63	0.29	0.99	-1.94 to 2.53
CTQ*Mid exposure*Haplotype	-0.87	0.94	-2.98 to 1.25	-0.24	0.88	-2.22 to 1.74	1.59	0.95	-0.55 to 3.74
CTQ*Early exposure*Haplotype	0.66	1.55	-2.82 to 4.14	-1.24	1.10	-3.72 to 1.24	-0.76	1.58	-4.32 to 2.81

N included	428	428	428
R^2 / R^2 adjusted	0.160 / 0.121	0.173 / 0.135	0.173 / 0.135

Log transformed PCL-5 scores as the outcome variable. [a]Childhood trauma questionnaire (log-transformed). [b]Long-term relationship used as the reference group for marital status; men used as the reference group for sex; non-exposed control group used as the reference group for prenatal famine exposure. [c]Minimization denial score of the childhood trauma questionnaire. [d]One or two copies of haplotype carried compared to no copies carried as a reference. BCA CI = bias corrected and accelerated confidence intervals.

Table 3. Results of linear regression analyses including the effects of GR haplotype carrier status, prenatal famine exposure and adulthood trauma on PTSD symptom severity in late adulthood, with separate models for the most common haplotype, the B*cl*I haplotype and the Exon 9β haplotype.

	Most common haplotype			B*cl*I SNP haplotype			Exon 9β SNP haplotype		
Predictors	*B*	*SE*	*BCA CI (97.5%)*	*B*	*SE*	*BCA CI (97.5%)*	*B*	*SE*	*BCA CI (97.5%)*
Intercept	1.58	0.14	**1.26 to 1.92**	1.82	0.12	**1.52 to 2.10**	1.67	0.10	**1.44 to 1.90**
LEC total score[a]	0.22	0.08	**0.02 to 0.44**	0.16	0.07	**0.01 to 0.30**	0.19	0.06	**0.06 to 0.33**
Sex (female)[b]	0.13	0.10	-0.07 to 0.26	0.13	0.10	-0.08 to 0.34	0.13	0.10	-0.09 to 0.34
Marital status (single)[b]	0.41	0.13	**0.10 to 0.71**	0.45	0.13	**0.15 to 0.73**	0.4	0.13	**0.10 to 0.70**
Marital status (widower)[b]	0.13	0.13	-0.14 to 0.41	0.16	0.13	-0.12 to 0.46	0.17	0.13	-0.13 to 0.47
Late exposure[b]	0.12	0.30	-0.75 to 0.75	-0.17	0.26	-0.73 to 0.38	-0.20	0.18	-0.61 to 0.20
Mid exposure[b]	-0.01	0.28	-0.75 to 0.74	-0.07	0.28	-0.69 to 0.54	-0.29	0.21	-0.77 to 0.18
Early exposure[b]	0.32	0.46	-1.81 to 1.70	-0.99	0.42	-2.02 to 0.18	-0.16	0.27	-0.78 to 0.46
Haplotype carrier status (≥1 copies)[c]	0.14	0.16	-0.24 to 0.54	-0.23	0.15	-0.59 to 0.14	0.07	0.17	-0.32 to 0.45
LEC*Late exposure	-0.11	0.20	-0.53 to 0.38	-0.06	0.12	-0.33 to 0.21	-0.05	0.10	-0.28 to 0.17
LEC*Mid exposure	-0.03	0.13	-0.37 to 0.58	0.09	0.18	-0.31 to 0.49	0.13	0.12	-0.13 to 0.39
LEC*Early exposure	-0.00	0.41	-1.49 to 1.44	0.58	0.20	**0.17 to 1.01**	0.08	0.16	-0.28 to 0.45
LEC*Haplotype	-0.03	0.10	-0.27 to 0.20	0.07	0.1	-0.16 to 0.30	0.03	0.10	-0.19 to 0.26
Late exposure*Haplotype	-0.33	0.35	-1.06 to 0.55	0.19	0.33	-0.53 to 0.89	0.4	0.42	-0.55 to 1.34
Mid exposure*Haplotype	-0.42	0.36	-1.35 to 0.55	-0.24	0.36	-1.04 to 0.90	0.22	0.38	-0.62 to 1.07
Early exposure*Haplotype	-0.55	0.55	-2.27 to 1.66	1.48	0.51	**0.05 to 2.72**	0.52	0.59	-0.81 to 1.85
LEC*Late exposure*Haplotype	0.03	0.22	-0.50 to 0.51	-0.07	0.17	-0.48 to 0.36	-0.17	0.21	-0.66 to 0.31
LEC*Mid exposure*Haplotype	0.32	0.19	-0.33 to 0.80	-0.03	0.21	-0.65 to 0.56	-0.12	0.19	-0.55 to 0.31
LEC*Early exposure*Haplotype	0.14	0.43	-1.43 to 1.67	-1.01	0.28	**-1.77 to -0.18**	-0.01	0.31	-0.71 to 0.69

N included	430	430	430
R^2 / R^2 adjusted	0.137 / 0.099	0.162 / 0.125[d]	0.141 / 0.103

Log transformed PCL-5 scores as the outcome variable. [a]Life events checklist for the DSM-5. [b]Long-term relationship used as the reference group for marital status; men used as the reference group for sex; non-exposed control group used as the reference group for prenatal famine exposure. [c]One or two copies of haplotype carried compared to no copies carried as a reference. [d]The model as a whole was significant (F(18, 403) = 4.23, p = 2.86e-08). BCA CI = bias corrected and accelerated confidence intervals.

Financial Support

This work was supported by the European Commission Horizon 2020, project LongITools [Grant number #874739]; and a Brain and Behavior Research Foundation NARSAD Young Investigator Grant awarded to dr. M. van Zuiden [#24928].

Conflict of Interest

None.

Acknowledgements

The authors thank all participants for their time and involvement, and are grateful for the Dutch famine birth cohort team for providing data.

5. Supplementary Materials

Supplementary Table 1. Results of exploratory linear regression analyses to investigate potential sex-specificity of the observed interactions between B*cl*I haplotype carrier status, prenatal famine exposure and adulthood trauma on PTSD symptom severity in late adulthood in men and women separately.

	Men			Women		
Predictors	***B***	***SE***	***BCA CI (97.5%)***	***B***	***SE***	***BCA CI (97.5%)***
Intercept	1.91	0.17	**1.54 to 2.29**	1.85	0.17	**1.47 to 2.24**
LEC total score[a]	0.09	0.11	-0.08 to 0.30	0.22	0.10	-0.04 to 0.40
Marital status (single)[b]	0.50	0.21	-0.06 to 0.97	0.48	0.17	**0.12 to 0.86**
Marital status (widower)[b]	0.17	0.26	-0.24 to 0.64	0.12	0.15	-0.21 to 0.46
Late exposure[b]	-0.45	0.36	-1.43 to 0.34	0.08	0.35	-0.55to 0.80
Mid exposure[b]	0.35	0.58	-1.18 to 1.42	-0.10	0.32	-1.08 to 0.89
Early exposure[b]	-0.80	0.58	-3.00 to 1.15	-1.18	0.61	-2.32 to 0.22
Haplotype carrier status (≥1 copies)[c]	-0.36	0.21	-0.83 to 0.14	-0.14	0.21	-0.62 to 0.34
LEC*Late exposure	0.06	0.19	-0.29 to 0.48	-0.14	0.16	-0.69 to 0.55
LEC*Mid exposure	0.27	0.37	-0.32 to 1.90	-0.02	0.21	-0.61 to 0.58
LEC*Early exposure	0.65	0.26	-0.01 to 2.40	0.45	0.32	-0.18 to 1.45
LEC*Haplotype	0.11	0.14	-0.22 to 0.46	0.03	0.12	-0.25 to 0.31
Late exposure*Haplotype	0.31	0.49	-1.02 to 1.60	-0.07	0.44	-0.92 to 0.74
Mid exposure*Haplotype	-0.98	0.65	-2.29 to 0.85	0.05	0.47	-1.12 to 1.30
Early exposure*Haplotype	1.89	0.74	-0.33 to 4.25	1.26	0.73	-0.50 to 2.94
LEC*Late exposure*Haplotype	-0.12	0.31	-0.92 to 0.70	-0.01	0.21	-0.76 to 0.65
LEC*Mid exposure*Haplotype	-0.11	0.39	-1.76 to 0.90	0.09	0.28	-0.67 to 0.82
LEC*Early exposure*Haplotype	-1.44	0.44	**-2.60 to -0.01**	-0.64	0.41	-1.92 to 0.39

N included		**197**		**233**
R^2 / R^2 adjusted	**0.225 / 0.152**		**0.140 / 0.072**	

Log transformed PCL-5 scores as the outcome variable. [a]Life events checklist for the DSM-V. [b]Long-term relationship used as the reference group for marital status; non-exposed control group used as the reference group for exposure to prenatal famine. [c]One or two copies of B*cl*I haplotype carried compared to no copies carried as a reference. BCA CI = bias corrected and accelerated confidence intervals


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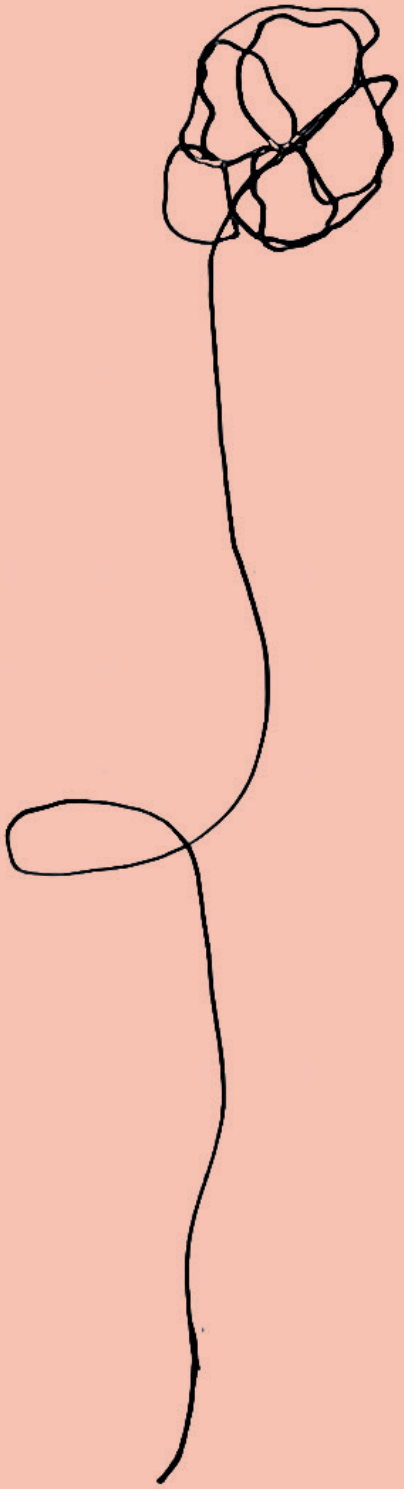

CHAPTER 4

Dysregulated functional brain connectivity in response to acute social-evaluative stress in adolescents with PTSD symptoms

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Published in European Journal of Psychotraumatology (2021), 12(1): 1880727, *https://doi.org/10.1080/20008198.2021.1880727*

Keywords: PTSD, adolescent, social-evaluative stress, functional connectivity, hippocampus, mPFC, amygdala, cortisol reactivity, cardiac reactivity

Abstract

Background Posttraumatic stress disorder (PTSD) is associated with dysregulated neural, cortisol, and cardiac stress reactivity and recovery. This understanding is predominantly based on studies in adults applying emotional-cognitive and trauma-related stimuli inducing negative emotions or perceived threat. Despite large numbers of adolescents with PTSD, few studies are available on neurobiological stress reactivity in this population. Moreover, no previous studies investigated neural reactivity to social-evaluative stress.

Objective To investigate functional brain connectivity, cortisol and cardiac reactivity to acute social-evaluative stress, and additional cortisol measures in trauma-exposed adolescents with and without high PTSD symptoms.

Method A speech preparation task to induce acute social-evaluative stress elicited by anticipatory threat, was used in a subsample of the Amsterdam Born Children and their Development (ABCD) birth cohort, consisting of trauma-exposed adolescents with (*n*=20) and without (n=29) high PTSD symptoms. Psychophysiological interaction analyses were performed to assess group differences in functional connectivity of the hippocampus, mPFC and amygdala during social-evaluative stress and recovery, as measured by fMRI. Additionally, perceived stress, heart rate and cortisol stress reactivity and recovery, cortisol awakening response and day curve were compared.

Results The stressor evoked significant changes in HR and perceived stress, but not cortisol. The PTSD symptom and control groups differed in functional connectivity between the hippocampus and cerebellum, middle and inferior frontal gyrus, and the mPFC and inferior frontal gyrus during social-evaluative stress versus baseline. Mostly, the same patterns were found during recovery versus baseline. We observed no significant group differences in amygdala connectivity, and cortisol and cardiac measures.

Conclusions Our findings suggest threat processing in response to social-evaluative stress is disrupted in adolescents with PTSD symptoms. Our findings are mainly but not entirely in line with findings in adults with PTSD, which denotes the importance to investigate adolescents with PTSD as a separate population.

Highlights

- Adolescents with PTSD symptoms showed different functional brain connectivity for the hippocampus and medial prefrontal cortex during social-evaluative stress.
- Amygdala connectivity was not different.
- Functional connectivity findings seem mostly similar to adults with PTSD.

1. Introduction

Approximately 16% of youth who experience a traumatic event subsequently develops posttraumatic stress disorder (PTSD) (Alisic et al., 2014), which is more than double the estimated lifetime prevalence of PTSD in trauma-exposed adults (de Vries & Olff, 2009). Evidence is mounting that PTSD is associated with, and potentially causally related to, dysregulated neural reactivity to a variety of stressors (Hayes et al., 2012a; Koch et al., 2016; Patel et al., 2012) and recovery from the stressors (Dickie et al., 2011). Despite the large numbers of adolescents affected by PTSD, most studies on neural stress reactivity in PTSD examined adults. As of yet, neural stress reactivity in adolescents with PTSD remains scarcely investigated.

Previous studies on neural stress reactivity in PTSD mainly focused on the functioning of specific brain regions of interest in response to various stimuli inducing negative emotions or feelings of threat, ultimately leading to a stress response. These stressors, for example, include emotional images to induce emotional distress and traumatic script-driven imagery to provoke PTSD symptoms. Meta-analyses of PTSD studies in adults previously demonstrated that activity and connectivity patterns between the hippocampus, medial prefrontal cortex (mPFC) and amygdala in response to these stressors were disrupted. Included studies used functional neuroimaging methods such as functional magnetic resonance imaging (fMRI), positron emission tomography (PET) and single-photon emission computed tomography (SPECT) (Hayes et al., 2012a; Koch et al., 2016; Patel et al., 2012). Resting-state studies have also shown that activity and connectivity within and between these regions differ between adult PTSD patients and trauma- and non-trauma-exposed controls during baseline conditions (Koch et al., 2016). The few studies available on adolescents with PTSD showed similar brain regions to be dysregulated. For example, PTSD in young adolescence was associated with increased left parahippocampal gyrus activity in response to trauma-related stimuli compared to trauma-exposed controls (Yang et al., 2004) and decreased amygdala-mPFC connectivity in response to threatening images compared to non-traumatized healthy controls (Wolf & Herringa, 2016). Also, resting-state connectivity findings in adolescent PTSD largely corroborate findings reported in adult PTSD (Viard et al., 2019).

Next to neural stress reactivity, endocrine and physiological stress regulation and reactivity have been found to differ between trauma-exposed individuals with and without PTSD as well. Findings on acute cortisol reactivity and its recovery in response to psychological stressors in adults with PTSD have been inconsistent thus far, demonstrating both increased and blunted responses (de Kloet et al., 2006; Elzinga et al., 2003; Zorn et al., 2017). Results from pharmacological challenge studies administering synthetic stress hormones, such as dexamethasone, are more consistent in finding PTSD-related enhanced suppression of cortisol secretion (Schumacher et al., 2019). Only two studies thus far focussed on cortisol stress reactivity in adolescents, and results were unequivocal. In one study, adolescents with (partial) PTSD showed a blunted cortisol response to trauma-related stimuli (Zantvoord et al., 2019). In the other study, female adolescents with PTSD symptoms showed blunted cortisol responses to a psychosocial stressor, whereas male

adolescents with PTSD symptoms showed increased cortisol responses (Zimmerman et al., 2020). Meta-analyses in adults demonstrated lower levels of cortisol in the morning and across the day in individuals with PTSD compared to trauma-exposed and non-exposed controls (Morris et al., 2012; Schumacher et al., 2019). Also, cortisol awakening responses (CAR) are likely attenuated in adults with PTSD compared to healthy controls (de Kloet et al., 2007; Wessa et al., 2006). The results available on basal cortisol in adolescent PTSD are mostly in line with studies in adults with PTSD. Adolescents with PTSD symptoms showed lower baseline and morning cortisol levels than healthy controls (Feldman et al., 2013; King et al., 2001; Pan et al., 2018). Also, the CAR was shown to be flattened in adolescents with PTSD or PTSD symptoms (Keeshin et al., 2014). However, in contrast to what was shown in adult PTSD, studies on diurnal cortisol showed an elevated day curve in young adolescents with PTSD symptoms (Carrion et al., 2002; De Bellis et al., 1999). These changes in endocrine output are thought to result from increased sensitivity of glucocorticoid receptors within the Hypothalamic Pituitary Adrenal (HPA) axis, which causes increased negative feedback by cortisol (Olff & van Zuiden, 2017). This dysfunction of the HPA axis is thought to underlie PTSD-related changes in hippocampus, amygdala and mPFC functioning and several symptoms, such as intrusions and hyperarousal (de Kloet et al., 2005; Herringa, 2017), although the observed correlation between endocrine and neural measures is generally only moderate (van Zuiden et al., 2019).

Another measure of physiological stress regulation and reactivity that has been studied in PTSD more rarely, is cardiovascular activity regulated by the autonomic nervous system. While adult PTSD has been associated with elevated heart rate in response to trauma-related stimuli in a meta-analysis (Pole, 2007), adolescents with PTSD symptoms showed no difference in heart rate reactivity to a social stressor or trauma-related stimuli compared to controls (Jones-Alexander et al., 2005; MacMillan et al., 2009).

In sum, it is still not certain if neural, cortisol and cardiac stress reactivity is dysregulated in adolescent PTSD and whether this is similar to adult PTSD (Leenarts et al., 2013). The brain undergoes extensive changes during development (Herringa, 2017; Heyn et al., 2019; Lupien et al., 2009; Weems et al., 2019), and thus findings from adult populations may not be directly generalizable to adolescent populations. Therefore, the aim of the present study was to investigate neurobiological stress reactivity in adolescents with PTSD symptoms. Specifically, we investigated differences in fMRI functional brain connectivity (using Psychophysiological Interaction analyses (PPI)), cortisol, and cardiac reactivity to a social-evaluative stress task between trauma-exposed adolescents with high PTSD symptom levels compared to trauma-exposed controls with no to mild PTSD symptoms.

To the best of our knowledge, neural reactivity to social-evaluative stress in PTSD has not been investigated yet, in either adults or adolescents. Nevertheless, as PTSD is associated with problems in social functioning (McLean et al., 2013; Schnurr et al., 2009), it is of interest to

investigate whether neural reactivity to this type of stressor is also dysregulated in individuals with PTSD, as was found for other types of stressors. Social-evaluative stressors are potent activators of central and peripheral stress systems (Dickerson & Kemeny, 2004; Ginty et al., 2019; Wager et al., 2009a). Furthermore, in both healthy adult and adolescent populations, involved brain regions and networks largely overlap between reactivity to and recovery from social-evaluative stressors and other types of stressors (Hermans et al., 2014; Liu et al., 2012; van Oort et al., 2017), specifically for the hippocampus, mPFC, and amygdala. Therefore, integrating previous findings on neurobiological mechanisms implicated in adults and adolescents with PTSD, combined with the available literature on neural regions involved in reactivity to social-evaluative stress in healthy populations, we expected differential functional connectivity involving the hippocampus, mPFC and amygdala, reduced cortisol reactivity but no differential heart rate reactivity in response to the social-evaluative stress task, as well as lower CAR and cortisol day curves in adolescents with high PTSD symptom levels compared to trauma-exposed controls.

2. Methods

2.1 Participants

In 2017, the ABCD-Early Life Stress and Obesity (ELSO) study was initiated; with the primary aim to investigate the potential mechanisms underlying the association between early life stress and childhood obesity. For the ABCD-ELSO study, a subsample of the Amsterdam Born Children and their Development (ABCD) cohort was invited to participate. The study design of this prospective population-based multiethnic birth cohort has been described in detail previously (van Eijsden et al., 2011).

Participants for the ABCD-ELSO study were selected based on criteria for four distinct groups – this information was collected 2 years earlier during the fourth follow-up assessment of the cohort in 2015; 1) children whose mother had high Body Mass Index (BMI; BMI>26.6 kg/m^2 (p50, i.e. 50th percentile of the study population)) pre-pregnancy; 2) children whose mother had high anxiety symptoms (State-Trait Anxiety Inventory scores >37 (p50)) during pregnancy; 3) trauma-exposed children with high PTSD symptom levels (PTSD symptom group); 4) a trauma-exposed healthy control group with no to mild PTSD symptom levels (TC). *N*=315 participants met criteria for one of these groups and were invited to participate. The subsample was invited by mail and a total of *n*=120 by then adolescent participants accepted the invitation (overall response rate: 38%; with a mean CRIES score for participants who accepted the invitation of 12.45 (SD 12.78) versus 12.30 (12.55) for those who declined the invitation, p = .732). For the present study, we specifically focused on two groups from the ABCD-ELSO subsample; the PTSD symptom and TC groups, a total of *N*=54 participants. Results within the other groups will be reported elsewhere. High PTSD symptoms were classified by a cut-off total score of ≥24 (p90) on the 13-item Children Revised Impact of Event Scale (CRIES-13) (Perrin et al., 2005) at the previous assessment at age 11-12. The CRIES-13 was filled out based on the experienced life event with the highest perceived impact (i.e.

index trauma) and subscale scores for symptom clusters *Intrusion, Avoidance* and *Arousal* as well as a total score were calculated. Children in the TC group had no or very mild PTSD symptoms (maximum CRIES total scores: 7 (p50)). Participants were excluded in case of claustrophobia, non-MRI compatible implants or braces, and severe neurological disorders. For female participants, hormonal contraception use was not an exclusion criterion, but upon finalizing the study, it appeared that only *n*=2 (3.9%) used hormonal contraception. As sensitivity analyses indicated that removing these participants from the analyses greatly influenced the neuroimaging results, it was decided to exclude these participants (see Supplementary Table S6-S7). The final sample consisted of *N*=49 participants (PTSD symptom: *n*=20; TC: *n*=29). Approval for this study was obtained from the local medical ethical committee, and the study was conducted following the Helsinki Declaration. All participants and their parents gave written informed consent prior to any study procedures.

2.2 Experimental session and fMRI task design

Enrolled participants were mailed questionnaires and a set of five salivettes for saliva collection prior to a clinic visit. All participants were asked to abstain from eating and drinking (except for water) 1.5 h before their visit. Upon arrival, participants handed in their collected salivettes and filled-out questionnaires. They received further instructions about the study procedure and MRI equipment and had the opportunity to ask questions. The scanning session consisted of a T1-weighted anatomical image, followed by a food-related Go/No-Go-task, a food reward- and anticipation-related Milkshake task, a 3D T2-weighted anatomical image, and finally a 9-min long social-evaluative stress task (*Stress*) to study acute neural, cardiac and cortisol stress reactivity in a laboratory setting (Kirschbaum et al., 1993; Wager et al., 2009a). We used a variation on the speech task from the Trier Social Stress Test eliciting anticipatory threat, which has been shown to generate acute stress responses on a neural, psychological, cardiac and endocrine level (Wager et al., 2009b). For a timeline of the used stress task, consisting of a *Baseline, Instruction, Stress* and *Recovery* phase, see Figure 1. For a detailed description of the stress task, see Supplemental S1.

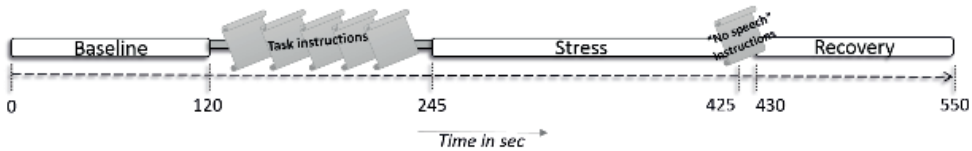


Figure 1. Overview of the timeline of the used stress task.

2.3 Psychological measures

To measure perceived stress participants were repeatedly asked to indicate how stressed they were during the experimental phases *Baseline*, *Stress* and *Recovery*. Possible answers ranged from 1 ('low perceived stress') to 7 ('high perceived stress'). The *Baseline* item was administered before

scanning, for *Stress* directly after scanning and for *Recovery* at the end of the experimental session. Additionally, perceived impact of the stress task was measured directly after scanning with a questionnaire consisting of four items; *Difficulty*, *Involvement, Expected performance* and *Control over the task*, scored using a 7-point Likert Scale.

General perceived stress experienced during the previous two weeks was measured using the 14-item Perceived Stress Questionnaire (PSQ14) (Cohen et al., 1983) on a 5-point Likert scale ranging from 0 ('Never') to 4 ('Very often'). Total scores (ranging from 0-56) were calculated, with higher scores indicating higher general perceived stress. To measure socio-emotional behaviour and problems, the Strengths and Difficulties Questionnaire (SDQ) (van Widenfelt et al., 2003) was administered on a 3-point Likert scale ranging from 0 ('Not true') to 2 ('Definitely true'). This self-report questionnaire measures five subscales; *Emotional symptoms*, *Conduct problems, Hyperactivity/Inattentio*n, *Peer problems*, and *Prosocial behaviour*. Total- and subscores were calculated representing psychosocial problems and behaviours, with higher scores indicating more socio-emotional problems and poorer psychosocial behaviour. Eating behaviour was measured using the self-report 33-item Dutch Eating Behaviour Questionnaire (DEBQ) (van Strien et al., 1986) on a 5-point Likert scale ranging from 0 ('Never') till 4 ('Very Often'). Scores on the three subscales *Emotional Eating, Restrained Eating* and *External Eating* were calculated, with higher scores indicating more problems in eating behavior. The PSQ14, SDQ and DEBQ were filled out at home prior to the experimental session. Sleep problems were measured using an adjusted version of the Children's Report of Sleep Patterns (CRSP) (Meltzer et al., 2013), filled out during the clinic visit. Mean total scores were calculated of 6 items following a 3-point Likert scale to represent sleep problems.

2.4 Biological measures

2.4.1 Cortisol

For saliva sampling, participants received salivettes with a biocompatible synthetic swab at home (Sarstedt, Germany). Participants were asked to place the salivette in their mouth for approximately one minute and lightly chew on the swab to absorb enough saliva, transfer it back into the tube and store it in the freezer until the day of the clinic visit. Sampling was instructed to take place on a weekend day (Stalder et al., 2016) according to a fixed schedule; directly after awakening, 30 and 45 minutes after awakening for CAR assessment (Pruessner et al., 1997) and additionally at 12:00 and 20:00 for assessment of the cortisol day curve. Participants were asked to abstain from food 30 minutes before sampling, abstain from dairy drinks one h before sampling, and not consume any products that contain caffeine on the collection day. During the clinic visit, saliva samples were collected right before scanning and 8, 14, and 23 minutes respectively after the social stressor started, for assessment of acute cortisol stress reactivity. The large majority (85.64%) of assessments took place between 12:00pm and 18:00pm. The earliest assessment started at 10:09 and the latest at 14:30. Time of the first cortisol assessment did not differ

between groups (mean(SD) PTSD symptom: 12:22:36 (1:29:34); TC: 11:42:25 (1:33:46); T_{48} = -1.49, p = .143). All salivette samples were centrifuged, pipetted, encoded and frozen (-80°C) before being sent to the Biological Psychology Laboratory in Dresden, Germany, for analysis. Samples were analyzed using a luminescence immunoassay (IBL International, Germany). Intra- and inter-assay variations are <4.5% and <4.3% respectively.

Cortisol output for CAR and acute cortisol stress reactivity (using all available samples from the clinic visit) were studied by calculating the 'Area under the Curve with respect to the Ground' (AUCg) and 'with respect to the Increase' (AUCi) following guidelines by Pruessner *et al.* (2003). In addition, the 'Maximum increase with respect to baseline' (MaxInc) was computed. For the cortisol day curve, AUCg (using the first, fourth and fifth sample (Pruessner et al., 2003)) and MaxInc (using all five samples) were calculated. All calculations were corrected for reported sampling time, and in case of missing sample values, imputation was applied a missing value by the mean of non-missing adjacent sample cases.

2.4.2 Heart rate

Heart rate (HR) was monitored continuously during fMRI acquisition using a wireless MR-compatible HR peripheral pulse monitor attached to the left index finger with a sampling rate of 500 Hz. R-wave peaks were identified using Matlab (version R2016a, The Mathworks, Inc., USA). Individual outlier R-wave peaks were identified and removed from the data. Mean HR was averaged across 1-min intervals (meanHR/min) using the remaining R-waves. All data was smoothed before selecting the corresponding meanHR/min peaks for every task phase separately (*Baseline, Instruction, Stress, Recovery*), and were time-locked to the functional scans. Finally, meanHR/min was averaged per phase. Due to technical failure, n=2 participants were excluded from HR analyses.

2.5 fMRI data analyses

2.5.1 fMRI data acquisition

Scans were acquired using a 3T Philips Ingenia MRI system (Philips, Best, The Netherlands) with a 32-channel head coil. To obtain functional images, a single-shot echo-planar imaging (EPI) sequence sensitive to blood oxygenation level-dependent contrast was used, and an additional EPI scan with opposite phase polarity was acquired for distortion correction with the following scan parameters: TR/TE=730/30 ms; flip angle=55°; multiband factor=4; SENSE=1.8; field of view=240 x 240 x 132 mm; voxel size=2.5 x 2.5 x 2.75 mm; 48 slices. A T1-weighted magnetization-prepared rapid acquisition gradient echo (MPRAGE) sequence was used for anatomical referencing with the following scan parameters: TR/TE=7/3 ms; flip angle=9°; field of view=240 x 180 x 256 mm; voxel size=1 mm isotropic; 180 slices. For every participant the last seven volumes were removed because they were obtained beyond the task duration.

2.5.2 Preprocessing and first-level analysis

Preprocessing was performed using FMRIPREP v1.2.3 (Esteban et al., 2018) and included motion correction, distortion correction, registration, and normalization to MNI space, smoothing and ICA-AROMA (for details see Supplemental S2). Framewise Displacement (FD) was calculated to add as a covariate to second-level analyses. Subsequently, a high pass filter (256s) was applied using FSL and grand-mean scaling was performed.

Seed-to-voxel functional connectivity was calculated using psychophysiological interaction (PPI) analyses in FSL FEAT (www.fmrib.ox.ac.uk/fsl (Woolrich et al., 2001)). Time-courses were extracted for each region of interest (ROI) based on previously identified relevant regions concerning stress reactivity in PTSD: 1) hippocampus, 2) mPFC, and 3) amygdala (Koch et al., 2016; Shin, 2006). For anatomical definition details, see Supplemental S3. In the first-level model, the contrasts for Stress vs. Baseline and Recovery vs. Baseline were used as psychological regressors of interest. In addition, for every ROI, individual time-courses were used as physiological regressors of interest. Time-series functional connectivity analyses were carried out using FILM with local autocorrelation correction (Woolrich et al., 2001).

2.5.3 Higher-level analysis

Due to excessive motion, *n*=10 (PTSD symptom group *n*=3; TC *n*=7) participants were excluded from the second level analyses (FD>0.3) (Achterberg & van der Meulen, 2019). First-level PPI maps were fed into higher-level analysis in FEAT to assess between and within-group differences. Additionally, demeaned FD (Power et al., 2014) and demeaned age values (Morris et al., 2012) were included as covariates. A 25% thresholded MNI avg152T1 gray matter mask was applied to Z statistic images before thresholding. First Z-statistic images were thresholded non-parametrically using clusters determined by Z>3.1 and a corrected cluster significance threshold of *P*=0.05 at the single-subject and group level (Worsley, 2001). Additionally, a False Discovery Rate (FDR) threshold of 0.01 was applied for between- and within-group results on the observed significant clusters within all test runs for ROIs and contrasts, to further control for multiple comparisons (Benjamini & Hochberg, 1995).

2.6 Statistical analyses

Independent samples *T*-tests, or in case of non-normally distributed data Mann-Whitney U tests, were performed to assess group differences in demographics, psychological characteristics, and questionnaire mean item, scale and delta scores. For categorical variables, Pearson Chi-square tests and Fisher's exact tests, or in case of >2 categories per variable Fisher-Freeman-Halton exact tests, were performed.

Furthermore, Linear Mixed Models (LMM) with Restricted Maximum Likelihood (REML) were performed to determine group differences in cortisol, HR levels, and perceived stress across the

experimental session, whilst controlling for sex and age. For details on these analyses, see Supplemental S4.

One-way analyses of covariance (ANCOVAs) were conducted for cortisol AUCg, AUCi, and MaxInc, including sex and age as covariates. Missing values and outliers (Z≥3.29) were excluded from the respective analyses.

Exploratory Pearson Correlations between cortisol AUCg, AUCi and MaxInc and questionnaire scores on sleep problems were performed. To explore potential effects of eating behaviour on our significant functional connectivity clusters, we extracted Functional Connectivity values (FC values; extracted from ROIs of our significant functional connectivity clusters using the CONN toolbox version 18b) of the significant clusters. One-way ANCOVAs were performed including questionnaire scores on eating behaviour that significantly differed between groups as covariate.

For all analyses, following significant main or interaction effects, post hoc *T*-tests with Bonferroni correction (α=5%) for multiple comparisons were performed.

3. Results

3.1 Demographics and psychological measures

By definition, groups differed significantly on PTSD symptom total score, which was reported at age 11-12 during the fourth follow-up assessment of the cohort in 2015 (Table 1). Current PTSD symptom scores were not measured during this substudy. Compared to the TC group, the PTSD symptom group reported significantly more types of traumatic life events and more general perceived stress, sleep problems, psychosocial problems and behaviours on the subscales *Emotional symptoms, Peer problems*, and *Total Difficulties*, and *Emotional Eating* problems.

3.2 Acute stress reactivity

3.2.1 Functional connectivity

We observed significantly increased functional connectivity for *Stress vs. Baseline* between the right hippocampus and right cerebellum in the PTSD symptom group compared to TC (Figure 2A, Table 2). For TC compared to the PTSD symptom group, significantly increased functional connectivity was found for *Stress vs. Baseline* between the right hippocampus and left middle frontal gyrus (MFG, Figure 2B); between the left hippocampus and left inferior frontal gyrus (pars opercularis; IFG) and right MFG (Figure 2C); and between the mPFC and left IFG (pars opercularis, Figure 2D). At a more liberal threshold for FDR correction of *p*=.05, for *Stress vs. Baseline* significantly increased functional connectivity was found between the mPFC and left insula in the PTSD symptom group compared to TC. Additionally, in TC compared to the PTSD symptom group, significantly increased connectivity was found between the mPFC and an additional cluster in the left IFG (pars opercularis; see Supplementary Table S1).

For *Recovery vs. Baseline* significantly increased functional connectivity was found between the left hippocampus and left IFG (pars opercularis), and right MFG; between the right hippocampus and left IFG (pars opercularis); and between the mPFC and left IFG (pars opercularis) in TC compared to the PTSD symptom group. At an FDR correction of p=.05 we found significantly increased connectivity between the left hippocampus and two extra clusters in the left IFG (pars opercularis and pars triangularis) in TC compared to the PTSD symptom group (see Supplementary Table S1).

Significant effects of the task on functional connectivity during *Stress vs. Baseline* and *Recovery vs. Baseline* in TC and PTSD symptom groups separately are summarized in Supplementary Table S2.

Additionally, our exploratory analyses showed no significant covariate effect of *Emotional Eating* problems on FC values.

3.2.2 Cortisol reactivity

No significant group difference were found in any of the cortisol stress reactivity measures (AUCg ($F(1,40) = 0.63$, $p = .433$, $\eta2 = 0.02$), AUCi ($F(1,39) = 0.22$, $p = .640$, $\eta2 = 0.01$), and MaxInc ($F(1,39) = 0.19$, $p = .667$, $\eta2 = 0.01$)). For both groups, cortisol levels did not change significantly throughout the experimental session (group ($F(42.11) = <0.01$, $p = .973$), time ($F(58.45) = 2.41$, $p = 0.076$),group*time ($F(58.45) = 0.14$, $p = .937$); Figure 3A, Supplementary Table S3).

3.2.3 Heart rate reactivity

HR significantly changed throughout the experimental session (time ($F(101.38) = 42.65$, $p = <.001$; Figure 3B); Supplementary Table S4). Post-hoc comparisons showed HR significantly increased after baseline, peaking during *Stress*, and significantly decreased again afterwards. There were no significant effects of group ($F(42.89) < 0.01$, $p = .949$) and group*time ($F(101.38) = 0.08$, $p = .971$) on HR.

3.2.4 Subjective perceived stress

Perceived stress significantly changed throughout the experimental session ($F(79.60) = 25.73$, $p = <.001$; Figure 4; Supplementary Table S5). Post-hoc comparisons showed perceived stress increased after *Baseline*, peaking during *Stress*, and decreasing again during *Recovery*, with least perceived stress during *Recovery*. There were no significant effects of group ($F(38.06) = 0.33$, $p = .571$) and group*time ($F(79.60) = 0.02$, $p = .985$). Subjective impact of the stressor did not differ between groups (Table 3).

3.3 CAR and cortisol day curve

There were no significant group differences for any of the CAR measures (AUCg ($F(1,30) = 0.04$, $p = .840$, $\eta2 < 0.01$), AUCi ($F(1,30) = 0.09$, $p = .766$, $\eta2 < 0.01$), MaxInc ($F(1,37) = 0.05$, $p = .822$, $\eta2$

< 0.01); Figure 3C). Additionally, for cortisol day curves no significant group difference in AUCg was observed (F(1,28) = 4.01, p = .055, η2 = 0.13; Figure 3C).

Sleep problems correlated moderately but non-significantly with CAR AUCg (r=.509, p = .075) and cortisol day curve AUCg (r=.441, p = .132) within the PTSD symptom group.

4. Discussion

To our best knowledge, this is the first study that examined functional brain connectivity in response to social-evaluative stress in trauma-exposed adolescents with and without high PTSD symptoms. Adolescents with high PTSD symptoms showed differential functional brain connectivity between the hippocampus and the cerebellum, MFG and IFG; and between the mPFC and IFG during acute social-evaluative stress compared to trauma-exposed controls. Mostly, the same patterns were observed during recovery. Contrary to our hypothesis, no group difference was found in functional brain connectivity for the amygdala during acute social-evaluative stress or recovery. We also investigated cortisol and cardiac reactivity and did not find an association between the presence of PTSD symptoms and cortisol reactivity, HR reactivity, CAR and cortisol day curve.

A neuroimaging study in adolescent PTSD demonstrated differential activity patterns in similar regions as dysregulated functional connectivity was observed in our study: Yang *et al.* (2004) found increased activity in hippocampal and cerebellar regions during trauma-related imagery compared to trauma-exposed controls. However, they did not examine functional connectivity between these regions. Moreover, a resting-state fMRI study in adults also demonstrated increased functional connectivity between the hippocampus and the cerebellum in PTSD patients compared to non-trauma-exposed healthy controls (Rabellino et al., 2018). The authors interpreted this to be associated with ongoing scanning of the environment for potential threat and, at the same time, storing this information and comparing it to past memories. It seems reasonable to expect this altered threat processing, i.e., increased attention to potential threat within the environment, to be similarly dysregulated during stressful conditions, as observed in our study during social-evaluative stress. Our finding of increased functional connectivity between the bilateral hippocampus and the bilateral MFG in response to stress in trauma-exposed controls compared to adolescents with high PTSD symptoms also suggests dysregulated threat processing under social-evaluative stress. Previously, Bremner *et al.* (2003a) proposed that both the hippocampus - essential in learning and memory processes (Squire & Zola-Morgan, 1991) - and MFG are involved in a dysfunctional emotional memory network in PTSD, associated explicitly with hypervigilance and impairments in inhibitory control of the fear response. In line with this model, O'Doherty *et al.* (2017) hypothesized that the reduction they found in MFG volumes could be related to PTSD-related hypervigilance, due to possible impairments in inhibitory modulation of the HPA axis and of fear responses.

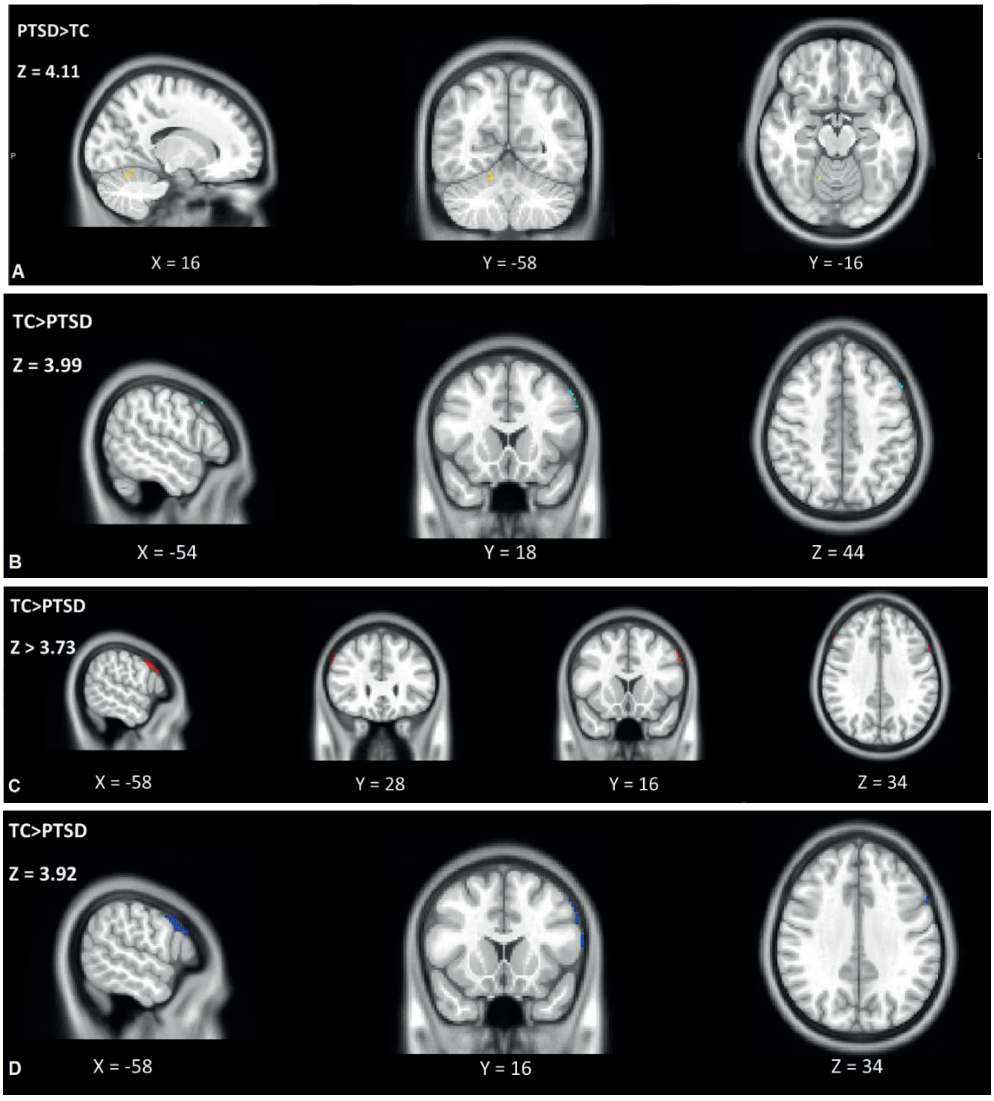


Figure 2. Representations of the significant increased functional connectivity clusters between adolescents with high PTSD symptoms vs. trauma-exposed controls for **A.** the right hippocampus with the right cerebellum during *Stress* vs. *Baseline* for PTSD symptom group>TC group; in yellow, **B.** the right hippocampus and left MFG during *Recovery* vs. *Baseline* for the TC>PTSD symptom group; in light blue, **C.** the left hippocampus and left IFG *and left hippocampus and right MFG* during *Recovery* vs. *Baseline* for the TC>PTSD symptom group; in red, and **D.** the mPFC and left IFG during *Recovery* vs. *Baseline* for the TC>PTSD symptom group; in dark blue. Significance level was defined as cluster p-values <.05 after FDR correction (α=0.01), PTSD symptom group *n*=17, TC *n*=22.

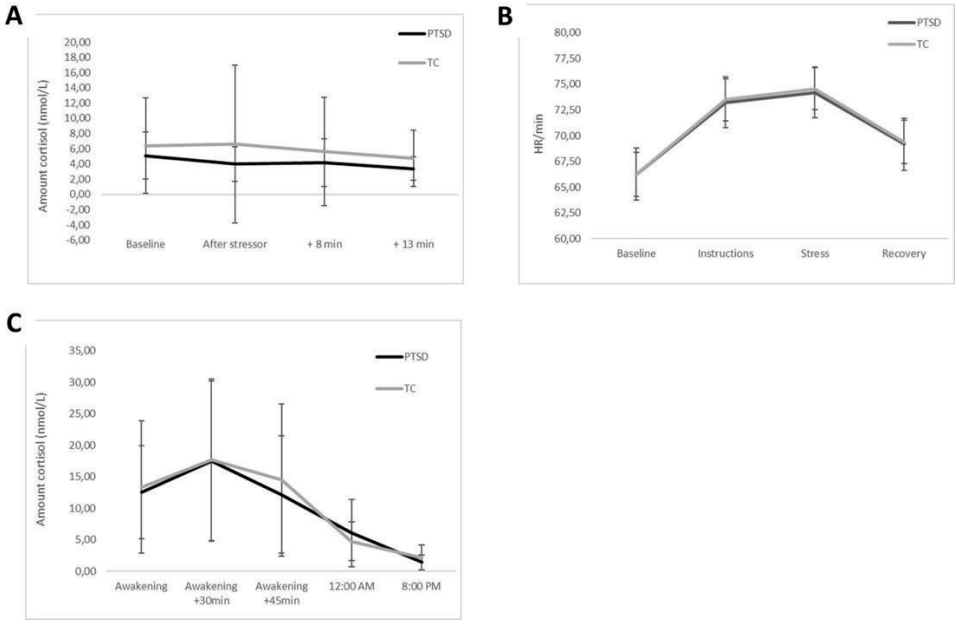


Figure 3. A. Cortisol acute stress reactivity. Displayed as means of cortisol levels across the experimental session. PTSD symptom group - Baseline *n*=19, After stressor *n*=19, +8:00 *n*=19, +13:00 *n*=19; TC – Baseline *n*=29, After stressor *n*=29, +8:00 *n*=29 +13:00 *n*=28, Error bars represent SDs. **B.** Heart rate reactivity. Displayed as estimated marginal means of heart rates per minute during the task. PTSD symptom group *n*=20, TC *n*=27, Error bars represent SEs. **C.** CAR and cortisol day curve. PTSD symptom group – CAR AUCg *n*=13, AUCi *n*=13, MaxInc *n*=14, cortisol day curve AUCg *n*=13; TC – CAR AUCg *n*=21, AUCi *n*=21, MaxInc *n*=27, cortisol day curve AUCg *n*=19. There were no effects of group on cortisol acute stress reactivity, heart rate reactivity, CAR or cortisol day curve. Error bars represent SDs.

Additionally, a magnetoencephalography study by Popescu *et al.* (2019) found that MFG activity during a working memory task was associated with false positive memories in adults with PTSD as well. This was thought to be related to hypervigilance and to reflect persistent retrieval of previously consolidated memory traces that are irrelevant or no longer relevant. Altogether, the differential connectivity that we observed in adolescents with high PTSD symptoms probably relates to difficulties in adequately focusing on and performing the stress task, without getting disrupted by potential threatening stimuli in the environment that attract attention, due to difficulties in separating irrelevant from relevant information.

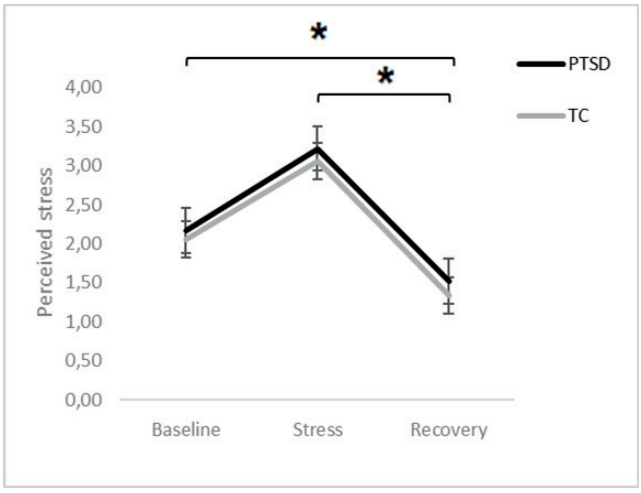


Figure 4. Perceived subjective levels for all participants during *Baseline, Stress,* and *Recovery* on perceived stress. Displayed as estimated marginal means perceived subjective levels across the experimental session PTSD symptom group n=20; TC n=29, *p<0.05, Error bars represent SEs.

We also observed increased functional connectivity between both the left hippocampus and mPFC and the left IFG - pars opercularis (also referred to as Broca's area) in response to stress in trauma-exposed controls compared to adolescents with high PTSD symptoms. The IFG is pivotal for multiple internal speech processes (Grèzes & Decety, 2001; Koski, 2002) and controlling motoric processes for a fight-or-flight response in a situation of stress because of threat (Kogler et al., 2015). As the IFG is also important for reconstructing semantic representations to be able to describe personal experiences (Rauch et al., 1996). Hull (2002) considered IFG activity disruptions in PTSD consistent with difficulties in restructuring traumatic experiences and memories. This reasoning was supported by Lindauer *et al.* (2004) reporting decreased activity in both the IFG and medial frontal cortex in police officers with PTSD in response to individualized trauma scripts compared to trauma-exposed controls. Thus, our findings could indicate that effective communication for the hippocampus and mPFC with the IFG is required in the presence of social-evaluative stress to prepare and practice the assigned speech internally, next to representing their personalized speech, whilst controlling a motoric stress response.

During recovery, we observed mainly the same differences in functional connectivity patterns between the groups with and without high PTSD symptoms as observed in response to the social-evaluative stress task, i.e., differential functional connectivity between the left hippocampus and left IFG, left hippocampus and right MFG, and mPFC and left IFG. Rauch *et al.* (2006) proposed a functional network in adult PTSD containing hippocampal and mPFC regions that may mediate PTSD-related deficits in threat-related fear extinction and associated attentional processes. They provided foundational reasoning for the necessity of proper functioning of these regions, not only for adequate functioning during acutely stressful or threatening situations, but also in its

immediate aftermath during recovery, which seems to be dysregulated in PTSD as also observed here.

Based on evidence from adults demonstrating the amygdala to be a key player in PTSD (Koch et al., 2016; Rauch et al., 2006), with amygdala functioning being linked to specific threat-sensitive behaviours related to PTSD symptoms such as threat detection and fear conditioning (Öhman, 2005), we expected to find differential amygdala connectivity patterns in response to stress in our adolescent sample as well. However, amygdala connectivity during and after the social-evaluative stress task did not significantly differ between adolescents with and without high PTSD symptoms. Previous studies on amygdala functional connectivity during a cognitive-emotional task in adolescents with PTSD have been inconsistent (for an overview see Weems et al., 2019). Puberty has also been found to be a critical period for changes in amygdala functioning in anxious adolescents (Ferri et al., 2014), who likely also deal with comparable dysregulated fear processing. Previous studies that did find amygdala connectivity alterations in adolescent PTSD included participants with more comprehensive ranges of pubertal stages (Cisler et al., 2013, 2018; Wolf & Herringa, 2016, in Weems et al., 2019). However, the age (14-15 years) and pubertal stage (mean pubertal stage 3.28: indicating mid pubertal stage) of our participants is quite similar to the previous studies, not finding dysregulated amygdala functional connectivity. This could suggest a developmental element underlying these differential findings and that the amygdala is not yet or differentially associated with PTSD symptoms during these pubertal stages.

Another alternative explanation for our amygdala null-result may be the type of stressor we used to induce social-evaluative stress. Social-evaluative stress was previously found to be able to induce stressor-evoked connectivity changes in the amygdala in healthy females (Ginty et al., 2019), possibly due to its role in autonomic changes such as HR in response to multiple types of stressors, which we also demonstrated in our participants. Nevertheless, it remains to be investigated how comparable the various paradigms of stress studies are, as social-evaluative stress may not provoke different amygdala responses between those affected and not affected by PTSD in the manner of trauma-related and emotional stimuli, because the latter induce traumatic memory recall.

We found no differences between adolescents with and without high PTSD symptoms in cortisol and cardiac reactivity to the social-evaluative stress task, and in the CAR and the cortisol day curve. Our findings may again be explained from a developmental perspective, as developmental timing of trauma was a significant moderator of morning and afternoon/evening cortisol levels in an extensive meta-analysis on cortisol in PTSD compared to trauma-exposed and non-trauma exposed controls (Morris et al., 2012). Additionally, it has been demonstrated that mid-puberty may be a recalibration window for HPA axis functioning in which cortisol-related impairments could potentially reset (Gunnar et al., 2019). On the other hand, there are quite a few studies on cortisol and cardiac activity that, similar to our results, also found no differences between adults

(Bremner et al., 2003b; Veazey et al., 2004) or adolescents (Jones-Alexander et al., 2005; Lipschitz et al., 2003) with and without PTSD, suggesting that other factors than developmental stage also may be at play, such as time since index trauma (Morris et al., 2012).

The main limitation to our study is that participants for the current study were selected based on their reported trauma exposure and PTSD symptom severity at age 11-12, without repeated assessment at the time of the completion of the stress protocol, approximately 2 years later. Although the participants in the PTSD symptom group on average reported more current PTSD-related behavioural problems, such as increased perceived stress, sleep problems and socio-emotional problem behaviours, than the trauma-exposed controls, we do not know whether their previously reported PTSD symptoms were still present (i.e. chronic) or had already remitted. This hampers the interpretation of our current findings. We cannot conclude whether the observed findings were associated with chronicity of PTSD symptoms or potentially were still present despite remitted symptoms. Additionally, we do not know whether the observed neuroimaging findings may actually reflect relatively stable vulnerability factors for development of PTSD symptoms upon trauma exposure. This is important as it has become increasingly clear that the course of PTSD symptoms upon their onset is heterogeneous (Santiago et al., 2013) and neurobiological correlates of PTSD may differ depending on the exact stage of the symptoms (McFarlane et al., 2017).

There were also some other limitations to our study. We had to exclude 20% of the participants due to excessive movement in the scanner, leading to reduced and relatively small sample sizes, limiting our statistical power and possibly resulting in not observing additional between-group differences in neurobiological stress reactivity. This may have been particularly relevant for our null-finding regarding amygdala connectivity, as our within-group results on functional connectivity do indicate some differences despite the absence of significant group differences: amygdala connectivity - with predominantly frontal regions - seemed to be present to a greater extent in trauma-exposed controls than in the group with PTSD symptoms. Additionally, both sex and hormonal contraception use have been increasingly associated with brain structure and functioning, including memory, emotion regulation, and behavioural, endocrine and physiological stress reactivity (Garza & Jovanovic, 2017; Haag et al., 2020; Pletzer et al., 2010). However, given our sample size, we were unable to investigate whether sex potentially moderated the associations between neural, cortisol and cardiac stress reactivity and PTSD symptoms. Also, as only two female participants used hormonal contraception, we could also not investigate this potential effect in more detail. Another limitation is the cross-sectional design of our study, which restricted us in investigating potential developmental changes and drawing conclusions on the causality of our findings. Thus, longitudinal studies are imperative to follow-up on these outstanding issues. Also, the stress task we applied in the MR scanner may also be considered a limitation. The social-evaluative stress task did not result in increased cortisol levels, possibly indicating that the acute stressor was not very powerful. However, we did observe an effect of

the stressor on perceived stress, cardiac reactivity and functional connectivity in both groups. The absence of the cortisol response may be due to high baseline levels induced by anticipatory anxiety and distress for the MRI experiment. Also, some may consider the 2-minute time length of the *Stress* phase of the social-evaluative stress task too short to exhibit an accurate stress response for PPI analyses. Although our experimental design was previously demonstrated to induce a robust neural and cardiac stress response (Ginty et al., 2019; Wager et al., 2009a, 2009b), Noack *et al.* (2019) stated that social-evaluative threat (SET) has not yet been sufficiently proven to be a well-suited and robust stressor paradigm for the MR scanner - despite its promising purpose - so therefore we recommend using a more extensively validated task for future PTSD-related research. Additionally, home saliva sampling was performed on one day instead of multiple days and on a weekend day, which could have caused variability of the CAR and diurnal cortisol profiles (Stalder et al., 2016). No information on bedtimes nor awakening times was collected, so we were not able to control for this in our cortisol analyses. During the scanning session the tasks prior to the stress task were both food-related. Since comorbidity between eating disorders and PTSD has been reported (Reyes-Rodríguez et al., 2011) and our participants with PTSD symptoms reported more problems in emotional eating behaviour, our adolescents with PTSD symptoms may have been emotionally aroused by the previous food-related tasks, ultimately probing the neural stress system differently and influencing our results. However, our exploratory analyses showed that eating problems did not influence our functional connectivity findings and therefore we can likely exclude this as a potential substantial confounder.

A significant strength of our study is that we focussed on adolescents with PTSD symptoms, which is a scarcely studied population. Also, our control group consisted of trauma-exposed controls instead of non-exposed controls or controls with unspecified exposure, which allows us to cautiously conclude that the observed group differences do not result from trauma exposure in general.

In conclusion, this is the first neuroimaging study on social-evaluative stress reactivity in adolescent PTSD. We found that the presence of PTSD symptoms was associated with differential functional connectivity of the hippocampus, mPFC, cerebellum, IFG and MFG during acute social-evaluative stress, and mostly the same patterns during recovery. Together, our findings indicated that neural threat processing in response to social-evaluative stress appears to be disrupted in adolescents with PTSD symptoms. Our findings are mainly but not entirely in line with functional connectivity findings in adults with PTSD. This denotes the importance of investigating adolescents with PTSD as a specific population, instead of generalizing findings from adult research. It also highlights that treatment guidelines in adolescents with PTSD symptoms ideally should be based on research uncovering dysregulated neurobiological mechanisms in adolescents with PTSD symptoms. This could eventually provide more specialized and targeted treatment possibilities, ultimately improving treatment effectiveness for adolescents with PTSD symptoms.

We strongly suggest that future studies focusing on neurobiological stress reactivity and recovery in adolescent PTSD adopt a longitudinal perspective, capturing potential developmental changes.

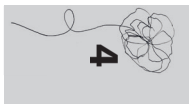
Table 1. Participant characteristics.

	PTSD symptom group (*n*=20)	TC (*n*=29)	Statistics
Boys (*n* (%))	12 (60.0%)	14 (48.3%)	X^2 = .65, *df* = 1, *p* = .419
Age (years)	14.25 (0.44)	14.79 (0.41)	*U* = 132.50, *p* < .001*
Puberty stage[1]	3.21 (0.71)	3.34 (0.81)	T_{46} = 0.59, *p* = .561
Index trauma (*n (%)*)			
Death	5 (25.0%)	13 (44.8%)	
Emotional Trauma	5 (25.0%)	-	
Medical Trauma	2 (10.0%)	8 (27.6%)	
Physical Trauma	1 (5.0%)	3 (10.3%)	
Accidents	1 (5.0%)	1 (3.4%)	
Other	6 (30.0%)	4 (13.8%)	
Total trauma exposure (nr of experienced event types)	5.65 (3.69)	2.86 (1.81)	*U* = 140.50, *p* = .002*
PTSD symptom severity (total CRIES score)[2]	32.30 (5.98)	2.34 (2.08)	$T_{22.188}$ = -21.54, *p* < .001*
Intrusion	8.20 (5.36)	0.34 (0.61)	*U* = 563.00, *p* < .001*
Avoidance	11.85 (3.90)	0.69 (1.20)	*U* = 579.50, *p* < .001*
Arousal	11.95 (4.72)	1.31 (1.95)	$T_{46.45}$ = -11.71, *p* < .001*
General functioning			
General perceived stress[3]	25.33 (4.77)	20.50 (4.35)	T_{44} = -3.54, *p* = .001*
Sleep problems[4]	0.95 (0.39)	0.68 (0.37)	T_{47} = -2.41, *p* = .020*
Socio-emotional behaviour and problems[5]			
Emotional symptoms	3.56 (2.77)	1.43 (1.29)	*U* = 142.00, *p* = .011*
Conduct problems	1.11 (1.13)	0.89 (0.96)	*U* = 225.50, *p* = .524
Hyperactivity/Inattention	4.17 (2.07)	2.82 (2.55)	*U* = 169.00, *p* = .059
Peer problems	1.22 (1.17)	0.50 (0.92)	*U* = 162.00, *p* = .023*
Prosocial Behaviour	8.56 (1.15)	8.89 (1.37)	*U* = 195.50, *p* = .185
Total Difficulties	10.06 (4.49)	5.64 (3.71)	*U* = 113.00, *p* = .002*
Eating behaviour and problems[6]			
Emotional eating	14.72 (9.77)	8.21 (7.60)	T_{44} = -2.53, *p* = .015*
Restrained eating	8.94 (6.94)	5.68 (6.12)	*U* = 325.50, *p* = .097
External eating	19.83 (4.27)	20.29 (5.75)	T_{44} = 0.29, *p* = .776
Ethnicity (*n* (%))			
Western European	19 (95.0%)	29 (100%)	*p* = 0.408
Non-Western European	1 (5.0)	-	
Hand preference (*right-handed* (*n (%)*))	17 (85.0%)	29 (100%)	*p* = 0.062

Scores are displayed as mean (SD) or *n* (%). [1]Categorical stage of puberty according to Petersen *et al.* (1988), PTSD symptom group *n*=19; TC *n*=29; [2]Children Revised Impact of Event Scale (CRIES-13) total score based on index trauma, PTSD symptom group *n*=20; TC *n*=29; [3]Perceived Stress Questionnaire (PSQ14), PTSD symptom group *n*=18; TC *n*=28; [4]Children's Report of Sleep Patterns (CRSP), PTSD symptom group *n*=20; TC *n*=29; [5]Strengths and Difficulties Questionnaire (SDQ), PTSD symptom group *n*=18; TC *n*=28, [6]Dutch Eating Behavior Questionnaire (DEBQ), PTSD symptom group *n*=18; TC *n*=28, *p < 0.05.

Table 2. Between-group cluster list of task effects for PTSD symptom group > TC and TC > PTSD symptom group.

							Stress vs. Baseline							
PTSD symptom group vs. TC								*TC vs. PTSD symptom group*						
	Region	X	Y	Z	Z-score	Cluster size	*p*	Region	X	Y	Z	Z-score	Cluster size	*p*
R Hippocampus	R Cerebellum	16	-58	-16	4.11	28	< .001	L Middle Frontal Gyrus	-54	18	44	3.99	20	.002
L Hippocampus								L Inferior Frontal Gyrus (pars opercularis)	-58	16	34	3.85	31	< .001
								R Middle Frontal Gyrus	54	28	38	3.73	18	.004
R Amygdala														
L Amygdala														
mPFC								L Inferior Frontal Gyrus (pars opercularis)	-58	16	34	3.92	42	< .001
							Recovery vs. Baseline							
PTSD symptom group vs. TC								*TC vs. PTSD symptom group*						
	Region	X	Y	Z	Z-score	Cluster size	*p*	Region	X	Y	Z	Z-score	Cluster size	*p*
R Hippocampus								L Inferior Frontal Gyrus (pars opercularis)	-58	16	34	3.80	22	.001
L Hippocampus								L Inferior Frontal Gyrus (pars opercularis)	-58	16	34	3.85	37	< .001
								R Middle Frontal Gyrus	54	28	38	3.68	24	< .001
R Amygdala														



L Amygdala							
mPFC	L Inferior Frontal Gyrus (pars opercularis)	-58	16	34	3.75	28	< .001

Significant results after correcting for multiple testing by FDR correction (α=0.01) according to Benjamini & Hochberg (1995). Regions were identified using the Harvard-Oxford Cortical and Subcortical Structural Atlases or if probability of the labels was low (<15%) using the MNI Structural Atlas. PTSD symptom group *n*=17, TC *n*=22.

Table 3. Perceived impact of the stress task.

Item	PTSD symptom group (*n*=20)	TC (*n*=29)	Statistics
Difficulty	3.40 (1.76)	3.14 (1.33)	T_{47} = -0.59, p = .555
Involvement	3.50 (1.99)	4.31 (1.31)	$T_{30.29}$ = -1.60, p = .120
Expected performance	3.80 (1.44)	4.34 (1.20)	T_{47} = -1.44, p = .157
Control over the task	4.70 (1.08)	5.10 (1.18)	T_{47} = -1.22, p = .229

Scores are displayed as mean (SD). PTSD symptom group *n*=20; TC *n*=29.

Financial Support

This work was supported by the Amsterdam Brain and Cognition Centre (ABC, University of Amsterdam), Nutricia Research Foundation, a Veni grant from ZonMw awarded to dr. M. van Zuiden, the Netherlands organization for Health Research and Development [Grant number 91617037] and a Young Investigator grant from Brain and Behaviour Research Foundation NARSAD [Grant number #24928].

Conflict of Interest

No potential conflict of interest was reported by the authors.

Data availability statement

The data that support the findings of this study are available on request from the corresponding author, CEH. The data are not publicly available due to their containment of information that could compromise the privacy of research participants.

Acknowledgements

The authors thank all participating children for their time and involvement.

5. Supplementary Materials

S1. Social stress task procedure

The Social stress task started with a *Baseline* phase during which participants were instructed to relax and lie still while focussing on a white cross on a black background that was displayed for 120 seconds. Next, during the *Instruction* phase, the participants viewed on-screen instructions to prepare a 4-min speech in silence for 3 minutes. With this speech, they had to defend themselves from being falsely accused of shoplifting a belt. The participants were informed that there was a chance that they would be selected to present their defense to a panel of judges (law students) immediately after the scanning procedure, which would be recorded. The instructions were written down on five slides presented automatically for 20 seconds each, to ensure the participants had enough time to read and understand all instructions. Then, participants were given 180 seconds to prepare their speech during the *Stress* phase. At the end of the *Stress* phase, every participant received a message on the screen, stating they were randomly selected for not giving the speech. Finally, participants were instructed to relax for the remaining 120 seconds during the *Recovery* phase. All task instructions were controlled by E-prime software (version 2.0., Psychology Software Tools Inc).

S2. Scan preprocessing

Preprocessing was performed using FMRIPREP v1.2.3 (Esteban et al., 2018). Each T1w scan was bias-corrected, skull-stripped and subsequently normalized to MNI space using non-linear registration (Tustison et al., 2010). Functional data preprocessing included motion correction using MCFLIRT and distortion correction using an implementation of the TOPUP technique using 3dQwarp (Andersson et al., 2003). This was followed by co-registration to the corresponding T1w using boundary-based registration with 9 degrees of freedom (Greve & Fischl, 2009). Motion correcting transformations, field distortion correcting warp, BOLD-to-T1w transformation, and T1w-to-template (MNI) warp were concatenated and applied in a single step using antsApplyTransforms (ANTs v2.1.0) using Lanczos interpolation. ICA-based Automatic Removal Of Motion Artifacts (AROMA) was used to generate data that was non-aggressively denoised (Pruim et al., 2015). Subsequently, data were spatially smoothed (6mm FWHM).

S3. Anatomical definition of ROIs

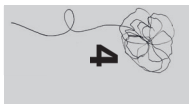
The amygdala and hippocampus (left and right separately) were anatomically defined using the Harvard-Oxford Subcortical probability atlas, thresholded 90% for the amygdala and 75% for the hippocampus. For the mPFC, a mask was created based on the 75% Harvard-Oxford Subcortical probability atlas distributed with FSL. Recommended labels for the superior frontal gyrus, paracingulate gyrus, and cingulate gyrus (anterior division) were selected and boundaries were restricting to MNI $-12 < x < 12$, $0 < y < 60$, and $z > -15$ (Jahn et al., 2016).

S4. Statistical analysis details for LMM

For linear mixed models (LMM) we first tested which standard error covariance structure with fixed effects fitted data best using Chi-square changes in -2 restricted log-likelihood (-2LL) (West, 2009): unstructured (UN), diagonal (DIAG) or first-order autoregressive covariance structure (AR1). Thereafter, we investigated whether adding random intercept and both random intercept and random slope improved model fit, using Akaike's Information Criterion (AIC) and Bayesian's Information Criterion (BIC) (West, 2009). Ultimately, the best-fitted model was used to investigate group differences in repeated measures of salivary cortisol levels, mean HR/min and perceived levels of stress across the experimental session whilst controlling for age and sex.

Table S1. Between-group cluster list of task effects for PTSD symptom group > TC and TC > PTSD symptom group.

Stress vs. Baseline														
PTSD symptom group vs. TC								*TC vs. PTSD symptom group*						
	Region	X	Y	Z	Z-score	Cluster size	*p*	Region	X	Y	Z	Z-score	Cluster size	*p*
R Hippocampus	R Cerebellum	16	-58	-16	4.11	28	< .001	L Middle Frontal Gyrus	-54	18	44	3.99	20	.002
L Hippocampus								L Inferior Frontal Gyrus (pars opercularis)	-58	16	34	3.85	31	< .001
								R Middle Frontal Gyrus	54	28	38	3.73	18	.004
R Amygdala														
L Amygdala														
mPFC	L Insula	-28	-28	14	3.98	16	.014	L Inferior Frontal Gyrus (pars opercularis)	-62	16	14	3.99	15	.021
								L Inferior Frontal Gyrus (pars opercularis)	-58	16	34	3.92	42	< .001
Recovery vs. Baseline														
PTSD symptom group vs. TC								*TC vs. PTSD symptom group*						
	Region	X	Y	Z	Z-score	Cluster size	*p*	Region	X	Y	Z	Z-score	Cluster size	*p*
R Hippocampus								L Inferior Frontal Gyrus (pars opercularis)	-58	16	34	3.80	22	.001
L Hippocampus								L Inferior Frontal Gyrus (pars opercularis)	-58	16	34	3.85	37	< .001
								L Inferior Frontal Gyrus (pars opercularis)	-62	16	14	3.82	14	.026
								L Inferior Frontal Gyrus (pars triangularis)	-60	26	20	3.78	13	.041
								R Middle Frontal Gyrus	54	28	38	3.68	24	< .001

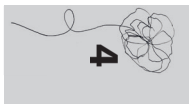


R Amygdala							
L Amygdala							
mPFC	L Inferior Frontal Gyrus (pars opercularis)	-58	16	34	3.75	28	< .001

Results after correcting for multiple testing by FDR correction (α=0.05) according to Benjamini & Hochberg (1995). Regions were identified using the Harvard-Oxford Cortical and Subcortical Structural Atlases or if probability of the labels was low (<15%) the MNI Structural Atlas was viewed. L = Left, R = Right; PTSD symptom group *n*=17, TC *n*=22.

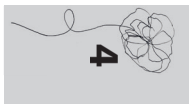
Table S2. Within-group cluster list of task effects for the PTSD symptom group (n=17) and TC group (n=22).

					Stress vs. Baseline										
PTSD symptom group								*TC*							
	Region	X	Y	Z	Z-score	Cluster size	*p*	Region	X	Y	Z	Z-score	Cluster size	*p*
R Hippocampus	R Thalamus	18	-24	0	4.25	18	0.005	L Anterior Supramarginal Gyrus	-60	-28	54	4.79	1290	< .001
	R Pallidum / Putamen	26	-12	0	3.85	19	0.003	R Postcentral Gyrus	68	-12	36	4.71	1291	< .001
								L Precuneous Cortex	-10	-62	32	4.45	36	< .001
								R Occipital Pole	4	-98	10	4.34	19	0.003
								R Superior Lateral Occipital Cortex	24	-72	32	4.33	49	< .001
								Lingual Gyrus	0	-74	-4	4.12	60	< .001
								R Pallidum / R Putamen	18	4	-4	4	22	0.001
								R Parietal Lobe	14	-40	22	3.94	19	0.003
								L Planum Polare	-36	-16	-10	3.92	29	< .001
								L Pallidum / L Putamen	-20	0	-4	3.91	24	< .001
								R Middle Frontal Gyrus	58	26	36	3.84	41	< .001
								R Inferior Lateral Occipital Cortex	46	-88	6	3.84	20	0.002
								R Posterior Superior Temporal Gyrus	72	-24	0	3.77	27	< .001
L Hippocampus	R Thalamus	18	-24	0	4.43	20	0.002	R Postcentral Gyrus	68	-12	36	4.51	937	< .001
	L Cerebellum	-28	-58	-38	3.61	28	< .001	L Frontal Orbital Cortex / L Inferior Frontal Gyrus (pars triangularis)	-58	28	-2	4.36	936	< .001
								R Pallidum	24	-8	-2	4.02	22	< .001



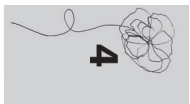
	R Angular Gyrus	68	-46	26	3.98	20	0.002
	R Frontal Lobe	50	4	58	3.94	23	< .001
	L Pallidum	-20	-10	-6	3.9	28	< .001
	R Pallidum / R Putamen	18	4	-4	3.87	32	< .001
	R Temporal Pole	60	16	-4	3.75	24	< .001
	R Frontal Pole	44	42	40	3.72	27	< .001
R Amygdala	L Anterior Supramarginal Gyrus	-60	-28	54	4.26	273	< .001
	R Temporal Pole	56	20	-10	4.16	55	5.96e -08
	R Postcentral Gyrus	64	-8	44	4.11	282	< .001
	R Posterior Cingulate Gyrus	12	-38	20	4.04	20	0.003
	L Anterior Superior Temporal Gyrus	-66	-2	-6	4.03	30	< .001
	L Occipital Pole	-34	-100	6	3.92	41	< .001
	L Superior Lateral Occipital Cortex	-62	-64	24	3.88	159	< .001
	R Angular Gyrus	60	-54	50	3.85	48	< .001
	R Lingual Gyrus	2	-76	-6	3.79	23	0.001
	R Inferior Frontal Gyrus (pars triangularis)	62	26	14	3.75	61	< .001
	R Occipital Pole	26	-96	28	3.71	19	0.005
	R Frontal Pole	40	40	46	3.63	29	< .001
	L Inferior Frontal Gyrus (pars opercularis)	-62	16	4	3.62	23	0.001
	L Postcentral Gyrus	-70	-20	18	3.54	19	0.005

L Amygdala								L Cerebellum	-26	-62	-38	4.37	21	0.002
								L Frontal Orbital Cortex / L Inferior Frontal Gyrus (pars triangularis)	-58	28	-2	3.94	33	< .001
								R Inferior Frontal Gyrus (pars triangularis)	60	26	8	3.84	22	0.002
								L Superior Lateral Occipital Cortex	-62	-64	24	3.83	28	< .001
								R Postcentral Gyrus	70	-16	24	3.79	33	< .001
								L Superior Lateral Occipital Cortex	-48	-82	32	3.77	63	< .001
								R Superior Lateral Occipital Cortex	54	-70	38	3.50	19	0.005
mPFC	R Pallidum	26	-12	-2	4.7	150	< .001	L Pallidum	-20	-10	-6	5.36	128	< .001
	L Brain-stem	-16	-34	-34	4.28	28	< .001	L Occipital Pole	-38	-96	4	5.01	1237	< .001
	R Planum Temporale	48	-26	6	4.27	56	< .001	R Precentral Gyrus	62	2	42	4.86	729	< .001
	R Postcentral Gyrus	18	-36	52	4.27	49	< .001	L Posterior Cingulate Gyrus	-10	-24	34	4.63	84	< .001
	R Putamen	28	-22	16	4.23	35	< .001	R Pallidum / R Putamen	26	-6	-2	4.62	29	< .001
	R Putamen	18	10	2	4.22	57	< .001	L Hippocampus	-30	-34	-12	4.55	30	< .001
	R Thalamus	18	-24	0	4.22	18	0.006	R Occipital Pole	32	-92	28	4.52	34	< .001
	R Precuneous Cortex	18	-56	42	4.16	20	0.003	L Putamen	-20	16	2	4.49	84	< .001
	L Cerebellum	-30	-62	-38	4.12	51	< .001	R Occipital Pole	36	-98	4	4.4	32	< .001
	L Cerebellum	-8	-50	-30	4.12	31	< .001	R Anterior Cingulate Gyrus	8	32	4	4.38	35	< .001
	R Cerebellum	24	-42	-34	4.01	25	< .001	R Cerebellum	12	-52	-32	4.35	40	< .001
	R Temporal Lobe	42	-26	-12	4	21	0.002	R Superior Lateral Occipital Cortex	46	-68	54	4.32	247	< .001
	R Superior Frontal Gyrus	20	-6	44	3.96	35	< .001	R Lingual Gyrus	2	-78	-10	4.32	41	< .001



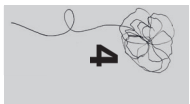
R Frontal Lobe	64	14	4	3.93	75	< .001	R Cerebellum	12	-52	-32	4.35	40	< .001
R Parietal Lobe	14	-36	34	3.9	26	< .001	R Cerebellum	44	-84	-30	4.31	18	0.006
R Lingual Gyrus	28	-44	-2	3.88	19	0.004	L Parietal Lobe	-22	-50	42	4.26	26	< .001
R Amygdala	14	-12	-10	3.85	22	0.001	R Occipital Pole	4	-98	10	4.21	36	< .001
Parietal Lobe	0	-62	6	3.8	28	< .001	R Anterior Cingulate Gyrus	4	10	22	4.18	27	< .001
R Occipital Lobe	32	-92	28	3.76	21	0.002	L Inferior Lateral Occipital Cortex	-36	-56	8	4.16	93	< .001
L Pallidum	-16	-2	2	3.74	21	0.002	R Caudate	22	28	0	4.11	79	< .001
L Inferior Frontal Gyrus (pars opercularis)	-62	18	4	3.67	29	< .001	L Brain-Stem	-4	-26	-18	4.1	25	< .001
L Posterior Cingulate Gyrus	-14	-26	32	3.67	20	0.003	R Temporal Occipital Fusiform Cortex	48	-62	-22	4.1	18	0.006
							L Lingual Gyrus	-10	-52	-4	4.03	20	0.003
							R Posterior Superior Temporal Gyrus	48	-20	0	3.92	21	0.002
							L Cerebellum	-8	-46	-28	3.89	22	0.001
							L Anterior Supramarginal Gyrus	-44	-38	34	3.79	18	0.006
							R Superior Lateral Occipital Cortex	24	-76	36	3.78	34	< .001
							R Lingual Gyrus	28	-52	0	3.74	20	0.003

						Recovery vs. Baseline								
PTSD symptom group								*TC*						
	Region	X	Y	Z	Z-score	Cluster size	*p*	Region	X	Y	Z	Z-score	Cluster size	*p*
R Hippocampus	R Thalamus	18	-24	0	5.00	25	< .001	R Postcentral Gyrus	68	-14	36	4.5	1041	< .001
	R Postcentral Gyrus	18	-34	50	4.49	45	< .001	L Occipital Pole	-38	-96	4	4.48	956	< .001
	R Superior Parietal Lobule	22	-52	56	4.17	27	< .001	L Precuneous Cortex	-10	-62	32	4.34	24	< .001
	R Hippocampus	26	-36	4	3.97	20	0.003	L Anterior Superior Temporal Gyrus	-68	-8	2	4.34	21	0.002
								L Cerebellum	-26	-62	-38	4.19	26	< .001
								R Anterior Cingulate Gyrus	8	10	24	4.14	24	< .001
								R Pallidum / R Putamen	18	4	-4	4.11	24	< .001
								R Superior Lateral Occipital Cortex / R Precuneous Cortex	20	-68	42	4.05	32	< .001
								L Pallidum	-20	-10	-6	4.05	26	< .001
								L Lingual Gyrus	-2	-66	0	3.84	45	< .001
								L Caudate	-20	24	0	3.82	19	0.004
								L Putamen	-20	16	2	3.78	21	0.002
								L Temporal Lobe	-40	-22	-10	3.72	27	< .001
								L Hippocampus	-28	-36	-10	3.59	18	0.006
L Hippocampus	L Cerebellum	-28	-58	-38	3.71	31	< .001	R Brain-Stem	2	-28	-18	4.47	28	< .001
								R Postcentral Gyrus	68	-12	36	4.42	251	< .001
								L Pallidum	-20	-10	-6	4.34	30	< .001
								L Frontal Orbital Cortex / L Inferior Frontal Gyrus (pars triangularis)	-58	28	-2	4.2	153	< .001



	L Precentral Gyrus	-64	10	28	4.07	315	< .001
	L Occipital Pole	-38	-96	4	4.07	36	< .001
	R Superior Lateral Occipital Cortex	54	-70	38	4.05	132	< .001
	R Frontal Pole	52	42	26	4.03	148	< .001
	L Postcentral Gyrus	-70	-20	18	3.94	32	< .001
	L Occipital Lobe	-34	-60	10	3.89	32	< .001
	R Posterior Supramarginal Gyrus	66	-48	38	3.85	86	< .001
	L Temporal Lobe	-70	-16	10	3.83	43	< .001
R Amygdala	L Anterior Supramarginal Gyrus	-60	-28	54	4.42	113	< .001
	R Postcentral Gyrus	64	-8	44	4.03	178	< .001
	L Occipital Pole	-42	-92	10	3.98	26	< .001
	L Inferior Frontal Gyrus (pars triangularis)	-58	26	-2	3.97	67	< .001
	R Inferior Frontal Gyrus (pars triangularis)	60	26	8	3.9	63	< .001
	L Temporal Pole	-58	16	-10	3.89	23	0.002
	R Angular Gyrus	60	-54	50	3.8	49	< .001
	L Superior Lateral Occipital Cortex	-42	-78	48	3.77	102	< .001
	R Frontal Lobe	50	4	58	3.76	19	0.006
	R Posterior Cingulate Gyrus	10	-42	20	3.74	22	0.002
	R Precentral Gyrus	64	0	38	3.68	20	0.004

								R Occipital Pole	30	-92	30	3.66	20	0.004
								R Temporal Pole	64	10	-2	3.64	21	0.003
L Amygdala								L Cerebellum	-26	-62	-38	5.5	38	< .001
								L Occipital Pole	-34	-100	6	4.13	72	< .001
								L Frontal Orbital Cortex / L Inferior Frontal Gyrus (pars triangularis)	-58	28	-2	4.06	22	0.002
								R Inferior Frontal Gyrus (pars triangularis)	60	28	8	3.78	22	0.002
								R Superior Lateral Occipital Cortex	60	-66	30	3.55	19	0.006
mPFC	R Occipital Pole	2	-100	2	4.34	20	0.003	R Precentral Gyrus	62	2	42	4.75	19	0.004
	L Posterior Temporal Fusiform Cortex / L Posterior Parahippocampal Gyrus	-28	-30	-28	4.21	25	< .001	L Occipital Pole	-38	-96	4	4.72	52	< .001
	L Parietal Lobe	-30	-70	2	3.99	22	0.001	L Insular Cortex / L Putamen	-26	22	0	4.53	48	< .001
	R Inferior Frontal Gyrus (pars triangularis)	60	30	0	3.96	60	< .001	L Superior Lateral Occipital Cortex	-40	-80	48	4.39	41	< .001
	R Precuneous Cortex	16	-60	40	3.96	39	< .001	L Precentral Gyrus	66	8	12	4.33	149	< .001
	Posterior Cingulate Gyrus	0	-36	24	3.95	19	0.004	L Subcallosal Cortex	-4	-26	-18	4.33	31	< .001
	R Putamen	18	10	6	3.94	34	< .001	R Lingual Gyrus	2	-78	-10	4.33	31	< .001
	L Cerebellum	-40	-62	-36	3.93	50	< .001	R Pallidum	14	8	0	4.29	69	< .001
	R Planum Temporale	48	-26	6	3.85	29	< .001	R Anterior Cingulate Gyrus	4	10	22	4.28	35	< .001
	L Brain-Stem	-14	-34	-32	3.85	20	0.003	R Precentral Gyrus	68	2	18	4.25	107	< .001



L Inferior Frontal Gyrus (pars opercularis)	-62	18	4	3.82	44	< .001	L Inferior Frontal Gyrus (pars opercularis)	-62	20	10	4.17	27	< .001
R Inferior Lateral Occipital Cortex	36	-58	6	3.81	24	< .001	L Frontal Orbital Cortex	-24	12	-14	4.15	25	< .001
R Postcentral Gyrus	70	-4	8	3.79	33	< .001	L Frontal Orbital Cortex / L Inferior Frontal Gyrus (pars triangularis)	-58	28	-2	4.14	59	< .001
L Lingual Gyrus	-2	-62	4	3.77	30	< .001	R Postcentral Gyrus / R Precentral Gyrus	60	-8	50	4.13	60	< .001
R Superior Parietal Lobule	32	-50	60	3.76	18	0.006	R Superior Lateral Occipital Cortex	46	-68	54	4.13	41	< .001
L Brain-Stem	-6	-44	-26	3.72	24	< .001	R Occipital Pole	30	-92	30	4.1	23	< .001
R Occipital Pole	32	-92	28	3.69	27	< .001	L Postcentral Gyrus	-62	-6	44	4.04	116	< .001
R Frontal Orbital Cortex	54	28	-12	3.66	20	0.003	R Precentral Gyrus	50	2	58	4.04	21	0.002
L Parietal Lobe	-70	-46	26	3.62	18	0.006	L Postcentral Gyrus	-70	-14	10	4.03	56	< .001
							L Posterior Cingulate Gyrus	-10	-24	34	3.93	45	< .001
							L Posterior Parahippocampal Gyrus	-22	-34	-12	3.93	18	0.006
							L Angular Gyrus / L Posterior Supramarginal Gyrus	-68	-50	28	3.92	138	< .001
							L Precentral Gyrus	-64	10	26	3.91	36	< .001
							R Lingual Gyrus	36	-42	-4	3.87	29	< .001
							R Cerebellum	32	-50	34	3.87	18	0.006
							R Superior Lateral Occipital Cortex	52	-74	36	3.86	31	< .001
							L Putamen	-28	-26	6	3.79	21	0.002
							R Lingual Gyrus	30	-52	-2	3.76	29	< .001

Results after correcting for multiple testing by FDR correction (α=0.01) according to Benjamini & Hochberg (1995). Regions were identified using the Harvard-Oxford Cortical and Subcortical Structural Atlases or if probability of the labels was low (<15%) the MNI Structural Atlas was viewed. L = Left, R = Right; PTSD symptom group *n*=17, TC *n*=22.

Table S3. LMM for cortisol levels (nmol/L) across the experimental session.

	Cortisol levels (nmol/L)					
	−2LL	AIC	BIC	DF	Δχ2	p
1. Fixed intercept and slope						
covariance structure (UN)	34.21	54.21	86.36	18	-	-
covariance structure (DIAG)	147.11	155.11	167.97	12	-	-
covariance structure (AR1)	66.87	70.87	77.30	10	-	-
UN > DIAG	-	-	-	-	112.90	<.001*
UN > AR1	-	-	-	-	32.66	<.001*
DIAG > AR1	-	-	-	-	80.24	<.001*
Best fitting covariance structure:			**DIAG** [b]			
2. Random intercept and fixed slope	53.81	63.81	79.89	-	-	-
3. Random intercept and slope	53.81	71.81	100.75	-	-	-
Final basic model			**Random intercept and fixed slope**			
4. Including covariates sex and age to the basic model	59.65	69.65	85.45		-	-

Final complex model with Random intercept and fixed slope including covariates sex and age

Results on LMM	*DF*	*F*	*p*
Intercept	42.75	62145.22	<.001*
Group	42.11	<0.01	.973
Time	58.45	2.41	.076
Sex	40.26	0.20	.660
Age	40.45	1.56	.219
Group*Time	58.45	0.14	.937

LMM: Linear Mixed Models; −2LL: restricted −2 log-likelihood; AIC: Akaike information criterion; BIC: Bayesian's Information Criterion; DF: degrees of freedom; Δχ2: Chi-square of change in the restricted −2 log-likelihood; UN: Unstructured covariance structure; DIAG: Diagonal covariance structure; AR1: First-order autoregressive covariance structure, *p<.05.

Table S4. LMM for mean HR per minute during the task.

	HR (mean/minute)					
	−2LL	AIC	BIC	DF	Δχ2	p
1. Fixed intercept and slope [a]						
covariance structure (UN)	1133.46	1153.46	1185.39	18	-	-
covariance structure (DIAG)	1364.28	1372.28	1385.05	12	-	-
covariance structure (AR1)	1207.59	1211.59	1217.98	10	-	-
UN > DIAG	-	-	-	-	230.82	<.001*
UN > AR1	-	-	-	-	74.13	<.001*
DIAG > AR1	-	-	-	-	156.68	<.001*
Best fitting covariance structure:			**AR1** [b]			
2. Random intercept and fixed slope [d]	1175.12	1181.12	1190.70	-	-	-
3. Random intercept and slope [d]	1200.80	1208.80	1221.58	-	-	-
Final basic model			**Random intercept and fixed slope**			
4. Including covariates sex and age to the basic model	1165.16	1171.16	1180.71	-	-	-

Final complex model with Random intercept and fixed slope including covariates sex and age

Results on LMM	*DF*	*F*	*p*
Intercept	42.89	2495.74	<.001*
Group	42.89	0.00	.949
Time	101.38	42.65	<.001*
Sex	42.89	1.53	.223
Age	42.89	0.16	.689
Group*Time	101.38	0.08	.971
	Estimated marginal mean (SE)		
Baseline	66.60 (1.51)		
Instructions	72.52 (1.51)		
Stress	75.25 (1.51)		
Recovery	68.95 (1.51)		

LMM: Linear Mixed Models; −2LL: restricted −2 log-likelihood; AIC: Akaike information criterion; BIC: Bayesian's Information Criterion; DF: degrees of freedom; Δχ2: Chi-square of change in the restricted −2 log-likelihood; UN: Unstructured covariance structure; DIAG: Diagonal covariance structure; AR1: First-order autoregressive covariance structure, *p<.05.

Table S5. LMM for subjective stress on feeling stressed during the task.

	Subjective perceived stress					
	−2LL	AIC	BIC	DF	Δχ2	p
1. Fixed intercept and slope						
covariance structure (UN)	430.85	442.85	460.54	12	-	-
covariance structure (DIAG)	433.54	439.54	448.39	9	-	-
covariance structure (AR1)	467.69	471.69	477.59	8	-	-
UN > DIAG	-	-	-	-	2.69	<.001*
UN > AR1	-	-	-	-	36.84	<.001*
DIAG > AR1	-	-	-	-	34.15	<.001*
Best fitting covariance structure:			**AR1** [b]			
2. Random intercept and fixed slope	467.03	473.03	481.87	-	-	-
3. Random intercept and slope	466.56	474.56	486.35	-	-	-
Final basic model			**Random intercept and fixed slope**			
4. Including covariates sex and age to the basic model	468.27	474.27	483.07		-	-

Final complex model with Random intercept and fixed slope including covariates sex and age

Results on LMM	***DF***	***F***	***p***
Intercept	37.66	377.88	<.001*
Group	38.06	0.33	.571
Time	79.60	25.73	<.001*
Sex	38.98	0.06	.802
Age	38.98	0.72	.401
Group*Time	79.60	0.02	.985
	Estimated marginal mean (SE)		
Baseline	2.10 (0.18)		
Stress	3.12 (0.18)		
Recovery	1.41 (0.18)		

LMM: Linear Mixed Models; −2LL: restricted −2 log-likelihood; AIC: Akaike information criterion; BIC: Bayesian's Information Criterion; DF: degrees of freedom; Δχ2: Chi-square of change in the restricted −2 log-likelihood; UN: Unstructured covariance structure; DIAG: Diagonal covariance structure; AR1: First-order autoregressive covariance structure, *p<.05.

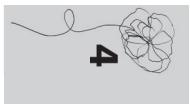
Table S6. Between-group cluster list of task effects for PTSD symptom group > TC and TC > PTSD symptom group, including *n*=1 participant using hormonal contraception.

Stress vs. Baseline														
PTSD symptom group vs. TC								*TC vs. PTSD symptom group*						
	Region	X	Y	Z	Z-score	Cluster size	*p*	Region	X	Y	Z	Z-score	Cluster size	*p*
R Hippocampus	R Cerebellum[1]	16	-58	-16	4.18	21	. 001							
L Hippocampus														
R Amygdala														
L Amygdala														
mPFC														
Recovery vs. Baseline														
PTSD symptom group vs. TC								*TC vs. PTSD symptom group*						
	Region	X	Y	Z	Z-score	Cluster size	*p*	Region	X	Y	Z	Z-score	Cluster size	*p*
R Hippocampus														
L Hippocampus								L Inferior Frontal Gyrus (pars opercularis)[2]	62	20	20	4.26	14	.022
R Amygdala														
L Amygdala														
mPFC														

Results after correcting for multiple testing by FDR correction [1](α=0.01) and [2] (α=0.05) according to Benjamini & Hochberg (1995). Regions were identified using the Harvard-Oxford Cortical and Subcortical Structural Atlases or if probability of the labels was low (<15%) the MNI Structural Atlas was viewed. L = Left, R = Right; PTSD symptom group *n*=17, TC *n*=23.

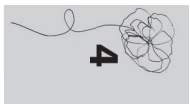
Table S7. Within-group cluster list of task effects for the PTSD symptom group (*n*=17) and TC group (*n*=23), including *n*=1 participant using hormonal contraception.

	Stress vs. Baseline													
	PTSD symptom group							*TC*						
	Region	X	Y	Z	Z-score	Cluster size	*p*	Region	X	Y	Z	Z-score	Cluster size	*p*
R Hippocampus	R Thalamus	18	-24	0	4.35	17	.006	R Parietal Lobe (Postcentral Gyrus)	68	-12	36	4.76	90	< .001
	R Pallidum	26	-12	0	3.78	18	.004	R Frontal Lobe (Precentral Gyrus)	68	2	16	4.52	42	< .001
								R Superior Lateral Occipital Cortex	24	-72	32	4.39	40	< .001
								R Parietal Lobe (Superior Lateral Occipital Cortex)	50	-78	32	4.23	141	< .001
								R Parietal Lobe (Angular Gyrus)	68	-46	26	4.21	40	< .001
								L Lingual Gyrus	-2	-66	0	4.16	43	< .001
								L Occipital Pole	-40	-94	10	4.11	18	.004
								L Precuneous Cortex	-14	-58	28	4.09	27	< .001
								R Frontal Lobe / Inferior Frontal Gyrus (pars opercularis/pars triangularis)	62	24	10	4.04	52	< .001



	R Parietal Lobe (Anterior Supramarginal Gyrus)	70	-28	30	3.9	17	.006
	L Pallidum, L Putamen	-20	0	-4	3.87	17	.006
	R Parietal Lobe (Postcentral Gyrus)	68	-12	36	4.76	90	< .001
L Hippocampus	R Parietal Lobe (Postcentral Gyrus)	68	-12	36	4.5	72	< .001
	R Parietal Lobe (Superior Lateral Occipital Cortex)	54	-70	38	4.43	160	< .001
	R Frontal Lobe (Precentral Gyrus)	68	2	16	4.27	46	< .001
	R Pallidum	24	-8	-2	4.07	17	.005
	R Parietal Lobe (Angular Gyrus)	68	-46	26	4.02	23	< .001
	R Frontal Lobe / Inferior Frontal Gyrus (pars opercularis/pars triangularis)	62	24	10	3.94	62	< .001
	L Pallidum, L Putamen	-24	-8	-4	3.92	21	< .001
	R Temporal Pole	58	14	-20	3.82	21	< .001
	Lingual Gyrus	0	-74	-4	3.79	19	.002
	R Pallidum	12	6	0	3.7	26	< .001
	L Lingual Gyrus	-2	-66	0	3.53	19	.002
R Amygdala	R Parietal Lobe (Postcentral gyrus)	68	-12	36	4.54	74	< .001

								R Parietal Lobe (Angular Gyrus)	68	-46	26	3.72	20	.003
L Amygdala								L Cerebellum	-26	-62	-38	4.41	22	.001
mPFC	R Pallidum	24	-8	-2	4.49	54	< .001	L Pallidum	-20	-10	-6	5	108	< .001
	L Brain Stem	-14	-34	-32	4.35	28	< .001	L Occipital Pole	-38	-96	4	4.71	22	< .001
	R Temporal Lobe (Planum Polare)	44	-30	8	4.29	37	< .001	R Frontal Lobe (Precentral Gyrus)	66	8	12	4.7	61	< .001
	R Putamen	18	10	6	4.22	45	< .001	L Insular Cortex / L Frontal Orbital Cortex	-26	22	0	4.53	45	< .001
	L Cerebellum	-30	-62	-38	3.87	22	< .001	L Posterior Parahippocampal Gyrus / L Hippocampus	-30	-34	-12	4.53	25	< .001
								R Parietal Lobe (Postcentral Gyrus, Anterior Supramarginal Gyrus)	68	-12	36	4.43	77	< .001
								R Frontal Lobe / Inferior Frontal Gyrus (pars opercularis/pars triangularis)	62	24	10	4.36	54	< .001
								R Pallidum	24	-8	-2	4.36	28	< .001
								R Cerebellum	12	-52	-32	4.31	38	< .001
								R Lingual Gyrus	2	-78	-10	4.21	37	< .001
								R Parietal Lobe (Angular Gyrus)	66	-50	36	4.2	125	< .001



								L Posterior Cingulate Gyrus	-8	-24	34	4.15	32	< .001
								L Brain Stem	-4	-26	-18	4.11	23	< .001
								R Occipital Pole	4	-98	10	4.1	19	.003
								R Parietal Lobe (Posterior Cingulate Gyrus)	10	-32	32	3.98	52	< .001
								R Temporal Lobe (Posterior Superior Temporal Gyrus)	48	-20	0	3.96	20	.002
								R Temporal Pole	58	14	-20	3.89	20	.002
								R Superior Lateral Occipital Cortex	24	-76	36	3.83	29	< .001
								R Pallidum	12	6	0	3.9	27	< .001
							Recovery vs. Baseline							
PTSD symptom group								*TC*						
	Region	X	Y	Z	Z-score	Cluster size	*p*	Region	X	Y	Z	Z-score	Cluster size	*p*
R Hippocampus	R Thalamus	18	-24	0	5.1	22	< .001	R Parietal Lobe (Postcentral Gyrus / Anterior Supramarginal Gyrus)	68	-12	36	4.54	74	< .001
								R Frontal Lobe (Precentral Gyrus)	68	2	16	4.47	38	< .001
								L Cerebellum	-26	-62	-38	4.24	28	< .001
								R Parietal Lobe (Angular Gyrus)	68	-46	26	4.19	27	< .001

	R Superior Lateral Occipital Cortex	52	-74	36	4.18	92	< .001
	R Superior Lateral Occipital Cortex	24	-72	32	4	24	< .001
	L Pallidum	-20	-10	-6	3.93	19	.003
	R Frontal Lobe / Inferior Frontal Gyrus (pars triangularis)	58	28	-4	3.74	24	< .001
	L Caudate	-12	4	8	3.73	18	.004
L Hippocampus	R Parietal Lobe (Postcentral Gyrus, Anterior Supramarginal Gyrus)	68	-12	36	4.39	60	< .001
	R Brain Stem	2	-28	-18	4.23	29	< .001
	R Parietal Lobe (Superior Lateral Occipital Cortex)	54	-70	38	4.11	91	< .001
	R Frontal Lobe (Precentral Gyrus)	68	2	16	4.1	19	.003
	R Parietal Lobe (Angular Gyrus)	68	-46	26	4.07	19	.003
	L Pallidum	-20	-10	-6	4.02	27	< .001
	R Frontal Lobe (Precentral Gyrus)	66	8	12	3.87	20	.002
	R Frontal Lobe / Inferior Frontal Gyrus (pars opercularis/pars triangularis)	62	24	10	3.84	56	< .001

R Amygdala								R Parietal Lobe (Postcentral Gyrus)	68	-12	34	3.96	81	< .001
								R Frontal Lobe / Inferior Frontal Gyrus (pars triangularis/pars opercularis)	62	24	14	3.73	20	.003
L Amygdala								L Cerebellum	-26	-62	-38	5.58	36	< .001
mPFC	R Putamen	18	10	6	4.04	22	.001	L Insular Cortex / L Frontal Orbital Cortex	-26	22	0	4.59	39	< .001
	L Lingual Gyrus	-2	-62	4	3.88	19	.003	L Brain Stem	-4	-26	-18	4.36	29	< .001
								R Caudate	18	18	2	4.33	36	< .001
								R Anterior Cingulate Gyrus	4	10	22	4.33	20	.002
								R Frontal Lobe (Precentral Gyrus)	68	2	18	4.32	27	< .001
								R Postcentral Gyrus	64	-10	44	4.08	23	< .001
								R Parietal Lobe / Inferior Frontal Gyrus (pars triangularis)	58	24	-4	4	22	.001
								R Parietal Lobe (Superior Lateral Occipital Cortex)	52	-74	36	3.91	21	.002
								R Parietal Lobe (Postcentral Gyrus)	70	-10	12	3.9	21	.002

Results after correcting for multiple testing by FDR correction (α=0.05) according to Benjamini & Hochberg (1995). Regions were identified using the Harvard-Oxford Cortical and Subcortical Structural Atlases or if probability of the labels was low (<15%) the MNI Structural Atlas was viewed. L = Left, R = Right; PTSD symptom group *n*=17, TC *n*=23.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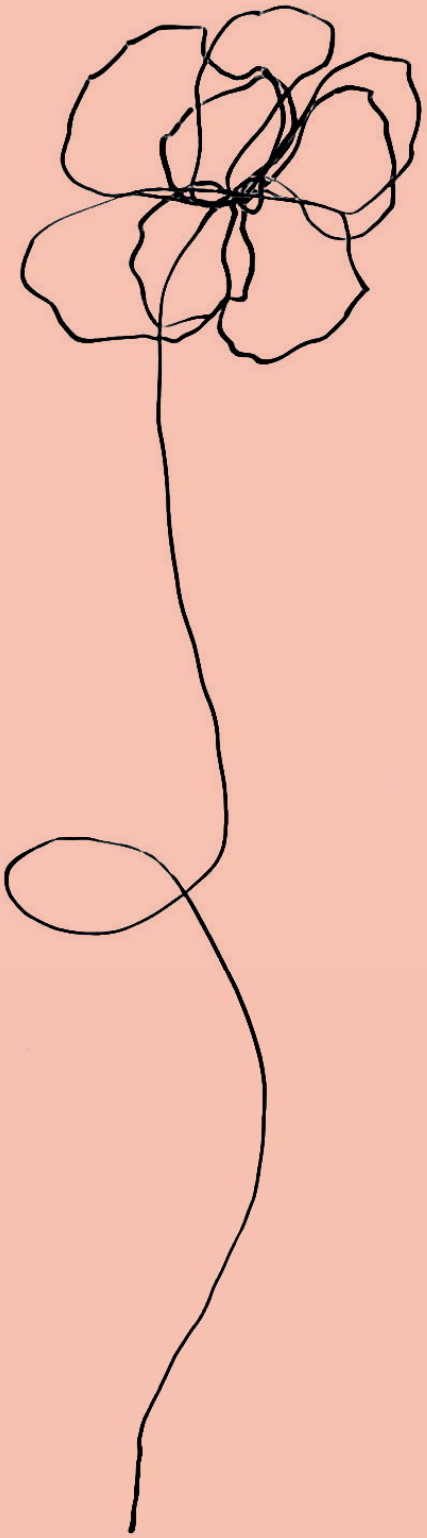

CHAPTER 5

Acute stress reactivity and intrusive memory development: a randomized trial using an adjusted trauma film paradigm

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Published in: Psychoneuroendocrinology (2022), 139: 105686, *https://doi.org/10.1016/j.psyneuen.2022.105686*

Keywords: PTSD, intrusive memories, stress reactivity, contextual memory, cortisol, alpha amylase

Abstract

Understanding the neurobiological and cognitive processes underlying the development of posttraumatic stress disorder (PTSD) and its specific symptoms may facilitate preventive intervention development. Severe traumatic stress and resulting biological stress system activations can alter contextual memory processes. This may provide a neurobiological explanation for the occurrence of intrusive memories following trauma. Investigating the associations between temporal aspects and individual variation in peri- and post-traumatic hypothalamic pituitary adrenal (HPA) axis and sympathetic nervous system (SNS) stress reactivity and memory processing may increase our understanding of intrusive symptom development. The experimental trauma film paradigm is commonly used for this purpose but lacks robust SNS and HPA axis activation. Here, we performed an RCT to investigate the effect of an adjusted trauma film paradigm containing an added brief psychosocial stressor on HPA and SNS stress reactivity throughout the experiment and intrusive memory frequency in the following week in healthy males (*N*=63, mean age=22.3). Secondary, we investigated effects on film-related declarative memory accuracy and intrusion-related characteristics, and associations between acute HPA and SNS stress reactivity, film-related memory, glucocorticoid receptor functioning and intrusion frequency and characteristics. Participants were randomized to the socially-evaluated cold pressor test (seCPT *n*=29) or control condition (warm water *n*=34) immediately prior to a trauma film. Linear Mixed Models revealed increased acute SNS and cortisol reactivity, lower recognition memory accuracy and more intrusions that were more vivid and distressing during the following week in the seCPT compared to control condition. Linear regression models revealed initial associations between cortisol and alpha amylase reactivity during the experimental assessment and subsequent intrusions, but these effects did not survive multiple comparison corrections. Thus, with this adjustment, we increased the translational value of the trauma film paradigm as it appears to elicit a stronger stress response that is likely more comparable to real-life trauma. The adapted paradigm may be useful to investigate individual variation in biological and cognitive processes underlying early post-trauma PTSD symptoms and could advance potential preventive interventions.

Highlights

- Experimental trauma can be used to study intrusive memory development
- The trauma film paradigm was adjusted to obtain more robust stress reactivity
- Adding a psychosocial stressor before the film increased HPA and SNS reactivity
- The stressor condition also reported more intrusive memories in the next week
- Acute sAA/cortisol reactivity may be associated with intrusion development

1. Introduction

Posttraumatic stress disorder (PTSD) is a psychiatric disorder occurring in approximately 10% of trauma-exposed individuals (de Vries & Olff, 2009). As PTSD is by definition preceded by traumatic events, this theoretically provides the opportunity for interventions early post-trauma to prevent PTSD development. To facilitate establishment of effective preventive interventions, further elucidation of the neurobiological and cognitive processes underlying the development of PTSD and its specific symptoms is warranted.

PTSD symptoms include intrusive re-experiencing of the traumatic event, in the form of recurrent distressing involuntary memories, nightmares or dissociative flashbacks. Trauma-related involuntary memory phenomena have been conceptualized to lie along a continuum, with overlapping and distinctive quantitative and qualitative characteristics between memory types. In this continuum, PTSD's intrusive re-experiencing symptoms are placed at its most severe end (Meyer et al., 2015). Yet, trauma-related involuntary memories, including intrusive re-experiencing, are not specific to (prodromal) PTSD and are common after trauma, especially in the first weeks (e.g. Michael et al., 2005). In a prospective study, the presence and frequency of trauma-related involuntary memories in the first weeks post-trauma had limited predictive value for PTSD symptom severity 6 months after assault. However, the extent of distress, feelings of 'nowness', and lack of context associated with these intrusive memories explained almost half of the variance in symptom severity (Michael et al., 2005). This latter observation fits with several cognitive PTSD models posing that intrusive re-experiencing results from poor contextualization during memory encoding and consolidation in the first hours post-trauma, which leads to fragmented ('disjointed') memories that are prone to spontaneous or triggered automatic retrieval (Brewin, 2015; Ehlers et al., 2004). Recent neurobiological PTSD models also have addressed the accumulating evidence for the importance of altered contextual processing in the pathophysiology of PTSD (Liberzon & Abelson, 2016). In line with these models, lower general ability to contextualize emotional memories predicted subsequent intrusive memories development following experimental trauma (Meyer et al., 2017). However, it remains unknown whether peri- and acute post-traumatic contextual memory processing is indeed associated with subsequent intrusive re-experiencing (van Rooij et al., 2021).

There is increasing evidence that severe stress and resulting sympathetic nervous system (SNS) and hypothalamic-pituitary-adrenal (HPA) axis activation together impact hippocampal-dependent contextual memory encoding (Lesuis et al., 2021; Schwabe et al., 2012). Specifically, non-genomic effects of cortisol on glucocorticoid receptors (GRs) within one hour post-stress decrease memory contextualization, while contrastingly later occurring genomic effects of cortisol increase memory contextualization (Sep et al., 2020). Thus, if contextual memory processing is indeed involved in intrusion development, these previous observations may provide a neurobiological explanation for the common occurrence of intrusive re-experiencing following traumatic stress. Yet, this provides no explanation for interindividual differences in the severity of

intrusive memories following trauma and why only a minority of trauma-exposed individuals experiences long-lasting intrusive re-experiencing symptoms and develop PTSD.

A growing number of prospective studies link individual differences in SNS and HPA axis reactivity around the time of traumatic stress to subsequent PTSD development. Higher GR signalling as measured before, two days and within 1,5 week post-trauma predicted subsequent high long-term PTSD symptom levels in predominantly male samples (Engel et al., 2020; McFarlane et al., 2011; Steudte-Schmiedgen et al., 2015; van Zuiden et al., 2013). Additionally, low cortisol in the first hours post-trauma was repeatedly observed to predict PTSD development, potentially as a result of enhanced negative feedback on cortisol release following initial cortisol release due to high GR signaling (e.g. Mouthaan et al., 2014; Schultebraucks et al., 2021). Regarding SNS reactivity, the most consistent associations with PTSD development have been found for higher heart rate within 72 hours post-trauma (Morris et al., 2016). Additionally, PTSD development was found to be associated with blood pressure (Schultebraucks et al., 2021) and skin conductance reactivity to trauma reminders in the immediate post-trauma period (Hinrichs et al., 2019).

Importantly, these prospective studies typically only used PTSD diagnostic status or total PTSD symptom severity as outcome, and it has rarely been investigated whether identified predictors were associated with development of specific PTSD symptoms in the early post-trauma period. It seems worthwhile to investigate whether HPA and SNS reactivity around the time of trauma is associated with subsequent intrusive re-experiencing in the early post-trauma period, and whether this is mediated via trauma-related contextual memory encoding. For this purpose, the associations between temporal aspects and individual variation in peri- and post-traumatic stress reactivity, various types of trauma-related declarative memory, and subsequent trauma-related intrusive memories should be studied in further detail.

As repeated in-depth biological and cognitive assessment is neither feasible nor ethical during real-life trauma and subjective characteristics of intrusions cannot be reliably assessed in animals, this currently can only be investigated in healthy participants using experimental trauma paradigms. The trauma film paradigm is a commonly used experimental trauma paradigm that consistently induces short-term mild intrusive memories that share characteristics with trauma-related intrusive symptoms (James et al., 2016). There commonly is considerable variation in induced intrusive memory frequency and characteristics (Clark et al., 2015), as is the case for intrusive memories and PTSD development upon real-life trauma-exposure. However, the previous studies using the trauma film paradigm have not shown robust and consistent SNS and HPA axis activation (Chou et al., 2014; Rombold et al., 2016a, 2016b; Weidmann et al., 2009). This lack of a reliably induced naturalistic stress response diminishes the paradigms' translational value as the magnitude, timing and duration of stress responses influences memory consolidation (Joëls et al., 2011; Schwabe et al., 2012) and intrusion development (Bryant et al., 2013).

In this randomized-controlled study in healthy male adults, our primary aim was to investigate the effect of an adjusted version of the trauma film paradigm containing a brief psychosocial stressor immediately prior the trauma film on HPA and SNS stress reactivity throughout the experimental paradigm as well as intrusive memory frequency in the following week. The socially-evaluated cold pressor test (seCPT) was used as psychosocial stressor, as it was previously shown to reliably induce HPA and ANS activation (Sänger et al., 2014; Schwabe et al., 2008). One previous study also adjusted the trauma film paradigm by adding a longer psychosocial stressor immediately prior to the trauma film in a female sample (Schultebraucks et al., 2019). This adjustment increased SNS reactivity prior to the trauma film and cortisol levels after the trauma film, but did not influence intrusion frequency. In contrast to this previous study, we additionally investigated the effects of the adjusted paradigm on declarative memory accuracy related to the trauma film and intrusion characteristics as a secondary objective. As a secondary aim, to further investigate the biological and cognitive processes underlying interindividual variability in trauma-related intrusive memories, we investigated whether acute SNS and HPA axis stress reactivity to the paradigm, acute film-related declarative memory accuracy, as well as salivary cortisol suppression upon oral dexamethasone ingestion as a measure of GR functioning were predictive of trauma film-related intrusion frequency and characteristics in the following week.

2. Methods

2.1 Participants

In this single-blind randomized-controlled trial (NL6550/NTR6739, Appendix A11), *N*=68 healthy males (aged 18-40, all university educated) were randomized to the experimental seCPT (n=34) or control condition (warm water condition; n=34; details regarding sample size calculations, blinding and randomization in Appendix A1). Ultimately, *N*=63 participants completed all procedures and were included in analyses (seCPT *n*=29, warm water *n*=34; Appendix A2 for flowchart). Inclusion criteria were Caucasian ethnic background (to prevent confounding of forthcoming genetic analyses), fluency in Dutch, Body Mass Index (BMI) of 18.5-30, and smartphone possession (required to report intrusions). Exclusion criteria were current (sub)clinical depressive, anxiety or PTSD symptoms; current major medical disorder; habitual smoking; use of medication known to impact HPA/ANS functioning; and lifetime diagnosis of any psychiatric disorder. Additionally, participants were excluded upon previous exposure to an event resembling trauma film-related events. Conditions did not differ regarding demographic characteristics, psychological screening or baseline GR functioning (Table 1). The Institutional Review Board of the Academic Medical Center approved the study, performed in accordance with the Medical Research Involving Human Subjects Act (WMO) and Declaration of Helsinki. All participants provided verbal and written informed consent and received a monetary reward (€40,-) or Student Course Credits.

2.2 Assessment procedures

2.2.1 Recruitment and screening

Participants were recruited through flyers and online advertisements targeted at university students. After indicating interest, a 10-minute screening took place by telephone. When eligible and upon continued interest, a face-to-face assessment (T1) was scheduled wherein current depressive, anxiety-related or PTSD symptoms and previous trauma exposure were screened using self-report questionnaires (details in Appendix A3). Weight was measured to determine BMI. Instructions for saliva collection and behavioural restrictions were provided and procedures practiced (Stalder et al., 2016). Behavioural restrictions for the experimental assessment (T2) included: no caffeine/nicotine/medication/drug use <24hrs; no alcohol use during the prior evening; no physical exercise on the T2 day; no brushing teeth <1hr. Participants were requested to eat a light lunch (low protein amount) before T2 began (details in Appendix A9).

2.2.2 Experimental assessment (T2)

The 95-minute T2 was scheduled in the afternoon to account for cortisol's diurnal rhythm (Figure 1 visualizes procedure). Firstly, participants ate a candy bar for glucose level standardization and collected saliva was handed in. Thereafter, two experimental manipulations (seCPT and trauma film, see below), two resting measurements (*Baseline* and *Recovery*) and film-related declarative memory assessment were performed. Furthermore, participants were instructed how to report film-related intrusions experienced in the following 7 days (day 1=T2 day). These procedures were interspersed with six 2.5-minute stress reactivity and recovery measurements.

2.2.3 Follow-up assessment (T3)

The 30-minute follow-up assessment (T3) took place exactly 7 days after T2. Film-related PTSD symptoms over the previous week were assessed using an adjusted PTSD Checklist for DSM-5 (PCL-5), followed by re-assessment of film-related declarative memory and debriefing (Appendix A3 for details). When intrusion validity was unclear, additional details of reported intrusions were inquired upon and video recorded for reliability assessment purposes.

2.3 Experimental manipulations

Socially-evaluated cold pressor test (seCPT)

The seCPT is a well-validated brief, mild experimental stressor that induces acute subjective and HPA responses up to 60 minutes and ANS responses up to 20 minutes (Sänger et al., 2014; Schwabe et al., 2008 (Appendix A4.1 for details). In the seCPT condition a female experimenter instructed participants to immerse their dominant hand up to their wrist into a plastic container filled with 0-3^{o}C ice water (mean(SD)=3.31^{o}C(1.26)) and to persist as long as possible or until they could no longer tolerate the cold, without knowing the exact test duration (maximum 3 minutes). The experimenter took an impatient and non-appeasing demeanor and recorded facial expressions during the seCPT with the stated purpose of later evaluation (although not truly

analysed). In the warm water condition, participants were instructed in a calm, friendly manner to immerse their dominant hand in water at body temperature (35-37^{o}C; mean (SD)=37.50^{o}C(1.08)), not inducing any stress responses. In both conditions systolic blood pressure (SBP) was measured 1-min after the seCPT started, unless participants withdrew their hand earlier (*n*=3 seCPT).

Trauma film paradigm

The well-validated trauma film paradigm was administered to induce intrusions (Holmes & Bourne, 2008). Participants watched a 15-minute aversive graphic scene from the movie *Irréversible* by Gaspar Noé (2002; Appendix A4.2 for details). The fragment displays a woman suffering severe sexual and physical violence, and was previously found to induce short-term mildly distressing intrusions, immediate distress and negative emotions equally in women and men (James et al., 2016; Weidmann et al., 2009).

2.4 Measures

2.4.1 Acute stress reactivity

During the six stress measurements at T2, we collected; 1) salivary cortisol and alpha amylase (sAA) reactivity, 2) cardiac reactivity, i.e. pre-ejection period (PEP), heart rate (HR) and heart rate variability (HRV), 3) SBP, and 4) subjective emotional states and distress (Appendix A10 for details).

Salivary cortisol levels were assessed as a marker of HPA reactivity and recovery and sAA levels as a marker of ANS reactivity and recovery. To enhance reliability of sAA analyses, the unstimulated spitting method was applied using a standardized timing (2 minutes) (Bosch et al., 2011). Cortisol levels were determined using ELISA (IBL International GmbH, Hamburg, Germany; Intra-assay variations<2.25%). For sAA a quantitative kinetic determination kit was used (Lyophilized, IBL International GmbH, Hamburg, Germany; Intra-assay variations<4.18%). Each sample was assayed in duplicate and their means were calculated. Additionally, 'Area under the Curve with respect to Ground' (AUCg) and 'with respect to Increase from baseline' (AUCi) were calculated for cortisol and sAA during stress reactivity (samples 2-3; after seCPT and trauma film) and recovery (samples 4-5-6; from *Recovery* measurement onwards (Pruessner et al., 2003)).

PEP, HR, and HRV were measured using the VU-Ambulatory Monitoring System. SBP was measured during each stress measurement using a separate monitor. PEP, HR and SBP were assessed to reflect SNS functioning. Root Mean Square of the Successive Differences (RMSSD) was assessed to measure vagal-parasympathetic modulation (Kleiger et al., 2005). Ultimately, mean RMSSD, PEP, HR (beats/min) for each separate stress measurement were averaged over three raters.

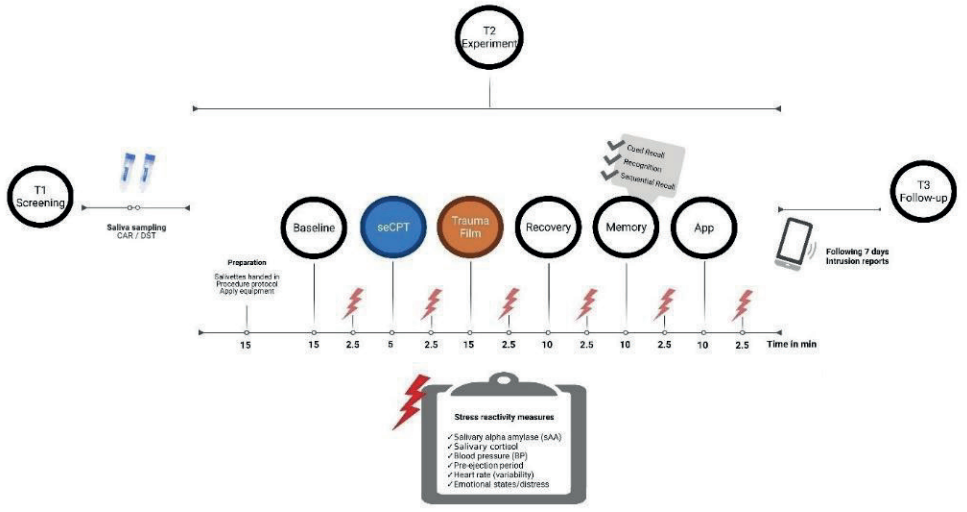


Figure 1. Overview of all assessments, including a detailed procedure of the experimental assessment (T2). CAR: cortisol awakening response, DST: dexamethasone suppression test, seCPT: socially-evaluated cold pressor test, sAA: salivary alpha amylase, BP: blood pressure, PEP: pre-ejection period, HR(V): heart rate (variability).

2.4.2 Intrusion frequency and characteristics

Participants were instructed to report all experienced film-related intrusions (i.e. involuntary, spontaneous memories) for 7 days in a smartphone app designed for this study (Appendix A5). The app was a digital version of the commonly used paper diaries in trauma film studies. For each intrusion, participants reported 1) date and time of occurrence, 2) short description of content, 3) *vividness* and *distress* (range 0-10), and 4) type (image-related, thought, mixture of both). To keep participants engaged, daily reminder notifications were sent (10am and 10pm, 13 total), upon which participants indicated if and how many intrusions they experienced since their last report. If participants did not respond>24hrs, they were contacted by the researchers.

In line with previous studies, reported intrusions were considered valid when their nature was intrusive; their content film-related; and both *vividness* and *distress*>0 (Ehlers et al., 2004; Schultebraucks et al., 2019). Additionally, participants needed to have rated their compliance at T3≥7. A second rater (blind to condition) scored reported intrusions of 20% randomly selected participants using app reports and videos of clarifying questions asked during T3. Interrater reliability was excellent (two-way mixed effects model, consistency, single measure interclass correlation=1.00, p<.01 (Koo & Li, 2016)).

For intrusion frequency, we counted the number of valid intrusions on every day separately and additionally calculated the total sum over 7 days. For intrusion characteristics *vividness* and *distress,* we calculated mean scores by dividing total *vividness/distress* scores by the number of

reported intrusions (valid and invalid) for each day separately. We included both valid and invalid intrusions to avoid overestimation of *vividness* and *distress* scores across all experienced intrusive memories. Furthermore, we calculated total *vividness* and *distress* sum scores over 7 days. Additionally, Visual Analogue Scales (VAS; all ranges 0-1) were administered at T3 to rate intrusion reporting compliance and characteristics of the most prominent intrusion (i.e. the intrusion indicated by the participants to be most significant, unpleasant and distressing in the past 7 days, (Davies & Clark, 1998)).

2.4.3 Declarative memory accuracy

Participants completed three commonly used film-related memory tasks in standardized order at T2 and T3 (James et al., 2016). To prevent learning effects, two versions consisting of different questions were administered in randomized and counterbalanced order between sessions and conditions. The *Cued Recall* task consisted of 9 open questions on details of the victim and surroundings portrayed in the film (e.g. '*What colour was the victim's purse?*'). For each item there was only one unambiguously correct answer. If the whole or part of the answer was wrong, the whole item was scored as incorrect. Total scores were calculated by counting the number of correct answers (range 0-9). The *Recognition* task consisted of 12 true/false statements regarding either film-related gist or peripheral/central details. Total scores were again calculated by counting the number of correct answers (range 0-12). The *Sequential Recall task* consisted of 10 film-related events that had to be placed in order of occurrence, measuring contextual memory. Accuracy was calculated per participant as Spearman's correlation between ranks of correct and recalled orders (Wegner et al., 1996).

2.4.4 Subjective experience of the experimental assessment

Digital VAS were used during stress measurements at T2 (range 0-1; PsychoPy (v1.81)) to assess emotional states (*Anxious, Angry, Happy, Sad, Disgust, Distress*). These specific states were selected based on previous studies using the trauma film paradigm observing an impact on these particular states (Clark et al., 2015; James et al., 2016; Schultebraucks et al., 2019; Weidmann et al., 2009), and for how *Painful, Unpleasant, Difficult* and *Stressful* the seCPT was. Also, using VAS, participants indicated how well they maintained their focus while viewing the film and how much they felt that they empathized with and were immersed in the film fragment ('To what extent were you able to focus on the film?', 'To what extent were you able to empathize with the film?', 'To what extent were you immersed in the film') as this may influence the feeling of realism (i.e. feeling of being physically present as if they were witnessing the events happening in the film) that is associated with eliciting emotional responses such as subjective distress and changed emotional state to a film (Visch et al., 2010).

2.4.5 GR functioning

Participants collected saliva at home for CAR assessment on two mornings between T1 and T2 on prescheduled time points: immediately upon awakening, 30 minutes and 45 minutes after

awakening (Stalder et al., 2016) using synthetic salivettes (Sarstedt, Rommelsdorf, Germany). Saliva from day 2 was used for GR sensitivity assessment using the dexamethasone suppression test (DST) (Yehuda et al., 1991). Participants were asked to administer 0.5mg dexamethasone (exogenous glucocorticoid) at 11pm on the evening before sample collection. To check compliance with prescribed time points, participants were asked to take time-stamped photos of themselves at time of assessment. Cortisol levels were determined as outlined above.

AUCg and AUCi were calculated for CAR (2x3 samples). Since potential delays between saliva collection time points might cause false-low estimates of CAR (Stalder et al., 2016), we included individual sampling times between time points in our calculations and excluded samples when awakening times were missing (*n*=2) or ≥5 minutes delayed (CAR *n*=7, DST *n*=8).

2.5 Statistical analyses

All data were analysed using SPSS 26 (IBM SPSS Statistics Software). When assumptions for normality of distributions were not met, data was log-transformed before presence of outliers was checked (Z≥3.29). Outliers were winsorized to obtain a normal distribution (Reifman & Garrett, 2016) and excluded if winsorizing was not successful.

To assess differences in cortisol and sAA reactivity and recovery, RMSSD, PEP, HR, SBP, and subjective emotional states and distress across the six assessments, Linear Mixed Models (LMMs) with Restricted Maximum Likelihood (REML) were performed. Standard error covariance first-order autoregressive covariance structure (AR1) with random intercept and fixed slope were used as this has been recommended for randomized-controlled trials (model details and formula are provided in Appendix A6).

An overdispersed Poisson Generalized Linear Mixed Model (GLMM) with canonical link function was performed to assess differences between conditions in intrusion frequency per day, due to multicollinearity and zero-inflation dependent overdispersion≈1.5 within Poisson-distributed count data (right-skewed approximating bi-nominal for days 1-7 with variance approximately equal to the mean (Crawley-Boevey, 2011; Payne et al., 2018)). Negative Binomial Regression GLMMs were used to assess differences in mean *vividness* and *distress* scores per day, due to zero-inflation dependent overdispersion<1.2 within Poisson-distributed count data. In all GLMMs, condition, time and the interaction effect between time and condition were included as fixed predictors, and intercept was included as the only random effect. Details on sensitivity analyses for intrusion frequency of all reported intrusions, including invalid intrusions with vividness and/or distress scores=0, are provided in Appendix A7.

Additionally, repeated measures Analyses of Covariance (rmANCOVAs) were performed to assess differences in memory task accuracy including condition as between-subject factor, time as

within-subject factor, an interaction effect between time and condition, and task version as covariate.

Differences in all other variables were assessed using independent samples *T*-tests and Mann-Whitney U tests for continuous variables and Pearson Chi-square tests, Fisher's or Fisher-Freeman-Halton exact tests (if>2 categories) for categorical variables.

Multiple Linear Regressions were performed to identify predictors of intrusion development (7-day frequency; *vividness* and *distress*; T3 PCL total score and Cluster B score) using the following separate models; baseline GR functioning: (1) DST AUCg, (2) DST AUCi; declarative memory accuracy at T2: (3) *Cued Recall*, (4) *Recognition*, (5) *Sequential Recall*; cortisol/sAA stress reactivity: (6) AUCg, (7) AUCi, and recovery (8): AUCg, (9) AUCi. Predictors were centered to prevent multicollinearity. For all models, main effects for condition and model predictors and interaction terms between condition and model predictors were included. Models 1 and 2 were corrected for CAR AUCg/AUCi and models 3, 4 and 5 for task version. To correct for multiple comparisons in the regression models, a False Discovery Rate threshold (5%) was applied (Benjamini & Hochberg, 1995).

3. Results

3.1 Primary outcomes

3.1.1 Acute stress reactivity

Biological stress reactivity in cortisol, sAA and all cardiac measures significantly changed across T2, with changes in cortisol and SBP differing between conditions.

For cortisol reactivity we found a significant interaction between condition and time ($F(5,292.04)=7.66$, $p<.01$) and main effect of time ($F(5,292.04)=13.89$, $p<.01$), but not condition ($F(2,69.64)=1.51$, $p=.22$; Figure 2A). The seCPT participants showed a stronger increase in cortisol levels compared to *Baseline* after the film ($B=0.14$, 95% CI 0.05-0.24, SE=0.05, $t=3.11$, $p<.01$) and during *Recovery* ($B=0.14$, 95% CI 0.03-0.25, SE=0.05, $t=2.59$, $p=.01$) than warm water participants, but not for the other stress measurements (all *p* values>.05).

A significant time effect was found for sAA reactivity ($F(5,172.32)=12.34$, $p<.01$; Figure 2B). *s*AA significantly increased from *Baseline* after the seCPT ($B=0.06$, 95% CI 0.01-0.12, SE=0.03, $t=2.29$, $p=.02$), after the film ($B=0.19$, 95% CI 0.13-0.25, SE=0.03, $t=6.16$, $p<.01$), after the memory tasks ($B=0.12$, 95% CI 0.05-0.18, SE=0.03, $t=3.73$, $p<.01$) and at the final assessment after explanation on how to report intrusions ($B=0.16$, 95% CI 0.10-0.23, SE=0.03, $t=5.27$, $p<.01$), and were only not significantly higher during *Recovery* ($B=0.04$, 95% CI -0.02-0.10, SE=0.03, $t=1.35$, $p=.18$). No significant interaction of condition and time ($F(5,172.32)=0.44$, $p=.82$) or main effect of condition ($F(1,61.07)=0.03$, $p=.86$) was found.

For SBP, a significant interaction effect between condition and time (F(5,185.13)=4.36, *p*<.01) and main time effect (F(5,185.13)=13.86, *p*<.01) was found, while the effect of condition was non-significant (F(1,60.98)=0.26, *p*=.61). The seCPT participants showed a stronger increase in SBP levels compared to *Baseline* after the seCPT (*B*=0.03, 95% CI 0.02-0.05, SE=0.01, *t*=4.05, *p*<.01) and the film (*B*=0.02, 95% CI <0.01-0.03, SE=0.01, *t*=2.02, *p*=.045) than warm water participants, but not for the other stress measurements (all *p* values>.05; Figure 2C). Moreover, the additional SBP measured during the seCPT was significantly higher in the seCPT (mean(SD)=141.32(18.17)) than warm water condition (123.85(11.34), *U*=166.50, *p*<.01).

For RMSSD, PEP and HR, significant time effects were found (RMSSD F(5,160.18)=5.11, *p*<.01; PEP F(5,137.22)=9.58, *p*<.01; HR F(5,162.91)=9.86, *p*<.01), without significant interactions between condition and time or main condition effects (RMSSD condition*time F(5,160.18)=1.80, *p*=.12, condition F(1,54.00)=0.03, *p*=.87; PEP condition*time F(5,137.22)=0.57, *p*=.72, condition F(1,54.25)=0.002, *p*=.97; HR condition*time F(5,162.91)=1.65, *p*=.15, condition F(1,54.16)=0.17, *p*=.68). RMSSD significantly decreased from *Baseline* after the film (*B*=-0.04, 95% CI -0.06--0.01, SE=0.01, *t*=-2.69, *p*<.01). PEP significantly increased from *Baseline* after the film (*B*=3.12, 95% CI 0.90-5.35, SE=1.13, *t*=2.77, *p*<.01), during *Recovery* (*B*=7.12, 95% CI 4.67-9.56, SE=1.23, *t*=5.76, *p*<.01), during the memory tasks (*B*=7.23, 95% CI 4.67-9.79, SE=1.29, *t*=5.61, *p*<.01), and final assessment *(B*=6.67, 95% CI 4.04-9.30, SE=1.32, *t*=5.06, *p*<.01). HR significantly increased from *Baseline* after the film (*B*=1.17, 95% CI -0.13-2.21, SE=0.53, *t*=2.22, *p*=.03), but significantly decreased from *Baseline* during the last assessment (*B*=-2.22, 95% CI -3.38--1.07, SE=0.58, *t*=-3.81, *p*<.01). During the other stress measurements RMSSD, PEP or HR did not significantly differ from *Baseline* (all *p* values>.05).

3.1.2 Intrusion frequency

Of all *n*=341 reported intrusions in the 7 days after T2, *n*=111 (32.6%) were excluded from our analyses based on validity according to our definition. Ten participants (15.9%) did not report any intrusions, either valid or invalid (see appendix A7 for results on sensitivity analyses with intrusion frequency including invalid reports). Seventeen (27.0%) participants reported no valid intrusions, which did not differ between conditions (Pearson's Chi-square (1)=1.08, *p*=.30). We found significant main effects of time (Wald Chi-square(6)=90.96, *p*<.01) and condition (Wald Chi-square(1)=5.11, *p*=.02), but no significant interaction between condition and time (Wald Chi-square(6)=3.38, *p*=.76) on intrusion frequency per day. The seCPT participants reported significantly more intrusions per day across the 7 days than warm water participants (*B*=0.44, 95% CI 0.10-0.78, SE=0.17, Wald Chi-square=6.31, *p*=.01; Figure 3A), and both conditions showed a steady decline in the number of reported intrusions per day across the week (*B*=-0.46, 95% CI -0.57--0.36, SE=0.05, Wald Chi-square=72.32, *p*<.01).

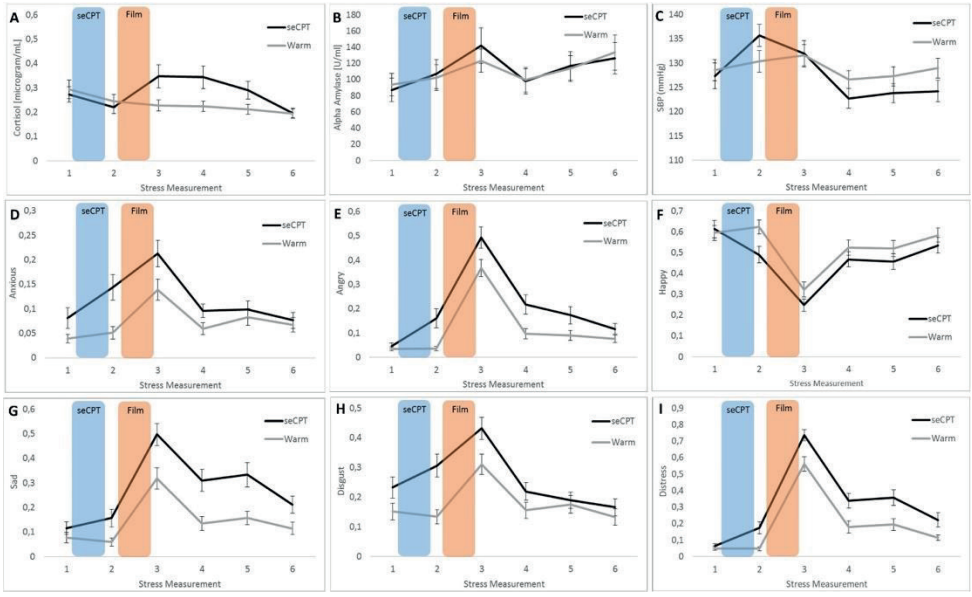


Figure 2. Biological reactivity and emotional states and distress across the experimental assessment (T2). **A.** Cortisol reactivity. SeCPT 1: *n*=29, 2: *n*=27, 3: *n*=29, 4: *n*=29, 5: *n*=29, 6: *n*=29, warm water all measurement times 1-6: *n*=34. **B.** Alpha amylase reactivity. SeCPT 1: *n*=29, 2: *n*=28, 3: *n*=29, 4: *n*=29, 5: *n*=29, 6: *n*=29, warm water 1: *n*=33, 2: *n*=34, 3: *n*=34, 4: *n*=34, 5: *n*=34, 6: *n*=34. **C.** Systolic BP reactivity. SeCPT *n*=29, warm water *n*=34. **D.** Anxious feelings. **E.** Angry feelings. **F.** Happy feeling. **G.** Sad feeling. **H.** Feeling distressed **I.** Feelings of disgust. For all emotional states: seCPT 1: *n*=29, 2: *n*=29, 3: *n*=29, 4: *n*=29, 5: *n*=29, 6: *n*=29, warm water 1: *n*=34, 2: *n*=33, 3: *n*=34, 4: *n*=34, 5: *n*=34, 6: *n*=34; Data are displayed as raw, non-transformed means(SE); sAA: salivary alpha amylase, SBP: systolic blood pressure.

However, a summation of all reported valid intrusions across the week did not differ significantly between conditions (seCPT 4.48(5.42), warm water 2.94(3.30); T_{61}=1.62, *p*=.11, *d*=.41). Notably, conditions differed significantly with regard to types of reported intrusions (Pearson's Chi-square(2)=15.13, *p*=.01). In the seCPT condition, most intrusions were image-based (48.5%) rather than thoughts (35.8%) or a mixture of both (15.7%), while in the warm water condition types were almost equally distributed with slightly more intrusions being a mixture of images and thoughts (mix 37.5%; image-based 31.3%; thought 31.3%).

3.2 Secondary outcomes

3.2.1 Intrusion characteristics

For intrusion characteristics *vividness* and *distress* per day across the 7 days after T2, we found significant main effects of time (*vividness* Wald Chi-square(6)=14.29, *p*=.03, *distress* Wald Chi-square(6)=26.60, *p*<.01) and condition (*vividness* Wald Chi-square(1)=6.82, *p*=.01, *distress* Wald Chi-square(1)=6.32, *p*=.01), but no significant interactions (*vividness* Wald Chi-square(6)=3.81,

p=.70, *distress* (Wald Chi-square(6)=1.63, *p*=.95). The seCPT participants reported more vivid (*B*=0.93, 95% CI 0.34-1.53, SE=0.30, Wald Chi-square=9.45, *p*<.01) and distressing intrusions (*B*=0.31, 95% CI 0.08-0.54, SE=0.12, Wald Chi-square=6.72, *p*=.01) than warm water participants. For both conditions the vividness and distress associated with the intrusions declined over time (*vividness B*=-0.33, 95% CI -0.47--0.18, SE=0.07, Wald Chi-square=19.08, *p*<.01; *distress B*=-0.12, 95% CI -0.18--0.06, SE=0.03, Wald Chi-square=14.97, *p*<.01; Figure 3B-C). Additionally, no differences between conditions were found in the summation of *vividness* (T_{61}=1.20, *p*=.24, *d*=.39) and *distress* (T_{61}=1.54, *p*=.13, *d*=.30) across the 7 days. Likewise, there were no significant difference between conditions in most prominent intrusion characteristics as reported during T3. seCPT participants had significantly higher PCL-5 total scores during T3, indicating more film-related PTSD symptoms than warm water participants, mainly due to significantly higher scores on Cluster B-*Intrusions* (details are reported in Table 1).

3.2.2 Memory accuracy

seCPT participants had significantly lower scores on the *Recognition* task than warm water participants across both assessments (main condition effect: F(1)=4.30, *p*=.04, η_2=0.07). No significant differences between conditions were found for *Cued Recall* (F(1)=1.00, *p*=.32, η_2=0.02) and *Sequential Recall* (F(1)=0.09, *p*=.77, η_2<0.01). On all tasks, memory accuracy significantly declined from T2 to T3 (main time effect: *Cued Recall* F(1)=5.28, *p*=.03, η_2=0.08; *Recognition* F(1)=7.03, *p*=.01, η_2=0.11; *Sequential Recall* F(1)=7.97, *p*<.01, η_2=0.12), without significant differences in this decline between conditions (condition*time: *Cued Recall* F(1)=1.00, *p*=.32, η_2=0.02; *Recognition* F(1)=1.81, *p*=.18, η_2=0.03; *Sequential Recall* F(1)=0.10, *p*=.76, η_2<0.01; Table 1).

3.2.3 Subjective experiences of the experimental manipulations

All assessed emotional states and subjective distress significantly changed across T2, with most changes differing between conditions (Figure 2D-I; Anxious: Condition*Time F(5,189.52)=2.29, *p*<.05, Condition F(1,61.68)=6.19, *p*=.02, Time F(5,189.52)=21.55, *p*<.01; Angry: Condition*Time F(5,188.04)=2.27, *p*<.05, Condition F(1,61.31)=11.21, *p*=.01, Time F(5,188.04)=88.15, *p*<.01; Happy: Condition*Time F(5,177.92)=2.63, *p*=.03, Condition F(1,61.23)=1.64, *p*=.21, Time F(5,177.92)=58.14, *p*<.01; Sad: Condition*Time F(5,188.12)=2.28, *p*<.05, Condition F(1,61.17)=13.00, *p*=.01, Time F(5,188.12)=47.87, *p*<.01; Distress: Condition*Time F(5,189.52)=2.29, *p*<.05, Condition F(1,61.68)=6.19, *p*=.02, Time F(5,189.52)=21.55, *p*<.01; Disgust: Condition*Time F(5,194.43)=2.01, *p*=.08, Condition F(1,63.31)=15.38, *p*<.01, Time F(5,194.43)=137.69, *p*<.01). The seCPT participants showed a stronger increase in negative emotions and distress from *Baseline* after the seCPT (*Anxious B*=0.02, 95% CI 0.01-0.04, SE=0.01, *t*=2.07, *p*<.04; *Angry B*=0.04, 95% CI 0.01-0.06, SE=0.01, *t*=2.95, *p*<.01; *Distress B*=0.03, 95% CI 0.01-0.06, SE=0.01, *t*=2.29, *p*=.02) and film (*Angry B*=0.03, 95% CI 0.01-0.06, SE=0.02, *t*=2.16, *p*=.03; *Sad B*=0.04, 95% CI 0.01-0.08, SE=0.02, *t*=2.69, *p*=.01), and during *Recovery* (*Angry B*=0.04, 95% CI 0.01-0.07, SE=0.02, *t*=2.35, *p*=.02; *Sad B*=0.04, 95% CI 0.01-0.08, SE=0.02, *t*=2.74, *p*=.01)

and the memory tasks (*Sad B*=0.04, 95% CI 0.01-0.07, SE=0.02, *t*=2.56, *p*=.01) than warm water participants, but not during the last assessment (all *p* values>.05). Positive emotions followed the opposite pattern, with seCPT participants showing stronger decreased happiness from *Baseline* after the seCPT (*B*=-0.04, 95% CI -0.07--0.02, SE=0.01, *t*=-3.61, *p*<.01) and film (*B*=-0.03, 95% CI -0.05-<-0.01, SE=0.01, *t*=-2.04, *p*=.04) than warm water participants. Only for disgust no interaction effects were found, but main effects indicated that feelings of disgust increased from *Baseline* after the seCPT (*B*=0.02, 95% CI 0.01-0.03, SE=0.01, *t*=2.41, *p*=.02), after the film (*B*=0.19, 95% CI 0.17-0.20, SE=0.01, *t*=20.87, *p*<.01), during *Recovery* (*B*=0.07, 95% CI 0.05-0.09, SE=0.01, *t*=7.10, *p*<.01), memory tasks (*B*=0.07, 95% CI 0.05-0.09, SE=0.01, *t*=7.52, *p*<.01), and the final assessment (*B*=0.04, 95% CI 0.02-0.06, SE=0.01, *t*=3.83, *p*<.01). Overall, the seCPT participants experienced more disgust across the experimental assessment than warm water participants (*B*=0.04, 95% CI 0.02-0.06, SE=0.01, *t*=3.58, *p*<.01).

The seCPT participants experienced the seCPT as significantly more painful, unpleasant, difficult and stressful than warm water participants (all *p* values<.01; Appendix A8). There were no significant differences between conditions in film-directed focus (T_{61}=0.41, *p*=.68, *d*=.10), empathizing (T_{61}=1.13, *p*=.27, *d*=.25) and immersion (T_{61}=-0.32, *p*=.75, *d*=-.08).

3.2.4 Predictors of intrusion development

We found initially significant interaction effects between condition and sAA AUCg during stress reactivity on intrusion frequency and associated vividness and distress (*frequency*: *β*=-0.362, *p*=.04, f^2=0.11; *vividness:* *β*=-0.366, *p*=.04, f^2=0.11; *distress* *β*=-0.355, *p*=.05, f^2=0.11), and between condition and sAA AUC during recovery on distress (*β*=-0.378, *p*=.05, f^2=0.13), indicating differential associations within both conditions (Figure 4; Table 2). However, these effects were no longer significant upon corrections for multiple comparisons. Significant main effects of sAA AUCg during reactivity and recovery on film-related PCL-5 Cluster B-*Intrusions* scores were initially found (stress reactivity: *β*=0.399, *p*=.02, f^2=0.21; recovery: *β*=0.408, *p*=.03, f^2=0.18), yet were also no longer significant upon multiple comparison corrections. Similarly, the interaction effect between condition and cortisol AUCg during stress reactivity on film-related PCL-5 total scores was also no longer significant upon multiple comparison corrections (*β*=-0.399, *p*=.02, f^2=0.23).

4. Discussion

As our primary aim, we investigated whether adding a brief psychosocial stressor immediately prior to a trauma film increased acute HPA and SNS axis reactivity and subsequent film-related intrusion frequency across the following week in healthy men. Secondary, we investigated the effects of adding the psychosocial stressor on intrusion characteristics and film-related declarative memory. Lastly, we investigated associations between sAA and cortisol stress reactivity to the experimental paradigm, film-related declarative memory accuracy, GR sensitivity and film-related intrusive memory development.

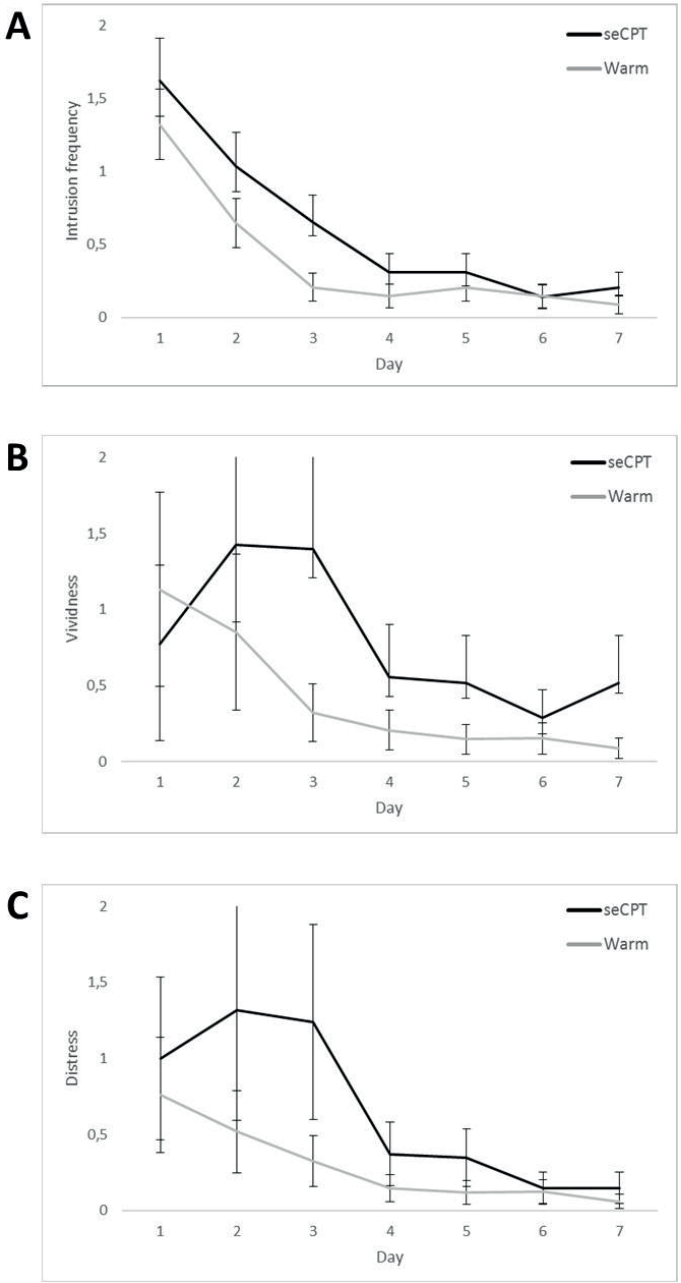


Figure 3. Intrusion development during the 7-days after T2. **A.** Intrusion frequency during 7-days after T2, **B.** *Vividness* of intrusions reported during 7-days after T2, **C.** *Distress* of intrusions reported during 7-days after T2. Data is displayed as estimated marginal means (SE); seCPT *n*=29, warm water *n*=34.

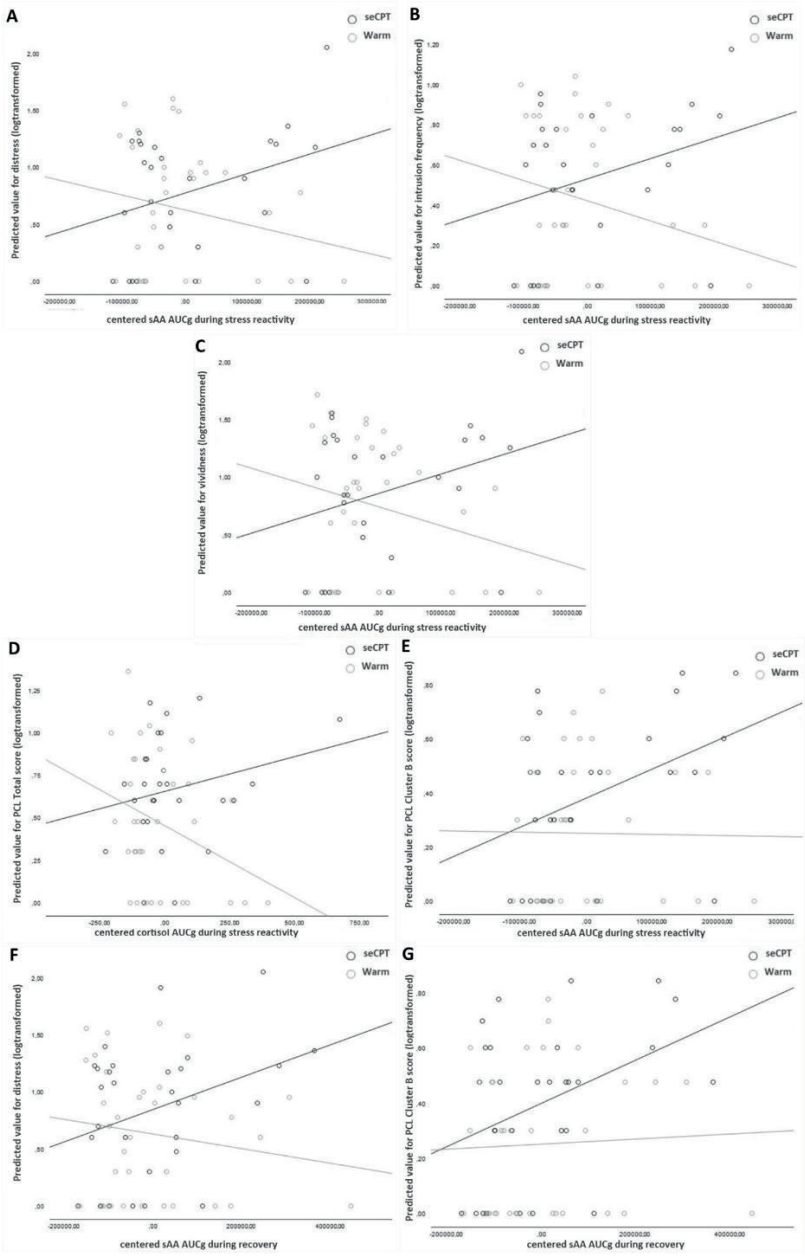


Figure 4. Biological stress reactivity of the HPA axis and ANS predicting intrusive memory development. Graphs display the significant (uncorrected) interaction effects between condition and sAA AUCg **(A-C)**; between condition and cortisol AUCg during stress reactivity **(D)**; between condition and sAA AUCg during recovery **(E)**, and main effect of sAA AUCg during stress reactivity **(F)** and recovery **(G)**. Scores are displayed as log transformed estimated marginal means, predictors were centered; seCPT *n*=29, warm water *n*=34; sAA: salivary alpha amylase; $p<0.05$.

We found stronger HPA and SNS reactivity and more intrusions in the psychosocial stressor condition compared to the warm water condition. Men undergoing the psychosocial stressor also had lower film-related recognition memory accuracy and their intrusive memories were associated with higher levels of vividness and distress than men in the warm water condition. Secondary, we found indications for associations between cortisol and sAA levels throughout the experimental session and subsequent intrusion development, but these predictive effects did not survive corrections for multiple comparisons.

We found a stronger increase in cortisol and SBP reactivity to the trauma film in the experimental condition compared to the control condition. The original trauma film paradigm lacked reliably induced biological stress responses (Chou et al., 2014; Rombold et al., 2016a, 2016b) and therefore has limited suitability for investigation of neurobiological and related cognitive processes underlying intrusive symptom development following traumatic stress. Our findings indicate that adjustment of the paradigm by adding the seCPT as a psychosocial stressor resulted in a more robust naturalistic biological stress response that is more similar to what is expected within a real-life trauma condition. This holds in particular for activation of the HPA axis, as we observed a less pronounced effect on SNS activation: we only identified a stronger increase in SBP, and not sAA, PEP or HR within the experimental condition.

Similar to (Schultebraucks et al., 2019), who previously added a longer lasting psychosocial stressor (Trier social stress test) immediately prior to the trauma film in university educated healthy females, we demonstrated a stronger increase in cortisol after the film in men receiving the psychosocial stressor. Our findings regarding the SNS parameters differed from Schultebraucks et al. (2019), as we observed a stronger increase in SBP both after the psychosocial stressor and the film in the seCPT condition, while Schultebraucks et al. (2019) observed differences in SBP and sAA immediately after the stressor but not after the film. Such seemingly inconsistent effects on different measures of the SNS within and between studies are not uncommon and may be explained by their rapid changes upon and in the aftermath of stress exposure, making it difficult to capture SNS activation with all these measures at exactly the right moment (Bosch et al., 2011; Nagy et al., 2015).

Additionally, in this previous study, addition of the psychosocial stressor did not influence intrusion frequency, whereas we did find a higher frequency of intrusions upon psychosocial stress exposure. Also, addition of the psychosocial stressor in our study resulted in more pronounced subjective distress and negative emotions during the experimental session, while this was not observed by Schultebraucks et al. (2019). The different applied psychosocial stressors (seCPT versus Trier social stress test) may obviously have impacted these differential findings. Moreover, differences may also be due to samples consisting of females versus males respectively. Women are at increased risk for PTSD development following most types of traumatic events (Olff, 2017) and the increased risk for PTSD one year after traumatic injury within

women was previously found to be mediated by higher initial PTSD symptom severity (Shalev et al., 2019). Although these findings concern PTSD diagnosis and not intrusive symptoms in particular, it seems counterintuitive at first sight that adjustment of the paradigm had a larger impact on our male sample than on the female sample of Schultebraucks et al. (2019) especially as our used film fragment portrays a victimized woman. Yet, previously no sex differences were observed in intrusion frequency after several commonly used trauma films including the fragment from '*Irreversible*' (Weidmann et al., 2009), used by both Schultebraucks et al. (2019) and ourselves. Moreover, there is increasing evidence that neurobiological processes underlying development of early post-trauma PTSD symptoms differ between males and females, and within females are also dependent on menstrual cycle phase, hormonal contraception and related estrogen and progesterone levels (Engel et al., 2020). Previous studies indicating predictive value of GR function and early post-trauma cortisol levels for early and long-term PTSD symptoms have been performed in predominantly male populations (Engel et al., 2020; McFarlane et al., 2011; Steudte-Schmiedgen et al., 2015; van Zuiden et al., 2013) and sex differences herein remain largely uninvestigated. Future studies using the (adjusted) trauma film paradigm to investigate intrusion development and its underlying processes should directly contrast male and female participants, preferably with various hormonal (estrogen and progesterone) statuses as well.

Contrary to our expectations, we did not observe differences between conditions in contextual film-related memory accuracy. We did however observe decreased accuracy on the *Recognition* task across T2 and T3 in the seCPT condition. In the absence of an effect on the other two memory tasks, at first sight this suggests poorer ability to recall facts about trauma film-related details and gist specifically. The used tasks were all based on previous trauma-film studies (James et al., 2016), but it is well possible that more complex tasks should be used to capture potential effects on contextual memory and recall, especially as the participants performed quite well and there was very limited variability across participants.

Our secondary aim was to further investigate the biological and cognitive processes underlying interindividual variability in development of intrusive memories following traumatic stress, by means of analyzing whether acute cortisol and sAA reactivity and recovery as well as declarative memory accuracy during the experimental assessment predicted trauma film-related intrusion frequency and characteristics in the week following the experimental assessment, including interaction effects with allocated condition. We found some initial associations between cortisol and sAA levels throughout the experimental assessment and subsequent intrusion development, but none of these predictive effects remained significant after our stringent multiple comparison corrections. Although this clearly urges caution in interpreting these findings, we believe that the observed predictive effects with moderate effect sizes are worth a brief mentioning in light of future research into these processes.

In an exploratory data-driven analysis across both conditions to predict intrusion frequency following the trauma film from several biological and psychological features, Schultebraucks et al. (2019) observed that higher cortisol increases during the experimental paradigm were associated with higher subsequent intrusion frequency. Here, we found tentative evidence that cortisol AUCg levels during specifically the acute stress reactivity phase predicted PTSD-related total symptom scores the following week, with the directionality of the associations differing between the seCPT and warm water conditions. These differential effects are noteworthy given the fact that the seCPT condition showed a stronger increase in cortisol levels in response to the experimental paradigm and warrant further investigation. Similar to Schultebraucks et al. (2019), we observed tentative associations between higher overall sAA AUCg levels during both reactivity and recovery phases and more self-reported film-related intrusive symptoms using the PTSD symptom questionnaire at follow-up across conditions, which is interesting in light of previous meta-analytic findings across observational cohort studies that high SNS activity within the first 72 hours post-trauma predicted subsequent PTSD symptom severity (Morris et al., 2016). Although both Schultebraucks et al. and we focused on intrusive memories within one week post-trauma and not on sustained intrusive memories nor long-term PTSD outcome, the combined findings tentatively indicate that this previously observed predictive effect with the cohort studies may not be merely associated with sustained high SNS activity during early recovery following trauma, but also with high peri-traumatic SNS reactivity.

We did not observe any associations between film-related declarative memory accuracy, including the sequential recall task thought to reflect contextual memory, and subsequent intrusion development. Thus, our findings do not support the hypothesized mechanism of decreased contextual encoding of traumatic memories mediating associations between HPA and ANS functioning and intrusive symptom development, but as stated above, it may be worthwhile to investigate contextual memory encoding using another more complex task and not only focus on sequential recall.

A methodological strength of our study is that we used a digital application to increase accurate real-time intrusion reports immediately upon occurrence and to miss fewer reports, thought to lead to less recall bias and more accurate measures of intrusion frequencies and their related characteristics (Moskowitz & Young, 2006). At first sight, the observation that 27% of our participants reported no valid intrusions may indicate that our adapted paradigm and used experimental procedures did not result in reliably induced intrusions. However, Laposa and Alden (2008) previously found a comparable percentage of 28% of their sample not reporting any distress-inducing intrusions after a trauma film. Also, if we take both valid and invalid intrusions into account, our observation that 15.9% of participants did not report any intrusions is highly similar to meta-analytic results of 15.5% of healthy individuals not reporting any intrusions (irrespective of vividness and distress) following trauma film viewing (Clark et al., 2015). Still, our strict in- and exclusion criteria, precluding all potential low-threshold psychological problems due

to ethical considerations (James et al., 2016), may have influenced the generalizability of our findings as pre-trauma psychopathology has been identified as a risk factor for not only trauma film-related intrusive memories (Clark et al., 2015) but also PTSD development (Sayed et al., 2015). While it has been found that the majority of trauma-exposed individuals reports intrusive memories in the first weeks following trauma, these memories commonly decrease within the first months after trauma. Only a minority experiences long-term or sustained intrusive memories, of which an even lower percentage will fulfil diagnostic criteria for PTSD (Iyadurai et al., 2019). Thus, including a more heterogeneous population in terms of pre-existing psychological problems could give important additional insights into the development of these longer-lasting intrusive memories, although this also brings along additional ethical considerations and should be very carefully considered. In addition, the generalizability of our findings may be limited by the fact that we included only university educated Caucasian men, while lower educational level, minority ethnic status and being women have been found to be risk factors for PTSD (Brewin et al., 2000; Olff, 2017). Furthermore, as only a minority of trauma-exposed individuals experiences sustained intrusive memories and eventually fulfil diagnostic PTSD criteria, it is important to be cautious in interpreting how our findings on the development of early post-(experimental) trauma intrusive memories may relate to sustained trauma-related intrusive re-experiencing, both in the absence or presence of PTSD diagnosis.

Several experimental procedure-related aspects may also have impacted subsequent intrusion development. These include the potential influence of the presence of the experimenter in the room during viewing of the trauma film, which has not been investigated currently. Studies using the trauma film paradigm have used a varying approach; in some studies the experimenter left the room (e.g. Holmes et al., 2009, 2010; James et al., 2015; Lau-Zhu et al., 2019), while in other studies the experimenter was present to monitor whether participants looked away from the screen (Chou et al., 2014; Meyer et al., 2017; Rombold et al., 2016a, 2016b). For this reason, as well as for safeguarding purposes in case of potential elicited distress, we also opted to have the experimenter in the room during film viewing (although fully out of sight). Furthermore, although common practice in studies using the trauma film paradigm, we cannot exclude that memory tasks and explanation on how to report intrusions during recovery at T2 – when it was not yet possible to report intrusions in the application – could have influenced intrusion development, particularly as we found increased sAA during recovery specifically during the memory tasks and intrusion reporting instructions. Finally, because of participant dropouts as well as technical issues with the VU-AMS that resulted in >10% of cardiac reactivity missing measures, our power was limited here.

5. Conclusions

We found that adding a brief psychosocial stressor prior to viewing a trauma film resulted in stronger increases in HPA and SNS axis activation during the experimental session, as well as increased intrusion frequency and associated vividness and distress during the following week in

healthy men. The elicitation of a more robust stress response in this adapted version, likely more comparable to real-life trauma exposure, increases the translational value of the trauma film paradigm. The adapted paradigm may be useful to investigate effects of individual variation in and potentially pharmacological manipulation of biological stress reactivity, as well as underlying cognitive processes, on development of intrusive symptoms, as more insights into the biological and cognitive processes underlying development of early post-trauma PTSD symptoms could advance future effective prevention.

Table 1. Participant characteristics, memory accuracy at the experimental (T2) and follow-up (T3) assessment, and intrusion characteristics of the most prominent intrusion and film-related PTSD symptoms assessed at follow-up (T3).

	SeCPT (n=29)	Warm-water (n=34)	Statistics
Age (years)	22.52 (4.83)	22. 90 (3.89)	U = 490.00, p =.97
BMI (kg/m^2)	22.53 (2.51)	22.75 (2.25)	T_{61} = -0.37, p =.72
Smoking behaviour (n (%))			
Occasional smoker	7 (24.1%)	9 (26.5%)	p = 1.00
Screening prior T2			
DASS-21[1]			
Depression	2.03 (2.28)	1.32 (1.80)	U = 379.500, p =.11
Anxiety	1.41 (1.24)	1.29 (1.29)	T_{61} = 0.37, p =.71
Stress	3.86 (3.29)	2.47 (2.25)	U = 372.00, p =.09
Total score	7.31 (5.75)	5.09 (3.62)	U = 393.00, p =.17
PCL-5[2]			
Total score	3.45 (3.45)	3.21 (3.89)	U = 451.00, p =.56
Cluster B–Intrusions	0.59 (0.98)	0.79 (1.43)	U = 523.50, p =.63
Cluster C–Avoidance	0.21 (0.49)	0.35 (0.60)	U = 553.00, p =.27
Cluster D–Negative Cognitions and Mood	1.24 (2.08)	0.74 (1.46)	U = 423.50, p =.27
Cluster E–Arousal and Reactivity	1.41 (1.78)	1.32 (1.59)	U = 486.00, p =.92
LEC-5[3]	4.90 (3.28)	6.47 (4.07)	T_{61} = -1.67, p =.10
GR functioning prior T2			
CAR[4]			
AUCg	1083.46 (61.59)	1051.83 (139.36)	$T_{38.85}$ = 0.65, p =.52
AUCi	303.28 (68.90)	309.73 (70.11)	T_{52} = -0.07, p =.95
DST[5]			
AUCg	105.20 (11.18)	144.97 (21.96)	$T_{40.81}$ = -1.62, p =.11
AUCi	8.95 (4.85)	26.82 (15.58)	$T_{32.06}$ = -0.84, p =.41

	SeCPT (n=29)	Warm-water (n=34)	Between-subject Statistics	Within-subject Statistics
Declarative memory accuracy			Condition effects	Time effects
At T2				
Cued Recall	6.40 (0.23)	6.82 (0.21)	F(1)=1.00, p=.32,	F(1)=5.28, p=.03
Recognition	9.46 (0.21)	10.24 (0.19)	F(1)=4.30, p=.04	F(1)=7.03, p=.01
Sequential Recall[6]	0.96 (0.01)	0.96 (0.01)	F(1)=0.09, p=.77	F(1)=7.97, p<.01
At T3				
Cued Recall	6.11 (0.21)	6.09 (0.20)		
Recognition	9.18 (0.26)	9.38 (0.24)		
Sequential Recall[6]	0.95 (0.01)	0.94 (0.01)		

	SeCPT (n=28)	Warm-water (n=24)	Statistics
Characteristics of most prominent intrusion at T3			
Vividness	0.41 (0.27)	0.47 (0.25)	
Anxiousness	0.28 (0.23)	0.35 (0.27)	T_{50} = -0.90, p =.37
Unpleasantness	0.40 (0.30)	0.39 (0.28)	T_{50} = -0.95, p =.35
Distress	0.26 (0.23)	0.26 (0.24)	T_{49} = 0.15, p =.88
Disjointedness/fragmentation	0.55 (0.35)	0.44 (0.35)	T_{50} = 0.14, p =.89
			T_{50} = 1.20, p =.23
Film-related PCL-5 at T3[2]			
Total score	4.93 (3.99)	3.38 (4.47)	T_{61} = 2.33, p =.02
Cluster B-Intrusions	2.03 (1.80)	1.15 (1.37)	T_{61} = 2.22, p =.03
Cluster C-Avoidance	0.38 (0.62)	0.35 (0.65)	U = 475.00, p =.75
Cluster D-Negative Cognition and Mood	1.45 (2.38)	0.85 (1.21)	T_{61} = 1.04, p =.30
Cluster E-Arousal and Reactivity	1.07 (1.19)	0.71 (1.22)	U =390.50, p =.12

Scores are displayed as raw, non-transformed mean(SD) and for memory tasks mean(SE) or *n* (%).[1]DASS-21: Depression, Anxiety and Stress Scale; [2]PCL-5: PTSD Checklist for DSM5; [3]LEC-5: Life Events Checklist, number of experienced traumatic event types when experienced personally, witnessed it, learned about it happening to close family members or friends, or if it happened at work; [4]CAR: cortisol awakening response; [5]DST: cortisol suppression using the dexamethasone suppression test; [6]Sequantial recall task accuracy was calculated by Spearman's Rank correlations (range 0-1); AUCg: area under the curve with respect to the ground, AUCi: area under the curve with respect to the increase; $p<0.05$.

Table 2. Results of linear regression analyses of GR functioning, biological stress reactivity and memory accuracy on intrusion frequency and intrusion characteristics reported during the 7 days after T2 and film-related PTSD symptoms administered during 7 day follow-up (T3).

Predictor variable	Intrusions						Film-related PTSD symptom scores								
	Frequency			*Vividness*			*Distress*			*Total*			*Cluster B-Intrusion*		
Model 1: GR functioning – AUCg *corrected for CAR*															
	β	*T*	*p*	β	*T*	*p*	β	*T*	*p*	β	*T*	*p*	β	*T*	*p*
(Constant)		8.096	<.001		7.938	<.001		7.652	<.001		9.454	<.001		7.317	<.001
Condition	-.211	-1.629	.109	-.153	-1.165	.249	-.190	-1.440	.155	-.304	-2.324	.024	-.275	-2.080	.042
DST	.090	0.324	.747	.065	0.233	.817	.102	0.362	.719	.011	0.040	.968	.145	0.512	.611
CAR	-.531	-1.570	.122	-.462	-1.350	.183	-.382	-1.112	.271	-.090	-0.263	.793	.019	0.057	.955
DST*Condition	-.278	-0.999	.322	-.244	-0.865	.391	-.292	-1.032	.306	-.014	-0.051	.959	-.215	-0.758	.452
CAR*Condition	.421	1.235	.222	.315	0.913	.365	.308	0.889	.378	-.100	-0.292	.771	-.122	-0.353	.726
Model 2: GR functioning – AUCi *corrected for CAR*															
	β	*T*	*p*	β	*T*	*p*	β	*T*	*p*	β	*T*	*p*	β	*T*	*p*
(Constant)		7.761	<.001		7.527	<.001		7.503	<.001		9.307	<.001		7.097	<.001
Condition	-.182	-1.400	.167	-.117	-0.886	.380	-.167	-1.287	.203	-.272	-2.094	.041	-.236	-1.820	.074
DST	-.033	-0.074	.942	-.204	-0.450	.654	-.007	-0.015	.988	-.326	-0.734	.466	-.370	-0.830	.410
CAR	.033	0.167	.868	.029	0.148	.883	.078	0.399	.691	-.048	-0.249	.804	.079	0.407	.686
DST*Condition	-.182	-0.411	.683	-.010	-0.023	.982	-.221	-0.498	.621	.270	0.610	.544	.222	0.501	.619
CAR*Condition	.156	0.805	.424	.107	0.544	.588	.113	0.582	.563	.060	0.309	.759	-.015	-0.080	.937

Model 3: Memory - Cued Recall
corrected for task version

	β	*T*	*p*	β	*T*	*p*	β	*T*	*p*	β	*T*	*p*	β	*T*	*p*
(Constant)		3.617	.001		3.422	.001		3.059	.003		2.335	.023		2.360	.022
Condition	-.224	-1.730	.089	-.170	-1.301	.198	-.206	-1.577	.120	-.269	-2.148	.036	-.269	-2.125	.038
Cued Recall	.201	1.015	.314	.183	0.911	.366	.104	0.521	.604	-.145	-0.759	.451	.084	0.434	.666
Task version	-.078	-0.506	.615	-.051	-0.325	.746	-.008	-0.051	.959	.241	1.617	.111	.110	0.727	.470
Cued*Condition	-.081	-0.457	.649	-.087	-0.482	.632	-.041	-0.230	.819	-.039	-0.225	.823	-.211	-1.215	.229

Model 4: Memory - Recognition
corrected for task version

	β	*T*	*p*	β	*T*	*p*	β	*T*	*p*	β	*T*	*p*	β	*T*	*p*
(Constant)		3.916	<.001		3.764	<.001		3.643	.001		3.678	.001		3.314	.002
Condition	-.145	-1.081	.284	-.098	-0.722	.473	-.124	-0.929	.357	-.247	-1.885	.064	-.244	-1.864	.067
Recognition	-.101	-0.539	.592	-.098	-0.517	.607	-.133	-0.714	.478	-.115	-0.628	.532	.072	0.394	.695
Task version	-.039	-0.299	.766	-.022	-0.165	.870	-.016	-0.122	.904	.113	0.891	.377	.047	0.373	.710
Recognition*Condition	-.109	-0.605	.548	-.098	-0.538	.593	-.121	-0.681	.499	-.038	-0.216	.829	-.248	-1.415	.162

Model 5: Memory - Sequential Recall
corrected for task version

	β	*T*	*p*	β	*T*	*p*	β	*T*	*p*	β	*T*	*p*	β	*T*	*p*
(Constant)		3.555	.001		3.451	.001		3.269	.002		3.333	.001		2.851	.006
Condition	-.204	-1.606	.114	-.153	-1.194	.237	-.194	-1.529	.132	-.297	-2.406	.019	-.280	-2.234	.029
Sequential Recall	-.157	-0.663	.510	-.194	-0.813	.420	-.214	-0.900	.372	.061	0.265	.792	.115	0.492	.624

Task version	.020	0.153	.879	.032	0.246	.807	.044	0.339	.736	.173	1.377	.174	.097	0.756	.453
Sequential *Condition	.282	1.197	.236	.310	1.304	.197	.301	1.274	.208	.089	0.389	.699	.011	0.049	.961
Model 6: Stress Reactivity AUCg															
	β	*T*	*p*	β	*T*	*p*	β	*T*	*p*	β	*T*	*p*	β	*T*	*p*
(Constant)		7.528	<.001		7.426	<.001		7.190	<.001		9.471	<.001		7.212	<.001
Condition	-.151	-1.167	.248	-.100	-0.773	.443	-.135	-1.037	.304	-.261	-2.122	.038	-.232	-1.869	.067
sAA	.279	1.593	.117	.292	1.663	.102	.311	1.772	.082	.261	1.568	.123	.399	2.379	.021
sAA*Condition	-.362	-2.074	.043	-.366	-2.097	.041	-.355	-2.033	.047	-.149	-0.901	.371	-.260	-1.556	.125
Cortisol	.060	0.339	.736	.082	0.462	.646	.074	0.418	.678	.220	1.310	.196	.184	1.087	.282
Cortisol*Condition	-.076	-0.433	.666	-.148	-0.843	.403	-.093	-0.530	.598	-.399	-2.395	.020	-.266	-1.586	.118
Model 7: Stress Reactivity AUCi															
	β	*T*	*p*	β	*T*	*p*	β	*T*	*p*	β	*T*	*p*	β	*T*	*p*
(Constant)		7.931	<.001		7.938	<.001		6.874	<.001		8.690	<.001		6.788	<.001
Condition	-.204	-1.518	.135	-.204	7.077	<.001	-.111	-0.800	.427	-.212	-1.569	.123	-.214	-1.585	.119
sAA	.180	1.039	.303	.180	-0.462	.646	.281	1.516	.135	.213	1.175	.245	.287	1.584	.119
sAA*Condition	-.206	-1.185	.241	-.206	1.512	.136	-.187	-1.013	.316	-.150	-0.830	.410	-.174	-0.964	.339
Cortisol	-.070	-0.367	.715	-.070	-1.052	.297	-.057	-0.277	.783	.156	0.776	.441	-.018	-0.088	.930
Cortisol*Condition	-.017	-0.091	.928	-.017	-0.274	.785	.043	0.210	.835	-.101	-0.508	.614	.015	0.076	.939
Model 8: Recovery AUCg															
	β	*T*	*p*	β	*T*	*p*	β	*T*	*p*	β	*T*	*p*	β	*T*	*p*
(Constant)		8.099	<.001		8.074	<.001		7.881	<.001		9.944	<.001		7.759	<.001
Condition	-.185	-1.425	.160	-.131	-1.003	.320	-.162	-1.260	.213	-.271	-2.175	.034	-.253	-2.005	.050

sAA	.314	1.660	.102	.317	1.671	.100	.360	1.926	.059	.350	1.929	.059	.408	2.219	.030
sAA*Condition	-.342	-1.820	.074	-.365	-1.930	.059	-.378	-2.029	.047	-.114	-0.633	.529	-.266	-1.457	.151
Cortisol	.042	0.269	.789	.043	0.273	.786	.080	0.513	.610	.166	1.100	.276	.084	0.552	.583
Cortisol*Condition	.033	0.215	.831	.047	0.302	.764	.054	0.356	.723	-.145	-0.980	.331	-.019	-0.124	.902
Model 9: Recovery AUCi															
	β	*T*	*p*	β	*T*	*p*	β	*T*	*p*	β	*T*	*p*	β	*T*	*p*
(Constant)		7.954	<.001		8.011	<.001		7.675	<.001		9.956	<.001		7.727	<.001
Condition	-.185	-1.378	.174	-.123	-0.916	.364	-.169	-1.256	.214	-.234	-1.838	.071	-.257	-1.969	.054
sAA	-.013	-0.073	.942	.032	0.187	.852	-.002	-0.014	.989	.110	0.664	.509	.063	0.370	.713
sAA*Condition	.063	0.363	.718	.090	0.517	.607	.073	0.419	.677	.120	0.722	.473	.028	0.163	.871
Cortisol	-.045	-0.171	.865	-.081	-0.309	.758	-.008	-0.031	.975	.046	0.185	.854	-.154	-0.604	.548
Cortisol*Condition	.077	0.296	.768	.188	0.725	.471	.069	0.266	.791	.146	0.596	.554	.240	0.952	.345

Significant results uncorrected for multiple testing. After False Discovery Rate correction (p=.05) for multiple comparisons according to Benjamini and Hochberg (1995) no results remained significant. sAA: salivary alpha amylase, AUCg: area under the curve with respect to the ground, AUCi: area under the curve with respect to the increase, DST: cortisol suppression using the dexamethasone suppression test, *β*: Standardized coefficients; seCPT *n*=29, warm water interaction with cortisol *n*=34, interaction with sAA *n*=33.

Financial Support

This work was supported by Veni grant from ZonMw awarded to dr. M. van Zuiden [Grant number #91617037, 2016].

Conflict of Interest

Declarations of interest: none.

Acknowledgements

The authors thank all participants for their time and involvement.

6. Supplementary Materials

A1. Sample size calculations, blinding and randomization

A1.1 Sample size

The sample size of N=68 (*n*=34 per condition) was based on sample size calculations for group differences in overall intrusion frequency over one week, and cortisol and sAA across six stress measurements between the seCPT and control condition, as based on the literature available in December 2016.

Total number of intrusive memories in the week post-experiment

Two previous studies using the same (Nixon et al., 2009) or a similar film (Chou et al., 2014) as analogue trauma provided adequate descriptives concerning frequency of intrusive memories in the subsequent week for sample size calculations. Participants not receiving any additional manipulations reported an average of 7.20 (SD:4.55) and 4.16 (SD:2.73) intrusions in the subsequent week respectively.

Several previous studies applied manipulations prior to or during the analogue trauma to increase the number of analogue trauma-related intrusive memories (for review see James et al., 2016). Importantly no studies had successfully manipulated the number of intrusions by providing a (non-trauma-related) stressor. Only one previous study showed successful manipulation of subsequent intrusive memory frequency using an experimental manipulation related to the processes of interest in this study (i.e. decreasing attentional capacity for contextual processing, interfering with memory consolidation) and also provided adequate descriptives (Bourne et al., 2010) (factor 1.6-1.8 increase in one-week frequency). Furthermore, one study showed successful manipulation of intrusion frequency by applying the same stressor as included in the proposed study, but then during reactivation of the traumatic memory two days after the analogue trauma. This is also thought to interfere with memory (re)consolidation but is considered a less ecologically valid representation of a real-life traumatic event (Cheung et al., 2015) (factor 1.4 increase in one-day intrusion frequency, using negative emotional pictures instead of a trauma film). Based on these previous studies, participants receiving the stressor prior to the analogue trauma were expected to report *1.5 times as many intrusive memories* in the subsequent week as reported by participants in the control condition. Sample size calculations for a two group t-test of equal means (with equal n's, alpha = 0.05, power = 80%), using the statistics on one-week intrusion frequency reported by the studies described above, resulted in required sample sizes of 34 per condition (Cohen's d 0.693) (Chou et al., 2014) and 32 per condition (Cohen's d: 0.720) (Nixon et al., 2009) respectively.

Cortisol

Three studies investigated cortisol reactivity to watching a trauma film, of which two reported a significant increase in cortisol levels after the film (Chou et al., 2014; Rombold et al., 2016b), but

only one study provided adequate descriptives for subsequent sample size calculation (Chou et al., 2014). Two analogue trauma studies applied direct manipulations of the cortisol stress responses by other means than administration glucocorticoids prior to or during the analogue trauma, reporting 1.9-fold increases in cortisol levels 20 minutes after viewing negative emotional pictures (Cheung et al., 2015) (effect in males after viewing negative emotional pictures) and on average 1.3-fold higher total cortisol output from baseline to 45 minutes after viewing a trauma film (Rombold et al., 2016a) compared to participants receiving placebo/control manipulation. Moreover, the seCPT in itself was found to result in approximately 2-fold increases in cortisol output up to 60 minutes post-stressor (Sänger et al., 2014; Schwabe et al., 2008). Therefore, participants receiving the stressor prior to the analogue trauma were expected to show cortisol output across the 4 repeated measures post-trauma film that are on average at least *1.5 times as high* as cortisol output of participants in the control condition with similar levels across the first two (pre-film) assessments. Sample size calculations for a two group univariate repeated measures ANOVA (with 6 repeated assessments with 0.3 correlations, greenhouse-geisser correction applied, alpha = 0.05, power to detect a significant interaction between condition*level (i.e. change in cortisol response over course of paradigm) = 80%), using the statistics on cortisol stress reactivity as observed by Chou et al. (2014), resulted in required sample sizes of 21 per condition.

Alpha Amylase

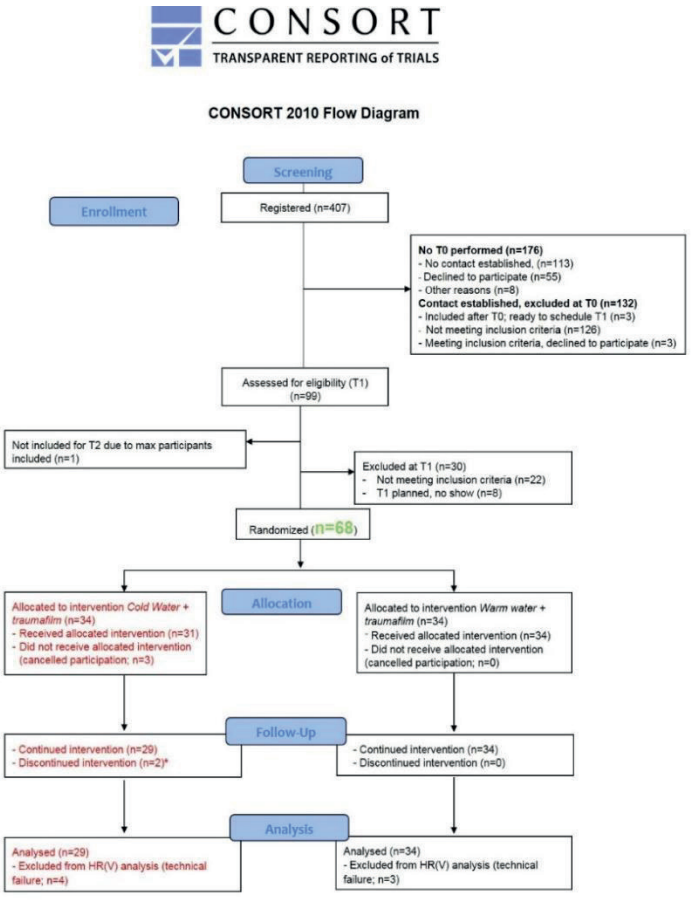
Watching a trauma film or other analogue trauma exposure is not consistently associated with pronounced increased salivary alpha amylase levels (e.g. Cheung et al., 2015; Chou et al., 2014). Also, there are currently no paradigms with additional manipulations prior to or during the analogue trauma that reliably show increases in alpha amylase levels, except for a study that pharmacologically administered a noradrenergic agonist prior to a trauma film (Rombold et al., 2016a) which is not preferable as this will override the naturalistic temporal course of stress response to a stressful or traumatic event. Previously, exposure to the SE-CPT was found to robustly activate the SNS system and increase alpha amylase levels (Schwabe et al., 2008), with immediate post-stressor levels 1.8-fold higher immediately and 1.6-fold higher 20 minutes later in a male population (Sänger et al., 2014). Extrapolating the results of Sänger et al. in our sample size calculations for a two group univariate repeated measures ANOVA (with 6 repeated assessments with 0.3 correlation, greenhouse-geisser correction applied, alpha = 0.05, power to detect a significant interaction between condition*level (i.e. change in alpha amylase response over course of paradigm) = 80%), using the statistics on baseline alpha amylase levels as observed by Chou et al. (2014), resulted in required sample sizes of 34 per condition.

A1.2 Blinding and randomization

The study was single-blinded for condition allocation, i.e. participants but not the researcher performing the assessments were blind to whether they are allocated to the stressor or control condition. This is not thought to bias study outcome as scoring of study parameters and endpoints

and parameters do not require experimenter judgment, except for rating of frequency of intrusive memories which was done according to a strict protocol and followed by interrater reliability evaluation (with excellent ICC). Randomization to the seCPT versus warm water condition occurred on a 1:1 allocation ratio between conditions using a block design (n=4 participants per block) generated by Nquery Advisor software by an independent researcher.

A2. Flowchart of participant inclusion of the Intrusion stud



**Reason for discontinuing intervention: n=1; Adverse event, n=1; Familiar film fragment, declined to watch it again*

A3. Details on screening at T1 and debriefing at the follow-up assessment (T3).

Screening

A face-to-face screening assessment (T1) was scheduled wherein current psychological problems and previous trauma exposure were assessed using three self-report questionnaires; 1) DASS-21: Depression, Anxiety and Stress Scale (21 items, Subscales: *Anxiety*, *Depression* and *Stress*) (Nieuwenhuijsen et al., 2003), 2) PCL-5: PTSD Checklist for DSM5 (20 items, Subscales: Cluster B-*Intrusions,* Cluster C-*Avoidance,* Cluster D-*Negative Cognitions and Mood alterations,* Cluster E-*Arousal and Reactivity alterations*) (Boeschoten et al., 2014b; Weathers et al., 2013b), 3) LEC-5: Life Events Checklist (17 items) (Boeschoten et al., 2014a; Weathers et al., 2013a). Participants were excluded in case of DASS21 subscores *Anxiety* ≥5 and/or *Depression* ≥12, or PCL-5 PTSD symptoms in at least 2 clusters - of which ≥1 symptom in Cluster B or C and ≥2 symptoms in Cluster D or E according to the DSM5 diagnostic rule (McLaughlin et al., 2015). Participants were also excluded if a LEC-5 event similar to the trauma film content (i.e. rape, several physical and sexual assault) was either personally experienced – or experienced by close others accompanied by significant distress of the participant when confronted with the details.

Debriefing

Additionally, participants were asked to contact the researcher at any time when encountering distress from the experimental paradigm or technical problems with the application, and asked not to discuss any content of the experiment with others to avoid interference with the experiment for subsequent participants.

Participants were encouraged to contact the researcher in case of questions or ongoing distress after participating, for which a standardized follow-up protocol including the possibility for psychological consultation within the department was in place. *n*=2 participants were followed-up by the researcher through telephone the week after completing the study because of ongoing distress from the experimental manipulations, and indicated not to experience any distress anymore.

A4. Details on experimental manipulations

A4.1 seCPT

An unfamiliar female researcher switched places with the general researcher to perform and instruct the participants about the test. As the seCPT took 3 minutes, the researcher informed participants about successfully completing the seCPT after 3 minutes. If participants withdrew their hand earlier than 3 minutes, the seCPT was aborted immediately.

seCPT condition:

- The unfamiliar researcher was professionally dressed wearing a badge and acted distant and neutral towards the participants. During the seCPT, questions were not answered, participants could not talk, and the researcher kept a close eye on the participant while taking fake notes.

- Participants additionally read written instructions.
- Participants were informed that facial expressions would be recorded during the seCPT and that they had to look straight into a video camera during the seCPT. An additional false informed consent had to be signed to agree with the fact that video recording during the seCPT may be used for scientific purposes. Facial expressions were not truly investigated, but the announcement of doing so is known to induce a socially-evaluative component to the stressor. When participants did not look straight into the camera they were corrected immediately.

Warm water condition:

- The unfamiliar researcher was casually dressed, did not wear a badge and acted friendly.
- Participants were able to ask questions.
- No video recordings were made and participants did not have to sign an additional informed consent.

In total 10.2% (*n*=3) participants of the seCPT condition withdrew their hand before experiment completion, with a mean duration of 166.21 sec (7.69). None of the participants of the control condition withdrew from the seCPT, with a mean duration of 180.06 sec (0.06).

A4.2 Trauma film paradigm

Participants first received brief instructions before the film. Both the instructions and the file were provided by PsychoPy (v1.81) on a computer screen in the lab room. Instructions included to imagine themselves being a close witness to the event in the film, to keep focussing on the film and on the screen without looking away or intentionally closing their eyes. Then, light was dimmed to a standardized setting and participants viewed the 15-minute short film wearing headphones with standardized audio settings. The researcher sat on the other side of the room to verify the participants watched the film as instructed.

A4.3 Ethical considerations

The trauma film paradigm is a well-controlled standardized and validated experiment that has been increasingly used since the early 21st century (with 74 studies alone published between 2008 and 2016 (James et al., 2016)). From these previous studies, it was already known that the induced negative emotions and intrusions are typically mildly distressing and transient; subsiding within a week after the experiment but usually already within the first few days (James et al., 2016; Rombold et al., 2016b, 2016a). Previous studies explicitly addressing remaining distress and symptoms after the 7th day of follow-up report no instances of participants requesting psychological support (Meyer et al., 2017; Weidmann et al., 2009).

We believe that the fact that this paradigm is currently the only feasible way to perform in depth and repeated assessment of psychological, cognitive and neurobiological mechanisms underlying intrusive memory development during and shortly after trauma in humans, albeit experimental,

justifies its use, as it could facilitate the much warranted development of future preventive interventions for in particular PTSD.

We would like to emphasize that we implemented several safeguarding strategies to make sure we further lowered the risk for severe or continuing distress and ongoing intrusive memories in individuals who participated in our study:

- We had very stringent in- and exclusion criteria. Pre-existing (subclinical) psychopathology is a risk factor tor PTSD development upon trauma exposure (Brewin et al., 2000). Furthermore, a meta-analysis on intrusive memory elicitation by the trauma film paradigm showed that there is substantial variance in intrusion frequency, with positive associations between pre-film anxiety and depression severity and subsequent frequency of intrusive memories (Clark et al., 2015). To minimize the potential for ongoing distress, we therefore excluded participants with self-reported lifetime psychiatric diagnoses and current (sub)clinical anxiety, depressive or PTSD symptoms. Furthermore, to prevent triggering of intrusive re-experiencing or distress related to previous trauma exposure, participants reporting previous exposure to the traumatic events portrayed in the film (either directly or by close friends or relatives) were also excluded.
- We informed participants several times about the aversive content of the film. In the recruitment information, participant information letter attached to the informed consent and during the baseline assessment, we carefully informed participants about the aversive nature of the film and that transient negative emotions, distress and intrusive memories were expected to occur. Additionally, we re-emphasized at the beginning of all assessments that participants could withdraw from the study at any time.
- Although ongoing or severe distress or intrusive symptoms following film viewing were not expected based on previous studies, we had several ways of monitoring and psychological support in place as a precaution.
    - At the end of both the experimental and follow-up assessments, participants were encouraged to contact the research team in case of any concerns regarding ongoing distress. Contact details were provided in the participant information and in the smartphone application that was used in the week after the experiment.
    - We included a very extensive follow up session, including a debriefing, (see Appendix A3). We included an adjusted film-related PTSD Checklist according to the DSM-5 (PCL-5) to check for any preclinical PTSD-related symptoms after film viewing. If participants experienced high symptoms or indicated lasting intrusions and feelings of distress related to the film, we actively offered psychological guidance (see bullet point below).

- Psychological guidance: In the unlikely situation that participants would have preferred or needed professional psychological guidance during or after the assessments, participants were able to see a psychologist or psychiatrist of our department within a few days. None of our participants expressed the need for this help.

In addition, we had several safeguarding measures in place during the experimental session:

- During film viewing, the experimenter was always present in the room in case of distress.
- During the designated moments during the experimental session when the experimenter left the room, we constantly monitored the participants by means of a live video stream. This way, the researcher could immediately intervene if needed or requested by the participant.

A5. Detailed information about the 'Intrude application' (Mecosud B.V.)

Regarding the smartphone application that participants used to report intrusions, the following measures were taken to safeguard the collected data:

- For each participant the app was activated once, using a unique user-activation code, generated by a random sequence generator. This code was used by the researcher to activate the app, and coupled to the numeric participant identification code that was used throughout the rest of the study in separate paper and digital files (stored on the AMC servers).
- All data reported in and saved by the app was marked with the user-activation code, as well as with a date and time stamp.
- No data allowing personal identification was reported via the app.
- All data was encrypted and stored on a SSL-secured server.
- After completion of each item in the app, data was stored to the server, to prevent loss of data should the app be closed in the midst of filling out the questionnaire.
- If participants were offline while filling out the app, data was locally stored on the smartphone and saved to the respective servers as soon as a connection with the internet is established.
- Upon storage at the server, data could not be modified.
- The researcher received a login code, to access the server and export the data in CSV format.
- Upon completion of the study, the AMC researchers stored the database onto the AMC servers and the database was removed from the external server.

Participants did not differ in responding to the notifications (mean(SD) well-responded notifications: seCPT 11.17(1.54), warm water 10.50(2.46), p=.55).

A6. Model equation for the Linear Mixed Models used to assess differences in stress reactivity and recovery across the six assessments during the experimental assessment (T2).

For the LMMs we used fixed slope as we expected the trajectory of the dependent variables for stress reactivity measures to be equal for both conditions, namely reverse U-shaped. However, these trajectories may not be fixed between participants within conditions and it is recommended for randomized-controlled trials to include random intercept in linear mixed models (Brauer & Curtin, 2018).

$$Y_{ij} = \beta_{0j} + \beta_{1j}\text{Condition}_{ij} + \beta_{2j}\text{Time}_{ij} + \beta_{3j}\text{Condition*Time}_{ij} + \varepsilon_{ij} \quad \text{Level 1 equation}$$

$$\beta_{0j} = \gamma_{00} + \mu_{0j}$$

$$\beta_{1j} = \gamma_{10}$$

$$\beta_{2j} = \gamma_{20} \quad \text{Level 2 equation}$$

$$\beta_{3j} = \gamma_{30}$$

$$Y_{ij} = \gamma_{00} + \gamma_{10}(\text{Condition})_{ij} + \gamma_{20}(\text{Time})_{ij} + \gamma_{30}(\text{Condition*Time})_{ij} + \mu_{0j} + \varepsilon_{ij.} \quad \textbf{Mixed model equation}$$

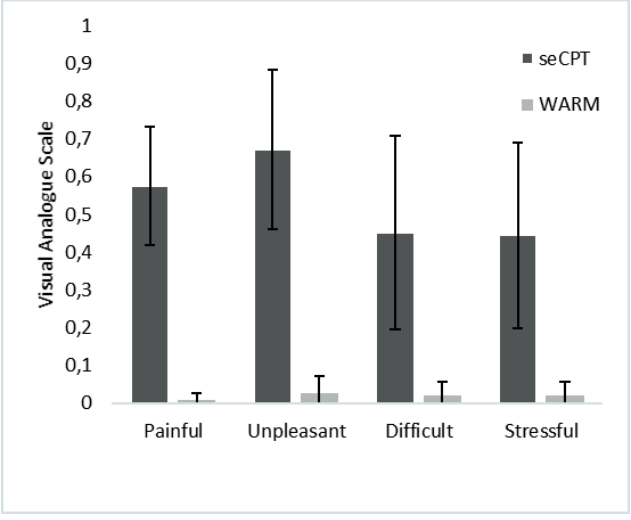
$_{ij}$ = person i in group j, Y_{ij} = score on dependent variable, γ_{00} = fixed effect of the intercept, γ_{10} = fixed effect of group, γ_{20} = fixed effect of time, γ_{30} = fixed effect of interaction Group*Time, μ_{0j} = random intercept, ε_{ij} = residual error.

A7. Sensitivity analyses frequency of all reported intrusions (valid and invalid).

Of all *n*=341 reported intrusions in the 7 days following the experimental assessment, *n*=111 (32.6%) were excluded from our analyses based on validity according to our definition. N=10 participants (15.9%) did not report any intrusions, either valid or invalid. A Negative Binomial Regression GLMM was used as sensitivity analysis to assess differences between conditions in intrusion frequency of all reported intrusions, also including invalid intrusions with *vividness* and/or *distress* scores=0, and showed that the effect of time remained significant (Wald Chi-square(6)=86.36, p<.01) and the interaction between condition and time remained non-significant (Wald Chi-square(6)=3.67, p=.72), similar to the previous findings regarding the frequencies of intrusions including the valid intrusions only. However, in contrast to the analysis including the valid intrusions only, the main effect of condition was no longer significant (Wald Chi-square(1)=3.24, *p*=.07). Additionally, similar to the findings regarding valid intrusions only, there was no significant group difference in the sum scores of all reported intrusions (including those with vividness and/or distress=0) across the 7 days after the experimental assessment ($T_{58.98}$= 1.77, p=.08, *d*=.45).

A8. Subjective experience of the seCPT at the experimental assessment (T2).

Painful: $U < 0.01$, $p<.001$, $r=-.87$; Unpleasant: $U = 2.50$, $p<.001$, $r=-.87$; Difficult: $U = 33.50$, $p<.001$, $r=-.81$; Stressful: $U = 36.00$, $p<.001$, $r=-.80$; Data is displayed as observed mean (SD); VAS: Visual Analogue Scale, seCPT $n=29$, warm water $n=33$.



A9. Sampling instructions and behavioural restrictions for saliva administration assessing Cortisol Awakening Responses (CAR) and GR sensitivity (DST).

Sampling for CAR assessments at home followed guidelines of Stalder et al. (2016); we broadly instructed the participants step-wise how and when to collect saliva samples, providing behavioural rules for the day and evening before and during collection, why it was important to follow instructions, to keep up a diary log, take a selfie with their smartphone at sampling times and practiced saliva sampling followed by feedback to increase adherence of the participants. Participants received premade envelopes for both CAR assessment days that included three marked salivettes for each collection day and a dexamethasone tablet (0.5mg). The day before the assessment was scheduled, the participants received a reminder email to highlight instructions and scheduled days.

BEHAVIOURAL RESTRICTIONS	RANGE	TEST STATISTIC OF ASSESSMENT DIFFERENCES	TEST STATISTIC OF CONDITION DIFFERENCE
TIME GOING TO BED			
CAR	21:00-04:00	$U = 479.50$, $p = .852$	$Z = -0.52$, $p = .607$
DST	22:00-03:25	$U = 480$, $p = .857$	
TIME FALLING ASLEEP			
CAR	21:04-04:05	$U = 653.00$, $p = .027$	$Z = -0.08$, $p = .936$
DST	23:00-05:30	$U = 580.00$, $p = .150$	
QUALITY OF SLEEP			
CAR		$p = .705$	$Z = -1.18$, $p = .238$
DST		$p = .154$	
TIME OF AWAKENING			
CAR	04:53-12:21	$T_{60} = 1.38$, $p = .172$	$Z = -0.52$, $p = .605$
DST	05:20-11:52	$U = 402.50$, $p = .210$	

DETAILS OF ASSESSMENT		%	TEST STATISTIC OF ASSESSMENT DIFFERENCE	TEST STATISTIC OF CONDITION DIFFERENCE
TYPE OF DAY				
DAY OF CAR ASSESSMENT	Day off/Weekend day	31.7%	$X^2 = .95$, $df = 1$, $p = .220$	$p = 1.000$
	Study/Work day	68.3%		
DAY OF DST ASSESSMENT	Day off/Weekend day	30.2%	$X^2 = .05$, $df = 1$, $p = .861$	
	Study/Work day	68.3%		
DAY BEFORE CAR ASSESSMENT	Day off/Weekend day	42.9%	$X^2 = .09$, $df = 1$, $p = .770$	$p = .210$
	Study/Work day	57.1%		
DAY BEFORE DST ASSESSMENT	Day off/Weekend day	33.3%	$X^2 = 1.57$, $df = 1$, $p = .211$	
	Study/Work day	66.7%		
KIND OF DAY				
DAY OF CAR ASSESSMENT	Normal day	58.7%	$X^2 = .69$, $df = 2$, $p = .707$	$p = .263$
	Quieter than normal	27.0%		
	Busier than normal	14.3%		
DAY OF DST ASSESSMENT	Normal day	52.4%	$X^2 = .50$, $df = 2$, $p = .779$	
	Quieter than normal	20.6%		
	Busier than normal	27.0%		

DAY BEFORE CAR ASSESSMENT	NORMAL DAY	57.1%	X^2 = 1.01, *DF* = 2, *P* = .604	*P* = .839
	QUIETER THAN NORMAL	23.8%		
	BUSIER THAN NORMAL	19.0%		
DAY BEFORE DST ASSESSMENT	NORMAL DAY	55.6%	X^2 = 1.68, *DF* = 2, *P* = .433	
	QUIETER THAN NORMAL	27.0%		
	BUSIER THAN NORMAL.	17.5%		
QUALITY OF SLEEP				
DAY BEFORE CAR ASSESSMENT	Very good	17.5%		
	Pretty good	57.1%		
	Pretty bad	23.8%		
	Very bad	1.6%		
DAY BEFORE DST ASSESSMENT	Very good	23.8%		
	Pretty good	60.3%		
	Pretty bad	12.7%		
	Very bad	3.2%		
WOKE UP BEFORE ALARM				
CAR	Woke up because of alarm	54.0%	X^2 = .03, *df* = 1, *p* = .859	*p* = 1.000
	Woke up before alarm	46.0%		
DST	Woke up because of alarm	52.5%	X^2 = .17, *df* = 1, *p* = .682	
	Woke up before alarm	47.6%		

A10. Additional information on methods

Cortisol

At the end of T2, all cortisol samples were centrifuged for 15-minutes at 4000xg, 4°C and slope 9°. Then, 300 mL of the sample was divided in duplo into cryovials and stored at -25°C until further analysis. Cortisol levels were determined using Enzyme-Linked Immynosorbent Assays (ELISA). Ultimately, means for cortisol values were calculated for the duplicate samples. Imputation was applied for missing sample values using the adjacent non-missing samples within cases for calculations (*n*=3).

sAA

For salivary alpha amylase (sAA) a quantitative kinetic determination kit was used (50 mL saliva starting dilution 1:11 in PBS); samples were mixed with 200 mL of amylase reagents and incubated for 10 minutes at room temperature. The increase in absorption (at 405nm) was measured and compared with the activity of a standard (Lyophilized, IBL International GmbH, Hamburg, Germany; Intra-assay variations<4.18%) in the same assay run after a quick spin to resolve potential air bubbles. Ultimately, means for sAA values were calculated for the duplicate samples and for sAA calculations deviations within plates were specifically taken into account for value calculations. When sAA values were missing due to the absorption detection limit (>1.2), values were calculated using this value.

Cardiac measures

Using the corresponding VU-DAMS software (Klaver et al., 1994), raw unfiltered interbeat (RR) intervals and peaks were exported and preprocessed for analyses. For every participant, the six stress measurements were time-locked and partitioned to RR -intervals from the whole recording. Artifacts in raw successive RR-intervals were calculated and checked using the default QRS detector by three individual raters (two-way random analysis of variance model, consistency, single measure Inter Class Correlation (IC)>0.97, p<.001) (Koo & Li, 2016). Automatic frequency-spectral analyses were performed with a low (0.04-0.15Hz) and high frequency band (0.15-0.40Hz). When suspicious artifacts, bias or misplaced R-peaks were detected, RR-peaks were discarded from intervals or impedance cardiogram scoring was manually corrected. PEP was measured as the time between R-peaks of left ventricular contraction and opening of the aortic valves, reflecting the time between electrical depolarization of the left ventricle and the beginning of the ventricular ejection in ms (automatically calculated by *Large Scale Ensemble Average* of the whole RR-interval and low-pass filtered (60Hz)) (Riese et al., 2003).

A11. CONSORT checklist for randomised trial.

CONSORT 2010 checklist of information to include when reporting a randomised trial*

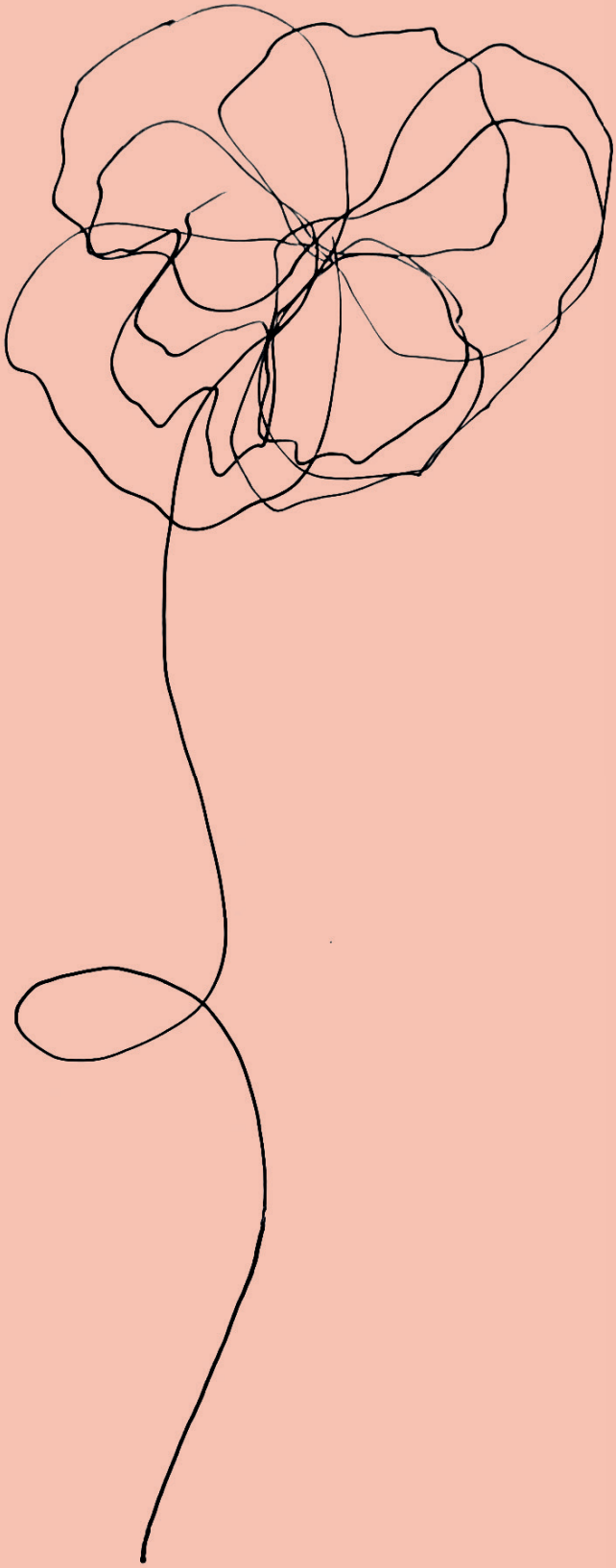
Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	1
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	2
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	3-6
	2b	Specific objectives or hypotheses	5-6
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	6-7, Appendix A1, A2
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	n.a.
Participants	4a	Eligibility criteria for participants	6
	4b	Settings and locations where the data were collected	7
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	8-9, Appendix A3, A4

Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	9-12, Appendix A10
	6b	Any changes to trial outcomes after the trial commenced, with reasons	n.a.
Sample size	7a	How sample size was determined	Appendix A1
	7b	When applicable, explanation of any interim analyses and stopping guidelines	n.a.
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	Appendix A1
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	Appendix A1
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	Appendix A1
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	6, Appendix A1
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and how	Appendix A1
	11b	If relevant, description of the similarity of interventions	8-9, Appendix A4
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	12-13
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	n.a.
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	6, Appendix A2
	13b	For each group, losses and exclusions after randomisation, together with reasons	6, Appendix A2

Recruitment	14a	Dates defining the periods of recruitment and follow-up	7-10, Appendix A3
	14b	Why the trial ended or was stopped	Appendix A2
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	33-34
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	12, 15, 30-37, Appendix A8
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	13-18, 30-37, Appendix A8, A9
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	15, 31, 33-34, Appendix A9
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	n.a.
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	7-8
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	19-23
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	19-24
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	18-24
Other information			
Registration	23	Registration number and name of trial registry	6

Protocol	24	Where the full trial protocol can be accessed, if available	6
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	24

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.



CHAPTER 6

GENERAL DISCUSSION

GENERAL DISCUSSION

The overarching aim of this thesis was to investigate whether and how the timing of adversity during different periods across the lifespan impacts the vulnerability for PTSD development and its potentially underlying stress-related neurobiological processes (**Chapter 2-5**). In this final chapter, we present a general discussion of our main findings, potential implications, strengths and limitations of this work, and suggestions for future research.

Time-dependent vulnerability for development of PTSD symptoms

Our research on psychological symptoms in men and women from the Dutch famine birth cohort revealed that vulnerability to develop psychological symptoms in late adulthood was increased after exposure to adversity, with differences in effects based on the timing of exposure during the prenatal period, childhood or mid-to-late adulthood (**Chapter 2**). Of the three adversity exposures, childhood adversity was the greatest risk factor for psychological symptoms in men, including symptoms of PTSD (**Chapter 2**). This is comparable to previous findings in adults and elderly, who showed increased PTSD risk after exposure to adversity during childhood rather than during adulthood (Dunn et al., 2017; Ogle et al., 2013; Schalinski et al., 2016). However, these studies did not take the impact of prenatal exposures into account. We found that exposure to adversity in early gestation was associated with an increased risk for mild comorbid symptoms including PTSD symptoms in men (**Chapter 2**), suggesting that early gestation might be a critical period for programming later susceptibility to PTSD. This is in agreement with pre-existing literature on self-reports of prenatal exposure to maternal infection and increased long-term risk for PTSD in 21 year old offspring (Betts et al., 2015), although our research focused on a broad range of symptom types aside from PTSD in participants during late adulthood. Hence, our evidence highlights that both the prenatal and childhood period seem to be sensitive periods for increased PTSD risk in later life following adversity. In addition, our findings emphasize the long-term ongoing impact of adversity, even when adversity occurred early in life (**Chapter 2**). The time-dependent effects could be explained by the fact that the brain is thought to be more susceptible to environmental stressors during the early prenatal, childhood and adolescent periods. This is likely because, during these periods, rapid development and maturation of brain structures and functional processes take place and brain plasticity is increased (Knudsen, 2004; Lupien et al., 2009; Tottenham & Sheridan, 2009). The fronto-limbic brain, for example, is still in development and processes of maturation appear till young adulthood, and therefore these regions are susceptible to stressors that might disrupt neurodevelopment (Casey et al., 2000; Tottenham & Sheridan, 2009). As a consequence, neural regions that regulate stress may experience long-lasting perturbations that ultimately influence the enduring susceptibility for later life health issues (Bosch et al., 2012; Heim et al., 2000; Lupien et al., 2009; Nemeroff, 2004; Seckl & Meaney, 2006; Sherin & Nemeroff, 2011). Thus, increased risk for the development of PTSD symptoms could be a result of potential brain programming and sensitization of biological stress systems after a

history of adversity in early life periods. Even during the prenatal period, exposure to adversity may lead to stress-induced *in utero* programming effects and neurobiological abnormalities may have persistent consequences for stress regulation and long-term psychopathology, for instance for the hypothalamic pituitary adrenal (HPA) axis and sympathetic nervous system (SNS) functioning (Pervanidou et al., 2020; Welberg & Seckl, 2001). This knowledge is of importance as it supports that developmental timing of exposure to adversity should be taken into account when investigating its effects on psychological health. As psychopathology risk appears not to be generalizable for adversity across the lifespan, exposure periods should be investigated separately.

Time-dependent neurobiological stress processes associated with risk for PTSD vulnerability

Previously identified differences in stress system functioning underlying PTSD's pathophysiology have been synthesized in a comprehensive evidence-based neurobiological framework in earlier research. This framework highlights the important role of fronto-limbic, HPA and autonomic functioning in eliciting PTSD symptoms to be associated with PTSD-related emotion regulation, threat and fear processes, and memory function (e.g. Hayes et al., 2012b, 2012a; Joshi et al., 2020; Liberzon & Abelson, 2016; Quinones et al., 2020; Sherin & Nemeroff, 2011). It mostly highlights a dysfunctional negative feedback loop of the HPA axis, increased responses of the amygdala and decreased reactivity of prefrontal regions in response to negative emotional and trauma-related stimuli, and structural changes and deficient activation and coupling of the hippocampus during rest or in response to memory- and fear-related tasks. What we do not know yet is what the exact neurobiological features are that may underlie the differences in PTSD symptom risk and whether they differ based on the timing of adversity at different life periods. We were able to shed some light on this with the studies in this thesis. In general, mostly but not always the same structures and systems that are part of this framework seem to be involved in PTSD symptom development across different life periods. Yet, adversity could have a differential impact on the regulation and functioning within these structures and systems that are related to PTSD risk depending on the timing of exposure across the lifespan (Agorastos et al., 2019; Sicorello et al., 2021; Tottenham & Sheridan, 2009). The life cycle model of stress, suggested by Lupien et al. (2009), is in line with our findings and proposes that stress-related neural systems each have their specific sensitive periods during development, with the effects of a stressor depending on its timing.

We identified differences in neural, HPA and ANS functioning that were associated with PTSD symptoms in an adolescent (**Chapter 4**) and adult population (**Chapter 5**). Within our adolescent population (**Chapter 4**), we found that experiencing high PTSD symptom levels was accompanied by alterations in neural functional connectivity within regions that were mostly part of the fronto-limbic brain circuitry in response to a socially-evaluative stressor and in recovery thereof. This largely supports previous results of common functional changes in adolescents and adults with PTSD (e.g. Kredlow et al., 2022; Sartory et al., 2013; Selemon et al., 2019; Wolf & Herringa, 2016; Yang et al., 2004) and suggests an important role of the fronto-limbic circuitry in stress regulation

processes related to threat processing in adolescent PTSD, mostly similar to adults with PTSD. Yet, we found some differences with regards to adult PTSD. We neither found changes in amygdala functional connectivity nor in cortisol and cardiac responses to a social-evaluative stressor in adolescents with high PTSD symptoms levels, or in baseline cortisol measures (**Chapter 4**). However, in our study in adults, we demonstrated that dysfunctioning of HPA and ANS systems in healthy men was related to the development of PTSD-related symptoms. Specifically, we found associations between higher (experimental) peri- and post-traumatic cortisol and alpha amylase reactivity and more subsequent intrusions, as well as more severe PTSD-related symptoms (**Chapter 5**). This adds to previous findings concerning affected HPA and ANS stress regulation around the time of (experimental) trauma that predict PTSD symptoms in adult men and women (Morris et al., 2016; Schultebraucks et al., 2019). Although we did not directly compare neural, endocrine or cardiac measures between adolescents and adults with PTSD symptoms in this thesis, our findings may suggest different vulnerability of these processes depending on the timing of exposure to adversity during adolescence or adulthood. Some processes might be less susceptible to adversity during these periods and might explain the null-findings in amygdala, cortisol and cardiac functioning in our adolescent population with PTSD symptoms (**Chapter 4**). In accordance, there are studies that similarly found no differences in amygdala functioning in childhood or adolescence (De Bellis et al., 2001; Weems et al., 2015). These differences in amygdala functioning could be explained by a time-dependent impact of adversity and because of critical periods of growth and development that the amygdala undergoes at the time of exposure (Gee et al., 2013; Hare et al., 2008; Knudsen, 2004).

Furthermore, Bremner et al. (2008) suggested that some alterations in HPA functioning may have the capacity to be reversible due to the brain's increased plasticity during early life periods. Indeed, reversible effects in glucocorticoid receptor (GR) functioning have been identified in an adult patient population receiving a high dose of hydrocortisone shortly after a traumatic event (Zohar et al., 2011). This intervention reduced the short- and long-term risk for PTSD symptoms. It could be that these positive effects result from a temporary overriding influence during a crucial period for symptom development, possibly through interacting with hippocampal GR receptors. A similar positive modulatory effect has been found in an aging combat veteran population, who showed enhanced episodic memory performance shortly after receiving hydrocortisone injections and even better working memory performance in those with current PTSD compared to those without current or past PTSD (Yehuda et al., 2007). Our reported differences in findings between our adolescent (**Chapter 4**) and adult (**Chapter 5**) studies, could also be explained by the conceptualized staging approach, as discussed by McFarlane et al. (2017), in which the range of emerging biological alterations should be differentiated from the stage of PTSD symptom progression. Some biological impairments may be present during the early post-trauma phase, but not during chronic PTSD or during the remitting phase when improvement of symptoms take place. Unfortunately, we had no information about the current status of PTSD symptoms in our adolescent population to know to which phase of PTSD development our findings apply to.

Overall, individuals with PTSD symptoms of various ages may be specific populations with different neurobiological vulnerabilities and findings should not be generalized. If we are able to uncover these specific vulnerabilities throughout all life periods, this could advance targeted and improved treatment that may be more effective and specialized for PTSD symptomatology based on the time when a traumatic event is experienced.

Individual differences in vulnerabilities in stress-related processes

Interindividual variability in stress system functioning

Identifying individual variations in neurobiological stress system functioning and associations with PTSD symptoms would benefit our understanding of why some individuals develop PTSD symptoms and others do not after exposure to the same adverse event. Findings of prospective studies and reviews supported the role of individual variability in PTSD risk, although some findings are conflicting (e.g. Bonne et al., 2001; Gilbertson et al., 2002; Hinrichs et al., 2019; McEwen et al., 2016; Morris et al., 2016; Schultebraucks et al., 2021; van Zuiden et al., 2013; Yehuda, 2006). We demonstrated that higher HPA and ANS reactivity during the peri- and post-traumatic period was related to the development of intrusive symptoms in healthy men (**Chapter 5**). These findings were similar to earlier work (Morris et al., 2016; Schultebraucks et al., 2019) and imply that factors related to stress system functioning around the time of an adverse event – not only during, but also in early recovery - may influence interindividual differences in PTSD risk after adversity. Evidence exists on different associations between neurobiological vulnerabilities and particular symptom clusters of PTSD. For example, differential associations were found between severity of the re-experiencing, avoidance and hyperarousal symptom clusters and increased or decreased cortical thickness and gray matter volumes of fronto-limbic brain regions in women with PTSD (Crombie et al., 2021). In accordance, a study in women with PTSD after intimate partner violence demonstrated that higher severity levels of specifically arousal and emotional numbing symptoms were associated with flatter baseline cortisol awakening response slopes compared to females with lower severity levels for these clusters (Garcia et al., 2020). This is in line with the finding in our study in healthy adult men, as we observed that salivary alpha amylase levels were positively associated with particularly intrusion-related symptom clusters of PTSD and not with the other symptom clusters (**Chapter 5**). This indicates that individual variation of specific HPA and ANS processes is related to particular PTSD symptoms, yet further investigation of potential symptom-specific associations is needed.

Genetic variation

We additionally found evidence that the association between famine exposure in early gestation and adulthood PTSD symptom severity was only detected when considered in interaction with genetic variation in a potential neurobiological pathway underlying PTSD susceptibility upon adulthood trauma exposure. We demonstrated that members of the Dutch famine birth cohort who were exposed to undernutrition during early gestation showed lower PTSD symptom severity

upon trauma exposure during adulthood when they carried the B*cl*I haplotype compared to those who were not exposed or who were exposed and did not carry this haplotype (**Chapter 3**). The *Bcl*I haplotype is a common functional single nucleotide polymorphism (SNP) within the NR3C1 gene that encodes the GR and is associated with hypersensitivity of these receptors to the effects of glucocorticoids (GCs) (DeRijk, 2009; Oakley & Cidlowski, 2013; van Rossum et al., 2003). Carriers of the *Bcl*I SNP repeatedly had increased PTSD susceptibility following childhood and following adulthood trauma (Castro-Vale et al., 2021; Hauer et al., 2011; Lian et al., 2014). Our findings thus indicate a gene-environment interaction for determining individual vulnerability for PTSD symptoms when faced with additional adult trauma after early life adversity. Previous research already identified genetic factors that predispose to PTSD symptom development through their prominent role in HPA and GR regulation (Carvalho et al., 2017; Sheerin et al., 2020). Importantly, our results especially showed that the B*cl*I haplotype only mediated PTSD severity in prenatally exposed individuals who were additionally exposed to trauma during adulthood, while this was not found for later life exposure to trauma during childhood. Therefore, it is important to consider genetic factors and environmental circumstances throughout life when aiming to elucidate individual PTSD susceptibility during various life periods.

Sex-specific effects

There is an increasing body of evidence showing that men and women might respond differently to exposures, with important consequences for health and wellbeing. These differences might partly be due to biological differences, societal differences and their interactions (Christiansen & Elkit, 2008; Crozier et al., 2014; Irish et al., 2011; Meewisse et al., 2007; Olff et al., 2007; Tolin & Foa, 2006). We found evidence for sex-specific effects of the type and severity of trauma-related psychological symptoms in late adulthood after exposure to adversity (**Chapter 2**). These sex-dependent differences could have possible clinical implications as women are at increased risk for developing lifelong PTSD symptoms compared to men after comparable traumatic events (e.g. Holbrook et al., 2002; Olff, 2017). This sex-specific risk may be due to differences in biological contributors. Sex differences in biological systems have been identified using animal models (Shansky, 2015) and in human studies, and include hormonal and genetic influences (Christiansen & Berke, 2020). For instance, a prospective study investigating emergency department patients found that women with PTSD experienced higher heart rate levels during a fear-potentiated startle paradigm in the first 2 weeks after trauma exposure compared to men with PTSD (Seligowski et al., 2021). These sex-differential effects in general and trauma-induced factors could therefore underlie divergent resilience and susceptibility for PTSD development between sexes, which highlights the fact that it is crucial to investigate men and women separately when studying the effects of adversity on mental health and their underlying neurobiological correlates. With this in mind, it is important to stress that our mixed-sex adolescent sample of the ABCD study was too small to investigate sex differences (**Chapter 4**) and therefore this study warrants replication in a larger sample in which differences between boys and girls can be studied.

Irrespective of sex-specific biological differences, it is noteworthy that gender-related factors may also influence the risk of PTSD in men and women, such as potential differences in the types of trauma they experience and coping mechanism to handle stress and trauma (Christiansen & Elklit, 2008; Olff et al., 2007; Tolin & Foa, 2006).

Strengths and limitations

Major strengths of the work described in this thesis include taking a lifecourse approach and looking at specific effects of gender and genetics, which offered us the opportunity to investigate differences in the impact of adversity on PTSD symptoms when exposure occurred during and across different periods of the lifespan. It is especially unique that we were able to examine the effects of adversity during early life periods that are still scarcely studied in relation to PTSD susceptibility; the prenatal period and adolescence. It is additionally a strength that our research included studies with a variety of study approaches. For example, we studied both distinct (**Chapter 2**) and cumulative impact of exposure to adversity (**Chapter 3**), which enabled us to provide insights into its differential time-dependent effects on PTSD risk. Furthermore, we did not solely investigate the risk for PTSD symptomatology after adversity, but also susceptibility for other trauma-related psychological symptoms and their comorbidity patterns (**Chapter 2**). This is especially important since comorbid symptoms might affect and worsen the progress of PTSD symptoms over time and could be associated with differential neurobiological processes (Breslau, 2001). We were also able to suggest a methodological improvement for experimental research on PTSD by adapting the original trauma film paradigm for investigation of intrusive memory development by adding a brief psychosocial stressor to the paradigm. This offered a paradigm that seems more comparable to real-life trauma exposure and thus increases translational value of the paradigm. We strongly recommend to use our adapted trauma film paradigm in future studies. Finally, to the best of our ability, we followed the guidelines concerning Sex and Gender Equity in Research (SAGER) (Heidari et al., 2016), as important sex-differential effects still remain largely uninvestigated, also in PTSD research. For instance, in all our studies we considered potential sex-specific effects by specifically investigating men and women in their late adulthood separately (**Chapter 2, Chapter 3**) or by noting the potential role of sex differences when we were not able to investigate this (**Chapter 4**, **Chapter 5**).

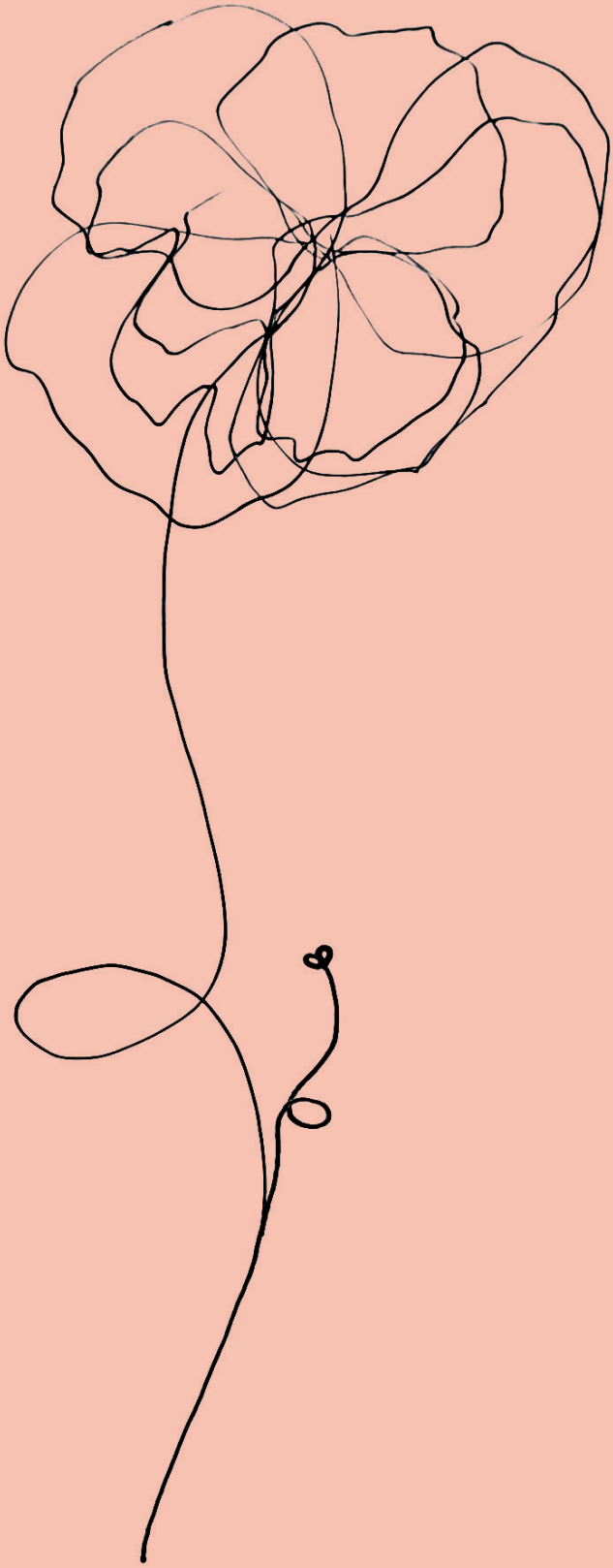
Our studies were also limited by a number of different factors. In our studies in which we investigated neurobiological alterations that were related to PTSD symptoms (**Chapter 4, Chapter 5**), we only investigated this within one specific life period. Consequently, we were unable to compare these findings on PTSD-related neurobiological processes between populations of different life periods. It therefore remains to be examined what the exact symptom course and their potential different underlying neurobiological background is in a real-life situation, where single and multiple exposure to adversities may occur across several periods throughout the entire lifespan. Also, it is important to keep in mind that our findings in adolescents are related to (pre)clinical PTSD symptoms (**Chapter 4**) and our findings in healthy adult men to very low

symptom levels or even absence of symptom levels (**Chapter 5**), indicating possible differences in PTSD status or progression. This could have influenced the generalizability of our findings between these two populations, particularly in light of McFarlane's suggested staging approach (2017). Furthermore, our study populations had limited sample sizes and, as a result, we could not always study sex differences and/or investigate genetic variation to explore potential effects in all our studies.

Future directions and overall conclusion

The research described in this thesis contributes to our understanding of neurobiological processes that may underlie the complexity of PTSD susceptibility depending on the timing of exposure to adversity across the lifespan, as well as other influencing factors. It offers promising insights to advance future prevention and treatment strategies for individuals who suffer from PTSD. We recommend future research to take the timing of exposure to adversity into account when investigating the (sex-dependent) impact of adversity. Ideally, future studies should investigate the impact of accumulated exposure to adversity on neurobiological processes that are related to PTSD symptom development throughout all periods across the lifespan if possible, also in early life periods. Furthermore, sex is an important factor and should be considered in the design of new studies, with a particular focus on individual variability of neurobiological processes in women with PTSD symptoms as this is a topic that received too little attention so far. The use of our adapted trauma film paradigm with improved translational value in future studies will be of value and will benefit investigation of individual variation in biological and cognitive processes underlying early post-trauma PTSD symptoms and advance future targeted interventions.

The poppies - that are often found on battlefields - that decorate this thesis, symbolize the hope that in the future, traumatic events will no longer have to have such a major impact on people's lives when through improved insight into prevention and treatment of trauma-related symptoms, we can work towards fewer and less severe trauma-related symptoms. The meaning of a poppy was poetically described in the poem "In Flanders Fields", written by Major McCrea during WWI, who himself died on the battlefields thereupon (see page 4). Poppies often grow in places where someone passed away. It was long thought that the poppy seeds absorbed the blood of the fallen soldiers, which then grew into red flowers explaining why so many poppies could be found on battlefields. However, the actual reason that so many poppies grow on battlefields is because those fields are destructed during battle. Grenades churn up the ground allow seeds that had been in the ground for years to grow on this barren soil - as a true pioneer. The strength and beauty of the poppy and its ability to renew in a situation of complete devastation symbolizes a potential positive future for the victims of trauma. Ultimately, the present work on the neurobiological understanding of PTSD symptomatology may contribute to improve future interventions that may - like poppies - help people to grow and flourish despite adversity.



CHAPTER 7

ENGLISH SUMMARY
NEDERLANDSE SAMENVATTING

ENGLISH SUMMARY

The neurobiological background of posttraumatic stress symptomatology throughout the lifespan

The majority of people experience at least one potentially traumatic event throughout their lives (Benjet et al., 2016; de Vries & Olff, 2009). It is common that individuals initially experience negative responses after experiencing a potential traumatic event that will usually fade away. Yet, in a minority of individuals these responses do not disappear and may result in development of posttraumatic stress disorder (PTSD) (Atwoli et al., 2015; Goldstein et al., 2016; McLaughlin et al., 2012, 2013), which is characterized by symptoms of re-experiencing the traumatic event, avoiding stimuli that are related to the traumatic event, exhibiting negative alterations in cognition and mood, and hyperarousal and –reactivity (American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5), The American Psychiatric Association, 2013). All these symptoms can be a considerable burden for the individual's wellbeing and for the larger society.

Over the past decades, many studies have demonstrated that dysfunctional behaviours of PTSD, such as impaired fear inhibition and extinction, and impaired memory processing, are potentially associated with disruptions in stress-related neurobiological processes. These include among others the fronto-limbic neurocircuitry, hypothalamic pituitary adrenal (HPA) axis and autonomic nervous system (ANS) functioning (e.g. Hayes et al., 2012a; Joshi et al., 2020; Liberzon & Abelson, 2016; Quinones et al., 2020; Sherin & Nemeroff, 2011). The impact of adversity on the risk to develop PTSD symptoms may differ based on the time of exposure to adversity across the lifespan, as there are indications of differences in the vulnerability and involvement of stress-related neurobiological mechanisms across distinct life periods (Herringa, 2017; Heyn et al., 2019; Lupien et al., 2009; Weems et al., 2019) and because the effects of adversities on psychological health seem to be time-dependent (Dunn et al., 2017; Herringa, 2017; Heyn et al., 2019; Lupien et al., 2009; Ogle et al., 2013; Shrira et al., 2012; Straussner & Calnan, 2014; Thornberry et al., 2001; Weems, 2019). Yet, the involved stress-related neural, endocrine, and cardiac mechanisms are still largely unknown and their underlying vulnerability may not be generalizable to all age populations throughout the entire lifespan, from early prenatal life to mid-adulthood. This lack of knowledge warrants improvement of our understanding of differential risk for PTSD symptom development after exposure to adversity. A better understanding of who is at risk to develop PTSD symptoms will ultimately promote early preventive interventions.

The aim of this thesis was to investigate how the timing of adversity during different periods across the lifespan impacts the development of PTSD symptomatology and the vulnerability of stress-related neurobiological processes (**Chapter 2-5**; Figure 1).

To examine the effects of timing of adversity, we studied the impact of adversity during the prenatal period, childhood, and mid-to-late adulthood on the vulnerability of trauma-related symptomatology regarding symptoms of anxiety, depression, psychotic and PTSD disorder during late adulthood in chapter 2.

We observed four different symptom profiles in men and five symptom profiles in women regarding symptoms of anxiety, depression, psychotic and PTSD disorder. In both men and women, we identified a profile for anxious/depressive symptoms and three profiles that included all symptom types of approximately equal severity within each profile, yet differentiating in overall severity between profiles in terms of low, mild and high severity. Additionally, we found a PTSD symptom profile in women. We observed that in men exposure to undernutrition during early gestation, traumatic maltreatment during childhood and trauma in mid-to-late adulthood were all associated with symptom profile classification, with differential effects depending on timing and effects being most profound for childhood maltreatment. In women, adversity in childhood and adulthood significantly increased classification probability in almost all profiles, with no significant effect of prenatal undernutrition.

The findings of this chapter indicate specific impact of adversity during different periods across the lifespan on psychological health later in life and support a time-dependent and sex-specific impact of adversity during late adulthood. Remarkably, the consequences of adversity during early life still appear to be visible in late adulthood, which suggests an ongoing lifelong impact of adversity occurring in early life.

To broaden our understanding of the effects of timing of adversity on PTSD vulnerability, in chapter 3, we investigated the impact of multiple trauma exposures across the prenatal period, childhood, and mid-to-late adulthood on PTSD symptom severity in late adulthood. We additionally examined if the impact of adversity is modulated by genetic variation in the glucocorticoid receptor (GR) for the common single nucleotide polymorphisms (SNPs) ER22/23EK, N363S, B*cl*I, exon 9β glucocorticoid, which are known to influence GR function and glucocorticoid signaling and are associated with PTSD susceptibility.

Participants exposed to famine during early gestation, but only those not carrying the GR B*cl*I haplotype, showed a stronger association between adulthood trauma and PTSD symptom severity compared to non-exposed participants.

These findings indicate potential different effects based on timing of exposure to famine within pregnancy, with strongest effects of exposure during early gestation. Findings additionally imply an influence of prenatal adversity on PTSD susceptibility following trauma in later life that extends well beyond the timeframe of early adulthood. Importantly, the effect of prenatal adversity on PTSD susceptibility after trauma in later life was dependent on common genetic variation

associated with GR hypersensitivity to GCs. This suggests that when aiming to elucidate how PTSD susceptibility evolves throughout life, an integrated approach that considers genetics and environmental context throughout various life periods is necessary, including the prenatal environment.

Furthermore, we aimed to offer more insight into neurobiological processes that are related to stress system functioning in adolescents with high levels of PTSD symptoms after exposure to trauma in **chapter 4.** We therefore studied functional brain connectivity, cortisol, and cardiac reactivity to a social-evaluative stress task using functional Magnetic Resonance Imaging (fMRI) to investigate differences between trauma-exposed adolescents with and without high PTSD symptom levels.

We were the first to investigate differential functional brain connectivity in response to social-evaluative stress between trauma-exposed adolescents with and without high PTSD symptom levels. We found that adolescents with high PTSD symptom levels showed differential functional brain connectivity between the hippocampus and the cerebellum, medial frontal gyrus (MFG) and inferior frontal gyrus (IFG); and between the middle prefrontal cortex (mPFC) and IFG during acute social-evaluative stress compared to trauma-exposed controls. Mostly similar patterns were observed during recovery. Contrary to our hypothesis, no group difference was found in functional brain connectivity for the amygdala during acute social-evaluative stress or recovery. We also investigated cortisol and cardiac reactivity and did not find an association between the presence of PTSD symptoms and cortisol reactivity, heart rate (HR) reactivity, cortisol awakening response (CAR) and cortisol day curve.

These findings suggest that the presence of PTSD symptoms in adolescents is associated with differential functional connectivity of the hippocampus, mPFC, cerebellum, IFG and MFG during acute social-evaluative stress, and mostly the same patterns during recovery. Our results could indicate that neural threat processing in response to social-evaluative stress is disrupted in adolescents with PTSD symptoms. Findings on functional connectivity in these adolescents are mainly but not entirely in line with findings in adults with PTSD, which denotes the importance of investigating adolescents with PTSD as a specific population, instead of generalizing findings from adult research. Treatment guidelines in adolescents with PTSD symptoms should ideally be based on research uncovering dysregulated neurobiological mechanisms in adolescents with PTSD symptoms, because it could eventually provide more specialized and targeted treatment possibilities and ultimately improve treatment effectiveness for adolescents with PTSD symptoms.

Lastly, to expand our knowledge on the individual variation in neurobiological and cognitive stress processes that underlies intrusive memory development, we performed a randomized-controlled trial study in chapter 5; participants were assigned to a stressor condition, consisting of an adapted trauma film paradigm with exposure to a psychosocial stressor, or to a control condition, without exposure to a psychosocial stressor. We adapted the original trauma film paradigm by adding a mild psychosocial stressor prior to watching a trauma film in a healthy adult male population for the purpose of increasing translational value and creating a more real-life trauma setting. We aimed to study whether acute sympathetic nervous system (SNS) and HPA axis stress reactivity to the paradigm, acute film-related declarative memory accuracy, and salivary cortisol suppression upon oral dexamethasone ingestion as a measure of GR functioning, were predictive of trauma film-related intrusion frequency and characteristics in the following week.

We demonstrated that participants in the stressor condition had stronger HPA axis and SNS reactivity and more intrusions compared to participants who were not stressed. Participants in the stressor condition also had lower film-related recognition memory accuracy and their intrusive memories were associated with higher levels of vividness and distress than men in the control condition. Secondly, we found associations between salivary cortisol and alpha amylase levels (indices for HPA and SNS reactivity) throughout the experimental session and subsequent intrusion development, although these predictive effects did not survive corrections for multiple comparisons.

Our findings indicate that adding a brief psychosocial stressor prior to viewing a trauma film elicited a more robust stress response that is likely more comparable to real-life trauma exposure and increases the translational value of the trauma film paradigm. Our results also suggest that the adjusted paradigm may be useful to investigate effects of individual variation in biological stress reactivity as well as underlying cognitive processes on the development of intrusive symptoms. It is important to offer more insights into the biological and cognitive processes underlying development of early post-trauma PTSD symptoms as it could advance future effective prevention.

Overall, our findings highlight time-dependent effects of adversity of which both the prenatal and childhood period seem to be sensitive periods for increased PTSD risk in later life following adversity, with an ongoing impact (Chapter 2, Chapter 3). This is likely because of the fact that the brain is more susceptible to environmental stressors early in life. Potential brain programming and sensitization of biological stress systems after a history of adversity in early life periods and long-lasting perturbations in these neurobiological processes may ultimately influence the susceptibility for PTSD symptom development. By shedding some light on changes in stress-related processes associated with differences in PTSD vulnerability, we were able to demonstrate that mostly but not always the same structures and systems seem to be involved in PTSD symptom development in adolescents and adults. This indicated that adversity might affect the regulation

and functioning of these structures and systems differently depending on the timing of exposure (Chapter 4, Chapter 5).

Furthermore, we were able to find interindividual differences in vulnerabilities in stress-related processes that benefit our understanding of varied PTSD risk (Chapter 5). Also, we demonstrated that genetic factors related to GR function could explain variation in PTSD risk and symptom outcome, in which accumulation of adverse events throughout life including early neurodevelopmental periods has to be considered as an important influencing factor (Chapter 3). Of important clinical relevance is that resilience and susceptibility for PTSD development also appeared to differ between sexes and is of important clinical relevance (Chapter 2, Chapter 3). In this thesis, we explored the complexities of the impact of adversity across the lifespan that underlie the development of PTSD symptomatology as well as other risk factors such as sex, and individual variability in stress system functioning and genetic factors. Ultimately, this work may contribute to improved future interventions that may like poppies help people to grow and flourish despite adversity.

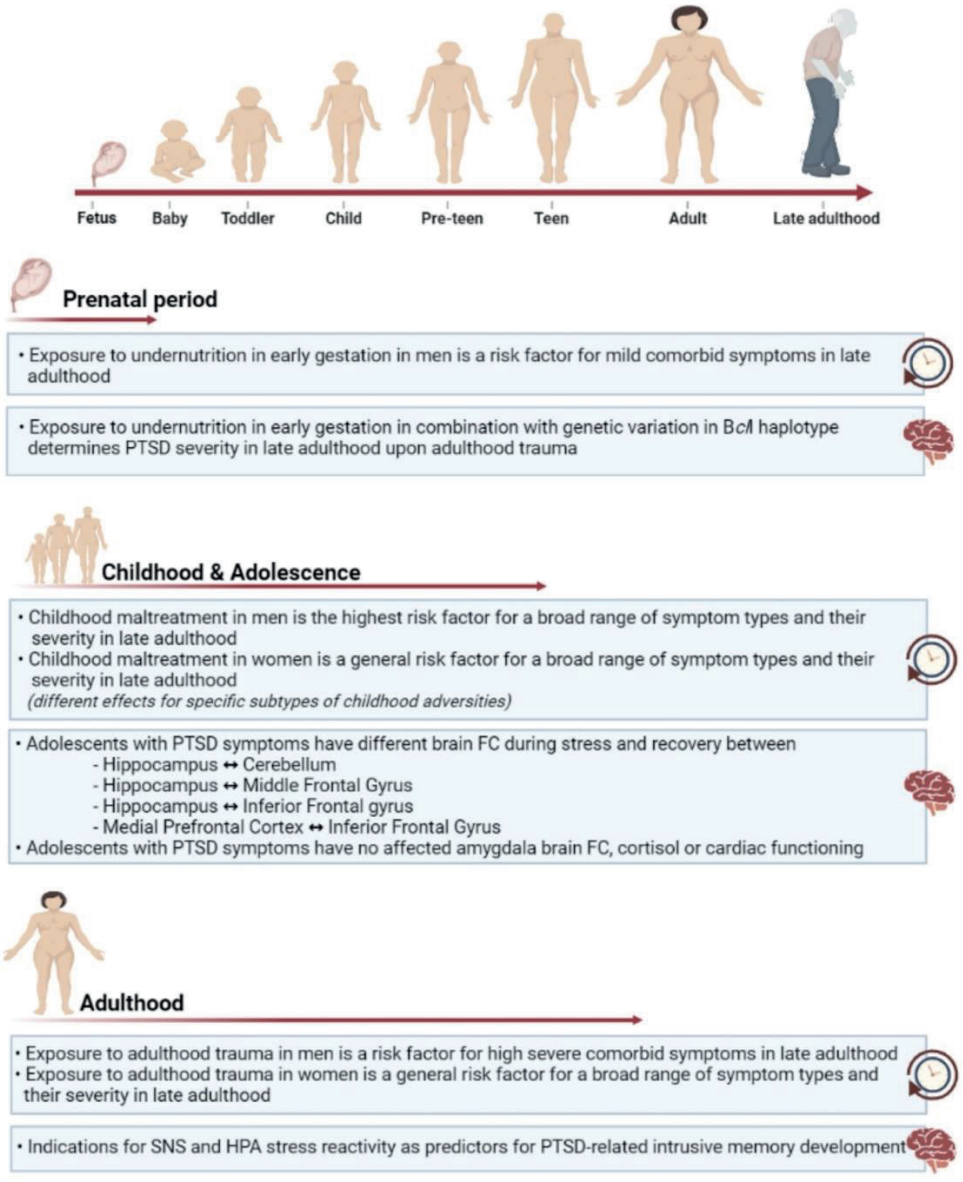


Figure 1. Overview of the time-dependent effects of adversity during the prenatal period, childhood and adulthood on psychopathological symptom development in later life. These effects are related to timing as well as to stress-related neurobiological processes regarding neural, HPA and ANS functioning related to PTSD symptomatology throughout the lifespan. PTSD: Posttraumatic stress disorder; FC: functional connectivity; SNS: Sympathetic nervous system; HPA: Hypothalamic pituitary adrenal axis.

NEDERLANDSE SAMENVATTING

De neurobiologische achtergrond van posttraumatische stress symptomatologie gedurende de levensloop

De meerderheid van de mensen maakt gedurende het leven minstens één potentieel traumatische gebeurtenis mee (Benjet et al., 2016; de Vries & Olff, 2009). In eerste instantie ervaren individuen in het algemeen negatieve reacties na het meemaken van een potentieel traumatische gebeurtenis, en deze reacties zullen meestal wegebben. Bij een minderheid van de individuen verdwijnen deze reacties echter niet en kunnen deze resulteren in de ontwikkeling van een posttraumatische stressstoornis (PTSS) (Atwoli et al., 2015; Goldstein et al., 2016; McLaughlin et al., 2012, 2013). Kenmerkende symptomen van PTSS zijn: het herbeleven van de traumatische gebeurtenis, het vermijden van stimuli die gerelateerd zijn aan de traumatische gebeurtenis, het vertonen van negatieve veranderingen in cognitie en stemming, en verhoogde prikkelbaarheid en reactiviteit (American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5), The American Psychiatric Association, 2013). Al deze symptomen kunnen een aanzienlijke last zijn voor zowel het welzijn van het individu als voor de algehele samenleving.

In de afgelopen decennia hebben vele studies aangetoond dat deze dysfunctionele gedragsprocessen, zoals verminderde remming van angst en extinctie, en verslechterde geheugenverwerking, mogelijk geassocieerd zijn met verstoringen in stressgerelateerde neurobiologische processen. Deze verstoringen omvatten onder andere het fronto-limbische neurocircuit, de hypothalamus-hypofyse-bijnier (HPA)-as en het autonome zenuwstelsel (ANS) (bijv. Hayes et al., 2012a; Joshi et al., 2020; Liberzon & Abelson, 2016; Quinones et al., 2020; Sherin & Nemeroff, 2011). De impact van een traumatische gebeurtenis op het risico om PTSS-symptomen te ontwikkelen, kan verschillen op basis van het tijdsmoment waarop men gedurende de levensloop aan trauma wordt blootgesteld, aangezien er aanwijzingen zijn voor variatie in de kwetsbaarheid en betrokkenheid van neurobiologische mechanismen tussen verschillende levensperioden (Herringa, 2017; Heyn et al., 2019; Lupien et al., 2009; Weems et al., 2019). Bovendien lijken de effecten van een traumatische gebeurtenis op de psychische gezondheid tijdsafhankelijk te zijn (Dunn et al., 2017; Herringa, 2017; Heyn et al., 2019; Lupien et al., 2009; Ogle et al., 2013; Shrira et al., 2012; Straussner & Calnan, 2014; Thornberry et al., 2001; Weems, 2019). De hierbij betrokken stressgerelateerde neurale, endocriene en cardiale processen zijn echter nog grotendeels onbekend en onderliggende kwetsbaarheid voor PTSS is wellicht niet te generaliseren naar alle leeftijdsgroepen binnen de levensloop, van het vroege prenatale leven tot het midden-tot-late volwassen leven. Dit gebrek aan kennis maakt het noodzakelijk door onderzoek meer inzicht te verkrijgen in de verschillende risico's, die een rol spelen bij het ontwikkelen van PTSS-symptomen na blootstelling aan trauma. Een beter begrip van wie risico

loopt op het ontwikkelen van PTSS-symptomen zal zodoende toekomstige vroegtijdige preventieve interventies kunnen bevorderen.

Het doel van dit proefschrift was te onderzoeken hoe de timing van trauma tijdens verschillende perioden in het leven de ontwikkeling van PTSS-symptomatologie beïnvloedt evenals de kwetsbaarheid van stressgerelateerde neurobiologische processen (**Hoofdstuk 2-5**; Figuur 2).

Om de effecten van de timing van blootstelling aan trauma te bestuderen, is in **hoofdstuk 2** de impact van trauma onderzocht tijdens de prenatale periode, de kindertijd, en het midden-tot-late volwassen leven op de kwetsbaarheid voor het ervaren van trauma-gerelateerde symptomen van angst, depressie, psychose en PTSS op late-volwassen leeftijd.

Vier verschillende symptoomprofielen konden worden aangetoond bij mannen en vijf verschillende symptoomprofielen bij vrouwen die symptomen van angst, depressie, psychose en PTSS bevatten. Bij zowel mannen als vrouwen identificeerden wij een profiel voor angst-/depressieve symptomen en drie profielen die alle symptoomtypen bevatten. De mate van ernst van de symptomen binnen elk van deze profielen was ongeveer gelijk, maar verschilde wel tussen de profielen, in termen van: een lage, milde of hoge mate van ernst. Daarnaast vonden wij een PTSS-symptoomprofiel bij vrouwen. Mannen die blootgesteld waren aan ondervoeding tijdens de vroege zwangerschap, of die trauma hadden ervaren tijdens de kindertijd of blootgesteld waren aan trauma tijdens het midden-tot-late volwassen leven hadden een significant verhoogd risico om geclassificeerd te worden in symptoomprofielen, waarbij verschillen in symptoomclassificatie afhankelijk leken te zijn van de timing van blootstelling aan trauma met de sterkste effecten na kindertrauma. Bij vrouwen kon worden vastgesteld, dat trauma tijdens de kindertijd en tijdens het midden-tot-late volwassen leven significant het risico op classificatie in bijna alle symptoomprofielen verhoogde, maar niet na blootstelling aan prenatale ondervoeding.

De bevindingen, beschreven in dit hoofdstuk, wijzen op een specifieke invloed van blootstelling aan trauma tijdens verschillende levensperioden op de psychische gezondheid op latere volwassen leeftijd en wijzen op tijdsafhankelijke en sekse-specifieke effecten. Opvallend is dat de gevolgen van trauma in het vroege leven nog steeds zichtbaar blijken te zijn op latere volwassen leeftijd, wat wijst op een levenslange impact van blootstelling aan trauma in het vroege leven.

Om ons begrip van de effecten van timing van trauma op de kwetsbaarheid voor PTSS te vergroten, is in **hoofdstuk 3** de impact van meervoudige blootstelling aan trauma onderzocht tijdens de prenatale periode, de kindertijd, en de late volwassenheid op de ernst van PTSS-symptomen op latere, volwassen leeftijd. Daarnaast werd ook onderzocht of de impact van trauma gemoduleerd wordt door genetische variatie in de glucocorticoïde receptor (GR) voor de single nucleotide polymorphisme (SNPs) ER22/23EK, N363S, B*cl*I, exon 9β glucocorticoïde,

waarvan we weten dat deze de GR-functie en glucocorticoïde signalering beïnvloeden en geassocieerd zijn met kwetsbaarheid voor PTSS.

Deelnemers blootgesteld aan hongersnood tijdens de vroege zwangerschap, maar alleen degenen die niet het GR B*cl*I haplotype droegen, vertoonden een sterkere associatie tussen trauma op volwassen leeftijd en ernst van PTSS symptoom in vergelijking met niet blootgestelde deelnemers.

Deze bevindingen wijzen op mogelijk verschillende tijdsafhankelijke effecten van blootstelling aan hongersnood, met de sterkste effecten van blootstelling tijdens de vroege zwangerschap. Bovendien impliceren deze bevindingen een invloed van trauma tijdens de prenatale periode op de kwetsbaarheid voor PTSS na blootstelling aan een trauma op latere leeftijd, die verder reikt dan de vroege volwassenheid. Belangrijk is dat het effect van prenatale blootstelling op PTSS-gevoeligheid na trauma in het latere leven afhankelijk blijkt te zijn van gemeenschappelijke genetische variatie die geassocieerd is met overgevoeligheid van GR voor GCs. Dit suggereert dat een geïntegreerde aanpak noodzakelijk is wanneer men probeert op te helderen hoe PTSS-gevoeligheid gedurende het hele leven evolueert, die rekening houdt met genetica en omgevingscontext gedurende verschillende levensperioden, met inbegrip van de prenatale omgeving.

Daarnaast is in **hoofdstuk 4** meer inzicht geboden in de neurobiologische processen die gerelateerd zijn aan het functioneren van stresssystemen bij adolescenten met ernstige PTSS-symptomen na blootstelling aan trauma. Als een van de eerste studies werden de potentiële verschillen onderzocht in functionele hersenconnectiviteit-, cortisol- en hart-responses op een sociaal-evaluatieve stresstaak met behulp van functionele Magnetic Resonance Imaging (fMRI) tussen blootgestelde adolescenten met en zonder ernstige PTSS-symptoomlevels.

Adolescenten met hoge levels van PTSS-symptomen vertoonden verschillen in functionele hersenconnectiviteit tussen de hippocampus en het cerebellum, middel frontale gyrus (MFG) en inferieure frontale gyrus (IFG); en tussen de mediale prefrontale cortex (mPFC) en IFG in reactie op een psychosociale stressor vergeleken met de adolescente controlegroep. Grotendeels vergelijkbare patronen werden ook waargenomen tijdens de directe herstelperiode na de stressor. In tegenstelling tot onze hypothese werd geen groepsverschil gevonden in functionele hersenconnectiviteit voor de amygdala in acute response op de stressor of tijdens de herstelperiode. Verder werden ook geen associaties gevonden tussen de aanwezigheid van ernstige PTSS-symptomen en cortisol- of hartslag (HR)-responses op de stressor, of voor cortisol levels gemeten tijdens het ontwaken (CAR) of gedurende de dag.

Deze bevindingen suggereren dat de aanwezigheid van ernstige PTSS-symptomen bij adolescenten geassocieerd is met een verschil in functionele connectiviteit van de hippocampus,

mPFC, cerebellum, IFG en MFG in respons op een psychosociale stressor, en met grotendeels dezelfde patronen tijdens de herstelperiode van de stressor. Onze resultaten zouden erop kunnen wijzen dat dreigingsverwerking in reactie op de stressor op neuraal niveau verstoord is bij adolescenten met ernstige PTSS. Deze bevindingen voor functionele connectiviteit bij adolescenten zijn grotendeels, maar niet geheel in overeenstemming met bevindingen zoals vastgesteld bij volwassenen met PTSS. Dit wijst erop dat adolescenten met PTSS als een specifieke populatie onderzocht moeten worden en dat bevindingen uit onderzoek bij volwassenen niet gegeneraliseerd moeten worden naar adolescenten. Behandelingsrichtlijnen bij adolescenten met ernstige PTSS-symptomen zouden idealiter gebaseerd moeten zijn op onderzoek dat specifiek de veranderingen in neurobiologische mechanismen bij adolescenten met PTSS-symptomen bestudeert. Dit zou namelijk meer gespecialiseerde en gerichte behandelingsmogelijkheden kunnen bieden en uiteindelijk de effectiviteit van de behandeling voor adolescenten met PTSS-symptomen kunnen verbeteren.

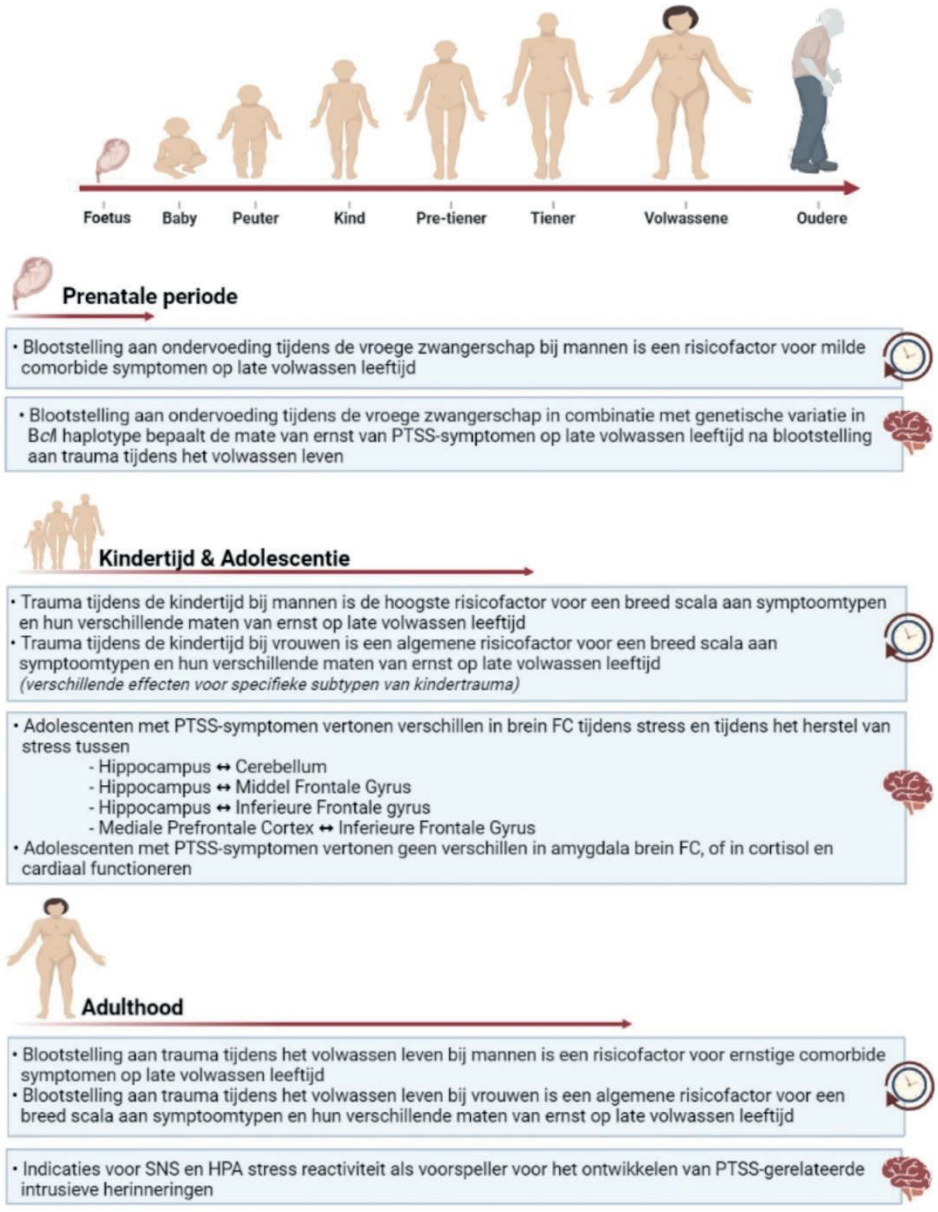
Om meer kennis te verkrijgen over individuele variatie in de neurobiologische en cognitieve stressprocessen die ten grondslag liggen aan de ontwikkeling van intrusieve herinneringen gerelateerd aan PTSS-symptomen, is ten slotte in hoofdstuk 5 een gerandomiseerd-gecontroleerd onderzoek uitgevoerd: deelnemers werden toegewezen aan een stressorconditie, bestaande uit een aangepast traumafilm-paradigma met blootstelling aan een psychosociale stressor, of aan een controleconditie, zonder blootstelling aan een psychosociale stressor. Wij pasten het originele traumafilm-paradigma aan door een milde psychosociale stressor toe te voegen vlak voor het bekijken van de traumafilm bij een gezonde volwassen mannenpopulatie met als doel om de translationele waarde te vergroten en een meer levensechte trauma-setting te creëren. Dit onderzoek bestudeerde of de acute respons van het sympathisch zenuwstelsel (SNS) en de HPA-as in reactie op een traumafilm-paradigma voorspellend waren voor de frequentie van intrusieve herinneringen aan de traumafilm, evenals de levendigheid en de mate van angst opgeroepen door deze herinneringen. Deze voorspelbaarheid werd ook bestudeerd voor de nauwkeurigheid van acute declaratieve herinneringen die gerelateerd waren aan de traumafilm en voor de mate van onderdrukking van de (speeksel) cortisol respons na orale inname van dexamethason als maat voor GR-functioneren.

Onze bevindingen toonden aan dat bij participanten in de stressor conditie een sterkere HPA-as en SNS-respons alsook meer intrusieve herinneringen werden opgewekt, vergeleken met de participanten die niet waren blootgesteld aan de stressor. Participanten in de stressor-conditie waren ook minder goed in staat zich film-gerelateerde kenmerken te herinneren en hadden intrusieve herinneringen die levendiger waren en meer angst veroorzaakten dan mannen uit de controle-conditie. Daarnaast werden associaties gevonden tussen cortisol en alpha-amylase speekselniveaus (indexen voor HPA- en SNS-reactiviteit) gedurende de experimentele sessie en de ontwikkeling van intrusies na de experimentele sessie, hoewel deze voorspellende effecten niet bleven bestaan na correctie voor het uitvoeren van meerdere statistische tests.

De bevindingen impliceren dat het toevoegen van een korte psychosociale stressor, voorafgaand aan het bekijken van een traumafilm, een meer robuuste stress respons opwekt, die waarschijnlijk meer vergelijkbaar is met de reactie na blootstelling aan een echte traumatische gebeurtenis. Dit vergroot de translationele waarde van het traumafilm-paradigma. Onze resultaten laten ook zien dat het aangepaste paradigma nuttig kan zijn voor onderzoek naar de effecten van individuele variatie in biologische stressreactiviteit en onderliggende cognitieve processen voor de ontwikkeling van intrusieve symptomen. Het is belangrijk om meer inzicht te krijgen in de biologische en cognitieve processen die ten grondslag liggen aan de vroege ontwikkeling van PTSS-symptomen vlak na blootstelling aan een trauma, aangezien dit toekomstige effectieve preventie zou kunnen bevorderen.

Deze bevindingen in dit proefschrift wijzen op tijdsafhankelijke effecten van trauma, waarbij zowel de prenatale periode als de kindertijd gevoelige periodes lijken te zijn voor een verhoogd PTSS-risico in het latere leven na blootstelling aan trauma, met een blijvend effect op de lange termijn (**Hoofdstuk 2, Hoofdstuk 3**). Dit is vermoedelijk het gevolg van het feit dat de hersenen gevoeliger zijn voor omgevingsstressoren in het vroege leven. Mogelijke hersenprogrammering en sensitisering van biologische stresssystemen na een geschiedenis van blootstelling aan trauma in vroege levensperioden en langdurige verstoringen in deze neurobiologische processen kunnen uiteindelijk de kwetsbaarheid voor het ontwikkelen van PTSS-symptomen beïnvloeden. Door licht te werpen op veranderingen in stressgerelateerde processen die samenhangen met verschillen in kwetsbaarheid voor PTSS, kon worden aangetoond dat meestal maar niet altijd dezelfde structuren en systemen betrokken lijken te zijn bij de ontwikkeling van PTSS-symptomen bij adolescenten en volwassenen. Dit wijst erop dat ongunstige gebeurtenissen de regulering en het functioneren van deze structuren en systemen op een verschillende manier kunnen beïnvloeden, afhankelijk van het tijdstip van blootstelling (**Hoofdstuk 4, Hoofdstuk 5**).

Verder werden interindividuele verschillen aangetoond in kwetsbaarheden van stress-gerelateerde processen die ons begrip van gevarieerde PTSS-risico's ten goede kunnen komen (**Hoofdstuk 5**). Ook werd aangetoond dat genetische factoren met betrekking tot GR-functie variatie in PTSS-risico en symptoomuitkomst kunnen verklaren, waarbij accumulatie van ongunstige gebeurtenissen gedurende het hele leven, met inbegrip van neuro-ontwikkelingsperioden in het vroege leven, als een belangrijke beïnvloedende factor moet worden beschouwd (**Hoofdstuk 3**). Het is van groot klinisch belang dat veerkracht en gevoeligheid voor de ontwikkeling van PTSS ook bleken te verschillen voor mannen en vrouwen (**Hoofdstuk 2, Hoofdstuk 3**). In dit proefschrift is de complexiteit van de impact van trauma gedurende de levensloop onderzocht, die ten grondslag ligt aan de ontwikkeling van PTSS-symptomatologie, evenals andere risicofactoren zoals geslacht en individuele variabiliteit in het functioneren van stresssystemen en genetische factoren. Uiteindelijk kan dit werk bijdragen aan verbeterde toekomstige interventies die net als klaprozen mensen kunnen helpen te groeien en te bloeien ondanks blootstelling aan trauma.



Figuur 2. Overzicht van de tijdsafhankelijke effecten van blootstelling aan trauma tijdens de prenatale periode, tijdens de kindertijd en in het volwassen leven op het ontwikkelen van psychopathologische symptomen in het latere leven. Deze effecten zijn gerelateerd aan timing en aan stressgerelateerde neurobiologische processen betreffende neuraal, HPA-as en ANS functioneren die geassocieerd zijn met PTSS-symptomatologie gedurende de gehele levensloop. PTSS: Posttraumatische stress stoornis; FC: functionele connectiviteit; SNS: Sympathische zenuwstelsel; HPA: Hypothalamus-hypofyse-bijnier-as.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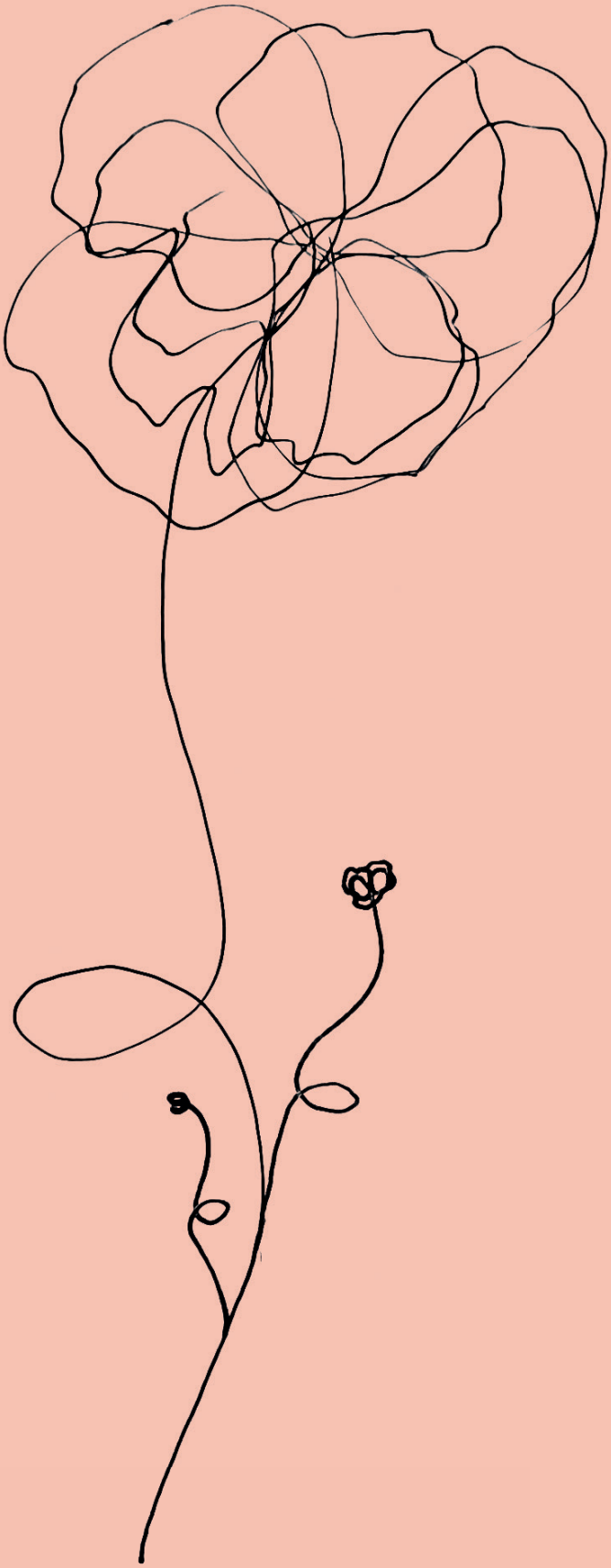

CHAPTER 8

REFERENCE SECTION

REFERENCE SECTION

A

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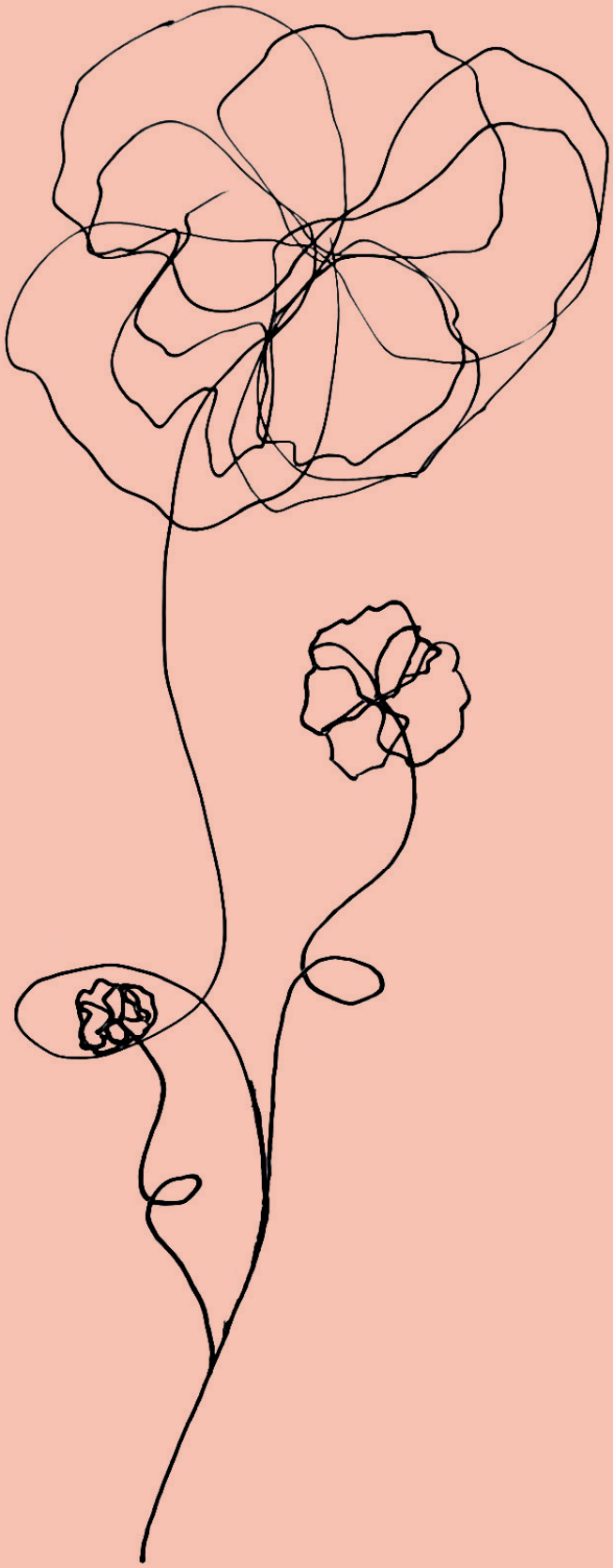

APPENDICES

AUTHOR CONTRIBUTIONS

The contributions below are specified according to the CRediT system (https://credit.niso.org/).

Chapter 2: The impact of adversities across the lifespan on psychological symptom profiles in late adulthood: a latent profile analysis

Charlotte E. Hilberdink: Methodology, Investigation, Software, Data Curation, Formal analysis, Writing – Original draft. **Mirjam van Zuiden:** Conceptualization, Methodology, Investigation, Data Curation, Writing – Review & Editing, Supervision, Project administration, Funding acquisition. **Miranda Olff:** Writing – Review & Editing. **Tessa J. Roseboom:** Writing – Review & Editing, Project administration. **Susanne R. de Rooij:** Conceptualization, Methodology, Data Curation, Writing – Review & Editing, Supervision, Resources, Funding acquisition.

Chapter 3: Effects of prenatal exposure to the 1944-45 Dutch famine and glucocorticoid receptor polymorphisms on later life PTSD susceptibility

Kayleigh Gultig: Software, Formal analysis, Writing – Original draft. **Susanne R. de Rooij:** Writing – Review & Editing, Resources, Funding acquisition. **Charlotte E. Hilberdink:** Investigation, Resources, Writing – Review & Editing. **Miranda Olff:** Writing – Review & Editing. **Tessa J. Roseboom:** Writing – Review & Editing, Project administration. **Mirjam van Zuiden:** Conceptualization, Methodology, Investigation, Writing – Original draft, Review & Editing, Supervision, Project administration, Funding acquisition.

Chapter 4: Dysregulated functional brain connectivity in response to acute social-evaluative stress in adolescents with PTSD symptoms

Charlotte E. Hilberdink: Methodology, Investigation, Software, Data Curation, Formal analysis, Writing – Original draft. **Mirjam van Zuiden:** Conceptualization, Methodology, Writing – Review & Editing, Supervision. **Anouk Schrantee:** Methodology, Writing – Review & Editing. **Aniko Korosi:** Methodology, Writing – Review & Editing. **Antonia Kaiser:** Methodology, Writing – Review & Editing. **Paul Zhutovsky:** Methodology, Writing – Review & Editing. **Annie T. Ginty:** Methodology, Writing – Review & Editing. **Judith B.M. Ensink:** Investigation, Writing – Review & Editing. **Ramon J.L. Lindauer:** Investigation, Writing – Review & Editing**, Tanja, G. Vrijkotte:** Writing – Review & Editing, Project administration. **Susanne R. de Rooij:** Conceptualization, Methodology, Data Curation, Writing – Review & Editing, Supervision, Resources, Project administration, Funding acquisition.

Chapter 5: Acute stress reactivity and intrusive memory development: a randomized trial using an adjusted trauma film paradigm

Charlotte E. Hilberdink: Methodology, Investigation, Software, Data Curation, Formal analysis, Writing – Original draft. **Susanne R. de Rooij:** Conceptualization, Methodology, Writing – Review & Editing, Supervision. **Miranda Olff:** Writing – Review & Editing. **Jos A. Bosch:** Methodology,

Resources, Writing – Review & Editing **Mirjam van Zuiden:** Conceptualization, Methodology, Data Curation, Writing – Review & Editing, Supervision, Project administration, Funding acquisition.

LIST OF PUBLICATIONS

In this thesis

Charlotte E. Hilberdink, Mirjam van Zuiden, Miranda Olff, Tessa J. Roseboom, Susanne R. de Rooij. The impact of adversities across the lifespan on psychological symptom profiles in late adulthood: a latent profile analysis.

Submitted for publication, Preprint: OSF (2022), 10.31219/osf.io/c6fqd

Kayleigh Gultig, Susanne R. De Rooij, **Charlotte E. Hilberdink**, Miranda Olff, Tessa J. Roseboom, Mirjam van Zuiden. Effects of prenatal exposure to the 1944-45 Dutch famine and glucocorticoid receptor polymorphisms on later life PTSD susceptibility.

Submitted for publication

Charlotte E. Hilberdink, Mirjam van Zuiden, Anouk Schrantee, Aniko Korosi, Paul Zhutovsky, Annie T. Ginty, Judith B.M. Ensink, Ramon J.L. Lindauer, Tanja G. Vrijkotte, Susanne R. de Rooij. Dysregulated functional brain connectivity in response to acute social-evaluative stress in adolescents with PTSD symptoms.

Published in European Journal of Psychotraumatology (2021), 12(1): 1880727, https://doi.org/10.1080/20008198.2021.1880727

Charlotte E. Hilberdink, Susanne R. de Rooij, Miranda Olff, Jos A. Bosch, Mirjam van Zuiden. Acute stress reactivity and intrusive memory development: a randomized trial using an adapted trauma film paradigm.

Published in: Psychoneuroendocrinology (2022), 139: 105686,

https://doi.org/10.1016/j.psyneuen.2022.105686

PhD PORTFOLIO

AMC Graduate School for Medical Sciences

Summary of PhD training, supervising and parameters of esteem

Name PhD student	Charlotte Elize Hilberdink
PhD period	September 2017 – November 2022
Names of PhD supervisors & co-supervisors	prof. dr. Tessa J. Roseboom, prof. dr. Miranda Olff, dr. Mirjam van Zuiden, dr. Susanne R. de Rooij

1. PhD Training

University of Amsterdam, Academic Medical Center, Department of Psychiatry, Amsterdam

- Assisting in designing, setting up and conducting a randomized-controlled trial, which included a study investigating the effects of stress on involuntary intrusive memory development after trauma in healthy male participants by using an adapted trauma film paradigm. This adaptation consisted of adding a psychosocial stressor (stressor condition) immediately before watching a trauma film versus healthy control males (warm water condition). For this study, programming and designing of behavioral tasks (e.g. PsychoPy) was done as well as online data entry (Castor); processing of saliva samples for endocrine data (ELISA kit), and performing data analyses of behavioral, cardiac (VU-AMS Ambulatory Monitoring System) and endocrine data (SPSS).
- Offering support and assistance in setting up and conducting a substudy for the Dutch famine birth cohort on the effects of undernutrition during pregnancy on health aspects in later life, collecting behavioral questionnaire data, and performing data analysis for questionnaire data (Microsoft Access, SPSS).
- Working as an fMRI operator for a substudy of the Amsterdam Born Children and their Development (ABCD) cohort on brain and physiological responses to psychosocial stress in adolescents with PTSD symptoms, collecting neuroimaging data (fMRI) and performing data analysis of neuroimaging (FSL, Matlab), behavioral (E-Prime), cardiac and endocrine data (SPSS).
- Assisting in setting up and offering advice for a new follow-up substudy for the ABCD cohort (2020-2021), which included online questionnaires through an application, assisting in building, coding and testing applications (Linux platform), and building, coding and testing online back-up questionnaires (Survalyzer).

General courses	*Year*	*ECTS*
Data Visualisation II, AMC Graduate School, Amsterdam	2022	0.1
Project Management, AMC Graduate School, Amsterdam	2022	0.6
Scientific Writing in English, AMC Graduate School, Amsterdam	2020	1.5
Practical Biostatistics , AMC Graduate School, Amsterdam	2020	1.1
Medical Literature: Zoeken voor een CAT, AMC Graduate School, Amsterdam	2020	0.1
Medical Literature: Searching for Evidence, AMC Graduate School, Amsterdam	2020	0.1
Privé en Werk tijdens Corona (e-course), AMC Graduate School, Amsterdam	2020	0.1
Writing for a scientific paper (e-course) , AMC Graduate School, Amsterdam	2020	1.5
AMC World of Science, AMC Graduate School, Amsterdam	2019	0.7
Medical Literature: PUBMED, AMC Graduate School, Amsterdam	2019	0.1
Specific courses	*Year*	*ECTS*
Computing in R, AMC Graduate School, Amsterdam	2022	0.4
Good Clinical Practice course 'Regulation and Organization in Clinical Research (eBROK, e-course), Dutch Federations of Universities (NFU), AMC, Amsterdam	2022	
Clinical Epidemiology: Observational Epidemiology, AMC Graduate School, Amsterdam	2021	0.6
3T Philips MRI scanner operating qualification, AMC Radiology & Nuclear Medicine, Amsterdam	2020	
MRI Basics: Basic understanding for (bio)medical research, AMC Graduate School, Amsterdam	2020	1.0
Seminars, workshops, master classes	*Year*	*ECTS*
PhD Workshop 'How to read a scientific paper'	2021	0.1
Benefietcursus Seksueel Geweld met Iva Bicanic	2021	0.1
Celebrating 10 years of European Journal of Psychotraumatology	2021	0.1
Wetenschapsfestival: Corona – straks & nu	2020	0.1
Netherlands Research Integrity Network	2020	0.1
Brain, Cognition and Behavior NL (BCB-NL) event	2020	0.1
COVID-19 event	2020	0.1
Anatomische Les with Dr. Fauci, AmsterdamUMC	2020	0.1
Global Mental Health webinar – In the era of COVID-19 Pandemic	2020	0.1

Several lectures of Amsterdam Brain and Cognition (ABC), AmsterdamUMC, Amsterdam, The Netherlands.	2019-2022	0.1
Several lectures of Amsterdam Public Health (APH), AmsterdamUMC, Amsterdam, The Netherlands.	2019-2022	0.1
Several webinars of Brain and Behavior Research Foundation (BBRF)	2019-2022	0.1
Several webinars of Statistical Analysis with Karen Grace-Martin	2019-2022	0.1
Several lectures of Nederlandstalige Vereniging voor Psychotrauma (NtVP)	2019-2022	0.1

Conference presentations	*Year*	*ECTS*
Hilberdink CE, de Rooij SR, Olff M, Bosch JA, van Zuiden M (2022). *Acute stress reactivity and intrusive memory development: a randomized trial using an adjusted trauma film.* **Anatomische Les with Sonia Lupien, AmsterdamUMC,** selected to participate in a live master class. *Cancelled due to COVID-19.*	2021	
Hilberdink CE, de Rooij SR, Olff M, Bosch JA, van Zuiden M (2022). *Acute stress reactivity and intrusive memory development: a randomized trial using an adjusted trauma film paradigm.* **Dutch Neuroscience meeting (DNM),** online, oral parallel session.	2021	0.5
Hilberdink CE, de Rooij SR, Olff M, Bosch JA, van Zuiden M (2022). *Acute biological stress reactivity and recovery influences intrusive memory development in healthy men.* **World Association for Stress Related and Anxiety Disorders (WASAD),** Vienna, Austria; (e- and in-person)poster presentation.	2021	1.5
Hilberdink CE, de Rooij SR, Olff M, Bosch JA, van Zuiden M (2022). *Indications for the involvement of individual variation in acute biological stress in intrusive memory development in healthy men.* **Amsterdam Neuroscience Annual Meeting,** Amsterdam, The Netherlands; (e)poster presentation.	2021	0.5
Hilberdink CE, de Rooij SR, Olff M, Bosch JA, van Zuiden M (2022). *Individual variation in biological stress reactivity and recovery influences intrusive memory development in healthy men: an experimental study using an adjusted trauma film paradigm.* **International Society of Traumatic Stress Studies (ISTSS),** online, (e)poster presentation.	2021	0.5

Hilberdink CE, van Zuiden M, Schrantee A, Korosi A, Kaiser A, Zhutovsky P, Ginty AT, Ensink JBM, Lindauer RJL, Vrijkotte TGM, de Rooij SR (2021). *Brain connectivity in response to social evaluative threat in adolescents with PTSD symptomatology.* **Amsterdam Neuroscience Annual Meeting,** Amsterdam, The Netherlands, poster presentation.	2019	0.5

(Inter)National conference attendance	*Years*	*ECTS*
World Association for Stress Related and Anxiety Disorders (WASAD), Vienna, Austria	2021	1.5
International Society of Traumatic Stress Studies (ISTSS), online	2020-2021	0.75
Imaging in Psychiatry (IiP), Eindhoven, The Netherlands. C*ancelled due to COVID-19*	2020	
European Society for Traumatic Stress Studies (ESTSS), online	2019-2020	1.0
Dutch Neuroscience meeting (DNM), online and Lunteren, The Netherlands	2019, 2021	0.75
Stress-NL, Amsterdam and Rotterdam, The Netherlands	2018-2019	0.5
Brain SIN-Posium, Amsterdam, The Netherlands	2018	0.25
Amsterdam Neuroscience Annual Meeting (ANS), Amsterdam, The Netherlands	2017-2021	1.75

Other	*Year*
Reviewer for Scientific Reports	2021
Reviewer for Clinical Psychology Review	2021
ABCD core team meetings, ABCD team, monthly, AMC and VUmc	2020-2021
ABCD wave 6 meetings, ABCD subteam follow up, weekly, AMC	2020-2021
DARE team meetings, Development and Reproductive Epidemiology team, 2-weekly, AMC and VUmc	Since 2019
Junior researcher group meetings, Psychotrauma team, weekly, AMC	Since 2019
Psychotrauma research group lab meetings, Psychotrauma team, weekly, AMC	Since 2017

2. Teaching

Lecturing

2021	**Guest lecture Amsterdam University College – Human Stress Research,** Biological stress reactivity and recovery in intrusive memory development: *How do we translate our research questions into a reliable and feasible study design*?

Supervising

2020 - 2021	**Second assessor for Bachelor thesis**
2019 - 2020	**Daily supervision for writing of Bachelor thesis**
Since 2017	**Daily supervision, training and mentoring of several Psychobiology, Biomedical Sciences, Clinical Psychology and Medicine Bachelor and Master students during their scientific internships**; supervising and training students in performing the research protocols, for example, conducting physiological measurements such as heart rate, collecting and preparing behavioral data and saliva samples; performing endocrine analyses, working with software, and other daily practical research activities.

3. Publications

Peer reviewed and submitted for publication – in this thesis

2022 Van Zuiden M, Gultig K, **Hilberdink CE**, Olff M, Roseboom TJ, De Rooij SR. Effects of prenatal exposure to the 1944-45 Dutch famine and glucocorticoid receptor polymorphisms on later life PTSD susceptibility. *Submitted for publication.*

Submitted for publication – in this thesis

2022 **Hilberdink CE**, van Zuiden M, Olff M, Roseboom TJ, de Rooij SR. The impact of adversities across the lifespan on psychological symptom profiles in late adulthood: a latent profile analysis.
Submitted for publication, Preprint: OSF (2022), 10.31219/osf.io/c6fgd.

CURRICULUM VITAE

Lotte Hilberdink was born on October 28th 1991, in Nunspeet, the Netherlands. In 2010, she completed her secondary education (VWO – Science & Health) at the Thorbecke Scholengemeenschap in Zwolle. After her graduation, she spent a gap year working at the Isala Klinieken Sophia Hospital in Zwolle as a nutrition, nurse and care assistant and travelled through South East Asia. Lotte obtained her Bachelor's degree in Psychobiology in 2014 and her Research Master's degree in Biomedical Sciences – Neurobiology (specialization in Psychopharmacology and Pathophysiology) in 2016, at the University of Amsterdam. During her master, she was doing research internships at the Swammerdam Institute for Life Sciences, the Research Center for Military Mental Health, and the Science & Research department of the Netherlands Institute for Forensic Psychiatry and Psychology (NIFP). After successfully completing her Master's degree, she continued to work temporarily as a research assistant for the NIFP. She also started working as a research assistant in the Cognitive and Affective Neuroscience group of the Donders Centre for Cognitive Neuroimaging (DCCN). Hereafter, as of September 2017, Lotte worked as a research assistant in the Psychotrauma team of the Department of Psychiatry in the Amsterdam University Medical Center, location AMC, University of Amsterdam, under supervision of dr. Mirjam van Zuiden. After six months, she additionally obtained a position as a functional magnetic resonance imaging operator for the Department of Epidemiology and Data Science of the AmsterdamUMC under supervision of dr. Susanne de Rooij. In 2019, her position as a research assistant was changed into a PhD trajectory at both departments, under supervision of prof. dr. Tessa Roseboom, prof. dr. Miranda Olff, dr. Mirjam van Zuiden, and dr. Susanne de Rooij. As she was already involved in two cohort studies and a cross-sectional study during her work as a research assistant, these projects became the main topic of her PhD project. For the Amsterdam Born Children and their Development cohort, Lotte was responsible for the fMRI data collection to investigate dysregulation of functional brain connectivity, endocrine and cardiac reactivity in response to a psychosocial stressor in adolescents with high levels of PTSD symptoms. She was also involved in setting up and performing the study procedures to collect data for a follow-up study for the Dutch famine birth cohort and a randomized controlled trial. In members from the Dutch famine birth cohort, the effects of malnutrition during pregnancy on health aspects in later life were investigated. In the randomized controlled trial, the effects of stress on posttraumatic stress-related involuntary intrusive memories after experimental psychotrauma were investigated. For her PhD thesis, Lotte focused on whether and how the timing of adversity during different periods across the lifespan impacts the vulnerability for PTSD development and its potential underlying stress-related neurobiological processes. Lotte started working as a postdoctoral researcher in the Neuropresage Schizophrenia and Affective Disorders group at the Cyceron Imagery Center in Caen, Normandy, France, in April 2022. Here, she investigates the effects of mindfulness training on symptom severity in patients with prolonged grief disorder and stress-related neurobiological mechanisms that underlie potential treatment-induced changes in these patients, together with prof. dr. Eric Bui. Lotte continues to focus on neurobiological stress functioning for trauma-induced psychopathology risk and its potential biomarkers using neuroimaging and physiological methods to increase fundamental and clinical knowledge, with an interdisciplinary approach.

ACKNOWLEDGEMENTS / DANKWOORD

IT IS DONE… Het schrijven van een proefschrift kan een redelijk stressvolle bedoeling zijn bij tijd en wijle. Zeker wanneer dit grotendeels plaatsvindt tijdens een pandemie in een appartementje van 29m2 en je tussentijds besluit een fulltime postdoc positie aan te nemen in een ander land waar je de taal niet spreekt. Maar ja... het is écht af! Dit was onmogelijk geweest zonder de steun, het vertrouwen en de positiviteit van de mensen om mij heen. *'Sans l'amitié la vie est un fardeau'.*

Allereerst wil ik graag **alle deelnemers** bedanken die hebben geparticipeerd aan de verschillende onderzoeksprojecten. Jullie deelname was essentieel voor dit proefschrift! Jullie openhartigheid en motivatie waren bewonderenswaardig voor de soms wat heftige en intense procedures. Het heeft ertoe geleid dat we nu meer weten over blootstelling aan potentieel traumatische gebeurtenissen.

Graag bedank ik ook mijn promotoren, **prof. dr. Tessa Roseboom en prof. dr. Miranda Olff**. Jullie zijn beiden inspiratiebronnen voor mij. Jullie begrip en luisterend oor wanneer de moed mij soms in de schoenen zonk, hebben mij altijd weer weten te motiveren. Jullie positieve aanpak, hulpvaardigheid en vertrouwen in mij zijn essentieel geweest en ik zie jullie als voorbeeld voor elke vrouwelijke wetenschapster. Miranda, ik ben blij dat Sint-Santa je computer altijd wist te vinden en een motiverend gedicht vol complimenten aan alle Psychotrauma teamleden achterliet. Tessa, het notitieboekje dat ik van je heb gekregen tijdens de Inspiration Day met het DARE-team bij Anna Haen haal ik nog steeds regelmatig uit de boekenkast in mijn Franse woonkamer.

Beste dr. Mirjam en dr. Susanne, bedankt dat jullie het vertrouwen in mij hadden voor dit unieke promotietraject en ervoor zorgden dat ik meer uit mijn werk heb kunnen halen. Jullie hebben het voor mij mogelijk gemaakt om mijn passie voor psychiatrie en mijn enthousiasme voor (neuro)biologisch onderzoek te kunnen combineren, waarbij beide afdelingen ook mooi samenvielen. Zonder jullie steun, geduld, kritische en scherpe blik was het eindproduct waarschijnlijk niet zo'n mooi geheel geworden. Jullie kennis en begrip zijn bewonderenswaardig en iets waar ik enorm veel van heb geleerd. Daar ben ik jullie dankbaar voor. Soms ging dat voor mij met enig vallen en opstaan, maar jullie meedenkende en bemoedigende woorden tijdens onze wekelijkse meetings brachten mij altijd weer op het rechte pad. Jullie betrokkenheid – op professioneel en persoonlijk vlak – en jullie daadkracht en enthousiasme zorgden ervoor dat ik door kon gaan. *"Just keep swimming!"* Jullie uitgebreide en leerzame feedback en begeleiding hebben mijn werk als wetenschapster naar een hoger niveau weten te tillen. **Mirjam**, ik zal nooit vergeten hoe wij op de terugweg van het WASAD congres in Wenen in de nachttrein door zaklampen en geschreeuw uit onze slaap werden gewekt. "Bundespolizei! Aufmachen!!" Ik zal mij ook altijd herinneren dat jij getuige was van mijn eerste opera 'Die Zauberflöte' in de Wiener Staatsoper. Deze was naar onze mening toch wel erg oubollig en leverde niet echt een bijdrage aan de emancipatie. **Susan**, bedankt voor alle kansen die je mij hebt aangereikt om mij te

ontwikkelen als onderzoekster. Van fMRI operator tot praktische ondersteuner bij de ABCD studie, elke uitdaging heb ik vol enthousiasme en een lading energie aangenomen.

Ook dank ik de leden van **de beoordelingscommissie**, dr. Van Ast, prof. dr. Reneman, prof. dr. mr. Vinkers, dr. Finken, prof. dr. Fliers en prof. dr. Lindauer. Bedankt dat jullie de tijd hebben gevonden om dit proefschrift te lezen en te beoordelen en mij bij mijn verdediging het vuur aan de schenen te leggen.

I would like to thank all wonderful (former) members of the **Psychotrauma team**. I had the privilege to be surrounded by many inspiring individuals and was lucky to work with most of you before the COVID-19 pandemic. I really appreciated all 'gezellige', educational and supporting meetings we had together. I would like to thank especially **Yulan and Paul**. The '*Best and Most Supporting Colleagues Award'* goes to…! Even after our 'office break', we always found each other back. I cannot express my appreciation for your kindness, support, compassion and all the help. Online writing sessions, TERMINAL issues and linear mixed models meetings helped me until the end of this journey. **Yulan**, thanks for drinking GTs with me and for your lovely pieces of advice. **Paul**, thanks for always being compassionate and occasionally sharing our realistic and less optimistic opinions about science and statistics. Because of you I will always continue to think critically. **Jeanet**, mijn loyale PhD mattie! Ik heb zoveel geleerd van jouw doorzettingsvermogen en optimisme. Samen kwamen wij deze PhD tijd wel door. Ik haalde veel steun uit onze wekelijkse meetings! Nu ik naar Frankrijk ben geëmigreerd, mis ik onze zelfgebakken (van mij zelf-meegebrachte) taartmomentjes natuurlijk wel, ondanks de consequentiële schade in kilo's. Ik ben blij met de vriendschap die het ons heeft gebracht! **Indira**, thank you for being the kindest and most wonderful office roomie! Your warm and lovely personality inspires me. Thanks for teaching me the Indonesian remedy to use a mix of sugar, ginger and water when I'm sick. **Ira**, hoe vaak kun je elkaar niet 'vinden' binnen een team, ook al werk je niet samen?! Je bent een prachtig persoon en ik heb veel geleerd van de rust die je uitstraalt en je brede kennis (ook de random feitjes ;)). **Anne**, ik wil je bedanken voor de shotjes die je mij hebt aangereikt in de club in Rotterdam tijdens de ESTSS. Zonder jou had ik het hotel of de McDonald's niet meer gevonden. Ik moet altijd enorm met je lachen en ik zal je leeuwenvisage nooit meer vergeten. Jouw vrolijkheid en optimisme zijn aanstekelijk! **Laura**, bedankt voor je droge humor en gezelligheid. Het was kort, maar krachtig! En bedankt voor het lenen van je BioRender account 8-). **Merel**, ik heb genoten van de dagen die we (vroegah) samen op het AMC waren. Jouw kritische en klinische vragen hielpen mij verder te kijken dan de wetenschap. Ik heb altijd genoten van onze gezamenlijke lunchmomentjes. **Bernadette**, de oprechte interesse die je altijd toonde in mij en mijn werk zeggen veel over jou. Ik zal onze korte brainstorm eBROK-meetings nooit vergeten (positief en enigszins negatief op mijn netvlies gebrand, brrr). **Chris**, bedankt voor je engelengeduld in het beantwoorden van mijn statistische vragen op 30 verschillende manieren tot ik het snapte. Je bent een topper en ik ben jaloers op je kennis. **Bruno**, thanks for your short contribution to the team. I appreciated you raising awareness about the socially relevant issues

in Brazil and sharing your great travel stories throughout Europe. You've seen more European countries within your few months' stay in The Netherlands than any Dutch person. Hopefully we will plan that visit to Brazil one day ;)! **Brinn**, I would like to thank you for teaching me how important teamwork and cozy coffee breaks are! **Thomas and Adria**, I really enjoyed your time in the team! Your cleverness amazed me and I enjoyed the fun we had together, even though I don't like cats. Too bad we were forced to limit our social real-life interactions to one drink in times of COVID-19. Daarnaast wil ik ook graag alle stagiaires bedanken die mij enorm hebben geholpen met de dataverzameling en invoer. Jullie bijdrage was cruciaal! Ik heb enorm veel van jullie geleerd en ben blij dat ik met jullie samen heb mogen werken. **Andrea**, ook jij enorm bedankt voor je steun en alle verrichte logistieke werkzaamheden om dit proefschrift tot een goed einde te brengen. Zelfs in ietwat bizarre uurtjes onder ietwat onverwachte omstandigheden was je niet te stoppen;).

Ik ben ook alle leden van het **DARE team** dankbaar. Ik keek altijd uit naar onze leuke en leerzame meetings en heb kennis gemaakt met veel uiteenlopende en voor mij nieuwe en relevante onderwerpen. Personen die ik in het bijzonder wil bedanken zijn Amber en Mandy. Megaslimme en lieve **Amber**, wat ben ik blij met jou als collega en vriendin! Je was mijn rots in de branding. Je werd het nooit beu om mijn random vragen te beantwoorden en mij te voorzien van eindeloze adviezen, ondanks je megadrukke schema! Jouw hulpvaardigheid, kennis en motivatie zijn grenzeloos en een enorm voorbeeld in de academische wereld. Ik keek en kijk nog altijd uit naar onze wekelijkse meetings, die eigenlijk vaker bestaan uit een bijklets-koffiemomentje met een schuimlaagje van SEM statische analyses en een koekje van neuroimaging software. **Mandy**, bedankt voor de prachtige cover die je voor mij hebt geschilderd. Ik ben je ontzettend dankbaar voor alle moeite die je erin hebt gestoken. Ik ben jaloers op jouw creativiteit. Het eindresultaat is nog mooier dan ik had durven dromen!

To all my new (and some already former) **French colleagues of Cyceron**, Thomas, Baptiste, Séverine, Elise, Annick, Maxime, Céline, Lucie et Maud. Merci pour votre accueil incroyablement chaleureux dans l'équipe et pour avoir parlé anglais avec moi xD. Sans votre hospitalité, Caen ne se serait pas sentie si vite comme ma (2ème) maison. Votre soutien sans faille et votre loyauté sont extraordinaires ! Vous me faites rire tous les jours et me motivez à monter la colline pour aller travailler sur mon vélo rose vintage à chaque fois. Un merci spécial à Thomas, pour m'avoir fait découvrir la Raclette #Shock. **Eric**, merci de m'avoir fait confiance en m'engageant comme chercheur postdoctoral alors que je terminais mon doctorat. Je me réjouis de notre future collaboration en France et vos suggestions de bon champagne !

In mijn sociale kring zijn er ontelbare vrienden en familieleden die ik niet vaak genoeg kan bedanken. De aandacht die jullie voor mijn werk hadden en de interesse die jullie toonden, maar ook de afleiding en ontspanning waar jullie voor hebben gezorgd, zijn enorm belangrijk voor mij geweest en hebben het mogelijk gemaakt om dit proefschrift af te ronden. Het is niet in woorden uit te drukken hoe ontzettend dankbaar ik ben met jullie aan mij zijde.

Lieve **Haarlemse klassieke balletdames en Nicolas**, bedankt voor de fijne afleidende en gezellige balletlessen tijdens mijn PhD jaren. Anneke, Ingrid, Eva, Marianne, Jacqueline, Bregje, Eveline, Iris, Erminia en Chantal, bij jullie kon ik mijn ei kwijt en de werkdag eruit 'dansen'. Ik mis jullie en onze borrels in 't Kantoor in Haarlem en kan niet wachten tot ik weer met jullie in de les sta.

Liefste **vrienden voor het leven**. Rigarda en André, 'Gouden Leeuwtjes' Lynn, Jorine, Suzanne, Iris, Linda, Renske, Martine, Monique, Sophie, Lindsay en Lorraine, Mark en Diaan, bedankt voor jullie altijd oprechte interesse en oneindige middagjes en avondjes vol met gezellige etentjes, wijntjes, uitjes, logeerpartijen, weekendjes weg, spelletjes, kletspartijen, filmavondjes, borrels, vakanties, energie, enthousiasme en nog meer wijntjes.

Allerliefste **Ester, Menno en baby Bódi**, bedankt voor de interesse en begrip die jullie altijd in mij en mijn werk toonden. Zelfs bij het ontmoeten van de pasgeboren Bódi was de eerste vraag "Hoe gaat het met je PhD, Lot?!". Ik haalde altijd veel ontspanning en afleiding uit onze gezellige en heerlijke borrelavondjes, vage stapavonden in de XO, vegan recepten die ik daarna MOEST hebben en slappe en serieuze series die we samen gebinge-watched hebben (waarbij M/Jenno bijna moest huilen van ellende door onze commentaren). Ik ben jullie zo dankbaar voor onze jarenlange vriendschap en alle lol die we samen hebben. Ik weet dat we altijd bij elkaar terecht kunnen (ook voor de ByLima taartjes).

Sweetest **Fabienne**, my lovely new neighbor and dear friend. Thank you for the lovely dinners and wonderful talks. Thank you for taking me on trips to get to know Normandy, for teaching me French and rescuing me from huge scary spiders in the apartment in the middle of the night. You're a lovely person and I am lucky to have you by my side. I am happy I have met you and our connection is very special to me. *'On ne va pas chez la reine, donc pas de chichis!'*

Lieve **Toke**, moeder van Mari, bedankt voor de altijd oprechte interesse die je hebt getoond in mij en in het verloop van mijn PhD. Ik heb veel aan je tips gehad en ik voel me vereerd dat je jouw ervaringen met mij hebt willen delen. Deze gesprekken zullen mij altijd bijblijven.

Lieve **Milan, Laura en Moniek**, 'de meiden'. Vanuit het diepste van mijn hart dank ik jullie voor onze jarenlange vriendschap, steun en toeverlaat. Onze ontelbare etentjes met enorme lachbuien, lekker eten, adviezen en dloge losé tijdens weekenden in Zwolle, Haarlem en Caen (en zélfs Den Haag), hebben mij altijd weer de energie en motivatie gegeven om optimistisch te blijven en er elke dag weer fris en fruitig tegenaan te gaan. Ik ben dankbaar dat onze vriendschap over alle afstanden met ons mee verhuist en ik bof ontzettend met jullie!

Allard, Ali, mijn beste male buddy. Wat had ik zonder jou gemoeten…! Op de momenten dat ik het nodig had, kwam jij aan met de juiste afleiding, een luisterend oor en zorgde voor intense lachbuien. Jouw energie, grenzeloze loyaliteit en interesse bewonderen mij. Bedankt voor het luisteren naar ONS eindeloze geklaag, het houden van politieke discussies gebaseerd op grotendeels onjuiste informatie, en onze ellenlange telefoongesprekken van over de hele wereld, je record aantal pizza's met extra p(l)akken salami en kaas, Pickle Rick, de literpakken wijn die we dronken in het park, in de Bijlmer, Utrecht, Haarlem, Amsterdam, Amstelveen, Heiloo en Caen. Jij maakte dat ik met beide benen op de grond bleef staan. Onze vriendschap is oneindig en ik ben ontzettend trots dat je nu ook zelf met een PhD bent gestart, ondanks onze toekomstplannen voor een eigen Disney Voice-Acting business die nu in het water valt. Ik ben super trots op jou! Ik zal altijd aan je zijde staan, ook tijdens jouw traject, zoals jij dat bij mij hebt gedaan!

Lieve **opa en oma**, bedankt voor jullie interesse in mijn promotie. Ook al kunnen jullie er helaas niet bij zijn, ik weet dat jullie achter de computer online meekijken en trots zullen zijn! Ik hoop hiermee de wetenschappelijke lijn in onze stamboom te hebben voortgezet.

Lieve **Cis, Jerrald en** (niet-meer-zo-kleine-baby) **Thomas**, bedankt voor jullie hulp en steun tijdens dit traject. Bij jullie met Thomas op mijn schoot, kon ik ontspannen. Cis(ter), ik prijs mij gelukkig met dat we toch zoveel meer op elkaar lijken dan wij zelf door hadden, in werk en privé! Jerrald, bedankt voor de Tawny portjes en mijn bijnaam als kersverse tante voor Thomas. En baby Thomas, wat je ook gaat doen in de toekomst, als trotse tante zal ik altijd een plekje voor je bezet houden om met mij de wetenschapswereld omver te blazen.

Allerliefste **papa en mama**, jullie zijn mijn oneindige steun en toeverlaat. Ik ben jullie eeuwig dankbaar voor jullie betrokkenheid, zorgzaamheid, vertrouwen en onvoorwaardelijke steun. Jullie hebben mij altijd gestimuleerd mijn eigen keuzes te maken en hebben mij vaak geholpen om die keuzes werkelijkheid te maken. Ik waardeer het ontzettend dat jullie altijd met mij meedenken en achter mij staan. Bedankt ook voor de genetische aanleg om het leuk te vinden nieuwe dingen te leren en spannende avonturen aan te gaan. Mam, bedankt voor het opnemen van mijn ontelbare telefoontjes vol getetter en voor het olifantengeduld waarmee je naar al mijn uitgebreide updates, dilemma's en ontwikkelingen hebt geluisterd. Niet alleen de afgelopen jaren. Pap, ik lijk steeds meer op jou. Bedankt voor al je tips, taalkundige hulp en steun tijdens mijn promotietraject. Jou aan mijn zijde als ervaringsdeskundige was een uiterst belangrijke en vooral bijzondere toevoeging. Ik prijs mij gelukkig met jullie als mijn ouders!

Carmen, Mari en Sanne, mijn basis. Woorden schieten tekort als ik mijn dankbaarheid probeer uit te drukken. We gaan al bijna 20 jaar terug, toen we onze intellectuele, sociale en avontuurlijke vaardigheden begonnen te ontwikkelen. Jullie staan altijd aan mijn zijde en zonder jullie waren alle fasen van mijn promotietraject onmogelijk geweest. Jullie hebben mij op de been gehouden tijdens de beste en moeilijkste momenten. Of jullie nou met een fles champagne voor de deur stonden nadat mijn eerste wetenschappelijke artikel werd gepubliceerd om ervoor te zorgen dat ik deze mijlpaal wel zou vieren ten tijde van corona, meelazen en taalkundige correcties maakten wanneer ik om hulp vroeg, kilometerslange wandelingen met mij maakten door de Kennemerduinen bedolven onder een waterval van ingewikkelde informatie over MRI data, mij hielpen lastige beslissingen te maken, van ontelbare (levens)adviezen voorzagen, de vele werkretraites in jullie huizen die mij uit mijn eenzame corona-thuiswerksituatie sleepten, ik zal het nooit vergeten. Het had ook niet gekund zonder het aangereikte Siciliaans wijntje van Walter en de pannenkoeken van Robert en de memory en wie-is-het spelletjes met James, Isé en Amélie die mijn werkgeheugen en andere executieve functies activeerden en trainden. Ik weet dat jullie deur altijd voor mij open staat, zoals hij bij mij open staat voor jullie. Zonder elkaar, was het ONS niet gelukt!

wat zal ik schrijven over 'ik'
'ik' ben een deel van 'jij'
en 'jij' bent 'ik', door dun en dik
en daarom zijn wij 'wij'

Toon Hermans – Ontbijten met jou, 1989, Baarn