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Publication date
2024

Document Version
Final published version

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Cover design: Jus Juchtmans, 20210610 (2021), acrylic on linen, 120 x 90 cm
Lay-out: Arina van Londen
Print: Ridderprint BV | www.ridderprint.nl

ISBN: 978-94-6483-679-0

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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 dinsdag 9 januari 2024, te 13.00 uur

door Jasper Brian Zantvoord
geboren te AMSTERDAM

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CHAPTER 1

General introduction

While trauma-focused psychotherapies are effective in the majority of youth suffering from posttraumatic stress disorder (PTSD), a substantial part of youth with PTSD does not benefit from current first line trauma-focused psychotherapies (Morina, Koerssen, & Pollet, 2016). The fundamental goal at the outset of this project was to improve understanding of neurobiological mechanisms of trauma-focused psychotherapies and to investigate whether these insights could eventually enhance treatment outcome in youth with PTSD. Before describing the aims and scope of the separate chapters of the thesis, this general introduction first addresses the burden of childhood trauma and PTSD in youth. Subsequently, evidence based trauma-focused psychotherapies in youth will be briefly introduced. Next, an overview of recent developments in clinical neuroscience relevant to PTSD as well trauma-focused psychotherapy will be presented and the question why youth with PTSD have not benefited from these developments will be discussed.

The individual and societal burden of childhood trauma and PTSD in youth

Childhood trauma imposes a substantial psychological burden on youth and accounts for 45% of the population-attributable risk of child-onset mental disorders and 32% of adolescent-onset mental disorders (Green et al., 2010), with the strongest correlates between childhood trauma and the onset of disruptive behavior disorders, mood disorders, substance use disorders as well as anxiety disorders including PTSD (Green et al., 2010). Trauma is defined as an event in which a person is directly or indirectly exposed to a life threating event, serious injury, or sexual violence, for example domestic violence, childhood sexual abuse, serious car accidents and physical assault (American Psychiatric Association, 2013). Approximately two-thirds of youth (defined here as the period spanning childhood and adolescence) are exposed to psychological trauma before they reach adulthood (Copeland, Keeler, Angold, & Costello, 2007). Although, most youth do not develop persistent posttraumatic stress symptoms in the aftermath of trauma, up to 16% of all youth develop a posttraumatic stress disorder (PTSD) by the age of 18 years (Alisic et al., 2014). The risk of PTSD is dependent on the timing and nature of trauma exposure with increased PTSD risk after interpersonal trauma, repeated trauma exposure and trauma exposure at a younger age (McLaughlin et al., 2013). Moreover, adequate social and caregiver support can have a mitigating effect on PTSD development (Berkowitz, Stover, & Marans, 2011). Girls are at increased risk of developing PTSD which may be due to the difference in the nature of trauma exposure, with more interpersonal and sexual trauma in girls, as well as peritraumatic responses with more dissociation and stronger perception of threat and loss of control in girls (Olff, 2017; Olff, Langeland, Draijer, & Gersons, 2007).

The daily lives of youth with PTSD are troubled by frequent re-experiencing of the traumatic event, persistent avoidance, negative alterations in cognition and mood, and hyperarousal (American Psychiatric Association, 2013). These symptoms often interfere with social functioning and school performance and have a negative effect on quality of life of the affected youth and their families (V. G. Carrion, Weems, Ray, & Reiss, 2002; Warshaw et al., 1993). In addition, PTSD in youth is associated with high rates of comorbidity, including anxiety and depressive disorders and is a crucial factor in shaping the vulnerability to substance abuse and suicidality in both adolescence and adulthood (Molnar, Berkman, & Buka, 2001; Sunley et al., 2020). Importantly, PTSD has the highest risk of all mental health disorders for first suicide attempt in adolescents and young adults (Miché et al., 2018). Moreover, PTSD exacts a substantial societal toll due to increased health care utilization and financial outlay, with an estimated annual cost of € 8.4 billion in the European Union (Gustavsson et al., 2011). Together, these figures emphasize the vital importance of effective treatments to mitigate the negative consequences of PTSD in youth.

Trauma-focused psychotherapy for youth with PTSD

With the absence of evidence-based pharmacological treatment options, current treatment guidelines recommend trauma-focused psychotherapies as the first-line treatment option for youth with PTSD (NICE, 2018). Multiple randomized controlled trials have shown efficacy of both trauma-focused cognitive behavioral therapy (TF-CBT) and Eye Movement and Desensitization and Reprocessing (EMDR) in youth with PTSD (Morina et al., 2016; Xiang et al., 2021). However, both treatments achieve only small to moderate effects sizes, as treatment response varies considerably among individuals, with 30-50% of youth not benefiting sufficiently (Diehle, Opmeer, Boer, Mannarino, & Lindauer, 2015; Gutermann, Schwartzkopff, & Steil, 2017). Treatment non-response is associated with prolonged suffering, longer treatment trajectories, demoralization and hopelessness (Dimidjian & Hollon, 2010). Although, different pre-treatment clinical and demographic factors have been associated with trauma-focused psychotherapy outcome, none have shown to reliably predict treatment response (Goldbeck, Muche, Sachser, Tutus, & Rosner, 2016). Clinical decisions making thus remains mostly based on trial-and-error, leaving a substantial portion of children unrecovered even with access to evidence-based trauma-focused psychotherapy. This underlines the need for strategies to improve treatment allocation and enhance efficacy of available evidence-based trauma-focused psychotherapies in youth with PTSD.

How can clinical neuroscience contribute to improve treatment outcome in PTSD?

Clinical neuroscience builds upon the assumption that advancing the understanding of the neurobiological mechanisms underlying mental health disorders and treatment response can eventually contribute to improve treatment efficacy (Linden, 2006). In PTSD, this line of research has focused on two different strategies. First, increasing efficacy by improving insight in the biological mechanisms underlying PTSD and treatment response, to develop novel treatment strategies or tailor existing treatments to (better) target the alternations associated with PTSD or treatment response (Cisler & Herringa, 2021). Second, identifying predictive biomarkers of treatment response, to enhance treatment efficacy by improving allocation to existing evidence based treatments (Paul Zhutovsky, 2021). This approach holds the promise of progressing from trial-and-error based treatment allocation to personalized treatment selection, in which selection of the treatment with the highest chance of response in the individual patient is based on pretreatment biomarkers (Cohen, Zantvoord, Wezenberg, Bockting, & van Wingen, 2021; P. Zhutovsky et al., 2019).

Alternations in brain structure and functional connectivity associate with PTSD and trauma-focused psychotherapy response

Regarding the former, research in PTSD has mainly focused on identifying dysfunctions in brain structure, activity and connectivity as well as autonomic nervous system (ANS) and hypothalamic-pituitary-adrenal (HPA) axis activity. Meta-analyses of structural neuroimaging studies found reduced grey-matter volume (GMV) of the hippocampus, ventromedial prefrontal cortex (vmPFC) including the anterior cingulate cortex (ACC) and insula in adults with PTSD relative to trauma exposed controls (Bromis, Calem, Reinders, Williams, & Kempton, 2018; Logue et al., 2018). These structures are strongly interconnected and are key nodes in multiple large-sale brain networks, particularly in the triple network model consisting of the salience network (SN), default mode network (DMN) and central executive network (CEN) (Vinod Menon, 2011; Vinod Menon & Uddin, 2010). Functional neuroimaging studies in adults with PTSD utilizing (resting state) functional MRI (rs-fMRI) have also found functional alterations in these networks (Wang et al., 2016). Adults with PTSD are characterized by decreased functional connectivity within the DMN, a network that is associated with internally focused thought as well as autobiographical memory (Vinod Menon, 2011; Sripada et al., 2012). Also, patients with PTSD demonstrated increased connectivity within the SN, a network that is responsible for detecting and orienting to salient stimuli and has a central role in fear conditioning and extinction learning, which are impaired in PTSD (Careaga, Girardi, & Suchecki, 2016; Amit Etkin & Wager, 2007). Finally, adults with PTSD demonstrated dysfunctional between

network connectivity between the DMN and CEN which are normally anti-correlated and involved in attentional control and behavioral response (Bressler & Menon, 2010; Daniels et al., 2010).

Although, evidence on structural and functional brain alternations associated with PTSD has accumulated rapidly over the past decades, longitudinal studies addressing the relationship between treatment response and brain structure as well as large-scale brain network connectivity remain relatively scarce (Colvonen et al., 2017; Pagani, Carletto, & Cavallo, 2021; Quidé, Witteveen, El-Hage, Veltman, & Olff, 2012). Longitudinal studies on brain structure have focused primarily on the hippocampus, with most (Laugharne et al., 2016; Lindauer et al., 2005; Van Rooij et al., 2015), finding no change with trauma-focused psychotherapy, while the few studies that have looked beyond hippocampus volume, showed amygdala (Laugharne et al., 2016), and PFC volume increase as well as ACC volume decrease (Boukezzi et al., 2017; Helpman et al., 2016) from pre- to posttreatment. Findings from longitudinal functional neuroimaging studies suggest that activity and connectivity within regions and networks involved in fear conditioning and extinction learning, (working) memory as well as emotional processing and modulation could be adaptively attenuated with successful trauma-focused psychotherapy (Fonzo et al., 2021; Korgaonkar et al., 2020; Lindauer et al., 2005).

Alternations in HPA axis and autonomous nervous system function associated with PTSD and trauma-focused psychotherapy response

As major stress regulating systems, both the HPA axis with its end product cortisol and the ANS, have also had considerable attention in PTSD research (Morris, Compas, & Garber, 2012; Pole, 2007). Although, findings regarding HPA function have been mixed, the majority of studies indicate lower 24-h cortisol secretion, flattened (morning) cortisol diurnal rhythms and enhanced glucocorticoid receptor negative feedback in adults with PTSD (Morris et al., 2012). Regarding ANS function, a mounting body of literature indicates alternations in both the sympathetic and parasympathetic branches of the ANS and their interaction in adults with PTSD (Michopoulos, Norrholm, & Jovanovic, 2015). Research suggests an overall lower parasympathetic activity (i.e. increased parasympathetic withdrawal) and heightened SNS activity in adults with PTSD (Schneider & Schwerdtfeger, 2020) reflected by increased HR (Pole, 2007), decreased heart rate variability (Campbell, Wisco, Silvia, & Gay, 2019) and heightened sympathetic indices (Keane et al., 1998; Michopoulos et al., 2015; Sheikh et al., 2022) during stress. Despite the large body of evidence on the relationship between HPA-axis and ANS function and PTSD, a limited number studies has investigated the prospective association between HPA-axis function as well as ANS function and treatment response (Colvonen et al., 2017; Schumacher, Niemeyer,

Engel, Cwik, & Knaevelsrud, 2018). Most of these studies found an association between trauma-focused psychotherapy response and reduction of heart rate and blood pressure (Zantvoord, Diehle, & Lindauer, 2013). The few studies that examined the association between treatment outcome and specific measures of PNS or SNS activity did not find an association between treatment response and PNS or SNS activity (D'Andrea & Pole, 2012; Sack, Lempa, Steinmetz, Lamprecht, & Hofmann, 2008). Regarding HPA-axis functioning, there is evidence that suggests an association between high pre-treatment cortisol (reactivity) and treatment response (Rapcencu, Gorter, Kennis, van Rooij, & Geuze, 2017; Rauch et al., 2015). Moreover, preclinical studies have shown enhancing effects of glucocorticoids (i.e. cortisol) on extinction learning (de Quervain et al., 2011).

Together, these studies have improved the understanding of the biological mechanisms underlying PTSD and treatment response and have enabled development of novel treatments targeting these mechanisms. Importantly, in adults this has indeed provided promising augmentation treatments to trauma-focused psychotherapy. For instance, repeated transcranial magnetic stimulation (rTMS) targeting the CEN trough dorsolateral prefrontal cortex (DLPFC) stimulation, the noradrenergic beta-receptor blocker propranolol targeting ANS function and glucocorticoid (i.e. cortisol) augmentation targeting HPA axis function have all shown efficacy in randomized controlled trials (Aerni et al., 2004; Brunet et al., 2018; de Quervain et al., 2011; Kan, Zhang, Zhang, & Kranz, 2020).

Predictive biomarkers of trauma-focused psychotherapy

Besides aiding in the identification of novel treatment targets, clinical neuroscience holds the promise of identifying predictive biomarkers of treatment response, that could guide treatment allocation to enhance treatment efficacy. Currently, no reliable markers are available to guide treatment allocation in PTSD. Previous studies in PTSD have traditionally used univariate data analysis methods to identify average differences between groups (i.e. responders vs. non-responders) or examine the association between biological data from baseline to a different variable (e,g, clinical improvement) at follow-up within the same sample. However, these strategies do not provide information for individual patients and may not generalize to new data (Arbabshirani, Plis, Sui, & Calhoun, 2017), which is necessary to allow clinicians to inform patients and to assist in clinical decision making. They have also ignored the possibility that a biomarker may predict response to one but not another treatment, hence precluding development of specific biomarkers for different treatments (Paul Zhutovsky, 2021). Predictions for individual patients can be made using multivariate supervised machine learning (ML) analysis which directly assesses generalization to new patients by means of cross-validation. Several studies have

utilized ML methods and resting-state functional magnetic resonance imaging (rs-fMRI) to predict treatment-response in adults with PTSD, with accuracies ranging between 71-90% (A. Etkin et al., 2019; Korgaonkar et al., 2020; P. Zhutovsky et al., 2019). The overall classification performance in these cross-validated studies can be considered substantial, which underscores the promise for potential clinical application. However, study samples were small and none of the studies employed out of sample validation which reduces generalizability and raise the possibility of inflated accuracy results through overfitting, hampering translation of research findings to clinical practice.

Why have youth with PTSD not benefited from recent developments in clinical neuroscience?

Despite the increased understanding of the underlying biological mechanisms of PTSD and treatment response and the identification of potential predictive biomarkers, treatment efficacy in youth with PTSD has not improved over the past decades. The paucity of neurobiological studies performed in youth and the inability to readily extrapolate neurobiological findings from adults to youth offer an explanation for this lack of progress. Extrapolation of results obtained in adults to youth with PTSD is impeded by ongoing development within brain structure and connectivity as well as HPA axis and ANS function throughout childhood and adolescence (Casey, Giedd, & Thomas, 2000; Giedd & Rapoport, 2010; Harteveld et al., 2021; Russell D Romeo & McEwen, 2006).

Brain structures associated in PTSD and trauma-focused treatment response in adults undergo substantial maturation throughout childhood and adolescence; with hippocampus volume increase in girls, amygdala volume increase in boys, as well as late maturation of the prefrontal cortex and insula volume increase in both girls and boys (Dennis et al., 2014; Lenroot & Giedd, 2006; Shaw et al., 2008; Tamnes et al., 2017; Zantvoord, Lindauer, Bakker, & Boer, 2013). The development of these brain structures coincide with considerable reorganization of large-scale brain networks involved in PTSD (e.g. DMN, SN and CEN) throughout childhood and adolescence (Weems, Russell, Neill, & McCurdy, 2019). Developmental change in large-scale brain organization is characterized by stronger within network connectivity and more efficient between-network connectivity, with a trend towards segregation (decrease in connectivity strength) between regions in close proximity and integration (increase in connectivity strength) between anatomically distant regions (V. Menon, 2013). Importantly, the ANS also undergoes substantial development throughout childhood and adolescence. PNS-activity follows a cubic trend, with an exponential increase from infancy, a plateau phase during middle childhood, followed by a decrease to

adolescence (Harteveld et al., 2021). SNS-activity shows a more linear trend, with a gradual decrease from infancy to adolescence (Harteveld et al., 2021). Although, basal levels of cortisol only vary slightly during the transition from childhood to adolescence and adulthood, there are distinct developmental changes in HPA axis stress reactivity (Elmlinger, Kühnel, & Ranke, 2002; Russell D Romeo & McEwen, 2006). Relative to adults, youth show a significantly extended cortisol stress response in which cortisol levels take longer to return to baseline after a stressor has been terminated (R. D. Romeo, Lee, & McEwen, 2004). Also, due to the shift in gonadal hormones during adolescents and the moderating effects of these gonadal hormones on the HPA function, adolescence is the period in which sex-differences in HPA axis functioning emerge, with testosterone reducing HPA axis stress reactivity in males and estradiol and progesterone typically heightening the stress response in females (R. D. Romeo, 2010).

These developmental processes provide a potential explanation for some of the contrasting neurobiological findings between youth and adults with PTSD which have been identified. For instance, cross-sectional studies using MRI in youth with PTSD did not find reduced hippocampal volume typical for adult PTSD and reported larger vmPFC volumes opposing findings in adult PTSD (Victor G Carrion et al., 2009; Keding & Herringa, 2015; Morey, Haswell, Hooper, & De Bellis, 2016). Also, Patriat and colleagues found that pediatric PTSD is characterized by increased connectivity within the DMN, contrasting the finding of decreased connectivity within the DMN in adults with PTSD (Patriat, Birn, Keding, & Herringa, 2016). Regarding HPA function, youth with PTSD have shown increased 24-h cortisol secretion and normal glucocorticoid receptor negative feedback (De Bellis et al., 1999; Duval et al., 2004; Lipschitz et al., 2003), opposing results in adults with PTSD. Contrary to results in adults, decreased SNS activity at rest corresponded to increased posttraumatic stress symptoms and the correlation between PNS measures and PTSD was non-significant (Siciliano, Anderson, & Compas, 2022). These divergent neurobiological findings between youth and adults with PTSD might indicate developmental effects that are not yet apparent until adulthood as well as plastic response to illness over time (Keding & Herringa, 2015; Weems et al., 2019). Importantly this also shows that results obtained in adults with PTSD cannot be readily extrapolated to youth with PTSD, explaining, in part, why youth with PTSD have not been able to benefit from progress made in clinical neuroscience in adults.

Together, this emphasizes the knowledge gap regarding developmentally informed neurobiological treatment outcome studies in youth with PTSD, and highlight the need for studies specifically designed to improve understanding of neurobiological mechanisms of trauma-focused psychotherapy and enhance treatment response prediction in youth with PTSD.

Outline of this thesis

The aim of the current PhD-thesis is therefore to investigate neurobiological measures and their relationship with trauma-focused psychotherapy outcome in youth with PTSD, in order to improve understanding of treatment mechanisms and successfully predict treatment response.

In part I of this thesis we address this aim by conducting a study examining the association between pretreatment cortisol and trauma-focused psychotherapy response in youth with PTSD (**chapter 2**). We hypothesize that, relative to psychotherapy non-responders treatment, responders display higher cortisol levels during script-driven imagery prior to eight sessions of trauma-focused psychotherapy and that higher pretreatment cortisol levels is associated with larger clinical improvement. In **chapter 3**, we perform a proof-of-concept study examining the utility of pretreatment rs-fMRI data together with machine learning to predict trauma-focused psychotherapy response in youth with PTSD on both the group- and individual-level.

In part II we describe a systematic review of neurobiological treatment outcome studies in PTSD **(chapter 4),** summarizing the effects of trauma-focused psychotherapies on brain structures and function, ANS and HPA axis as well as reviewing neurobiological treatment outcome prediction studies in PTSD. In **chapter 5** we use repeated structural MRI along with voxel based morphometry (VBM) in youth with PTSD to compare brain-wide structural changes in trauma-focused psychotherapy responders relative to non-responders, we hypothesize that treatment response is associated with volume increases in brain areas associated with PTSD, while non-response is characterized by volume decreases in these areas. In **chapter 6** we investigate the association between ANS function and PTSD and examine the relationship between trauma-focused psychotherapy response and changes in ANS in youth with PTSD. We hypothesize that youth with PTSD relative to TEC show an overall increased ANS stress reactivity as indicated by greater increase of SNS reactivity together with a greater vagal withdrawal during script driven imagery and hypothesize that trauma-focused psychotherapy response is associated with a normalization of ANS stress reactivity. In the final chapter **(chapter 7)**, we will summarize the main results of this thesis and in **chapter 8** integrate our findings into a neurodevelopmental framework.

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CHAPTER 2

Pretreatment cortisol predicts trauma focused psychotherapy response in youth with (partial) posttraumatic stress disorder

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Psychoneuroendocrinology 2019; 109: 104380

ABSTRACT

Background: Despite availability of effective trauma-focused psychotherapies, treatment non-response in youth with (partial) posttraumatic stress disorder remains substantial. Studies in adult PTSD have suggested that cortisol is associated with treatment outcome. Furthermore, cortisol prior to treatment could be used to predict treatment success. However, there is a lack of comparable studies in youth with (partial) PTSD. The objective of the current study was therefore to test whether cortisol prior to treatment would differ between treatment responders and non-responders and would positively predict the extent of clinical improvement in youth with (partial) PTSD.

Methods: Youth aged 8-18 with PTSD (79.2%) or partial PTSD (20.8%) were treated with 8 sessions of either trauma focused cognitive behavioral therapy (TF-CBT) or eye movement desensitization and reprocessing (EMDR). Prior to treatment initiation, salivary cortisol was measured in treatment responders (n = 23) and treatment non-responders (n = 30) at 10 and 1 min before and 10, 20 and 30 min after personalized trauma script driven imagery (SDI). The cortisol stress response (> 1.5 nmol/L increase from baseline) and basal cortisol secretion was assessed during the SDI procedure. We hypothesized that treatment responders would display higher cortisol levels caused by increased cortisol reactivity prior to trauma-focused psychotherapy relative to psychotherapy non-responders and higher cortisol levels would positively predict the extent of clinical improvement.

Results: Script driven imagery did not induce a cortisol stress response in all but two participants. Prior to treatment responders showed significantly higher basal cortisol secretion during SDI compared to treatment non-responders. This effect remained significant after controlling for gender. Higher pre-treatment basal cortisol secretion further positively predicted the extent of clinical improvement during trauma-focused psychotherapy.

Conclusion: Because SDI failed to provoke a cortisol stress response in our sample, the question if cortisol reactivity differs between treatment responders and non-responders remains inconclusive. However, our results do suggest that higher pretreatment basal cortisol secretion forms a potential indicator of prospective trauma focused psychotherapy response in youth with (partial) PTSD. Although, the amount of uniquely explained variance in clinical improvement by pre-treatment cortisol secretion is limited and still renders insufficient basis for clinical applicability, these findings do suggest directions for future studies to delineate the mechanisms of treatment success in youth with (partial) PTSD

INTRODUCTION

Posttraumatic stress disorder (PTSD) is a mental health disorder that develops in approximately 16% of youth exposed to traumatic events, which may include domestic violence, sexual abuse and accidents (Alisic et al., 2014). The daily lives of youth with PTSD are hampered by frequent re-experiencing of the traumatic event, persistent avoidance, hyperarousal and negative alterations in cognition and mood (AmericanPsychiatricAssociation, 2013). These symptoms can interfere with social functioning and school performance and have a deeply negative effect on the quality of life of the affected youth and those around them (Carrion, Weems, Ray, & Reiss, 2002). Moreover, they are a crucial factor in shaping the vulnerability to depression and suicidality later in life (Molnar, Berkman, & Buka, 2001). Furthermore, youth exposed to traumatic events with clinically important symptoms of PTSD but with subthreshold criteria for PTSD (partial PTSD) also demonstrate substantial functional impairment and distress which do not differ significantly from youth meeting full PTSD criteria (Carrion et al., 2002). Current practice parameters therefore recommend that in youth, patients with partial PTSD should also be provided with evidence-supported treatment options ("Post-traumatic stress disorder," National Institute for Health and Care Excellence 2018). All of the above highlight the vital importance of effective treatment for youth with (partial)PTSD.

Although, multiple randomized controlled trials have shown efficacy of trauma focused psychotherapies in youth with (partial) PTSD, treatment non-response remains a major clinical challenge (Gillies, Taylor, Gray, O'Brien, & D'Abrew, 2012; Leenarts, Diehle, Doreleijers, Jansma, & Lindauer, 2013). Treatment non-response leads to persistent symptoms, prolonged suffering and longer treatment trajectories. Objective and reliable predictive markers of treatment response are needed to guide treatment selection and improve treatment efficacy. Investigation of biological factors associated with effective treatment may help to identify biomarkers of treatment response (J. B. Zantvoord, Diehle, & Lindauer, 2013).

As the major neuroendocrine stress regulating system, the hypothalamic-pituitary-adrenal (HPA) axis has had considerable attention in PTSD research (Morris, Compas, & Garber, 2012). Cortisol, the end product of HPA-axis activation, plays a central role in an individual's response to threat. Cortisol promotes survival by mobilizing energy resources contributing to a state of increased vigilance and arousal. Through a negative feedback system, cortisol terminates the stress response by inhibiting the HPA-axis through binding to the glucocorticoid receptors. Furthermore, cortisol is crucially involved in (emotional) memory consolidation, retrieval of information from long-term memory, memory reconsolidation and extinction learning. These learning

and memory processes have been shown pivotal in PTSD and may be involved in trauma focused psychotherapies (de Quervain, Schwabe, & Roozendaal, 2017).

Findings regarding HPA function in adults with PTSD have been mixed. Meta-analyses of Morris and colleagues showed enhanced glucocorticoid receptor negative feedback, lower 24-h cortisol secretion and flattened (morning) cortisol diurnal rhythms in PTSD patients compared to (non-traumatized) controls (Morris et al., 2012). However, two other meta-analyses did not show differences in HPA function between PTSD patients and (traumatized) controls (Klaassens, Giltay, Cuijpers, van Veen, & Zitman, 2012; Meewisse, Reitsma, de Vries, Gersons, & Olff, 2007). Finding from these and other meta-analyses do highlight the importance of age, gender, comorbid depression, time since trauma, type of control group (trauma exposed or non-trauma exposed), differences in assessment method and timing of trauma exposure as moderators of HPA function in PTSD patients (Doom, Cicchetti, Rogosch, & Dackis, 2013; Friedman, Jalowiec, McHugo, Wang, & McDonagh, 2007; Klaassens et al., 2012; Kuhlman, Geiss, Vargas, & Lopez-Duran, 2015; Meewisse et al., 2007; Morris et al., 2012; Schumacher et al., 2019).

Studies on HPA function in youth suffering from posttraumatic stress symptoms are altogether scarce. They pose additional methodical challenges because of heterogeneity associated with ongoing developmental change in HPA function throughout childhood and adolescence (Lupien, McEwen, Gunnar, & Heim, 2009). Furthermore, clinical heterogeneity is increased, as some studies only include youth with a full PTSD diagnosis while others also include patients with (partial) PTSD. Findings on HPA function in youth with (partial)PTSD are therefore not necessarily in line with findings in adults (Kirsch, Wilhelm, & Goldbeck, 2011). For instance, youth with (partial)PTSD have shown increased 24-h cortisol secretion and normal glucocorticoid receptor negative feedback (De Bellis et al., 1999; Duval et al., 2004; Lipschitz et al., 2003). Together, these findings suggest that results on HPA function obtained in adults with PTSD cannot be readily extrapolated to youth with (partial) PTSD. This emphasizes the need for studies on HPA function specifically performed in youth.

Interestingly, relatively few prospective studies have investigated the association between HPA function and the course of illness or treatment efficacy (Schumacher, Niemeyer, Engel, Cwik, & Knaevelsrud, 2018; J. B. Zantvoord et al., 2013). Studies in adults with PTSD suggest lower cortisol levels in the acute aftermath of trauma exposure to be associated with an increased risk of developing PTSD over time (Walsh et al., 2013). Furthermore, lower hair cortisol concentrations and lower cortisol stress reactivity before deployment were predictive of a greater PTSD symptom increase in soldiers who had experienced new-onset traumatic events (Steudte-Schmiedgen

et al., 2015). In youth, some studies have also found decreased afternoon cortisol in the aftermath of trauma to be associated with an increased risk of developing post-traumatic stress symptoms (Pfeffer, Altemus, Heo, & Jiang, 2007), while others have indicated increased afternoon cortisol to be predictive of symptom development (Pervanidou et al., 2007). The association between cortisol and treatment outcome was examined in adults with PTSD, for a recent and comprehensive overview see Schumacher et al. 2018 (Schumacher et al., 2018). Studies on basal cortisol secretion prior to treatment as a predictor of treatment outcome were mostly negative (Nijdam, van Amsterdam, Gersons, & Olff, 2015; Rauch et al., 2015; Yehuda et al., 2014). However, increased cortisol reactivity prior to treatment was shown to be predictive of PTSD symptom reduction, both in the form of the cortisol awakening response (*CAR*) (Pacella, Feeny, Zoellner, & Delahanty, 2014; Rapcencu, Gorter, Kennis, van Rooij, & Geuze, 2017), suppression of the *CAR* by dexamethasone challenge and activation by script-driven imagery (Nijdam et al., 2015; Rauch et al., 2015). Together these findings suggest that in adult PTSD, higher cortisol reactivity and not basal cortisol secretion is a predictive biomarker of treatment response. However, the number of studies is still small and heterogeneity in design considerable, precluding a strong synthesis of the current evidence.

Surprisingly however, no prior study has specifically examined the relationship between HPA function and treatment outcome in youth with (partial)PTSD. Thus, it is unknown whether HPA function in general and cortisol in particular prior to treatment is predictive of treatment response. Therefore, we examined the association between salivary cortisol prior to treatment and trauma-focused psychotherapy outcome in youth with (partial)PTSD, while accounting for relevant clinical and demographic confounders. First, we hypothesized that treatment responders would display higher cortisol levels caused by increased cortisol reactivity prior to trauma-focused psychotherapy relative to psychotherapy non-responders. Furthermore, we hypothesized that higher pretreatment cortisol levels would positively predict the extent of clinical improvement in youth with (partial)PTSD.

METHODS

Participants

Participants were recruited between April 1, 2011 and June 1 2017 at the outpatient child psycho-trauma center of the department of child and adolescent psychiatry, de Bascule as part of a larger randomized controlled trial on efficacy of trauma-focused psychotherapies in youth with (partial) PTSD (Diehle, Opmeer, Boer, Mannarino,

& Lindauer, 2015). Youth were referred for assessment and treatment by child welfare services, physicians or their general practitioner. Youth were eligible when fulfilling the following inclusion criteria: age of 8 through 18 years, mastery of the Dutch language and a PTSD or partial PTSD diagnosis. PTSD was diagnosed by a clinician according to the DSM-IV criteria using both child reports on the Clinician-Administered PTSD Scale for Children and Adolescents (CAPS-CA) semi-structured interview and caregiver reports on the PTSD scale of the Anxiety Disorders Interview Schedule – Parent Version (ADIS-P). Partial PTSD was defined as either fulfilling two of the three PTSD symptom clusters or one symptom present in each of the three symptom clusters (Stein, Walker, Hazen, & Forde, 1997). Exclusion criteria were acute suicidality, IQ < 70, pregnancy, meeting the criteria of one of the following diagnosis (assessed using the relevant screening questions of the ADIS child and parent versions): psychotic disorders, substance use disorder or pervasive developmental disorder, presence of an endocrine disorder or other significant medical illness potentially influencing HPA function, the use of oral glucocorticoid medication or other medication potentially influencing HPA function within the 2 weeks before study entry (Stalder et al., 2016). In accordance with procedures approved by the Institutional Review Board of the Amsterdam University Medical Center and the declaration of Helsinki, written informed consent was obtained from all parents or legal guardians and youth aged 12 years and over. For youth aged 11 and under assent from the youth was obtained. All participants received a monetary incentive.

Clinical assessment

After study-entry and after eight sessions of trauma focused treatment, all participants were repeatedly assessed with a semi-structured clinical interview and self-report questionnaires. Semi-structured interviews were administered by trained psychologists.

PTSD diagnosis and PTSD severity were confirmed using the Clinician-Administered PTSD Scale for Children and Adolescents (CAPS-CA) semi-structured interview (Nader et al., 1996). To complement PTSD diagnosis based on youth's reports, we administered the PTSD scale of the Anxiety Disorders Interview Schedule – Parent Version (ADIS-P) (Verlinden et al., 2014). Parents or caregivers rated every symptom of PTSD as either absent or present. The CAPS-CA assesses all possible symptoms of PTSD according to DSM-IV-TR and has become the gold standard for the assessment of pediatric PTSD. For every symptom, frequency of occurrence was rated from 0 to 4 (0 = never in the past month, 4 = four or more times a week in the past month). Intensity was also rated from 0 to 4 (0 = not a problem, 4 = extreme). A PTSD case was identified if participants met the requisite DSM-IV symptoms at least at a

frequency of 1 and at an intensity of 2 (Frank W Weathers, Ruscio, & Keane, 1999). Partial PTSD was identified with the same "frequency at least 1 and intensity at least 2" scoring rule by either fulfilling two of the three PTSD symptom clusters or one symptom present in each of the three symptom clusters (Stein et al., 1997). In addition, a suffering score of at least 4 on a 0-to-8 scale (0 = no impairment, 8 = a lot of impairment) was needed for a participant to fulfill a full or partial PTSD diagnosis. The overall PTSD severity score on the CAPS-CA was measured by adding the total frequency and intensity score and could range between minimal (<20) and extreme (80–136). The Dutch version of the CAPS-CA has shown good psychometric properties in a sample overlapping the current sample (Cronbach's alpha's: 0.62-0,83; ICC for interrater reliability: 0.97-0.99) (Diehle, de Roos, Boer, & Lindauer, 2013; Diehle et al., 2015). Both CAPS-CA and ADIS-P were administered by trained psychologists.

Depressive and anxiety symptoms were assessed using the subscales of the Dutch Revised Child Anxiety and Depression Scale (RCADS), a 47 item questionnaire that measures the reported frequency of various symptoms of social phobia (SP), obsessive compulsive disorder (OCD), generalized anxiety disorder (GAD), panic disorder (PD), separation anxiety disorder (SAD) and depression (MDD) (Chorpita, Yim, Moffitt, Umemoto, & Francis, 2000). Both youth (RCADS) and their caregiver (RCADS-P) filled out the questionnaires. Items were scored on a four-point Likert scale ranging from 0 (never) to 3 (always). Cronbach's alpha's for the Dutch RCADS (-P) were 0.93 for the pediatric version and 0.90 for the parent version.

Script driven imagery (SDI) and cortisol collection

All study participants performed a standardized protocol for script driven imagery (SDI) (Shalev, Orr, & Pitman, 1992) within two weeks before the start of trauma-focused psychotherapy. Youth were instructed to abstain from intensive physical exercise the day before and during the day of SDI. Also, youth were instructed to abstain from eating, drinking (except water) and smoking 3 hours before SDI and during the remaining sampling period. Compliance to the instructions was checked by the examiner before the start of the SDI procedure, in case of non-compliance the procedure was postponed. During the previous CAPS-CA interview all participants provided a detailed contextual description of their most disturbing traumatic event, including physical sensations that were experienced during the event. This information was used to compose and record a 30 s (range 29 - 31 s) personalized trauma audio script prior to the SDI procedure. A general 30 s neutral audio script describing tooth brushing was also composed and recorded. Both scripts were recorded in present tense by a member of the research team with corresponding sex as a given participant. The SDI procedure was performed on weekdays, at the end of morning or early afternoon (Table 1), in a room with constant temperature and light

exposure. During the SDI procedure the neutral script always preceded the trauma script condition, both scripts were preceded by a three min movie clip depicting neutral landscapes. Participants were instructed to vividly imagine the events described in the scripts with their eyes closed during and 30 s after the scripts. For the remainder of the cortisol sampling period participants remained seated in the examination room together with the examiner while reading or engaging in neutral conversation with the examiner.

Five saliva samples were collected using Salivettes (Sarstedt Inc. Newton, NC, USA) 10 min and 1 min prior to trauma script imagery as well as 10 min, 20 min, and 30 min after trauma script imagery. Salivettes were stored at -20 °C at the Amsterdam University Medical Center until transport to Universitaet Trier for processing and analysis. After transport samples were centrifuged at 2000 g for 10 min. Duplicate analysis was executed using 100ul saliva. Salivary free cortisol concentrations were determined using a competitive solid phase time-resolved fluorescence immunoassay with fluorometric endpoint detection (DELFIA) (Dressendorfer, Kirschbaum, Rohde, Stahl, & Strasburger, 1992). The intra-assay coefficient of variation was between 4.0% and 6.7%, and the corresponding inter-assay coefficients of variation were between 7.1 -9.0%.

Table 1 Subject characteristics.

	Responders (*n*= 23)	**Non-responders (*n*= 30)**	***p*-value[a]**
Sociodemographic characteristics			
Female (%)	43.5	66.7	**.091**
Age (years; mean, SE)	12.3 (0.64)	12.6 (0.54)	.686
West European Ethnicity (%)	45.8	42.8	.509
Current educational level (%)			.614
Elementary school	56.2	43.3	
Middle/High school lower level	8.7	3.3	
Middle/High school middle level	21.7	40.0	
Middle/High school higher level	8.7	6.7	
Vocational school	4.3	6.7	
Weight (kg; mean, SE)	49.2 (5.19)	50.2 (2.27)	.863
Current psychotropic medication (%)	8.7	10	.911
Smoking (%)	5%	4%	.697
Alcohol >1 consumption/day (%)	0%	0%	N/A
Time of cortisol assessment (hrs:min; mean, SE)	13:22 (0:28)	13:36 (0:27)	.712
Trauma characteristics			
Index trauma (%)			.178
Sexual abuse	34.7	20.0	
Domestic violence	21.7	13.3	
Community violence	13.0	33.3	
Accidents/Medical	17.4	6.7	
Other	13.0	26.7	
Repeated trauma exposure (%)	43.5	56.6	.341
Age at index trauma (years; mean, SE)	8.4 (0.97)	9.2 (0.73)	.525
Time since index trauma (years; mean, SE)	3.9 (0.81)	3.4 (0.62)	.669
Clinical characteristics			
CAPS-CA study entry (mean, SE)[b]			
Total	46.3 (4.26)	52.7 (4.73)	.322
Re-experiencing	13.9 (1.92)	18.1 (2.17)	.154
Avoidance	17.7 (1.83)	18.8 (2.13)	.707
Hyperarousal	14.9 (1.84)	17.2 (1.84)	.272
Full PTSD diagnosis (%)	78.3	80.0	.877
RCADS study entry (mean, SE)[b]			
MDD	10.1 (1.31)	12.0 (1.21)	.301
GAD	6.9 (1.02)	6.8 (0.75)	.936
OCD	5.4 (0.67)	6.7 (0.69)	.205
PD	6.7 (1.30)	8.4 (1.06)	.305
SAD	5.5 (0.90)	6.2 (0.89)	.589
SP	10.3 (1.44)	11.9 (1.52)	.452

Abbreviations: CAPS-CA, Clinician-Administered PTSD Scale for Children and Adolescents; RCADS, Revised Child Anxiety and Depression Scale; MDD, major depressive disorder; GAD, general anxiety disorder; OCD, obsessive compulsive disorder; PD, panic disorder; SAD, separation anxiety disorder; SP, social phobia; SE, standard error.

[a] *p*-values <0.1 shown in bold. Independent samples t-test for continuous and x^2 tests for categorical variables.

[b] Ranges: CAPS-CA total, 0-139; RCADS MDD, 0-30; RCADS GAD, 0-18; RCADS OCD, 0-18; RCADS PD, 0-27; RCADS SAD, 0-21; RCADS SP, 0-27.

Treatment

After study-entry all patients were randomized to receive either trauma-focused cognitive behavioral therapy (TF-CBT) or Eye movement desensitization and reprocessing (EMDR). In a previous manuscript we showed that TF-CBT and EMDR are effective in youth with (partial) PTSD with no significant differences in efficacy between TF-CBT and EMDR (Diehle et al., 2015). Both TF-CBT and EMDR consisted of eight sessions with 60-min duration, which were given on a weekly basis. Treatment was delivered by experienced therapists who were all trained in TF-CBT and EMDR before the initiation of the study. Supervision by experts on TF-CBT and EMDR were provided throughout the study. In short, the EMDR treatment protocol consisted of the following components: psychoeducation and preparation, assessment, desensitization, installation, body scan, positive closure and enhancing future safety (Shapiro, 2001). TF-CBT consisted of psychoeducation and parenting skills, relaxation, affective modulation, cognitive coping and processing, trauma narrative, in vivo mastery of trauma reminders, conjoint child-parent sessions, enhancing future safety and development (Deblinger, Mannarino, Cohen, Runyon, & Steer, 2011).

Pre- to posttreatment symptom change was calculated by subtracting the baseline from the posttreatment CAPS-CA total score (ΔCAPS-CA) for each participant. There is no established definition of a response criterion or a consensus definition of response terms in the child trauma treatment field. Based on the psychometric properties of the CAPS(-CA) and previous treatment outcome studies using the CAPS-CA, a tentative response criterion of >50% reduction of CAPS-CA total score was a priori adopted as reflecting a substantial and clinically meaningful improvement (Diehle et al., 2013; F. W. Weathers, Keane, & Davidson, 2001). We chose not to employ presence of a PTSD diagnosis at posttreatment (e.g. remission) as an outcome measure, as patients with partial PTSD did not meet full PTSD diagnostic criteria at baseline which would make it impossible for these patients to 'improve'.

Data cleaning and handling of missing data

Cortisol data were positively skewed and therefore log-transformed to approximate a normal distribution. Extreme outlying cortisol values were identified as log-transformed values that were located more than three standard deviations from the mean (Stalder et al., 2016). No outlying cortisol values were identified.

Data for missing cortisol concentrations was interpolated by using the mean value of the preceding and following sample concentrations (Yehuda, Golier, & Kaufman, 2005). In the case that the first or last sample was missing, the second and fourth samples were used respectively. In total 15 out of 265 (5,7%) cortisol concentrations

were missing and interpolated. The number of missing cortisol concentrations did not differ between responders and non-responders ($p > 0.10$).

Statistical analyses

Statistical analyses were performed using SPSS version 24.0 (SPSS Inc., Chicago IL, USA). In order to detect potential confounders, the distribution of characteristics at study entry across responders and non-responders was examined using two-tailed X^2-tests for categorical variables, independent sample *t*-tests for normally distributed continuous variables and Mann-Whitney test for non-normally distributed continuous variables. In case of significant group differences, variables were subsequently included as covariates.

The cortisol stress response was defined as an increase of at least 1.5 nmol/l compared to baseline levels. For each participant we determined if SDI induced a cortisol stress response (Miller, Plessow, Kirschbaum, & Stalder, 2013). Area under the curve with respect to ground (AUCg) and increase (AUCi) were derived using the trapezoidal formulas (Pruessner, Kirschbaum, Meinlschmid, & Hellhammer, 2003). As the SDI failed to induce a cortisol stress response in all but two participants, we did not use the AUCi as measure of cortisol responsivity in further analyses. The AUCg was used as a measure of total cortisol output which captures basal cortisol secretion as opposed to stressor reactivity. We therefore excluded the two participants who did have a cortisol stress response during SDI from further analysis. AUCg was examined for normality of distribution within each group. Non normally distributed data were log-transformed.

To test our first hypothesis, a ANCOVA was conducted to determine group differences between responders and non-responders on log- transformed AUCg, gender was added as an additional covariate. To test the robustness of findings a sensitivity analysis was performed with a more lenient response criterion (> 30% instead of > 50% reduction in CAPS-CA total scores).

Furthermore, a sequential multiple regression analysis was performed in order to test the second hypothesis that cortisol can uniquely predict treatment response. First, we examined zero-order correlations of the treatment outcome variable (posttreatment CAPS-CA), with the log- transformed cortisol AUCg as well as with a priori selected putative confounders of treatment outcome (baseline CAPS-CA score, baseline RCADS-MDD scores, age, gender and type of index trauma) to test for (multi)collinearity using Pearson's r (Deblinger, Mannarino, Cohen, & Steer, 2006; Goldbeck, Muche, Sachser, Tutus, & Rosner, 2016). There was an intercorrelation between baseline CAPS-CA and RCADS-MDD scores ($r(48) = 0.71$ $p < .001$). To prevent multicollinearity RCADS-MDD score was excluded as a covariate in the subsequent

regression analysis. Among the possible confounders pretreatment CAPS-CA total scores, age and gender were selected as most relevant. A sequential multiple linear regression model was constructed with posttreatment CAPS-CA as outcome measure, with gender, age and pretreatment CAPS-CA at the initial step and with log-transformed cortisol AUCg as predictor at the second step. Finally, because we included youth with a broad age range (8 - 18 years), we tested if age moderated the relationship between log-transformed cortisol AUCg and treatment outcome by adding the interaction term age x log- transformed cortisol AUCg in the final step (Aiken, West, & Reno, 1991; Lupien et al., 2009).

RESULTS

Participant characteristics

A summary of participant characteristics is shown in Table 1. Responders and non-responders did not differ in baseline sociodemographic, trauma or clinical characteristics apart from female to male ratio ($p < 0.10$), for which was controlled in further analyses. The time of cortisol assessment did not differ between responders and non-responders. At baseline 79.2% of participants met the full DSM-IV diagnostic criteria for PTSD, the remaining 20.8% met criteria for partial PTSD. The average baseline CAPS-CA score was $M = 49.9$ points, $SD = 23.7$, which is indicative of moderately severe PTSD. The most common index trauma was sexual abuse, followed by community violence and witnessing domestic violence.

Treatment outcome

Fig. 1 shows a flow diagram of participants. Of 70 patients randomized to EMDR or TF-CBT, 17 (24%) were lost to follow-up (reasons described in Fig.1). Treatment completers and non-completers did not differ in baseline sociodemographic, trauma or clinical characteristics and cortisol measures apart from age, in which treatment completers' mean age was lower ($M = 12.5$, $SD = 2.97$) compared to non-completers ($M = 14.8$, $SD = 2.80$); $t(69) = 2.90$, $p = 0.005$. Setting a cutoff of 50% reduction of CAPS-CA total score as the response criterion, overall treatment response rate was 43.4%. 23 participants were responders and 30 were non-responders. Using a 30% CAPS-CA reduction response criterion, overall response rate increased to 62.3%. Posttreatment mean total CAPS-CA scores improved ($M = 19.8$ points, $SD = 21.9$ $t(52) = 9.8$, $p < .001$).

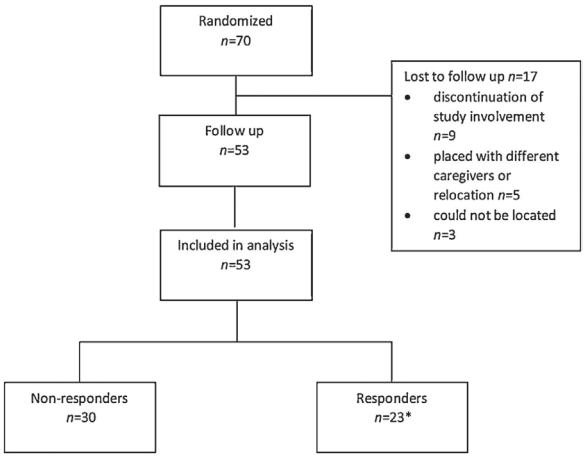

Fig. 1. Flow diagram of included patients.
* Response = >50% reduction in CAPS-CA total score

Salivary cortisol response during script driven imagery

Fig. 2 shows mean levels of cortisol measured 10 min and 1 min prior to trauma script imagery as well as 10 min, 20 min, and 30 min after trauma script imagery for both treatment responders and non-responders. All but two participants (one treatment responder and one treatment non-responder) failed to show a cortisol stress response (>1.5 nm/L salivary cortisol increase from baseline) during SDI. Both cortisol stress responders were excluded from further analyses.

Differences between treatment responders and non-responders in pretreatment salivary cortisol

An ANCOVA with log- transformed AUCg showed a significant effect of treatment response group after controlling for gender $F(1, 48) = 4.46$, $p = .040$, partial $\eta^2 = .085$, showing a higher pre-treatment log-transformed cortisol AUCg in responders ($M = 1.0$, $SD = 0.27$) compared to non-responders ($M = 0.85$, $SD = 0.25$).

A sensitivity analysis, using a more lenient response criterion (>30% CAPS reduction), also showed a significant effect of treatment response group $F(1, 48) = 5.50$, $p = .023$, partial $\eta^2 = .10$, showing a higher pre-treatment log transformed cortisol AUCg in responders ($n = 31$, $M = 0.98$, $SD = 0.29$) compared to non-responders ($n = 20$, $M = 0.81$, $SD = 0.19$).

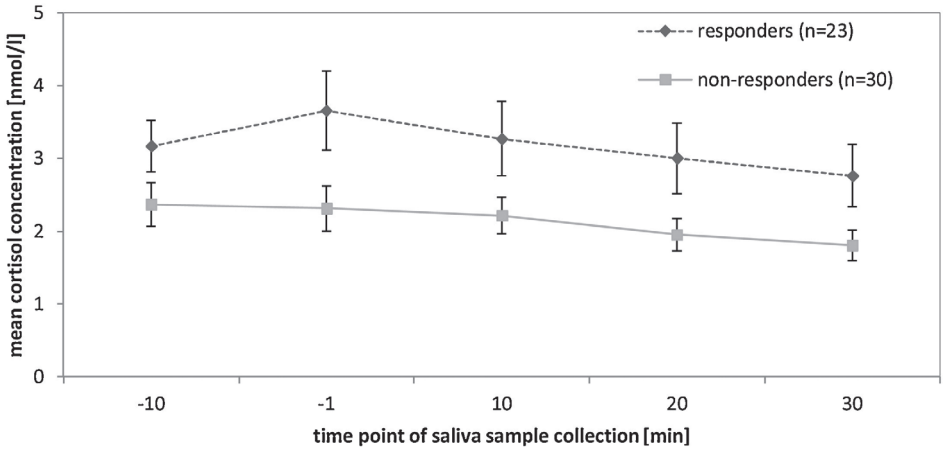

Fig. 2. Mean (±SE) saliva cortisol concentrations (original units) of pediatric posttraumatic stress disorder treatment responders and non-responders.

Regression analysis

In Table 2, the results of the sequential multiple regression analysis are presented. Among the possible confounders gender, age and baseline CAPS-CA scores were selected as most relevant. At the first step of the model gender, age together with baseline CAPS-CA scores explained 35.5% of the variance in posttreatment PTSD severity (posttreatment CAPS-CA) (Adjusted R^2 = 0.31, F(3 47) = 8.61, p < .001). At the second step, there was a positive significant contribution of the log- transformed AUCg in explaining variance in posttreatment PTSD severity. Log- transformed cortisol AUCg prior to treatment significantly explained 5.70% of the variance in PTSD severity after treatment above and beyond gender, age and baseline CAPS-CA scores (R^2 change = 0.073 F change(1, 46 = 4.45 Sig F change = .040. At the final step the interaction term age x log- transformed cortisol AUCg did not make a significant contribution to R^2 (R^2 change = 0.009 F change (1, 45) = 0.731, Sig F change = .397. A final model containing gender, age, baseline CAPS-CA and log-transformed AUCg was chosen. The final model explained 41.2% of the variance in posttreatment CAPS-CA scores (Adjusted R^2 = 0.36, F(4, 46) = 8.05, p < .001). The log- transformed AUCg (β = -.266, p =.040), gender (β = - .316, p = .021) and baseline CAPS-CA scores (β = .336, p .016) showed a positive independent contribution to the final model.

There were no violations of regression assumptions in this analysis. In Fig. 3, a scatterplot is given to show that the pretreatment log- transformed AUCg was linearly related to posttreatment PTSD severity.

Table 2 Results of regression analysis (*N*=51) with outcome variable posttreatment CAPS-CA total score.

	B [95% CI]	*SE B*	Beta	*p*
Step 1				
Constant	6.59 [-16.5, 29.6]	11.44		.569
Gender	15.0 [2.72, 27.3]	6.10	.34	**.018**
Pretreatment CAPS-CA	0.36 [0.08, 0.63]	0.14	.36	**.012**
Age	-0.23 [-2.14, 1.69]	0.95	-.030	.813
Step 2				
Constant	17.0 [-7.4, 41.4]	12.1		.167
Gender	14.1 [2.21, 26.0]	5.91	.316	**.021**
Pretreatment CAPS-CA	0.33 [0.06, 0.59]	0.13	.336	**.016**
Age	0.72 [-1.34, 2.75]	1.02	.096	.485
Log transformed AUCg	-22.1 [-43.2, -1.02]	10.48	-.266	**.040**
Step 3				
Constant	16.5 [-7.9, 41.00]	12.15		.180
Gender	16.0 [3.25, 28.7]	6.31	.358	**.015**
Pretreatment CAPS-CA	0.33 [0.07, 0.60]	0.13	.340	**.016**
Age	0.64 [-1,43, 2.71]	1.03	.085	**.**535
Log transformed AUCg	-20.8 [-42.2, 0.57]	10.6	-.250	.056
Age x Log transformed AUCg	-3.27 [-10.8, 4.3]	3,78	-.106	.397

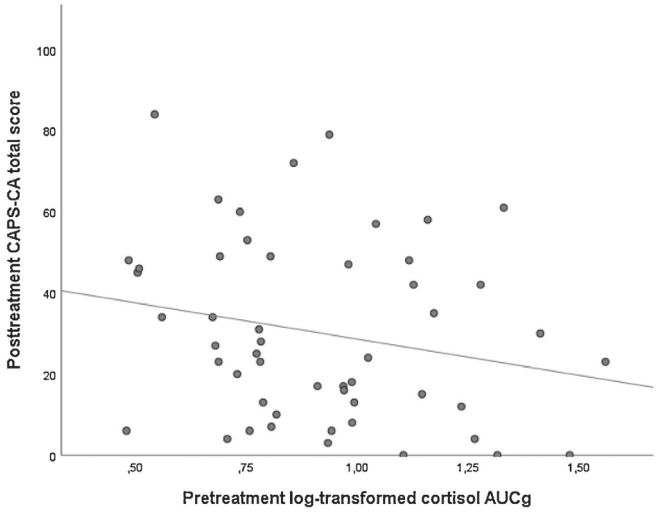

Fig. 3. Association of posttreatment PTSD symptoms (CAPS-CA total) with pretreatment log-transformed cortisol area under the curve with respect to ground (AUCg).

DISCUSSION

We examined the association between salivary cortisol prior to treatment and trauma-focused psychotherapy outcome in youth with (partial) PTSD. We hypothesized that treatment responders would display higher pretreatment cortisol levels caused by increased cortisol responsivity to trauma script driven imagery relative to psychotherapy non-responders. Contrary to our expectations, we found that trauma script driven imagery did not induce a cortisol stress response in the vast majority of youth in our sample. Therefore, we cannot conclude if cortisol responsivity prior to treatment differs between treatment responders and non-responders. However, we did find that basal cortisol secretion prior to treatment, as measured with the total cortisol AUCg during the SDI procedure, did differ between responders and non-responders. Consistent with our hypothesis, our study revealed higher cortisol levels in treatment responders relative to non-responders. Higher pre-treatment cortisol levels further positively predicted the extent of clinical improvement during trauma-focused psychotherapy. Although we included patients with a broad age range (8- 18 years), age did not moderate the relationship between pretreatment cortisol and treatment outcome. Together, these novel findings suggest cortisol prior to treatment is associated with and may predict the efficacy of trauma-focused psychotherapy in youth with (partial) PTSD.

There are several possible explanations why trauma script driven imagery did not elicit a cortisol stress response in most youth in our sample. First, effectiveness of stressor paradigm to elicit a stress response is subjective to developmental change. Throughout childhood and adolescence, marked changes are found in the ability of the same stressor paradigm to provoke elevations in cortisol. These changes in stressor paradigm effectiveness may be related to (neuro)developmental and psychological changes during childhood and adolescence. Gunnar et al. (2009) showed that threat to the social self is a critical psychological factor in determining the ability of a stressor paradigm to elicit cortisol elevation in youth, especially in older children and adolescents (Gunnar, Talge, & Herrera, 2009). Although the SDI contains a detailed contextual description of the patients' most disturbing traumatic event, it does not necessarily contain a threat to the social self. Furthermore, the examiner was present with the participants during the whole SDI period, which could have been perceived as social support. This is further supported by findings from a study by Dieleman et al. (2016) in youth with anxiety disorders treated with CBT. They did find pretreatment cortisol responsivity during a social stress task, which contains potential threat to the social self, to be associated with symptom improvement at one year follow-up (Dieleman, Huizink, Tulen, Utens, & Tiemeier, 2016). A second possible explanation of the absence of a cortisol stress response

in our sample is that participants knew they would be exposed to a personalized trauma script during the procedure before the start of the SDI procedure. This could have induced high anticipatory anxiety which may have led to a ceiling effect in cortisol levels. Our findings emphasize the complex challenges in studying cortisol reactivity during development in youth with anxiety and trauma related disorders. Future research in youth should therefore also include stress paradigms with a social stress component.

There are several possible explanations how higher basal cortisol secretion might be associated with better psychotherapy outcome. First, trauma-focused psychotherapies require that patients confront, rather than avoid, traumatic memories (Foa, Keane, Friedman, & Cohen, 2008; Shapiro, 2001). By confronting traumatic memories and associated distress in a safe therapeutic environment, a gradual decrease of the patients' distress to the traumatic memory can begin through habituation. However, patients may engage in avoidance before habituation can commence which hampers treatment success. Cortisol has been shown to have anxiolytic effects, increasing tolerance for distressing recollection of the traumatic memory (de Quervain et al., 2017). It might thus minimize avoidance and promote engagement during treatment sessions (Yehuda et al., 2009). Second, cortisol has been demonstrated to directly enhance memory reconsolidation of emotionally arousing experiences and extinction learning (de Quervain et al., 2017). This is relevant for trauma-focused treatments as emotionally arousing experiences are discussed to provide corrective information through cognitive restructuring and support extinction learning (Foa et al., 2008). Higher cortisol levels during treatment could thus improve treatment outcome by contributing to reduce avoidance and by promoting memory reconsolidation and extinction learning.

The results presented here show partial overlap with the few existing prospective studies on the relationship between cortisol and treatment efficacy in adults with PTSD. Studies in adult PTSD so far provide preliminary evidence that higher cortisol levels are related to increased treatment gains (Pacella et al., 2014; Rapcencu et al., 2017; Rauch et al., 2015; Yehuda et al., 2014). However, studies in adults suggest that higher cortisol levels are mainly driven by higher cortisol reactivity and not basal cortisol secretion. Higher reactivity as measured through the cortisol awakening response (CAR) and suppression of the CAR by dexamethasone challenge was found in responders (Nijdam et al., 2015; Pacella et al., 2014; Rapcencu et al., 2017). Furthermore, the only prospective study using script driven imagery to assess cortisol reactivity in adult PTSD showed increased post script cortisol levels in treatment responders prior to treatment (Rauch et al., 2015). Contrary to our findings however, in adults with PTSD basal cortisol secretion was not associated with treatment outcome (Nijdam et al., 2015; Rauch et al., 2015; Yehuda et al., 2014).

Together these findings again emphasize that findings on HPA function in adults with PTSD are not readily translated to youth with (partial) PTSD.

Our findings indicate pretreatment cortisol AUCg explained 5.70% of the variance in posttreatment symptom severity. The unique predicting value of cortisol in our study can be considered as rather small when compared to the predicting value of clinical and demographic variables in our study. Baseline symptom severity, age and gender accounted for 35.5% of the explained variance in posttreatment PTSD symptom severity, contributing considerately more to the final prediction model. The predictive value of cortisol measures in the current study is also limited compared to cortisol biomarkers in adult PTSD studies. Previous studies in adults reported effect sizes for treatment biomarkers ranging from 10.7% for CAR and 35% for CAR response following dexamethasone administration (Nijdam et al., 2015; Rapcencu et al., 2017). The limited amount of explained variance could reflect increased complexity in treating youth with (partial) PTSD in which treatment outcome is dependent on a more extensive and heterogeneous set of (developmental) factors. Indeed, youth with (partial) PTSD have shown increased heterogeneity in both clinical expression and biological characteristics, reflecting developmental differences across age groups (Scheeringa, Zeanah, & Cohen, 2011; Jasper B Zantvoord, Lindauer, Bakker, & Boer, 2013).

In the current study the average total CAPS-CA reduction from pre- to post treatment was 20 points. Previous trials using CAPS-CA as an outcome measure have shown comparable (Diehle et al., 2015; Ertl, Pfeiffer, Schauer, Elbert, & Neuner, 2011) and higher (Goldbeck et al., 2016; Smith et al., 2007) CAPS-CA reductions after trauma-focused psychotherapy in youth with (partial) PTSD. With a 50% reduction of CAPS-CA total score as response criterion, overall treatment response rate was 43.4%. Several factors could have contributed to the relative low response in the current study. First, our sample had a relative underrepresentation of youth exposed to single non-interpersonal traumatic events compared to previous trials (Gillies et al., 2012). Youth were predominantly exposed to repeated interpersonal trauma in which first trauma exposure often was at a young age and in a domestic setting. Furthermore, some participants were exposed to ongoing stressful life events such as ongoing third custody cases and out-of-home placement during the course of treatment. These trauma characteristics are associated with a poorer treatment response compared to single non-interpersonal trauma (Ertl et al., 2011; Smith et al., 2007). Second, although at pretreatment the majority (79.2%) of included youth had a full PTSD diagnosis, the remaining 20.8% had a partial PTSD diagnosis. Patients with partial PTSD show a more diffuse pattern of symptoms across different disorders and higher rates of comorbidity with depression, anxiety and externalizing disorders. In these patients treatment gains are not necessarily reflected in a reduction of

CAPS-CA scores, treatment gains could be defined as a reduction of symptoms and problems in multiple domains beyond PTSD diagnostic criteria (Carrion et al., 2002). Furthermore, change scores in the partial PTSD group could have been relatively low due to a floor effect. Including youth with partial PTSD in our sample thus increased clinical heterogeneity and might have lowered overall reduction of CAPS-CA total score during treatment. On the other hand, by including youth with partial PTSD our sample better reflects the real-life clinical setting, which adds to the ecological validity of our findings. Finally, our sample received a limited number of treatment sessions compared to most previous trials and had a short posttreatment follow-up period. In the current study both the EMDR and TF-CBT protocols consisted of eight weekly treatment sessions. Both protocols have shown efficacy in previous trials, however, conventional treatment protocols tent to contain more sessions. Furthermore, a previous trial using an eight session protocol has shown that symptom reduction continues even in the period after treatment sessions have stopped (Ertl et al., 2011). Youth included in our study thus had fewer sessions and less time to respond compared to most previous trials. Therefore, it cannot be ruled out that some non-responders would have shown response if they received more treatment sessions or had a longer posttreatment follow-up period. We have tried to account for this possibility by performing a sensitivity analysis with a more lenient response criterion (30% CAPS-CA reduction) which confirmed our finding of higher pre-treatment cortisol levels in treatment responders. Nevertheless, future studies using more treatment sessions are warranted to compare cortisol levels in short term treatment responders and delayed responders.

The present study details novel findings regarding the relationship between salivary cortisol and treatment outcome in youth with (partial) PTSD. It is not, however, without limitations. First, the trauma script driven imagery procedure did not elicit a cortisol stress response in all but two participants. Therefore cortisol reactivity could not be addressed in the current sample, precluding conclusions on differences in cortisol reactivity between treatment responders and treatment non-responders. The potential reasons for the lack of cortisol response are discussed above. Furthermore, the lack of a circadian cortisol secretion measure mitigates interpretation of our results. Inclusion of measures of cortisol levels throughout the day (awakening, morning, noon, afternoon, evening) would have provided the opportunity to distinguish between differences in basal circadian cortisol levels and anticipatory cortisol reactivity to the SDI. Future treatment studies in youth with (partial) PTSD should therefore ideally also include stress paradigms with a social threat component and measures of circadian cortisol levels. Second, salivary cortisol levels are related to many state and trait factors, we have tried to account for most of these confounding factors in the current study to prevent unwanted

influences on our results. However, we omitted inquiry on menstrual status, pubertal stage and (oral) contraceptive use, which are known factors to influence cortisol levels and may influence cognitive and emotional processing (Stalder et al., 2016; Sundström Poromaa & Gingnell, 2014). In theory, this could thus have created a spurious relationship between cortisol and treatment outcome. Obviously future studies should try to include these variables as well. Third, the current analyses on differences between treatment responders and non-responders are based on cross-sectional data, highlighting the need for future longitudinal treatment studies in which cortisol is measured before, during and after treatment. This would enable examination of common and differential longitudinal trajectories of cortisol in treatment responders and non-responders. This could render insight in the working mechanisms of treatments and might provide novel ways to improve treatment efficacy. Fourth, youth were randomized to receive either TF-CBT or EMDR, for the current analysis both treatment conditions were collapsed. Due to limited power it was not feasible to examine differences between treatment responders and non-responders separately for both treatment conditions or examine specific predictors for each treatment separately. Larger RCT's with more statistical power using cortisol measures are therefore warranted. Finally, our study had considerable drop-out rate, as 24% of randomized patients were lost to follow-up. Although, dropout rates in our study reflect routine clinical practice and cortisol measures did not differ between treatment completers and non-completers, there is a possibility that drop-out could have influenced our main findings through attrition bias.

CONCLUSIONS

To our knowledge, this is the first report of differences in cortisol levels prior to treatment between treatment responders and non-responders in youth with (partial) PTSD. Because the trauma script driven imagery procedure failed to provoke a cortisol stress response in our sample the question if cortisol reactivity differs between treatment responders and non-responders remains inconclusive. Our results do however show that higher pretreatment basal cortisol secretion is associated with higher treatment response. This may suggest that cortisol forms a potential indicator of prospective trauma focused psychotherapy efficacy in youth with (partial) PTSD. However, the current study does not provide clinically applicable biomarkers yet, as our findings do not provide markers which are predictive for individual patients. Future studies should therefore use stress paradigms with a social threat paradigm and data-analysis methods specifically aimed at maximizing classification accuracy combining cortisol data with other clinical and neurobiological data. Furthermore, longitudinal cortisol measures during the course of treatment

contribute to delineate the association between cortisol over time and treatment response. This could help to determine if cortisol may be a potential target as add-on therapy to improve trauma focused psychotherapy efficacy in youth with (partial) PTSD.

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CHAPTER 3

Individual prediction of trauma-focused psychotherapy response in youth with posttraumatic stress disorder using resting-state functional connectivity

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Neuroimage: Clinical 2021; 32: 102898

ABSTRACT

Randomized controlled trials have shown efficacy of trauma-focused psychotherapies in youth with posttraumatic stress disorder (PTSD). However, response varies considerably among individuals. Currently, no biomarkers are available to assist clinicians in identifying youth who are most likely to benefit from treatment. In this study, we investigated whether resting-state functional magnetic resonance imaging (rs-fMRI) could distinguish between responders and non-responders on the group- and individual patient level. Pre-treatment rs-fMRI was recorded in 40 youth (ages 8-17 years) with (partial) PTSD before trauma-focused psychotherapy. Change in symptom severity from pre- to post-treatment was assessed using the Clinician-Administered PTSD scale for Children and Adolescents to divide participants into responders (≥30% symptom reduction) and non-responders. Functional networks were identified using meta-independent component analysis. Group-differences within- and between-network connectivity between responders and non-responders were tested using permutation testing. Individual predictions were made using multivariate, cross-validated support vector machine classification. A network centered on the bilateral superior temporal gyrus predicted treatment response for individual patients with 76% accuracy (p_{FWE} = 0.02, 87% sensitivity, 65% specificity, area-under-receiver-operator-curve of 0.82). Functional connectivity between the frontoparietal and sensorimotor network was significantly stronger in non-responders (t = 5.35, p_{FWE} = 0.01) on the group-level. Within-network connectivity was not significantly different between groups. This study provides proof-of-concept evidence for the feasibility to predict trauma-focused psychotherapy response in youth with PTSD at an individual-level. Future studies are required to test if larger cohorts could increase accuracy and to test further generalizability of the prediction models.

INTRODUCTION

Posttraumatic stress disorder (PTSD) is a common mental health disorder that develops in approximately 16% of youth exposed to traumatic events (Alisic et al., 2014). Youth with PTSD are troubled by frequent re-experiencing of the traumatic event, persistent avoidance, hyperarousal and negative alterations in cognition and mood (American Psychiatric Association, 2013). These symptoms can interfere with social functioning and school performance, have a negative effect on quality of life (Carrion, Weems, Ray, & Reiss, 2002) and are a crucial factor in shaping the vulnerability to depression and suicidality later in life (Molnar, Berkman, & Buka, 2001). Randomized controlled trials (RCTs) have demonstrated the efficacy of trauma-focused psychotherapies in youth with PTSD (Morina, Koerssen, & Pollet, 2016), but response varies considerably among individuals, with 30-50% of youth not benefiting sufficiently (Diehle, Opmeer, Boer, Mannarino, & Lindauer, 2015; Morina et al., 2016). Different pre-treatment clinical and demographic factors have been associated with trauma-focused psychotherapy outcome, but none have shown to reliably predict treatment response (Goldbeck, Muche, Sachser, Tutus, & Rosner, 2016). This underlines the need for the identification of reliable (bio)markers of treatment response which could assist clinicians to optimize treatment allocation and improve clinical outcome.

Previous studies have shown that adult PTSD is characterized by functional alterations in brain regions which are key nodes in multiple large-scale brain networks, including the insula and medial prefrontal cortex (Wang et al., 2016). The insula is part of the salience network (SN) that is responsible for detecting and orienting to salient stimuli (Vinod Menon, 2011), and the medial prefrontal cortex is part of the default mode network (DMN) that is associated with internally focused thought as well as autobiographical memory (Vinod Menon, 2011). Results from studies examining large-scale network connectivity in youth with PTSD have not always corresponded with results obtained in adults (Weems, Russell, Neill, & McCurdy, 2019). Patriat and colleagues, for instance, found that pediatric PTSD is characterized by increased connectivity within the DMN, contrasting the finding of decreased connectivity within the DMN in adults (Patriat, Birn, Keding, & Herringa, 2016). This could be related to considerable reorganization of large-scale brain networks throughout childhood and adolescence (Weems et al., 2019). Developmental change in large-scale brain organization is characterized by stronger within network connectivity and more efficient between-network connectivity, with a trend towards segregation (decrease in connectivity strength) between regions in close proximity and integration (increase in connectivity strength) between anatomically distant regions (V. Menon, 2013). These developmental processes provide a potential explanation for the contrasting

findings between youth and adults with PTSD and emphasize the need for studies on large-scale brain networks specifically performed in youth with PTSD.

Few studies have investigated the relationship between large-scale brain network connectivity and treatment-response. In adults, neuroimaging studies have observed pre-treatment differences between responders and non-responders to trauma-focused psychotherapy (Duval et al., 2020; Fonzo, Goodkind, Oathes, Zaiko, Harvey, Peng, Weiss, Thompson, Zack, & Lindley, 2017; Fonzo et al., 2021; Korgaonkar et al., 2020; Jasper B Zantvoord, Diehle, & Lindauer, 2013; Zhutovsky et al., 2019). Findings from these studies suggest that activity and connectivity within regions and networks involved in working memory as well as emotional processing and modulation differed between responders and non-responders at baseline (Duval et al., 2020; Zhutovsky et al., 2019) and could be adaptively attenuated with successful trauma-focused psychotherapy (Fonzo et al., 2021). A study in adolescent girls reported greater pre-treatment bilateral amygdala activation during emotion processing in treatment responders and differences in large-scale brain network connectivity (Cisler et al., 2016). These studies provide initial evidence for group-differences in pre-treatment brain activity and connectivity between treatment responders and non-responders.

The studies reported above used univariate analysis to detect group-differences. However, this does not provide information for individual patients and may not generalize to new data (Arbabshirani, Plis, Sui, & Calhoun, 2017), which is necessary to allow clinicians to inform patients and to assist in clinical decision making. Predictions for individual patients can be made using multivariate supervised machine learning (ML) analysis which directly assesses generalization to new patients by means of cross-validation. Several studies have utilized ML methods and resting-state functional magnetic resonance imaging (rs-fMRI) to predict treatment-response in adults with PTSD, with accuracies ranging between 71 and 90% (Etkin et al., 2019; Korgaonkar et al., 2020; Zhutovsky et al., 2019). However, no studies are available that have investigated the utility of ML and rs-fMRI to predict treatment-response in youth with PTSD. Therefore, we collected pre-treatment rs-fMRI data of 40 youth with PTSD/partial-PTSD (age 8-17) to predict treatment response on the group- and individual-level.

MATERIALS AND METHODS

Participants

Our initial sample consisted of 61 participants (39 girls/22 boys) diagnosed with PTSD or partial PTSD. Participants entered trauma-focused psychotherapy as part of an RCT comparing trauma-focused cognitive behavioral therapy (TF-CBT) and eye movement desensitization and reprocessing (EMDR) (Diehle et al., 2015). Of these, 50 completed treatment as well as pre- and post-treatment assessment (see flow diagram in Figure S1). After data quality control 40 participants (26 girls/14 boys) were included in the final analysis. All participants were Dutch speaking, and 8-17 years old. Gender categories were based on the personal identification of participants' own gender. Participants were recruited between June 2011 and September 2018 at the outpatient child psycho-trauma center of the department of child and adolescent psychiatry, de Bascule in Amsterdam, The Netherlands. Youth were referred by child welfare services, physicians or general practitioners. Diagnoses for PTSD or partial PTSD were established clinically by an experienced child and adolescent psychiatrist or psychologists according to the DSM-IV-TR criteria using joint child and caregiver reports on individual symptoms on the Clinician-Administered PTSD Scale for Children and Adolescents (CAPS-CA) semi-structured interview (Nader et al., 1996) and the caregiver reports from the PTSD scale of the Anxiety Disorders Interview Schedule – Parent Version (ADIS-P) (Verlinden et al., 2014). A symptom was established as present, if either child or caregiver reported its presence. Partial PTSD was defined as either fulfilling two of the three PTSD symptom clusters or having one symptom present in each of the three symptom clusters (Stein, Walker, Hazen, & Forde, 1997). Furthermore, participants were required to have a CAPS-CA total score indicating at least mild PTSD symptom severity (>20 points). Exclusion criteria were: acute suicidality, IQ < 70, pregnancy, neurological disorders or serious medical illnesses or meeting the criteria of the following diagnosis: psychotic disorders, substance-use disorder or pervasive developmental disorder. If participants were taking psychotropic or central nervous-active medication, medication was required to be stable for at least three weeks before and during trauma-focused psychotherapy. In our sample one participant was taking sertraline and two methylphenidate. In accordance with procedures approved by the Institutional Review Board of the Amsterdam University Medical Center and the declaration of Helsinki, written informed consent was obtained from all parents or legal guardians. Written informed consent from youth aged 12 years and older and assent from youth aged 11 and younger, was also obtained from the youth themselves. All participants received a monetary incentive for participation (€5 for each assessments).

Trauma-focused psychotherapy

Participants were randomly assigned to weekly protocolized sessions for a total of 8 weeks of either TF-CBT or EMDR. The data reported here were obtained as part of a larger study on the efficacy of TF-CBT and EMDR. Treatment was delivered by experienced trauma therapists who were trained in TF-CBT and EMDR before study initiation. Supervision by TF-CBT and EMDR experts was provided throughout the study. Treatment protocols, training and supervision of therapists, as well as treatment fidelity have been described in detail previously (Jasper B Zantvoord et al., 2019).

Trained psychologists administered the CAPS-CA and the PTSD scale of the ADIS-P to measure PTSD symptoms before and after treatment. Caregiver reports on the ADIS-P were used to complement child reports and clinical observation. The Dutch Revised Child Anxiety and Depression Scale (RCADS(-P)) questionnaires was administered to assess depressive and anxiety symptoms (Chorpita, Yim, Moffitt, Umemoto, & Francis, 2000). Symptom change was calculated by subtracting the pre-treatment from the post-treatment CAPS-CA total score. We used ≥30% reduction of CAPS-CA total score as response criterion for clinically meaningful improvement (J. B. Zantvoord et al., 2021).

The distribution of baseline clinical, trauma and demographic characteristics across responders and non-responders was examined using X^2-tests, independent sample *t*-tests or Mann-Whitney tests as appropriate. Paired sample *t*-test were used to examine pre- to post-treatment symptom change. Statistical analyses were performed using SPSS version 26 (SPSS Inc., Chicago IL, USA).

Imaging data acquisition

High-resolution T1 and rs-fMRI data were acquired using a 3T Philips Achieva scanner (Philips Healthcare, Best, The Netherlands) equipped with a SENSE eight-channel receiver head coil. For each participant, a T1-weighted structural MRI image was acquired with the following parameters: TE: 3.527 ms, TR: 9 ms, slice thickness: 1 mm, 170 slices, flip angle: 8° and image matrix 256 x 256 that covert the entire brain. 200 blood oxygen level dependent rs-fMRI scans were acquired with a repetition time of 2.3s and a voxel size of 2.3x2.3x3mm^3. For rs-fMRI, participants were instructed to remain still with their eyes closed.

Imaging data preprocessing

All (f)MRI preprocessing was performed utilizing a singularity image container running fMRIPrep (v1.5.3[1]).

Structural data preprocessing

Structural MR images were corrected for intensity non-uniformity and brain-extracted using the ANTs toolbox (v2.2.0[2]). Brain tissue segmentation of cerebrospinal fluid (CSF), white-matter (WM), and gray-matter (GM) was performed on the brain-extracted T1w images using FSL FAST (v5.0.9). Volume-based spatial normalization to MNI space (MNI152NLin6Asym) was performed through nonlinear symmetric normalization with ANTs.

Functional data preprocessing

Preprocessing of rs-fMRI data followed the standard procedure implemented in fMRIPrep involving generation of a reference volume, co-registration to the T1w scan, motion correction (before any spatiotemporal filtering) and normalization to MNI space in one step using a combination of all spatial transformations (see Supplementary Materials for details). Normalizations and co-registrations were assessed visually and four PTSD patients were excluded due to poor normalization quality. We excluded five additional participants with high spikes of motion identified from visual inspection of plots of the realignment parameters (volume-to-volume changes >2mm). Therefore, after quality control of the structural MRI and rs-fMRI data the final sample included 40 patients. These remaining participants did not differ in overall motion levels according to their framewise displacement (Power et al., 2014) (see Table 1). Data were spatially smoothed with an isotropic, Gaussian kernel of 6mm full-width-at-half-maximum. To further address motion contamination, we applied ICA-AROMA (Pruim et al., 2015) (in MNI space) to remove additional motion sources from the data. Data was then resampled to $4mm^3$ to speed-up additional procedures. We addressed further structured noise present in the data by regressing out average WM and CSF signals using masks calculated in T1w space, transformed to rs-fMRI space. We combined this regression step with highpass filtering by a discrete cosine set with 128s cut-off. To avoid reintroducing already removed nuisance signal into the data by applying a sequential pipeline, both WM/CSF and cosine regressors were denoised with the previously identified ICA-AROMA regressors (Lindquist, Geuter, Wager, & Caffo, 2019). As a final step the rs-fMRI data were grand-mean scaled with a factor of 10000.

1 https://fmriprep.org/en/1.5.3/

2 https://stnava.github.io/ANTs

Identification of intrinsic connectivity networks

To identify a set of robust intrinsic connectivity networks (ICNs) we employed a meta-independent component analysis (ICA) (Biswal et al., 2010) utilizing FSL MELODIC (v3.15) (Beckmann & Smith, 2004). To ensure that the identification of ICNs was independent from their use in the analysis, which may introduce a positive bias (Poldrack, Huckins, & Varoquaux, 2019), we included rs-fMRI data of 17 trauma-exposed controls (TEC) who did not differ in age, gender, or motion from the included patients (see Supplementary Materials for further details). The number of components was fixed to 70 as it has been successful in the identification of treatment-related PTSD biomarkers for veterans in our previous study and in addition has been shown to be repeatable, able to well separate signal sources and be optimal in detecting disease-related group-level differences (Abou-Elseoud et al., 2010; Abou Elseoud et al., 2011; Zhutovsky et al., 2019). To identify ICNs, we employed a semi-automatic approach (Cerliani et al., 2015) which led to the inclusion of 48 ICNs (see Supplementary Materials). Both ICNs and excluded components are shown in Figures S2 and S3, respectively.

To reconstruct individual-level representations of the group-level ICNs and their time-courses we applied group-information guided ICA (GIG-ICA) to the preprocessed data of the PTSD patients (Du & Fan, 2013). GIG-ICA computes a spatially constrained individual-level ICA which estimates individual ICNs which are maximally spatially correlated with a group-map. This procedure is repeated for each group ICN and each participant, generating a set of individual-level ICN representations and their corresponding time-courses. GIG-ICA has been shown to outperform conventional reconstruction methods like dual regression in identifying reliable biomarkers for psychiatric disorders and to produce spatially independent components (Du & Fan, 2013; Salman et al., 2019). GIG-ICA was applied utilizing MATLAB code (R2018b, The Mathworks, Natick, MA) distributed with the GroupICA toolbox (v4.0b[3]).

To investigate between-ICN connectivity we applied the FSLnets toolbox (v0.6.3[4]) to the individual-level ICN time-courses estimated via GIG-ICA. We estimated full- and partial-correlation matrices between all identified ICNs and converted all correlation coefficients to z-scores for further analyses. Full-correlation matrices were estimated using Pearson correlation while partial-correlation matrices were calculated from regularized Ridge regressions (rho=0.1) as is the default in the FSLnets package.

3 https://trendscenter.org/software/gift

4 https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FSLNets

Group-level analyses

We tested for group-differences across ICNs (within-ICN connectivity) between responders and non-responders using permutation testing implemented in PALM (a117[5]). We included demeaned age, gender and pre-treatment CAPS-CA total scores as covariates-of-no-interest into a general linear model (GLM). Familywise error (FWE) correction of p-values across the whole-brain, 48 ICNs and two-sided tests of the threshold-free-cluster-enhancement (TFCE) statistic (Smith & Nichols, 2009) was performed using synchronized permutations (n = 10000) of the maximum statistic.

The same procedure, involving permutation testing (n = 10000), and the same covariates-of-no-interest was utilized to investigate group-difference in between-ICN connectivity across responders and non-responders. The FWE-correction of p-values of the t-statistic was performed across all connections, two-sided contrasts and the full- and partial correlation matrices utilizing the maximum statistic. Alpha was set to 0.05 in both analyses.

Individual-level analyses

To investigate whether within- or between-ICN connectivity could predict treatment-response for the individual patient, we applied multivariate, cross-validated linear-kernel support vector classifiers (SVM) (Cortes & Vapnik, 1995) to our data. For that we considered every ICN (n = 48) and their connectivity profiles (full- and partial-correlation matrices, n = 2) separately, resulting in 50 separate multivariate classification analyses. We divided our data into 5-folds (each fold containing 20% of the data) ensuring (approximate) balance of responders and non-responders per fold. Data of 4-folds was used as training set for rescaling all features to - 1 to 1 range and fitting the SVM. The fifth fold served as the test set and we calculated balanced accuracy (average between sensitivity and specificity), area-under-the-receiver-operator-curve (AUC), sensitivity (of identifying responders), specificity (of identifying non-responders) and negative/positive predictive value (NPV/PPV) as performance measures of the trained SVM classifier. We repeated the procedure five times, each time retraining the classifier and utilizing a different fold as the test set. Finally, to ensure a reliable average measure of classification performance we repeated the random division of the data across the five folds 50 times and repeated the entire analysis, yielding a 50-times-repeated-5-fold cross-validation procedure (Varoquaux et al., 2017). In the end, we averaged the performance measures across the 250 test set evaluations, providing a set of measures estimating the generalizability of our classifier to new data.

5 https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/PALM

To assess statistically whether the estimated average accuracies provided better-than-chance performance and to correct for the total number of classifications performed (48 ICNs + 2 correlations matrices = 50), we used synchronized permutation tests (n = 2000, see Supplementary Materials). Alpha was set to 0.05.

We also assessed which features were important for the classification by calculating p-values for each weight of the SVM using a novel permutation-based procedure (Gaonkar, Shinohara, Davatzikos, & Alzheimers Disease Neuroimaging, 2015) (see Supplementary Materials). The p-values were computed after the classifier was applied to the entire data set and are intended for visualization purposes only.

All individual-level analyses were implemented in the Python programming language (v3.8.2) utilizing the scikit-learn ML toolbox (v0.22.1).

RESULTS

Demographic and clinical characteristics

A summary of participant characteristics is shown in Table 1. Treatment responders and non-responders did not differ in demographic, trauma and clinical characteristics at baseline apart from separation anxiety symptoms which were (marginally significantly) higher in responders (p = 0.048). Based on joint child (CAPS-CA) and caregiver (ADIS-P) reports 82.5% of all participants met the full DSM-IV diagnostic criteria for PTSD at baseline, the remaining 17.5% met criteria for partial PTSD. The average baseline CAPS-CA score was 56.13 (SD = 23.25), which is indicative of moderately severe PTSD. The most common index trauma was sexual abuse, followed by community violence, accidents and domestic violence. 57.5% of participants were exposed to multiple-event trauma. Average age at trauma exposure was M = 9.95 years, SD = 3.89 (range 2-16) and average time since trauma was M = 2.82 years, SD = 2.52 (range 0-10).

Table 1 Subject characteristics.

	Overall (n=40)	Responders (n = 21) ≥30% CAPS-CA	Non-responders (n = 19) <30% CAPS-CA	*p*-value[a]
Sociodemographic characteristics (pre-treatment)				
Girls (%)	65.0	57.1	73.7	.273
Age (years; mean, SD)	12.6 (2.91)	12.5 (2.64)	12.7 (3.25)	.820
West European Ethnicity (%)	47.5	52.4	42.1	.413
Current educational level (%)				.557
Elementary school	47.5	52.4	42.1	
Middle/High school lower level	7.5	9.5	5.3	
Middle/High school middle level	27.5	28.6	26.3	
Middle/High school higher level	12.5	9.5	15.8	
Vocational school	5.0	0	10.5	
Household Income (€; %)				.622
<25000	27.5	28.6	26.3	
25000-35000	12.5	19.0	5.3	
>35000	20.0	23.8	15.8	
Weight (kg; mean, SD)	51.1 (10.94)	51.3 (12.67)	50.7 (8.46)	.875
Current psychotropic medication (%)	7.5	9.5	5.3	.609
Smoking (%)	7.5	9.5	5.3	.702
Alcohol >1 consumption/day (%)	0	0	0	N/A
Imaging Data (pre-treatment)				
Framewise displacement (mean, SD)	0.20 (0.11)	0.21 (0.11)	0.20 (0.12)	.820
Trauma characteristics (pre-treatment)				
Index trauma (%)				.971
Sexual abuse	32.5	28.6	36.8	
Domestic violence	12.5	14.3	10.5	
Community violence	25.0	23.8	26.3	
Accidents/Medical	12.5	14.3	10.5	
Other	17.5	19.0	15.8	
Repeated trauma exposure (%)	57.5	61.9	52.6	.554
Age at index trauma (years; mean, SD)	9.9 (3.89)	10.0 (3.43)	9.9 (4.42)	.824
Time since index trauma (years; mean, SD)	2.8 (2.52)	2.7 (2.00)	2.9 (3.03)	.773
Clinical characteristics (pre-treatment)				
CAPS-CA (mean, SD)[b]				
Total	56.1 (23.25)	55.5 (23.95)	56.8 (23.09)	.856
Re-experiencing	17.8 (10.37)	16.7 (10.04)	18.9 (10.92)	.532
Avoidance	21.5 (10.02)	22.8 (9.62)	20.1 (10.54)	.422
Hyperarousal	17.8 (8.96)	16.8 (9.49)	18.8 (8.48)	.515
Full PTSD diagnosis (%)	82.5	85.7	78.9	.574
RCADS (mean, SD)[b]				
MDD	12.0 (6.08)	11.7 (6.11)	12.5 (6.29)	.729
GAD	7.2 (4.30)	8.4 (4.56)	5.6 (3.48)	.089
OCD	6.8 (3.35)	7.3 (3.84)	6.2 (2.59)	.407
PD	8.4 (6.25)	9.2 (6.65)	7.4 (5.81)	.469
SAD	6.1 (4.24)	7.4 (3.91)	4.3 (4.12)	**.048**
SP	12.2 (6.76)	13.3 (7.28)	10.8 (6.00)	.339
Administered Psychotherapies				
TF-CBT/EMDR	24/16	11/10	13/6	.301

Clinical characteristics (post-treatment)				
CAPS-CA (mean, SD)[b]				
Total	38.0 (25.70)	22.3 (19.58)	55.2 (20.14)	**<.001**
Re-experiencing	10.6 (10.17)	5.4 (16.93)	16.9 (10.66)	**.001**
Avoidance	12.1 (9.14)	9.4 (15.27)	15.3 (7.52)	.062
Hyperarousal	12.2 (9.14)	6.4 (6.38)	19.2 (6.81)	**<.001**

Abbreviations: CAPS-CA, Clinician-Administered PTSD Scale for Children and Adolescents; RCADS, Revised Child Anxiety and Depression Scale; MDD, major depressive disorder; GAD, general anxiety disorder; OCD, obsessive compulsive disorder; PD, panic disorder; SAD, separation anxiety disorder; SP, social phobia; SD, standard deviation; TF-CBT, trauma-focused cognitive behavioral therapy; EMDR, eye movement desensitization and reprocessing.

[a] *p-values <0.05 shown in bold. Independent samples t-test for continuous and X2 tests for categorical variables between responders and non-responders.*

[b] *Ranges: CAPS-CA total, 0-139; RCADS MDD, 0-30; RCADS GAD, 0-18; RCADS OCD, 0-18; RCADS PD, 0-27; RCADS SAD, 0-21; RCADS SP, 0-27.*

Changes in psychopathology

Treatment completers and non-completers did not differ in baseline sociodemographic, trauma or clinical characteristics. Across the completer sample, we found significant reductions in CAPS-CA total score ($t(39) = 5.65$, $p < 0.001$, Cohen's effect size (d) = 0.89), re-experiencing ($t(39) = 4.39$, $p < 0.001$, d = 0.71), avoidance ($t(39) = 4.10$, $p < 0.001$, d = 0.68) and hyperarousal clusters ($t(39) = 2.935$, $p = 0.006$, d = 0.55. Twenty-one fulfilled the criterion for treatment response (≥30% PTSD symptom reduction on CAPS-CA), and nineteen were non-responders.

Resting-state fMRI

Group-level analyses

Within-network analyses

There were no group-differences surviving FWE-correction between responders and non-responders for any of the 48 ICNs. Because the number of investigated components was large, requiring stringent correction for multiple comparisons, we also provide the results of the analyses when FWE-correction was only applied for each network separately (see Figure S4). Within-network connectivity of two ICNs (left frotoparietal network (FPN) and a visual ICN) was increased in responders over non-responders in this exploratory analysis.

Between-network analyses

Between-network analyses showed a significantly larger Pearson correlation between the (predominantly) left FPN and a sensorimotor network in non-responders over responders (t=5.35, p_{FWE}=0.012, Figure 1).

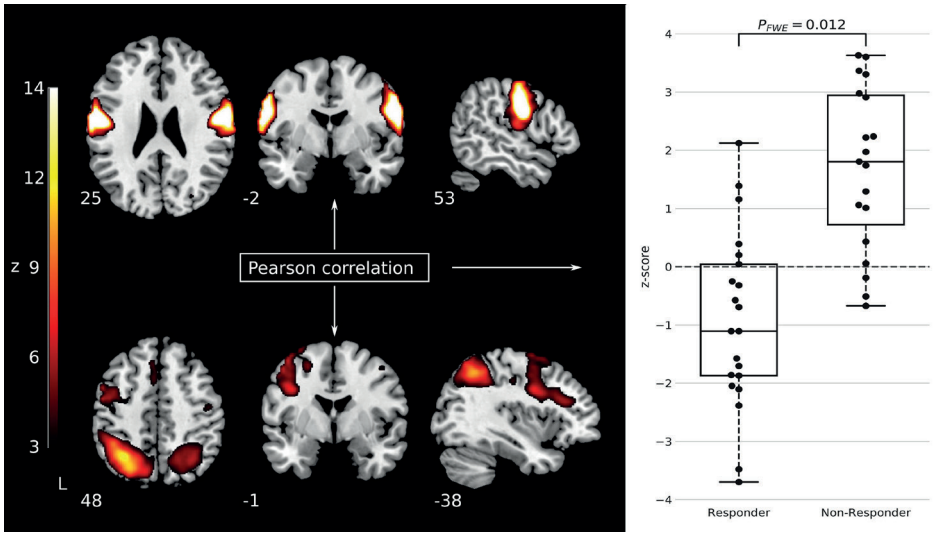

Figure 1: Stronger Fisher r-to-z transformed Pearson correlation between a sensorimotor network and the (predominantly) left frontoparietal network was observed for non-responders over responders. Boxplots show median and interquartile range of the distribution of responders/non-responders. The dots show the individual z-transformed correlation values of the individual patients. It is important to note that the individual correlation values shown in the boxplot cannot be directly used to infer the performance in the classification analysis as this would constitute 'double dipping' (Kriegeskorte, Simmons, Bellgowan, & Baker, 2009).

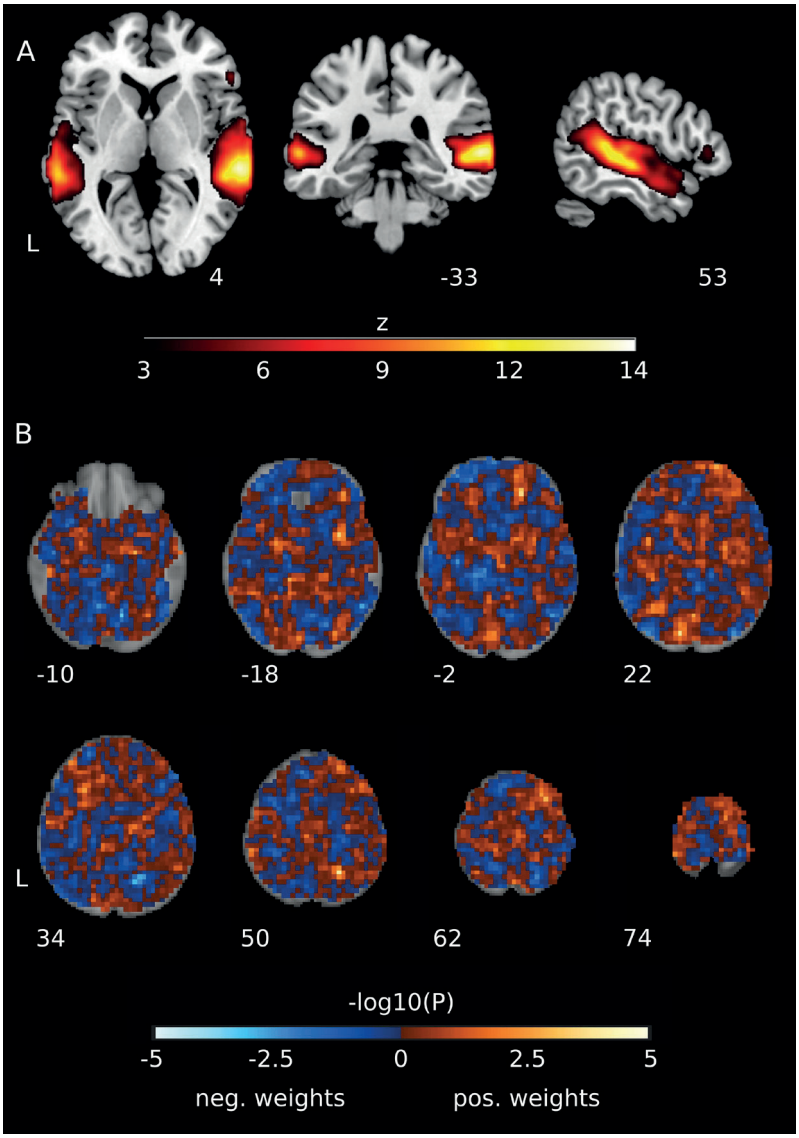

Figure 2: A. A network centered on the bilateral superior temporal gyrus which provided the best performance during the multivariate classification of responders and non-responders. The network was part of the 70 networks computed by means of meta-ICA on the (independent) HC sample. B. p-values of the individual voxel weights of the SVM estimated using the margin-aware statistic and analytical approximation of the null-distribution (Gaonkar et al., 2015) for classification using the individual-level representation of the group network in A. p-values are shown unthresholded as the analysis is multivariate and therefore all voxels – and not only the most significant ones – always contribute to the classification task.

Individual-level analyses

SVMs trained on data from an ICN centered on the bilateral superior temporal gyrus (STG) provided an average cross-validated accuracy of 76.17% (SD=12.58%, p_{FWE} = 0.018, Figure 2(A) and Figure 3). The network achieved an AUC of 0.82 (SD = 0.16), with a sensitivity of 87.14% (SD = 16.56%) and a specificity of 65.20% (SD = 21.44%). The PPV/NPV was 0.75/0.85 (SD = 0.14/0.19). To explore whether test accuracy was comparable for TF-CBT and EMDR, we also tested the same model for this ICN for the different treatments separately. The cross-validated performance for the TF-CBT subgroup showed a balanced accuracy of 76.16% (AUC: 0.82, sensitivity: 85.97%, specificity: 64.3%). For the EMDR subgroup a balanced accuracy of 78.56% (AUC: 0.83, sensitivity: 83.43, specificity: 52.0%) was observed. We also investigated how the usage of a different validation approach (leave-one-out cross-validation) would influence the performance estimates of the best performing network: we observed an accuracy of 69.42%, AUC of 0.77, sensitivity of 80.95%, and specificity of 57.89%. However, given that there are theoretical and empirical reasons for why leave-one-out cross-validation is not recommended – especially in the case of small sample sizes – the reported results from this validation scheme should not be the focus of this study (Flint et al., 2021; Poldrack et al., 2019). To illustrate that there is a significant advantage in utilizing multivariate instead of univariate models we performed an experiment in which we selected the best separating voxel (according to a t-test performed on the training set) of the above network and trained and tested our linear SVM models using only this one voxel. The obtained performance dropped significantly to an averaged accuracy of 50.99%, AUC of 0.54, sensitivity of 53.12% and specificity of 48.87%. No other network showed classification accuracies exceeding chance-level when FWE-correction was applied.

p-values corresponding to the voxel-weights of the SVM classifier when trained on data of all patients of the STG ICN can be seen in Fig. 2 (B), showing a diffuse whole-brain pattern required to successfully perform the classification.

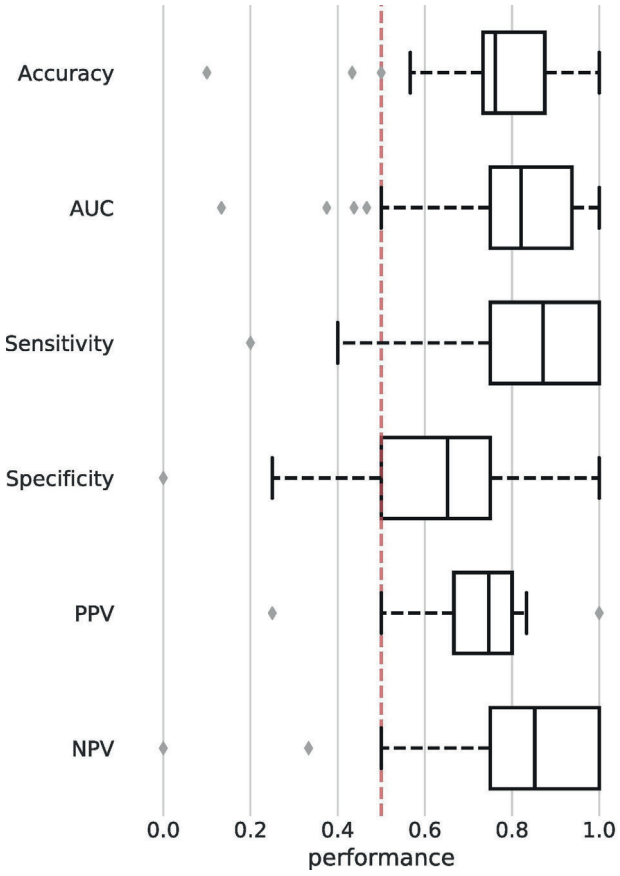

Figure 3: Cross-validated performance estimates of the best performing network during classification (Figure 2). Boxplots show the mean and interquartile range (IQR) of the individual performance distributions. The mean instead of the median is shown because it was also used and reported as final performance of the network. Red dotted line indicates approximate chance-level. However, statistically, deviation from chance-level and FWE-correction were estimated through synchronized permutations.

DISCUSSION

In this study we investigated the possibility of using pre-treatment rs-fMRI data as a biomarker to predict trauma-focused psychotherapy response in youth with (partial) PTSD. We examined prediction both on the group- and individual-level. In our study, a network centered on the bilateral STG could distinguish between responders and non-responders on the individual-level, with an accuracy of 76.2%. We further found increased connectivity between the left FPN and a sensorimotor network in non-responders on the group-level. To our knowledge this is the first study to examine the prediction of individual treatment-response using rs-fMRI data in youth with PTSD. Together our results provide a first proof-of-concept for the utility of rs-fMRI as a biomarker for treatment-response in youth with PTSD.

Our findings indicate increased pre-treatment connectivity between the left FPN and sensorimotor network in trauma-focused psychotherapy non-responders. The FPN is highly integrated with other brain networks and has a comprehensive role in attention, working memory and decision making by flexibly interacting with other brain networks(Vinod Menon, 2011). Abnormal recruitment of other brain networks into the FPN is linked with deficits in these cognitive processes and has been associated with multiple psychiatric disorders (Vinod Menon, 2011). More specifically, increased connectivity between the FPN and a sensorimotor network has been found in youth with autism (ASD) and attention-deficit/hyperactivity disorder (ADHD) (Cerliani et al., 2015). While speculative at this point, abnormal recruitment of the sensorimotor network into the FPN in non-responders might be related to deficient cognitive processes resulting in suboptimal engagement in trauma-focused psychotherapy and poor treatment response. To test this hypothesis, future research could address functional connectivity patterns of the FPN together with neurocognitive tests before and after treatment and use repeated transcranial magnetic stimulation to directly influence FPN connectivity (Etkin et al., 2019). Such an approach could eventually delineate clinical relevance and might identify promising targets for non-invasive stimulation-based interventions (Fonzo, Goodkind, Oathes, Zaiko, Harvey, Peng, Weiss, Thompson, Zack, Mills-Finnerty, et al., 2017).

Our group-level analysis did not identify networks which were previously found to distinguish between trauma-focused psychotherapy responders and non-responders in adult PTSD. More specifically, we did not find group differences in connectivity between the SN and DMN which was found to differentiate veterans who responded to prolonged exposure (PE) therapy compared to non-responders (Sheynin et al., 2020). Additionally, previous findings of lower within Ventral Attention Network (VAN) connectivity and increased connectivity in the frontal pole in adults

with poor trauma-focused psychotherapy response were also not replicated (Etkin et al., 2019) (Zhutovsky et al., 2019). One possible explanation for these divergent findings could be that most previous studies used a region of interest approach focusing on predefined networks contrary to our whole-brain analysis. In addition, different types of psychotherapies and clinical as well as trauma characteristics could have accounted for these differences. And finally, developmental processes could have contributed as large-scale brain networks undergo considerable reorganization throughout childhood and adolescence (V. Menon, 2013).

The ICN yielding significant classification performance was centered on the STG. A growing number of studies have shown structural and functional abnormalities in the STG in PTSD patients (Engdahl et al., 2010; Lanius et al., 2002; Lindauer et al., 2008). Based on electrical stimulation of the area, Engdahl and colleagues (Engdahl et al., 2010) have suggested that STG abnormalities may be associated with re-experiencing symptoms. Others have suggested a relationship between STG abnormalities and dissociative symptoms in PTSD patients (Lanius et al., 2002). Interestingly, we have previously shown a positive correlation between STG activation and trauma-focused psychotherapy response in adults with PTSD (Lindauer et al., 2008).

Previous studies utilizing ML methods, however, did not identify network connectivity of the STG as an accurate predictor of treatment response. In adults treated with PE, Etkin and colleagues, found a classification accuracy of >85%, using a combination of pre-treatment rs-fMRI connectivity within the VAN and delayed recall performance in a verbal memory task (Etkin et al., 2019). In another study, pre-treatment functional connectivity within- and between- the default mode, dorsal attention, cingulo-opercular, salience, and central executive network during task-free fMRI predicted response to TF-CBT with an accuracy of 71.4% (Korgaonkar et al., 2020). Finally, we have previously shown the feasibility of the same approach as outlined here to predict response to trauma-focused therapy in veterans with PTSD with 81.4% accuracy (Zhutovsky et al., 2019), with an ICN centered on the pre-supplementary motor area providing the best predictive accuracy.

At present, it remains unclear why our findings on classification accuracy differ from findings in adult PTSD. Studies in adults have reported different networks/ functional connectivity estimates than identified here and have found classification accuracies which mostly exceeded accuracy found in the current study. One possibility is that, with inclusion of both PTSD and partial PTSD patients, clinical heterogeneity increased, resulting in lower classification accuracy. Another possibility is that neurodevelopmental trajectories add to heterogeneity and might reduce classification accuracy, as previous studies in youth with PTSD using rs-fMRI have shown neurodevelopmental effects on network connectivity and we

included youth with a relatively wide age range. These hypotheses require further investigation, including longitudinal studies of youth with PTSD which develop into adulthood. While the current individual-level classification findings differ from adults, it is reassuring that the application of the same approach to treatment-response classification as reported here has been associated with significant classification accuracies in adults multiple times, even for a different disorder (van Waarde et al., 2015; Zhutovsky et al., 2019).

There is a difference between the findings observed on the group- and on the individual-level. While there was no difference in within-network connectivity for any ICN between responders and non-responders on the group-level, there was a network significantly predictive on the individual-level. The opposite was true for the between-network connectivity. These discrepancies can be explained by the fact that a significant p-value in group-comparisons does not have to imply the ability to distinguish between patients on the individual-level because of low effect sizes of the difference (Arbabshirani et al., 2017). In addition, both analyses have different goals and therefore can identify different ICNs: group-level analyses focus on determining localized average differences between groups while individual-level analyses utilize all multivariate data to determine a model which provides the highest prediction (Bzdok & Ioannidis, 2019). This clearly marks the importance of performing individual-level prediction studies as these may improve clinical decision making in the future and may lead to independent results from group-level studies.

Although classification accuracy exceeded chance-level performance, it still falls below the APA proposed threshold for clinical applicability of biomarkers (First et al., 2018). The suggested combination of >80% sensitivity, specificity, and PPV is useful as guidance for research, but clinical utility should preferably be based on cost-benefit analyses (Pepe et al., 2016). As the current clinical standard is to offer trauma-focused psychotherapy to all youth with PTSD, a biomarker which reliably identifies non-responders could aid clinical decision making. This would correspond to a classifier with high specificity, but also reasonably high sensitivity to prevent classifying all patients as non-responders. If a-priory chances of treatment non-response are high, clinicians together with patients and their caregivers, could decide to abstain from initiating trauma-focused psychotherapy and search for alternative treatments with higher chances of success. This may help to prevent the unnecessary burden of failed treatment trials.

Several limitations of this study should be noted. First, the sample size in the current study is low. This has an impact on the certainty of the estimated performance of the individual-level analysis. Cross-validation can lead to high variance in performance estimates if applied to studies with low sample sizes (Varoquaux, 2018). To increase

the confidence in the presented results, we followed best-practices for the field (Poldrack et al., 2019), utilizing a permutation test corrected for multiple comparisons to provide a valid statistical control of the observed performance (Varoquaux, 2018). However, only with larger sample sizes can these problems be fully addressed and therefore the current study can only be regarded as a first step for further individual-level prediction studies in youth with PTSD. Larger sample sizes at the same time may increase clinical heterogeneity, limiting classification performance as well (Arbabshirani et al., 2017). Second, although the majority (82.5%) of included youth had a full PTSD diagnosis, the remaining 17.5% had a partial PTSD diagnosis. Including youth with partial PTSD increased clinical heterogeneity. Increased clinical heterogeneity might have lowered overall treatment response due to a floor effect and might have lowered prediction accuracy. However, by including youth with partial PTSD, our sample better reflects the real-life clinical setting, which adds to the ecological validity of our findings. Third, youth were randomized to receive either TF-CBT or EMDR, and both treatment conditions were collapsed for the current analysis. Due to limited power it was not feasible to examine differences between treatment responders and non-responders separately for both treatments or examine specific predictors for each treatment separately. However, we exploratively investigated the selective performance of the STG ICN for the different treatment groups in our sample which showed a similar performance to the classifier applied to the combined group. This indicates that the network is predictive of treatment-response in both treatment groups. Importantly, efficacy of both treatments has been shown to be comparable in an RCT with considerable sample overlap with the current study (Diehle et al., 2015). In addition, three of the 40 included patients were taking psychotropic medication. While one of the inclusion criteria of the study was that medication usage had to be stable for at least three weeks before and during trauma-focused psychotherapy this could have influenced our results. However, excluding more patients would have limited our sample size even more which is why we chose not to do it. Furthermore the relatively wide age range (8-17) of the included patients might have influenced the results of the current study as functional networks may be represented differently across development of youth (V. Menon, 2013). Finally, our study had substantial drop-out, as 18% of randomized patients were lost to follow-up. Although such dropout rates reflect routine clinical practice and treatment completers and non-completers did not differ on baseline characteristics, there is a possibility that drop-out could have influenced our findings through attrition bias.

CONCLUSIONS

The present study demonstrates that increased resting-state connectivity between the FPN and a sensorimotor network can distinguish trauma-focused psychotherapy responders from non-responders on the group-level. Future studies could examine if these network patterns are potential targets for (non-invasive) neuromodulation interventions to reduce PTSD symptoms in afflicted youth. We further show that resting-state connectivity patterns in a network centered on the bilateral STG are capable of predicting trauma-focused psychotherapy response in youth with PTSD. These proof-of-concept findings emphasize the feasibility of combining ML analysis and rs-fMRI to identify predictive biomarkers for treatment response. However, before translation to clinical practice can commence, future research should aim to test the robustness and generalizability of these findings in larger independent cohorts.

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SUPPLEMENTARY MATERIAL

Acknowledgements

This study was supported by the Netherlands Organization for Scientific Research (GvW, NWO/ZonMW Vidi 016.156.318) and the AMC Research Council (GvW, 150622).

Supplementary Methods

Trauma-exposed controls

To prevent overfitting in the main analyses, we utilized an independent sample of trauma-exposed controls (TEC) to identify our intrinsic connectivity network (ICN) templates (Poldrack et al., 2019). TEC were aged between 8 and 18 years and were able to understand the Dutch language. TEC were recruited between June 2011 and September 2018 through local elementary- and high schools by researcher JBZ, RodK and JBME. Exposure to traumatic events were validated according to A1 and A2 criteria of DSM-IV-TR (American Psychiatric Association, 2000) using the life-events checklist of the Clinician-Administered PTSD Scale for Children and Adolescents (CAPS-CA) semi-structured interview (Nader et al., 1996). TEC were excluded if they met PTSD or partial PTSD diagnosis using both the CAPS-CA and caregiver reports from the PTSD scale of the Anxiety Disorders Interview Schedule – Parent Version (ADIS-P) (Verlinden et al., 2014) or had a CAPS-CA total score of >20 points. Additional exclusion criteria were: acute suicidality, IQ<70, pregnancy, neurological disorders or serious medical illnesses or meeting the criteria of one of the following diagnosis: psychotic disorders, substance-use disorder or pervasive developmental disorder. 21 TEC were scanned on the same scanner using the same parameters and protocol as the (partial)-PTSD patients described in the main manuscript.

The resting-state functional magnetic resonance imaging (rs-fMRI) data of the TEC was preprocessed according to the exact same procedures as described in the main manuscript. Application of the same quality control procedures led to the exclusion of two TEC due to registration failures and two TEC due to excess motion, leading to a final included sample of 17 TEC as reported in the main manuscript. The included TEC did not differ from the included PTSD patients in age (M: 14, SD: 3.57, $t(55) = 1.53$, $p = 0.133$), gender (64.7% female, $X^2(1) = 0.0005$, $p = 0.983$), age at trauma (M; 12.13, SD 3.52, $t(52) = 1.87$, $p = 0.063$), time since trauma (M; 2.07, SD 1.98, $t(52) = 1.039$, $p = 0.304$), and motion as estimated via mean framewise displacement(Power et al., 2014) (M: 0.15, SD: 0.04, $t(55) = -1.77$, $p = 0.08$) but did differ in type of trauma exposure $X^2(4) = 15.998$, $p = 0.003$ with relatively more accidents and other trauma and less sexual abuse and domestic/community violence in the TEC group.

Functional data preprocessing

For each participant, a reference rs-fMRI volume and its skull-stripped version were generated using custom methodology of fMRIPrep. This reference image was then co-registered to the corresponding MRI scan using boundary-based registration (Greve and Fischl, 2009). Head-motion correction with respect to the reference was estimated before any spatiotemporal filtering using MCFLIRT (Jenkinson et al., 2002). The rs-fMRI scans were normalized to MNI space combining all spatial transformations (head-motion correction, co-registration and normalization) into one single step using Lanczos interpolation (Lanczos, 1964).

Identification of intrinsic connectivity networks

The meta-ICA procedure was implemented as follows: we repeatedly (n=25) and randomly selected 15 out of our 17 TEC and computed a temporally-concatenated ICA to identify spatially independent components (70 components each, i.e. 25 * 70 = 1750 spatial components in total). Then, we concatenated all spatial components across all individual ICA runs and calculated a final meta-ICA (70 components) leading to a set of robust spatial components to consider for identification of ICNs.

To identify valid ICNs, we employed a semi-automatic approach (Cerliani et al., 2015). In a first stage, we assessed all spatial components visually, focusing on overlap with GM and overlap with previously identified ICNs. This led to the exclusion of 20 components. In a second stage, we computed the average spatial correlation between each of the meta-ICA components and the maximally correlated spatial components of each individual ICA run (Cerliani et al., 2015). Such a measure represents the reproducibility of the meta-ICA components across all individual ICA runs. We excluded all components with a correlation <0.6, leading to the exclusion of 2 additional components.

Individual-level analyses

To assess statistically whether the averaged, cross-validated balanced accuracies allowed for better-than-chance performance and to correct for the number of classifications performed, we utilized synchronized permutation tests of the maximum statistic. To this end we randomly permuted the classification labels associated with our data (same permutations for each ICN and the between-ICN connectivity, n=2000) (Ojala and Garriga, 2010), estimated the maximum performance for each of the permutations across all the included ICNs/between-ICN connectivity measures and used this estimated null-distribution of the maximum statistic to correct for familywise-error of the p-values of the individual performances of each of our classifications.

We also assessed which features (voxel values of individual ICNs or individual between-ICN connectivity) were important for the SVM classification by calculating p-values for each weight of the classifier. The p-values were estimated using an analytical approximation of a permutation procedure from a combination of the weights and the size of the margin of the SVM (Gaonkar et al., 2015). The p-values were computed after the classifier was applied to the entire data set (no cross-validation) and are intended for post-hoc visualization purposes only.

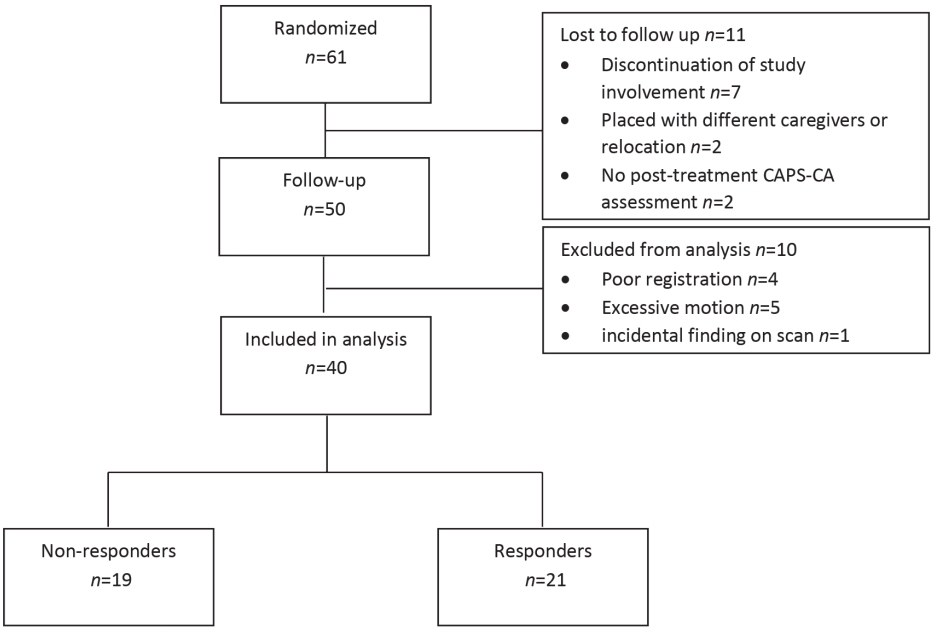

Figure S1: Flow diagram of included patients. Response is defined as ≥30% reduction in CAPS-CA total score from pre- to post-treatment.

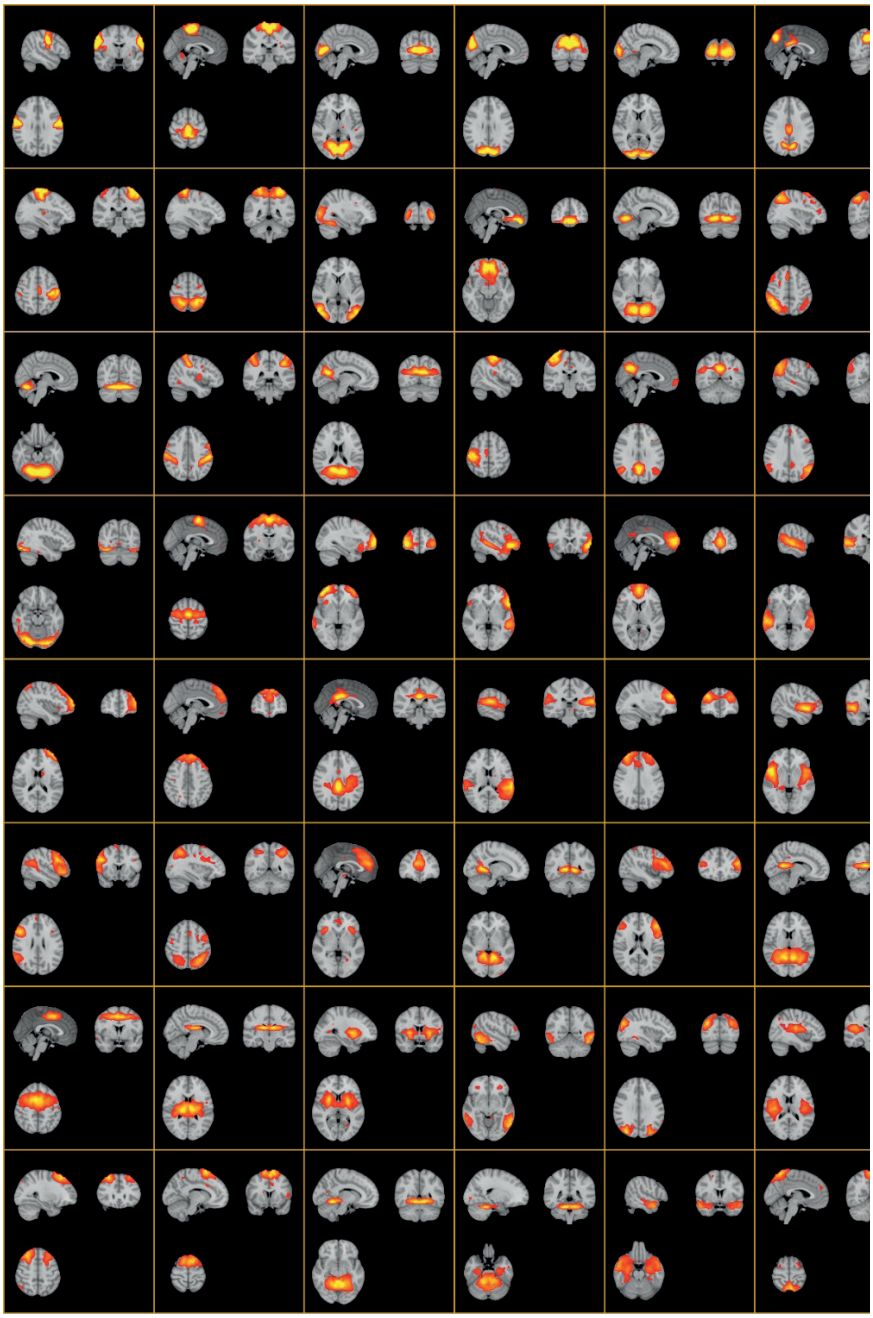

Figure S2: All 48 included intrinsic connectivity networks (ICNs) estimated through the application of meta-independent component analyses (ICA) with 70 (group-)components. ICNs were identified utilizing a semi-automatic approach consisting of visual assessment and calculation of average spatial correlation coefficients between each of the meta-ICA spatial components and their corresponding maximally correlated individual-ICA components. Components with an average correlation <0.6 were removed. The right hemisphere is plotted on the left.

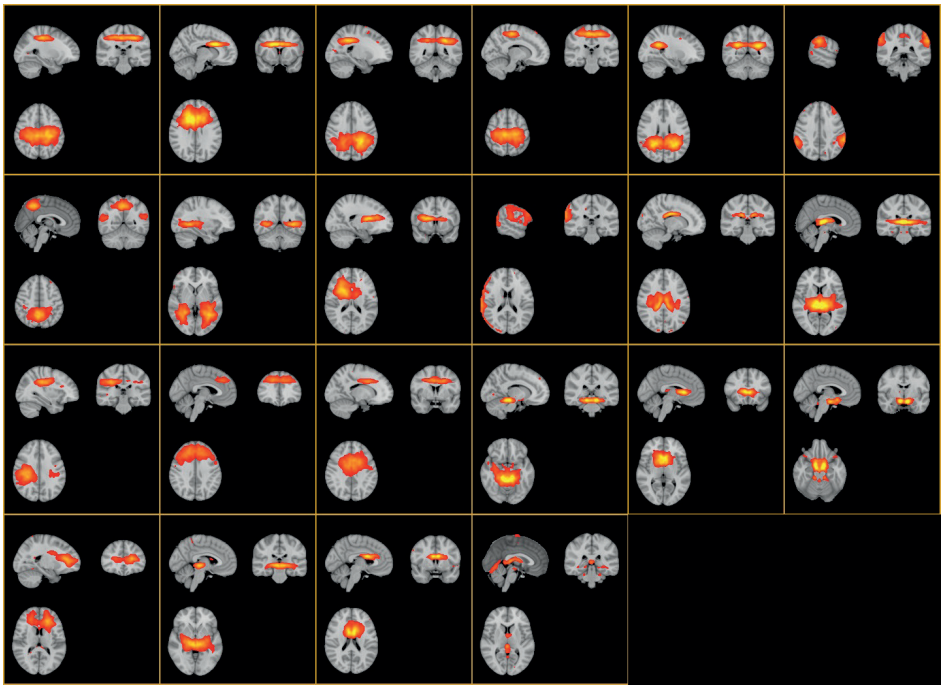

Figure S3: All 22 excluded components. Either excluded because of their overlap with white matter/cerebrospinal fluid or because of their low average spatial correlation (<0.6) between the meta-ICA group-component and its maximally correlated individual-ICA components. The right hemisphere is plotted on the left.

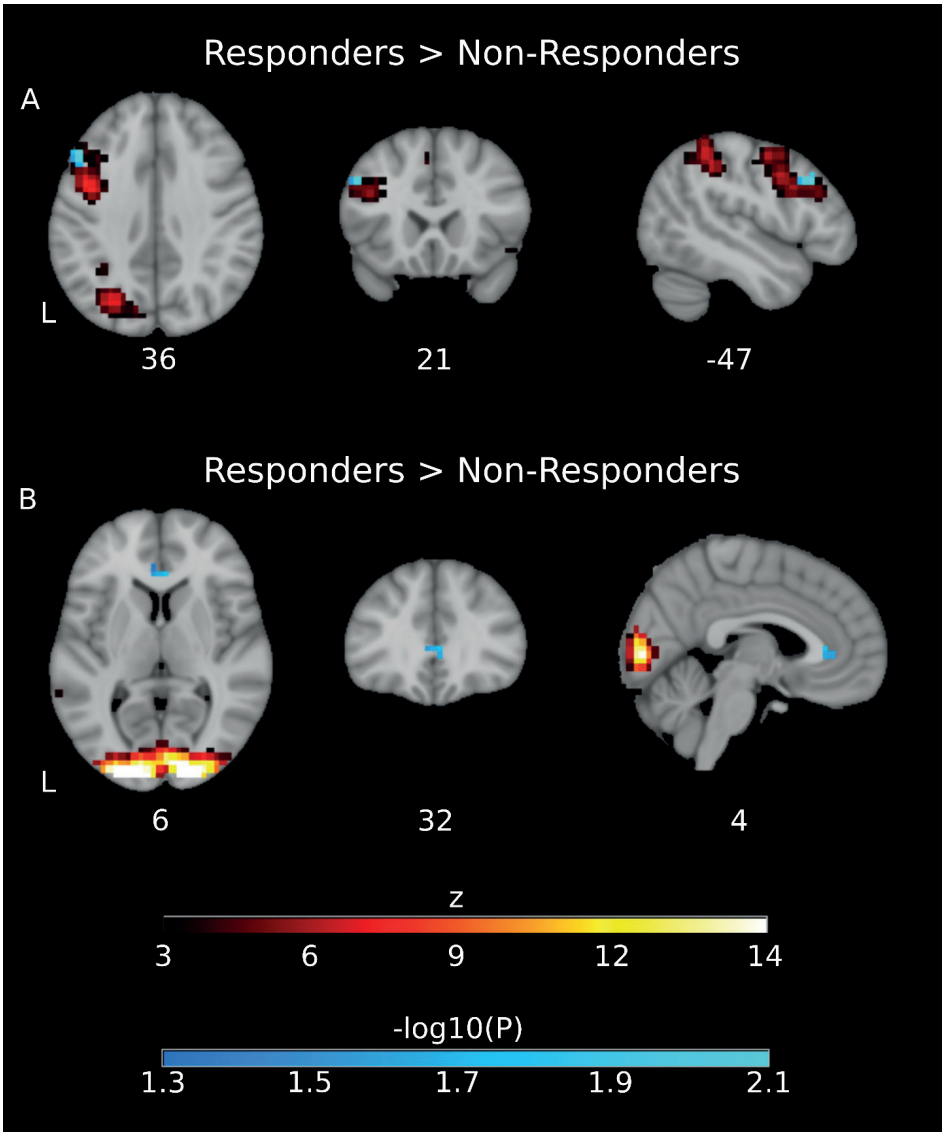

Figure S4: Exploratory group-level analyses comparing responders to non-responders for within-ICN connectivity without correction for multiple-comparisons for the number of investigated components. A. increased within-ICN connectivity in the left frontoparietal ICN (same as in Figure 1 of the main manuscript) for responders over non-responders. B. increased within-ICN connectivity in the visual (occipital) ICN for responders over non-responders.

CHAPTER 4

Using neurobiological measures to predict and assess treatment outcome of psychotherapy in posttraumatic stress disorder: systematic review

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Psychotherapy and Psychosomatics 2013;82(3):142-51

ABSTRACT

Background: Trauma-focused cognitive-behavioral therapy (TF-CBT) and eye movement desensitization and reprocessing (EMDR) are effective treatments for posttraumatic stress disorder. However, little is known about their neurobiological effects. The usefulness of neurobiological measures to predict the treatment outcome of psychotherapy also has yet to be determined.

Methods: Systematic review of randomized controlled trials (RCTs) focused on neurobiological treatment effects of TF-CBT or EMDR and trials with neurobiological measures as predictors of treatment response.

Results: We included 23 publications reporting on 16 separate trials. TF-CBT was compared with a waitlist in most trials. TF-CBT was associated with a decrease in heart rate and blood pressure and changes in activity but not in volume of frontal brain structures and the amygdala. Neurobiological changes correlated with changes in symptom severity. EMDR was only tested against other active treatments in included trials. We did not find a difference in neurobiological treatment effects between EMDR and other treatments. Publications on neurobiological predictors of treatment response showed ambiguous results.

Conclusion: TF-CBT was associated with a reduction of physiological reactivity. There is some preliminary evidence that TF-CBT influences brain regions involved in fear conditioning, extinction learning and possibly working memory and attention regulation; however, these effects could be nonspecific psychotherapeutic effects. Future trials should use paradigms aimed specifically at these brain regions and physiological reactivity. There are concerns regarding the risk of bias in some of the RCTs, indicating that methodologically more rigorous trials are required. Trials with neurobiological measures as predictors of treatment outcome render insufficient results to be useful in clinical practice.

INTRODUCTION

Posttraumatic stress disorder (PTSD) is a common, disabling and often chronic anxiety disorder (R. C. Kessler, 2000; Ronald C. Kessler, Chiu, Demler, Merikangas, & Walters, 2005), which can develop after exposure to a traumatic event. It is characterized by symptoms of re-experiencing, avoidance and hyperarousal (American Psychiatric Association, 2013). In recent decades, accurate and minimally invasive genotyping, neuroimaging, physiological and endocrinological techniques have emerged, and studies using these together with preclinical studies have facilitated a better understanding of the underlying mechanisms of PTSD (Rachel Yehuda & LeDoux, 2007). Four cardinal findings have emerged: (1) PTSD is associated with changes in the neural circuitry involving the prefrontal and limbic structures (Francati, Vermetten, & Bremner, 2007; Karl et al., 2006), (2) changes in the neural circuitry correlate with changes in the autonomous nervous system (ANS) (Charney, 2004) and hypothalamus pituitary adrenal (HPA) axis activity (Carrion, Weems, Richert, Hoffman, & Reiss, 2010; Rodrigues, LeDoux, & Sapolsky, 2009), (3) changes in the neural circuitry, ANS and HPA axis arise from an interaction between environmental factors and a genetic profile (Koenen, Amstadter, & Nugent, 2009; Rachel Yehuda & LeDoux, 2007) and (4) these changes play a crucial role in the development and maintenance of PTSD (Charney, 2004).

Persistent PTSD leads to considerable suffering and disturbances of social and work-related functioning (R. C. Kessler, 2000), which underlines how important effective treatments can be. Several psychological treatments for PTSD have been developed (Foa, 2011). Of these, trauma-focused cognitive-behavioral therapy (TF-CBT) and eye movement desensitization and reprocessing (EMDR) have been shown to be effective in reducing symptoms (Bisson & Andrew, 2007). Studies measuring the effects of these psychotherapies on the neural circuitry, ANS and HPA axis activity are underrepresented in comparison to analogous studies of pharmacotherapy (Roffman, Marci, Glick, Dougherty, & Rauch, 2005). Biological measures, however, render important insights into the working mechanisms of psychotherapy, although it is not yet sure if these insights can indeed improve treatment strategies (Linden, 2006). Biological measures may be useful to predict treatment outcome (Siegle, Carter, & Thase, 2006) and may contribute to psychoeducation through outcome feedback (Knaup, Koesters, Schoefer, Becker, & Puschner, 2009). Given this background, we conducted a systematic review following the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) statement (Moher, Liberati, Tetzlaff, Altman, & Group, 2009) with the following objectives: (1) to examine the effects of TF-CBT and EMDR on the (re)activity of the limbic and frontal structures, ANS and HPA axis, (2) to examine if neurobiological changes correlate with changes in PTSD severity and (3) to examine if pretreatment neurobiological measures can predict treatment outcome.

METHODS

Search Strategy

We conducted searches in MEDLINE, Embase, PsycINFO, PILOTS and the Cochrane register of controlled trials and database of systematic reviews. Each database was searched from inception to the second week of February 2012. A clinical librarian experienced in conducting systemic reviews assisted us with the formation of appropriate search terms for each of the databases. The following terms were used: posttraumatic stress disorder and eye movement desensitization and reprocessing OR cognitive behavioral therapy OR processing therapy OR exposure therapy OR brief psychotherapy OR short-term psychotherapy (a complete list of search terms for each individual database can be found in the supplementary material). Given the broad range of possible outcome measures, including the search terms for each outcome is undesirable because it would narrow our search and risk omitting relevant studies. The reference lists of selected articles were also monitored for relevant studies.

Retrieved studies were imported into Reference Manager (version 12, for Windows, Thomson Reuters, New York, N.Y., USA). Duplicates were identified and eliminated. Thereafter, the first 2 authors reviewed all titles and abstracts independently. Articles were selected for full-text review if based upon the title and abstract, inclusion criteria were met or uncertainty regarding eligibility persisted. Disagreements were discussed and resolved after the title abstract review and after full text reviews. Final agreement was reached in all cases.

Quality Assessment

In accordance with the PRISMA statement and the Cochrane handbook (Higgins, 2011), included randomized controlled trials (RCTs) were assessed for potential risk of bias on the following 5 domains: whether or not (1) the study used a randomized sequence of assignments, (2) the allocation sequence was concealed from those involved in the enrolment and assignment of participants, (3) people who determined the outcome measurements were aware of intervention assignments (blinding of outcome assessment), (4) outcome data were missing due to attrition during the study or exclusion from analysis and (5) selective reporting of outcomes occurred (for the detailed assessment criteria for each individual domain, see the Cochrane handbook, chapter 8 (Higgins, 2011). All RCTs were assessed separately on these 5 domains by both reviewers. Disagreements were discussed in order to reach one final judgment. We did not address the blinding of participants or therapists, given the obvious difficulties regarding blinding during psychotherapy.

Inclusion Criteria

Design

In order to differentiate neurobiological treatment effects from time effects, the studies used for objectives (1) and (2) had to be randomized and controlled. For objective (3), the studies did not have to meet these conditions in order to be included. Considering the difficulties of blinding participants and practitioners during psychotherapy, studies did not have to be blinded to be included. Single-case studies were excluded, and only studies published in English were included.

Participants

Participants had to fulfil the criteria for PTSD or partial PTSD at the beginning of treatment. People with partial PTSD are some- what less impaired than individuals with (full) PTSD; however, symptoms also cause clinical meaningful levels of functional impairment in these individuals (Stein, Walker, Hazen, & Forde, 1997). To fulfil the diagnosis of partial PTSD, individuals needed to meet the A, E and F DSM-IV criteria for PTSD in combination with one or more symptoms in each of the 3 symptom groups (criteria B, C and D) or meeting 2 of 3 symptom clusters for criteria B, C or D. There was no restriction on the basis of the type of traumatic event. Since comorbidity is common in PTSD (Ronald C. Kessler et al., 2005), studies involving individuals with comorbid psychiatric disorders besides PTSD were not excluded; the primary diagnosis for participants had to be PTSD, however.

Interventions

We included studies if participants had been treated with either EMDR or TF-CBT. EMDR had to involve bilateral stimulation by means of eye movements, beeps or taps whilst patients focused on a traumatic image, thought, emotion or bodily sensation. TF-CBT was defined as each treatment which involved both (1) deliberate systematic confrontation with trauma-related stimuli through imaginal or in vivo exposure and (2) therapists assisted identification and disconfirmation of distorted thought patterns and beliefs regarding oneself, traumatic events and the world. In line with the most recent Cochrane review on psychological interventions in PTSD (Bisson & Andrew, 2007), this group also included (prolonged and narrative) exposure (Foa, 2011) and brief eclectic psychotherapy (which also includes psychodynamic treatment elements) (R. J. L. Lindauer, Gersons, et al., 2005).

Comparison

For treatment outcome studies, the following comparisons were included: comparison to a waitlist condition, delayed-treatment conditions, routine clinical care or other active treatments.

Outcome Measures

Studies were included if one or more of the following measures were performed: hormonal levels, brain activity or volume(s), activity of the ANS or if genotyping was performed. For the correlation analysis – objective (2) – the severity of a posttraumatic stress symptom had to be either rated by a clinician using a standardized measure or by a standardized self-report measure.

Data Analysis

Because of the large clinical heterogeneity between studies, calculation of the standardized mean difference was judged unreasonable by our consulting statistician.

Outcome data were extracted independently by the first two authors. Reported measures only included continuous neurobiological outcomes. To minimize the heterogeneity of outcomes, we translated continued measures to a standardized effect size (i.e. posttreatment mean of intervention group minus posttreatment mean of control group, divided by the pooled standard deviation). Calculations were performed on a PC in Microsoft Office Excel 2007.

RESULTS

Search Findings

We included a total of 23 publications from 16 separate trials (fig. 1). Sixteen concerned 11 RCTs and were used for objectives (1) and (2)(Adenauer et al., 2011; Blanchard et al., 2002; Fecteau & Nicki, 1999; Gerardi, Rothbaum, Astin, & Kelley, 2010; Hinton, Hofmann, Pollack, & Otto, 2009; Karl, Malta, Alexander, & Blanchard, 2004; R. J. Lindauer et al., 2008; R. J. L. Lindauer, Gersons, et al., 2005; R. T. Lindauer et al., 2006; Peres et al., 2011; Peres et al., 2007; Rabe, Dörfel, Zöllner, Maercker, & Karl, 2006; Rabe, Zoellner, Beauducel, Maercker, & Karl, 2008; Renfrey & Spates, 1994; Rogers et al., 1999). The remaining 7 publications reported on 5 trials which were used for objective (3) (Abou Elseoud et al., 2011; Blanchard et al., 2003; Richard A. Bryant et al., 2008; Bryant et al., 2010; Nardo et al., 2010; Tarrier et al., 1999; R. Yehuda et al., 2009). All studies applied DSM-IV criteria except Renfrey and Spates (Renfrey & Spates, 1994) and Rogers et al. (Rogers et al., 1999), who applied DSM-III criteria. Studies included both people with full PTSD and partial PTSD. The age of participants ranged from 18 to 71 years. Most studies included both males and females and were conducted on an outpatient basis. The most common traumatic events were combat-related events, accidents and interpersonal violence. Participants were recruited through advertisement, (self-)referrals and inpatient programs. Baseline PTSD severity ranged from moderate to severe with most participants suffering from chronic PTSD (symptoms lasting 3 months or more). The most common exclusion criteria were substance-use disorder, personality disorder and psychotic disorders. Figure 2 summarizes the risk of bias of the included RCTs.

In the 11 included RCTs, 321 patients were analyzed. Different types of outcome data of a portion of these patients were reported in separate publications (R. J. Lindauer et al., 2008; R. J. L. Lindauer, Vlieger, et al., 2005; R. T. Lindauer et al., 2006) and (Blanchard et al., 2002; Karl et al., 2004; Rabe et al., 2006; Rabe et al., 2008). The number of people randomized in trials ranged from 12 to 78.

In addition, 143 unique nonrandomized participants were included for objective (3). Sample sizes varied between 13 and 45. Again, different outcome data of a portion of participants were presented in separate publications ((R. A. Bryant et al., 2008; Richard A. Bryant et al., 2008) and (Blanchard et al., 2003; Blanchard et al., 2002)).

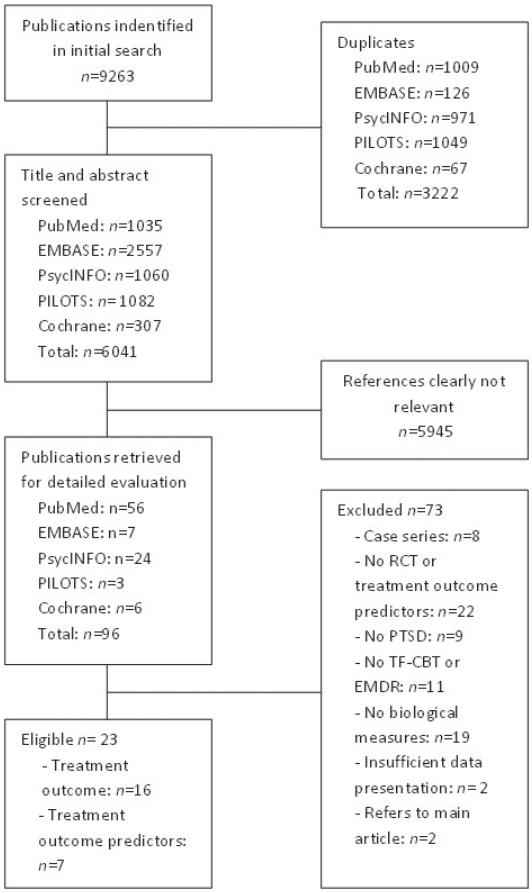

Fig. 1 Flow diagram of trials included and excluded in systematic review

Effects of TF-CBT and EMDR on the (Re)Activity of the Limbic and Frontal Structures, the ANS and the HPA Axis (1)

TF-CBT versus Waitlist or Support

Twelve publications out of 7 trials described a comparison between TF-CBT and a waitlist condition or supportive psychotherapy (table 1) (Adenauer et al., 2011; Blanchard et al., 2002; Fecteau & Nicki, 1999; Hinton et al., 2009; Karl et al., 2004; R. J. Lindauer et al., 2008; R. J. L. Lindauer, Vlieger, et al., 2005; R. T. Lindauer et al., 2006; Peres et al., 2011; Peres et al., 2007; Rabe et al., 2006; Rabe et al., 2008). In 6 of these publications, TF-CBT was compared to waitlist or support using psychophysiological measures as a treatment outcome (table 1) (Blake et al., 1995; Blanchard et al., 2002; Fecteau & Nicki, 1999; Hinton et al., 2009; Karl et al., 2004; R. T. Lindauer et al., 2006;

Rabe et al., 2006). One of these described effect sizes but did not report sufficient data for effect- size recalculation (Karl et al., 2004). In the other 6 publications, TF-CBT was compared to waitlist using brain activity and brain volume as an outcome measure (table 1)(Adenauer et al., 2011; R. J. Lindauer et al., 2008; R. J. L. Lindauer, Gersons, et al., 2005; Peres et al., 2011; Peres et al., 2007; Rabe et al., 2008).

EMDR versus Active Treatment or Routine Clinical Care

In 3 trials, EMDR was either compared with active treatment(s) or routine clinical care (table 1) (Carlson, Chemtob, Rusnak, Hedlund, & Muraoka, 1998; Renfrey & Spates, 1994; Rogers et al., 1999). All 3 used psychophysiological measures as a treatment outcome. One publication did not report sufficient data to calculate effect sizes (Carlson et al., 1998).

EMDR versus Prolonged Exposure

One trial compared the treatment effects of EMDR with prolonged exposure on HPA-axis activity (table 1) (Gerardi et al., 2010).

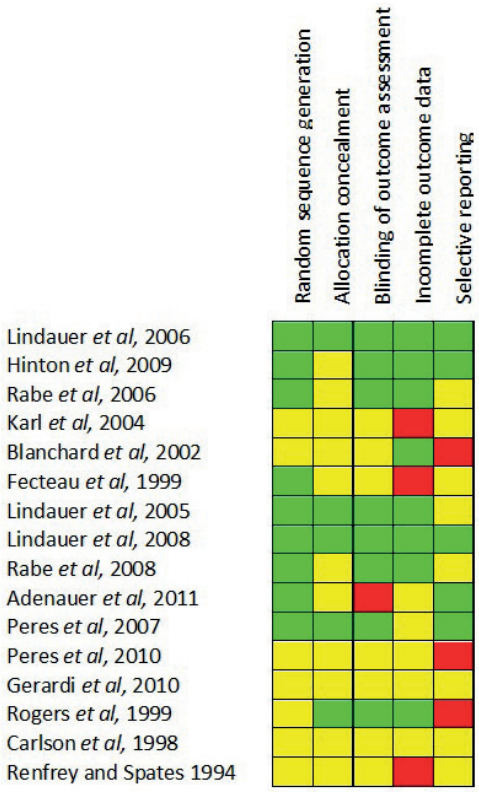

Fig. 2. Risk of bias of included randomized controlled trials

Correlation between Biological Treatment Outcome and Change in Symptom Severity (2)

In a total of 7 publications out of 4 trials, change in biological variables was correlated with change in PTSD symptoms (table 2) (Blanchard et al., 2002; R. J. Lindauer et al., 2008; R. T. Lindauer et al., 2006; Peres et al., 2011; Peres et al., 2007; Rabe et al., 2006; Rabe et al., 2008). Correlations between a change in neurobiological variables and a change in symptom severity scores were obtained using the Pearson product-moment correlation coefficient in all the publications. In 4, outcome data from the intervention group and the control group(s) were combined to calculate correlations (Blanchard et al., 2002; R. T. Lindauer et al., 2006; Rabe et al., 2006; Rabe et al., 2008); in the remaining 3, only outcome data from the intervention group were used (Blanchard et al., 2002; R. J. Lindauer et al., 2008; Peres et al., 2011; Peres et al., 2007). Symptom severity was measured with either the Clinician-Administered PTSD scale (CAPS) or the Structured Interview for PTSD (SI-PTSD) (Blake et al., 1995; Davidson, Kudler, & Smith, 1990)[45, 46]. In 3 publications, a change in psychophysiological variables was correlated with a change in symptom severity (Blanchard et al., 2002; R. T. Lindauer et al., 2006; Rabe et al., 2006) and in 4, a change in brain activity was correlated with a change in symptom severity (R. J. Lindauer et al., 2008; Peres et al., 2011; Peres et al., 2007; Rabe et al., 2008).

Pretreatment Neurobiological Measures to Predict Treatment Outcome (3)

Seven publications on 5 studies assessed if pretreatment biological and genetic variables could predict treatment outcome (table 3) (Blanchard et al., 2003; R. A. Bryant et al., 2008; Richard A. Bryant et al., 2008; Bryant et al., 2010; Nardo et al., 2010; Roffman et al., 2005; Tarrier et al., 1999; R. Yehuda et al., 2009). One reported that genotyping was used (Bryant et al., 2010), 3 reported neuroimaging (R. A. Bryant et al., 2008; Richard A. Bryant et al., 2008; Nardo et al., 2010), 1 reported endocrine measures (R. Yehuda et al., 2009) and 2 reported the use of psychophysiological measures (Blanchard et al., 2003; Tarrier et al., 1999).

Table 1. Summary of findings: Neurobiological treatment effects of TF-CBT and EMDR in PTSD.

Study	n participant; n treatment n controls (*n sessions*)	Measures; provocation	Results
Lindauer *et al*, 2006	20 PTSD; 9 BEP 11 WL (*16*)	HR and BP; Baseline, SDI, recovery	↓ HRR score in BEP>*WL (d=1.02); ↓ SBP baseline and DSBP recovery in BEP>*WL
Hinton *et al*, 2009	24 PTSD with orthostatic panic attacks; 12 TF-CBT 12 delayed TF-CBT (*12*)	HR and BP; Standing up provoking orthostatic panic attack	↓ SBP in immediate TF- CBT>** delayed TF-CBT at 2nd assessment (end immediate TF-CBT) (d=1.38); DSBP and HR = between groups at 2nd assessment; SBP, DSBP and HR = between groups at end delayed TF-CBT
Rabe *et al*, 2006	35 (partial) PTSD; 17 TF-CBT 18 WL (*8-12*)	HR; Baseline, positive, negative and trauma related pictures	↓ HRR trauma related pictures in TF-CBT >* WL (d=0.78); HRR pos. and neg. pictures = between groups
Karl *et al*, 2004	9 (partial) PTSD; 6 TF-CBT/SUP 3 WL (*8-12*)	EMG musculus orbicularis oculli; neutral, startle and trauma sounds	↓ EMG reactivity to startle and trauma sounds in TF-CBT/SUP group>** WL (d=2.89/2.8); ↓EMG reactivity to neutral sounds TF-CBT/SUP group >* WL (d=1.67)
Blanchard *et al*, 2002	73 (partial) PTSD; 25 TF-CBT 26 SUP 22 WL (*8-12*)	HR, BP, SCL; Baseline, mental arithmetic, SDI, relaxation	↓ HRR in TF-CBT group >*** both SUP (d=0.77) and WL (d=0.51); BP and SCL reactivity = between groups
Fecteau *et al*, 1999	20 PTSD; 10 TF-CBT 10 WL (*4*)	HR; Baseline and trauma script	Trend towards ↓ HRR in TF-CBT group > WL (p=<0.1, d=0.75)
Lindauer *et al*, 2005	18 PTSD; 9 BEP, 9 WL (*16)*	MRI	= in VOI's: amygdala, hippocampus and parahippocampal gyrus in both groups
Lindauer *et al*, 2008	20 PTSD; 10 BEP, 10 WL (*16)*	[99mTc]HMPAO SPECT; Trauma SDI	↓ rCBF in r. middle frontal gyrus (dorsolateral prefrontal cortex) and r. uncus in BEP>**WL
Peres *et al*, 2007	27 partial PTSD; 16 TF-CBT, 11 WL (*8)*	[99mTc]HMPAO SPECT; Trauma SDI	↑ rCBF in l. anterior cingulate cortex, l. prefrontal cortex, thalamus, l. parietal lobe, l. hippocampus and l. Broca's area in TF-CBT >*** WL; ↓ rCBF in l. amygdala in TF-CBT >*** WL
Peres *et al*, 2010	24 (partial) PTSD; 12 TF-CBT, 12 WL *(8)*	fMRI; Cued recall during positive, negative and trauma sounds	↑ BOLD in mid prefrontal cortex and ↓ BOLD in l. amygdala in TF-CBT >*** WL; = between groups in ROI's (anterior cingulate cortex, orbito frontal cortex, thalamus, insula, parietal lobe, hippocampus) or whole-brain analysis
Rabe *et al*, 2008	35 (partial) PTSD; 17 TF-CBT, 18 WL (*8-12)*	EEG; Baseline, positive, negative and trauma pictures	Trend towards ↓reactivity in r. anterior region in TF-CBT group > WL (p=0.07); = reactivity in posterior region in TF-CBT compared to WL
Adenauer *et al,* 2011	19 PTSD; 11 NET, 8 WL *(12)*	MEG; Steady state visual evoked fields during trauma and aversive pictures	↑ activity in superior parietal cortex and l. occipital brain regions in NET>(* and **) WL
Gerardi *et al*, 2010	50 PTSD; 25 EMDR 25 PE(*9*)	Salivary cortisol; baseline, post exposure	= salivary cortisol level between groups
Rogers *et al*, 1999	12 PTSD; 6 EMDR 6 Exposure (*1*)	HR; Baseline, trauma imagery	= in HR(R) between groups
Carlson *et al*, 1998	27 PTSD; 8 EMDR 7 RCC 12 Biofeedback (*12*)	HR, SCL, EMG, skin temperature; Baseline and SDI	= in EMG (bilateral frontalis, trapezius, l. sternomastoid and l. forearm flexor), HR, SCL and skin temperature between groups between pre and post treatment and between post treatment and follow up
Renfrey and Spates 1994	23 (partial) PTSD; 8 EMD 8 EMD with automated EMs 7 with visual fixation (*6*)	HR(R); Baseline, positive and negative imagery	= in HR(R) between groups between pre and post treatment and between post treatment and follow up

TF-CBT=trauma focused cognitive-behavioural therapy; BEP=brief eclectic psychotherapy; WL=Waitlist; SUP=support; EMD(R)=Eye movement desensitisation (and reprocessing); PE=prolonged exposure; NET=narrative exposure therapy; RCC=routine clinical care; HR(R)=heart rate (reactivity); BP=blood pressure; (D)SBP=(dia)systolic blood pressure; SCL=skin conductance level; EMG=electromyogram; SDI=script driven imagery; (f)MRI=(functional) magnetic resonance imaging; SPECT= single photon emission computerised tomography; MEC=magnetoenchephalography; rCBF=regional cerebral blood flow; BOLD= blood oxygen level dependence; R/VOI=region/volume of interest; l.=left; r.=right; EEG=electro encephalogram; *<0.05; **<0.01;***<0.001

DISCUSSION

This is the first systematic review assessing both the biological treatment outcome of psychotherapy and biological predictors of treatment outcome in PTSD. We identified 6 controlled neuroimaging trials of TF-CBT, but none of EMDR. Overall, these trials did not yield unambiguous findings. Two showed that after TF-CBT, activity in the mid-prefrontal cortex increased while activity in the amygdala decreased (Peres et al., 2011; Peres et al., 2007). Both structures are involved in fear conditioning and extinction learning and show disturbed activity in people with PTSD (LeDoux, 2000). Lindauer et al. (R. J. Lindauer et al., 2008) demonstrated that after TF-CBT, activity had decreased in the dorsolateral prefrontal cortex, which is part of the neural circuitry underlying working memory function. Disturbances in this neural circuitry seem to be involved the development and maintenance of PTSD (Shaw et al., 2009; Veltman, Rombouts, & Dolan, 2003). However, in the single-photon emission computed tomography trial of Peres et al. (Peres et al., 2007), no changes in activity of the dorsolateral prefrontal cortex were found. Contrasting findings were also found regarding the role of the anterior cingulate cortex, orbitofrontal cortex, thalamus, insula, Broca's area, parietal lobe and hippocampus in TF-CBT treatment response. These areas are all implicated in the processing and integration of (sensory) information and the formation of structured memories and narratives. Deviations in both structure and function of these areas have been found in people with PTSD (Bremner et al., 2003; Lanius et al., 2004; Lanius et al., 2003; R. J. L. Lindauer et al., 2004). In their single-photon emission computed tomography Study, Peres et al. (Peres et al., 2007) found that after treatment, activity in these regions increased, while in their functional magnetic resonance imaging (fMRI) study (Peres et al., 2011) activity did not change. In a magnetoencephalography study, Adenauer et al. (Adenauer et al., 2011) demonstrated that after TF-CBT treatment, activity increased in the parietal and occipital brain regions which are involved in attention regulation towards aversive stimuli. However, a substantial amount of magnetoencephalography outcome data was lost during this trial. Given the relatively small number of neuroimaging trials and mostly divergent findings in these trials, no conclusion can yet be drawn on the effects of TF-CBT (or EMDR) on neural activity in PTSD. Furthermore, because TF-CBT was compared to a waitlist condition and not to an active treatment in all trials, results may be regarded as nonspecific (psychotherapeutic) effects rather than specific effects of TF-CBT.

Table 2. Summary of findings: Correlation between change in neurobiological variables and change in symptom severity scores.

Study	Sample	Pre-Post change in Biological variable	Δ on Psycho-metric scale	Correlation
Lindauer *et al*, 2008	9 BEP	rCBF l. superior temporal gyrus, l.+ r. superior/middle frontal gyrus	SI-PTSD total	+ l. superior temporal gyrus z(9)=3.1*; + l.+r. superior/middle frontal gyrus: l. z=3.36***, r. z=3.52*
Lindauer *et al*, 2006	9 BEP + 11 WL	HRR to neutral, stressful and trauma scripts	SI-PTSD total	+ HRR trauma script: r(20)=0.56**; = HRR neutral and stressful scripts
Peres *et al*, 2010	12 TF-CBT	BOLD l. amygdala, mid prefrontal cortex cued recall trauma sounds	CAPS	+ mid prefrontal cortex r(12)= 0.82*; - l. amygdala r(12)= 0.86*
Peres *et al*, 2007	16 TF-CBT	rCBF l. prefrontal cortex, l. amygdala and other brain regions	CAPS	+ l. prefrontal cortex z=3.79**; - l. amygdala z=3.12**; = other brain areas
Rabe *et al*, 2006	17 TF-CBT + 18 WL	HRR to positive, negative and trauma related pictures	CAPS	+ HRR trauma pictures r(35)=0.30* =HRR positive/negative pictures
Rabe *et al*, 2008	17 TF-CBT + 18 WL	Activity within l/ r. hemisphere and activation asymmetry	CAPS	+ r. anterior activation r(35)=0.39* = l. hemisphere activation, posterior or anterior asymmetry
Blanchard *et al*, 2002	25 TF-CBT + 26 SUP + 22 WL	HRR to trauma imagery	CAPS	+ HRR: r(73)=0.29**

TF-CBT=trauma focused cognitive-behavioural therapy; BEP=brief eclectic psychotherapy; WL=Waitlist; SUP=support ; rCBF=regional cerebral blood flow; BOLD= blood oxygen level dependant; l.=left; r.=right; HRR=heart rate reactivity; SBP=systolic blood pressure; SI-PTSD=structured interview post traumatic stress disorder; CAPS=clinician administered posttraumatic stress disorder scale; *<0.05; **<0.01; ***<0.001

In contrast to the included neuroimaging trials, physiological-treatment outcome trials showed less ambiguous results. Regarding TF-CBT, all included publications showed a reduction of posttreatment physiological reactivity compared to waitlist conditions. PTSD is associated with a heightened physiological reactivity (Orr, Metzger, & Pitman, 2002). A heightened physiological reactivity to traumatic cues seems to reflect an elevated sensitivity to unconditioned aversive stimuli (Phelps & LeDoux, 2005). We found that after TF-CBT, heart rate reactivity, systolic blood pressure and electromyogram (EMG) reactivity decreased. These findings indicate that successful treatment reduces physiological reactivity to traumatic cues and thus decreases sensitivity to aversive unconditioned stimuli. Again, since in almost all trials TF-CBT was compared to waitlist, this might be a non-specific psychotherapeutic effect. We did not identify trials in which EMDR was compared to a waitlist condition. Our review showed no differences in the physiological treatment effects of EMDR compared to other active treatments (Carlson et al., 1998; Gerardi et al., 2010; Renfrey & Spates, 1994; Rogers et al., 1999). People treated with EMDR or other active treatments had both a reduction of heart rate and EMG reactivity. These findings show that active treatment (or time) reduces physiological reactivity but that the reduction is not specific for EMDR.

Table 3. Summary of findings: biological predictors of treatment outcome.

Study	Biological measure	Treatment	Responders/ non responders	Outcome
Bryant *et al, 2010*	5-HTTLPR genotype	TF-CBT	45 PTSD CAPS scores	↑ CAPS scores at follow up in low expression 5-HTTLPR expression group than in high expression group (F= 1.38**)
Bryant *et al, 2008*	MRI VOI ACC	TF-CBT	7 R / 6 NR	Pre-treatment rACC in NR < R (z=4.42***)
Bryant *et al, 2008*	fMRI neutral emotional faces ROI: amygdala, ACC	TF-CBT	7 R / 7 NR	Pre-treatment bilateral amygdala in NR > R (r. z=1.85*, l. z= 2.13*);Pre-treatment r. ventral ACC in NR > R (z=2.23*); Pre-treatment bilateral dorsal ACC R > NR (z=3.1**)
Nardo *et al, 2009*	MRI gray matter density	EMDR	10 R / 5 NR	Pre-treatment gray matter density in frontal and limbic structures R > NR (z score range= 3.0-4.54 ***)
Yehuda *et al, 2009*	5α-THF, total glucocorticoids, 5α-reductase activity	PE	14 R / 14 NR	Pre-treatment 5α-reductase activity NR < R (F=6.43*); = R, NR in pre-treatment 5α-THF and total glucocorticoids
Blanchard *et al, 2003*	Baseline HR and HR during SDI	TF-CBT/ support	57 PTSD Correlation CAPS scores	= correlation between pre-treatment HR and post-treatment CAPS scores in support
Tarrier *et al, 2002*	SCL neutral, startle and trauma scripts	Exposure/ CT	42 PTSD Correlation CAPS scores	= correlation between pre-treatment SCL and post-treatment CAPS scores

(f)MRI = (functional) magnetic resonance imaging; V/ROI=volume/region of interest; HR(R)=heart rate; SCL=skin conductance level; SDI=script driven imagery; 5α-THF=5α-tetrahydrocortisol; ACC= anterior cingulated cortex; TF-C(B)T=trauma focused cognitive-(behavioural) therapy; EMDR=eye movement desensitization and reprocessing; PE=prolonged exposure; R=responder; NR=non-responder; CAPS= clinician administered posttraumatic stress disorder scale; r.= right; l.= left; *<0.05; **<0.01; ***<0.001

We included 7 trials which use pretreatment biological measurements to predict treatment outcome. Bryant et al. (R. A. Bryant et al., 2008; Richard A. Bryant et al., 2008) found that decreased rostral anterior cingulate cortex volumes and increased amygdala/ventral anterior cingulate cortex activity were associated with a poor response to TF-CBT. The findings of Nardo et al. (Nardo et al., 2010) stand in contrast to this. They found that reduced amygdala gray matter volume predicted a poor response to EMDR. However, it would be premature to draw conclusions based on these differential findings because both studies had small sample sizes and used different paradigms. The 2 included trials which used physiological measures to predict treatment outcome had a relatively large sample size; nevertheless, no association was found between pre- treatment physiological measures and treatment outcome (Blanchard et al., 2003; Tarrier et al., 1999). We included only 2 trials which used either genetic measurements (5-HT receptor gene variants) or pretreatment endocrinological measurements as predictors of treatment outcome; this makes it too early to make general conclusions about both measurements. There is only a limited number of trials in which biological measures are used to predict treatment outcome. Trials using somewhat comparable measurements yield negative or partially contrasting findings, making the use of biological predictors of treatment outcome not (yet) suitable for clinical practice.

Taken together, these findings indicate that more high-quality research is necessary before we can infer any firm conclusions on the neurobiological working mechanisms of psychotherapy in PTSD or start to think of implementing neurobiological measures in clinical practice. Against this background, there is a great need for biological-treatment outcome studies of psychotherapies, especially EMDR, and RCTs comparing multiple active treatments, as well as studies assessing predictors of treatment response to different treatments using neuroimaging, endocrinological or genetic measurements or combinations of multiple measures (Bisson & Andrew, 2007).

Seven of the 16 included publications on RCT outcome data had a high risk of bias on one of the five domains (fig. 2). The remaining publications of RCTs almost all had a certain degree of uncertainty regarding risk of bias due to the inadequate reporting of data and the methodologies used. These findings stress the need for methodologically more rigorous trials and improvement of reporting, for instance, by using intent-to-treat analysis, by not omitting reports of negative findings and by using Cochrane criteria for reporting on RCTs (Higgins, 2011). The large heterogeneity between studies made it not reasonable to pool the study results. To enhance the comparability of studies, future research should make use of standardized treatment and measurement protocols. Measurement protocols should include standardized paradigms which directly assess systems involved in PTSD. Paradigms aimed at fear conditioning (Wessa & Flor, 2007), extinction learning, attention regulation and working memory (Oei, Tollenaar, Spinhoven, & Elzinga, 2009) might be profitable.

Finally, we did not find any study that assessed biological-treatment effects of psychotherapy in children with PTSD. This group deserves special attention because early disruptions of the biological fear systems increase the risk of PTSD and other anxiety disorders in later life (McLaughlin et al., 2010). The brain's fear circuitry contains a high rate of plasticity during childhood and adolescence (Pynoos et al., 2009). Long-term treatment benefits in this group could thus be very substantial.

The populations included in our review varied considerably with regard to types of trauma and PTSD severity. Included trials also had a wide variety of measurement procedures, number of treatment sessions, comparison conditions and trial durations. Including trials which recruited individuals with partial PTSD made sure that results related to a wide range of individuals suffering from posttraumatic stress symptoms but added to the heterogeneity. This heterogeneity meant it was not reasonable to perform a meta-analysis. Most publications reported sufficient data to calculate effect sizes, but this was not possible in all of them.

We chose to follow the example of the most recent Cochrane review on psychological interventions in PTSD and present prolonged exposure, narrative exposure therapy

and brief eclectic psychotherapy in the TF-CBT group instead of presenting them independently of one another. These and other trauma-focused cognitive behavioral therapies share certain treatment modules but also have distinct treatment components. As we did not differentiate between different TF-CBT components, it is not yet possible to attribute neurobiological effects to specific treatment components.

Seven of the included publications on RCT outcome data had a high risk of bias, mainly regarding incomplete outcome data or selective reporting (fig. 2). Incomplete outcome data raise the risk of attrition bias and an exaggeration of the effect size (Higgins, 2011). Selective reporting can lead to an imbalance of negative and positive findings.

The total number of people included in this review was relatively small. If non-controlled trials were included, it would increase this number, but would make it impossible to differentiate between treatment effects and time effects, which is an important concern, given the considerable time effects observed in the waitlist groups of the RCTs that were included.

Our review did not account for the possibility of a publication bias and we included only studies that were published in English.

However, the strength of this systematic review lies in the fact that it follows the PRISMA statement for systematic reviews and meta-analyses and has a robust and comprehensive search strategy, an extensive assessment of risk of bias and is of an innovative character.

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SUPPLEMENTARY MATERIAL

Acknowledgments

This study was supported by the University of Amsterdam and by funding from the Frijling Prins Fonds. We would like to thank Joost G. Daams (clinical librarian) for assisting with the construction of our search strategy and Brent C. Opmeer (clinical epidemiologist) for his advice regarding data analysis. We also thank Marinus F. Zantvoord (psychophysiologist) for his critical comments and assistance with managing the reference database.

Supplementary methods

List of search terms

PubMed

Post Traumatic Stress Disorder OR PTSD OR Posttraumatic Stress Disorder* OR PTSS[tiab] OR posttraumatic stress[tiab] OR post traumatic stress[tiab]

AND

emdr OR emd[tiab] OR Eye Movement Desensitization and Processing OR Eye Movement Desensitization and reprocessing OR "Desensitization, Psychologic"[Mesh] OR cognitive therapies OR cognitive psychotherapy OR cognitive psychotherapies OR processing therapy OR processing therapies OR (exposure therapy OR exposure therapies) OR short term psychotherapy

EMBASE

Posttraumatic Stress Disorder/ OR (Posttraumatic Stress Disorder? OR post traumatic stress disorder?).ab,ti OR (post traumatic stress symptom? OR posttraumatic stress symptom?).ab,ti (ptsd OR ptss).ab,ti

AND

emdr.ab,ti OR emd.ab,ti OR eye movement/ OR desensitization/ OR eye movement desensiti?ation.ab,ti. OR behavior therapy/ OR cognitive therapy/ OR (cognitive ADJ1 therap#).ab,ti OR (cognitive ADJ1 treatment#).ab,ti OR processing therap#.mp OR exposure therap#.mp OR brief psychotherap#.mp OR short term psychotherap#.mp

PSYCINFO

Posttraumatic Stress Disorder/ OR (Posttraumatic Stress Disorder? OR post traumatic stress disorder?).ab,id,ti OR (post traumatic stress symptom? OR posttraumatic

stress symptom?).ab,id,ti (ptsd OR ptss).ab,id,ti OR (posttraumatic syndrome? or post traumatic syndrome?).id

AND

emdr.ab,id,ti OR eye movement desensiti?ation.ab,id,ti OR eye movement desensitization therapy/ OR eye movement?.ab,id,sh,ti OR exp cognitive behavior therapy/ OR cognitive behavio?r therap#.ab,id,ti OR cognitive therap#.ab,id,sh,ti OR (cognitive adj1 treatment?).ab,id,ti. OR processing therap#.ab,id,ti OR exposure therap#.ab,id,ti OR exp exposure therapy/ OR brief psychotherap#.ab,id,sh,ti OR short term psychotherap#.ab,id,sh,ti

CHAPTER 5

Trauma-focused psychotherapy response in youth with posttraumatic stress disorder is associated with changes in insula volume

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Journal of Psychiatric research 2021; 132: 207-214

ABSTRACT

Randomized controlled trials have shown efficacy of trauma-focused psychotherapies in youth with posttraumatic stress disorder (PTSD), but little is known about the relationship between treatment response and alternations in brain structures associated with PTSD. In this study, we longitudinally examined the association between treatment response and pre- to posttreatment changes in structural magnetic resonance imaging (MRI) scans using a voxel-based morphometry approach. We analyzed MRI scans of 35 patients (ages 8 - 18 years, 21 female) with PTSD (80%) or partial PTSD (20%) before and after eight weekly sessions of trauma-focused psychotherapy. PTSD severity was assessed longitudinally using the Clinician-Administered PTSD scale for Children and Adolescents to divide participants into responders and non-responders. Group by time interaction analysis showed significant differences in grey-matter volume in the bilateral insula due to volume reductions over time in non-responders compared to responders. Despite the significant group by time interaction, there were no significant group differences at baseline or follow-up. As typical development is associated with insula volume increase, these longitudinal MRI findings suggest that treatment non-response is associated with atypical neurodevelopment of the insula, which may underlie persistence of PTSD in youth. The absence of structural MRI changes in treatment responders, while in need of replication, suggest that successful trauma-focused psychotherapy may not directly normalize brain abnormalities associated with PTSD.

INTRODUCTION

Posttraumatic stress disorder (PTSD) is a common mental health disorder that develops in approximately 16% of youth exposed to traumatic events, which may include domestic violence, sexual abuse and accidents (Alisic et al., 2014). Youth with PTSD are troubled by frequent re-experiencing of the traumatic event, persistent avoidance, hyperarousal and negative alterations in cognition and mood (American Psychiatric Association, 2013). These symptoms can interfere with social functioning and school performance and have a negative effect on the quality of life of the affected youth and their families (V. G. Carrion, Weems, Ray, & Reiss, 2002). Moreover, they are a crucial factor in shaping the vulnerability to depression and suicidality later in life (Molnar, Berkman, & Buka, 2001; Sunley et al., 2020). Furthermore, youth exposed to traumatic events with clinically important symptoms of PTSD but with subthreshold criteria for PTSD (partial PTSD) also demonstrate substantial functional impairment and distress which do not differ from youth meeting full PTSD criteria (V. G. Carrion et al., 2002). All of the above highlight the vital importance of effective treatment for youth with PTSD and partial PTSD.

Multiple randomized controlled trials (RCTs) have shown efficacy of trauma-focused psychotherapies in youth with PTSD (Morina, Koerssen, & Pollet, 2016). However, less is known about the association between treatment response and morphometric brain changes in youth with PTSD (J. B. Zantvoord, Diehle, & Lindauer, 2013). Examining changes in structural magnetic resonance imaging (MRI) measurements associated with treatment response can provide a better understanding of why some youth recover after trauma-focused psychotherapy while in some PTSD persists (J. B. Zantvoord et al., 2013). This is important because, response varies considerable among individuals, with up to 30-50% of youth who do not benefit sufficiently from treatment (Bradley, Greene, Russ, Dutra, & Westen, 2005; Diehle, Opmeer, Boer, Mannarino, & Lindauer, 2015), leading to persistent PTSD symptoms and longer treatment trajectories.

Meta-analyses of structural neuroimaging studies in adults with PTSD found differences in MRI measurements possibly suggestive of reduced grey-matter volume (GMV) of the hippocampus, ventromedial prefrontal cortex (vmPFC) including the anterior cingulate cortex (ACC) and insula relative to adults exposed to trauma without PTSD (Bromis, Calem, Reinders, Williams, & Kempton, 2018; Logue et al., 2018). These interconnected structures have a critical role in fear conditioning, extinction learning and emotional processing which are impaired in PTSD (Careaga, Girardi, & Suchecki, 2016; Etkin & Wager, 2007). Importantly, these structures undergo substantial maturation throughout childhood and adolescence; with hippocampus volume increase in girls, amygdala volume increase in boys, as well as late maturation

of the prefrontal cortex and insula volume increase in both sexes (Dennis et al., 2014; Lenroot & Giedd, 2006; Shaw et al., 2008; Tamnes et al., 2017; Jasper B Zantvoord, Lindauer, Bakker, & Boer, 2013).

Interestingly, cross-sectional studies in youth with PTSD did not find reduced hippocampal volume and findings on vmPFC volume have been mixed, with some reporting larger vmPFC volumes, while others reported smaller vmPFC and ACC volumes or no difference relative to healthy controls (Victor G Carrion et al., 2009; Keding & Herringa, 2015; Morey, Haswell, Hooper, & De Bellis, 2016). Divergent structural brain findings between youth and adults with PTSD might indicate developmental effects that are not yet apparent until adulthood as well as plastic response to illness over time (Keding & Herringa, 2015; Weems, Russell, Neill, & McCurdy, 2019). A recent naturalistic longitudinal study in youth aged 8-18 years with PTSD, indeed suggests that PTSD persistence is characterized by sustained reduced PFC volume, abnormal prefrontal cortex (PFC) development and abnormal development of amygdala-hippocampus and amygdala (vm-)PFC connectivity (Heyn et al., 2019).

There remains a debate as to whether successful trauma-focused psychotherapy may reverse structural abnormalities associated with PTSD and might influence development of brain structures associated with PTSD persistence. Results from longitudinal morphometric studies in adult patients with PTSD have been mixed. The majority of these studies have used small samples and focused on the hippocampus, with most (Laugharne et al., 2016; Lindauer et al., 2005; S. Van Rooij et al., 2015), but not all (Levy-Gigi, Szabó, Kelemen, & Kéri, 2013) suggesting that hippocampal volume does not change with trauma-focused psychotherapy. Few studies have looked beyond hippocampus volume, showing amygdala (Laugharne et al., 2016), parahippocampal gyrus (Bossini et al., 2017) and prefrontal volume increase and ACC volume decrease (Boukezzi et al., 2017; Helpman et al., 2016) from pre- to posttreatment. To the best of our knowledge, no study has been published which examined morphometric changes associated with treatment response in youth with PTSD.

In the present longitudinal study, we investigated longitudinal changes in MRI measurements in youth with PTSD or partial PTSD treated with trauma-focused psychotherapy. We used pre- and posttreatment 3T MRI scans along with voxel-based morphometry (VBM) to compare brain-wide changes in treatment responders relative to non-responders. Overall, we hypothesized that treatment response would be associated with changes in MRI measurement suggestive of volume increases in brain areas associated with PTSD (hippocampus, insula and vmPFC including the ACC), while non-response would be characterized by volume decreases in these areas.

MATERIALS AND METHODS

Participants

Our initial sample consisted of 61 participants (39 female) diagnosed with PTSD or partial PTSD. Participants entered trauma-focused psychotherapy as part of a larger RCT comparing trauma-focused cognitive behavioral therapy (TF-CBT) and eye movement desensitization and reprocessing (EMDR) (Diehle et al., 2015). Of these participants, 40 (34 right-handed) completed treatment as well as pre- and posttreatment scans (Fig. S1 in Supplementary material). All participants were Dutch speaking, and aged 8-18 years. Participants were recruited between April 2011 and September 2018 at the outpatient child psycho-trauma center of the department of child and adolescent psychiatry, de Bascule in Amsterdam, The Netherlands. Youth were referred by child welfare services, physicians or their general practitioner. For eligibility diagnoses for PTSD or partial PTSD were established clinically by an experienced child and adolescent psychiatrist or psychologists according to the DSM-IV-TR criteria using both child reports on the Clinician-Administered PTSD Scale for Children and Adolescents (CAPS-CA) semi-structured interview (Nader et al., 1996) and caregiver reports from the PTSD scale of the Anxiety Disorders Interview Schedule – Parent Version (ADIS-P) (Verlinden et al., 2014). (Partial) PTSD diagnosis was determined using joint- child and caregiver reports on individual symptoms. A symptom was established as present, if either child or caregiver reported its presence. Partial PTSD was defined as either fulfilling two of the three PTSD symptom clusters or having one symptom present in each of the three symptom clusters (Stein, Walker, Hazen, & Forde, 1997). Furthermore, participants were required to have a CAPS-CA total score indicating at least mild PTSD symptom severity (>20 points). Exclusion criteria were: acute suicidality, IQ < 70, pregnancy, neurological disorders or serious medical illnesses (one patient was excluded due to an incidental finding on the MRI, see Fig. S1) or meeting the criteria of one of the following diagnosis: psychotic disorders, substance use disorder or pervasive developmental disorder. If participants were taking psychotropic or central nervous-active medication, medication was required to be stable for at least three weeks (five weeks for fluoxetine) before and during the trauma-focused psychotherapy. In our sample three participants were taking psychotropic medication (one sertraline and two methylphenidate). In accordance with procedures approved by the Institutional Review Board of the Amsterdam University Medical Center and the declaration of Helsinki, written informed consent was obtained from all parents or legal guardians. Written informed consent from youth aged 12 years and older and assent from youth aged 11 and younger, was also obtained from the youth themselves. All participants received a monetary incentive for participation (€5 for each assessments).

Treatment

All participants received eight weekly protocolized sessions of either TF-CBT or EMDR. Treatment was delivered by experienced therapists who were all trained in TF-CBT and EMDR before the initiation of the study. Supervision by experts on TF-CBT and EMDR was provided throughout the study. Treatment protocols, training and supervision of therapists, as well as treatment fidelity have been described in detail previously (Deblinger, Mannarino, Cohen, Runyon, & Steer, 2011; Shapiro, 2001; Jasper B Zantvoord et al., 2019).

Trained psychologists administered the CAPS-CA and the PTSD scale of the ADIS-P semi-structured interviews to measure PTSD symptom severity before and after treatment. Caregiver reports on the ADIS-P were used to complement child reports and clinical observation (e.g. when a child is unable to disclose certain symptoms due to avoidance). Participants and their caregivers additionally completed the Dutch Revised Child Anxiety and Depression Scale (RCADS(-P)) questionnaires to assess depressive and anxiety symptoms (Chorpita, Yim, Moffitt, Umemoto, & Francis, 2000). Symptom change was calculated by subtracting the baseline from the posttreatment CAPS-CA total score. There is no established definition of a response criterion or a consensus definition of response terms in the child trauma treatment field. Based on the psychometric properties of the CAPS (-CA) and previous treatment outcome studies using the CAPS-CA, we used ≥30% reduction of CAPS-CA total score as response criterion for clinically meaningful improvement, and ≥50% reduction of CAPS-CA total score as response criterion for substantial clinical improvement (Diehle, de Roos, Boer, & Lindauer, 2013; Weathers, Keane, & Davidson, 2001).

Imaging data acquisition and preprocessing

All scans were acquired at the Amsterdam University Medical Center using a 3T Philips Achieva scanner (Philips Healthcare, Best, The Netherlands). Please refer to Supplementary material for technical details of the applied MR sequences.

MRI images were preprocessed with the CAT12 toolbox (r1446, http://www.neuro.uni-jena.de/cat/) and SPM12 (r7487, https://www.fil.ion.ucl.ac.uk/spm/software/spm12/) in MATLAB R2017a (The Mathworks, Natick, MA). Preprocessing was performed by running the longitudinal segmentation pipeline of CAT12 with default parameters. Detailed preprocessing procedures are described in Supplementary material (Ashburner & Friston, 2011). Each segmentation was checked visually and using the quality measures provided by the CAT12 toolbox. This led to the exclusion of four participants due to poor segmentation quality and one participant due to the presence of an incidental finding. These procedures led to a total sample of 35 participants in the longitudinal analysis.

To facilitate the analyses of the group by time interaction for GMV and white-matter volume (WMV), difference images were computed by subtracting the posttreatment scans of each patient from their corresponding pretreatment scans.

Statistical analysis

Clinical and demographic data

The distribution of baseline clinical, trauma and demographic characteristics across responders and non-responders was examined using X^2-tests for categorical variables, independent sample *t*-tests for normally distributed continuous variables and Mann-Whitney tests for non-normally distributed continuous variables. Paired sample *t*-test were used to examine pre- to posttreatment symptom change. Statistical analyses were performed using SPSS version 24.0 (SPSS Inc., Chicago IL, USA).

MRI data

We investigated the group by time interaction of GMV/WMV images using a two-sample test of the within-subject difference images. Family-wise error (FWE) correction for whole-brain, two-sided tests as well as the two investigated segmentations of the threshold-free cluster enhanced (TFCE) statistic were performed using synchronized permutations (n=10000) (Smith & Nichols, 2009; Winkler et al., 2016). Alpha was set to 0.025, additionally Bonferroni-correcting for the two response criteria investigated. To correct for the effect of gender and age on total brain volume, obtain valid localized GMV/WMV estimates, and control for baseline PTSD symptom severity, we included mean-centered total intracranial volume (TIV), age, gender and baseline CAPS-CA total scores as covariates for each patient in all analyses. A detailed justification of the included covariates can be found in the Supplementary material. All statistical tests utilized the PALM toolbox (a115, https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/PALM).

In addition, we performed a region of interest (ROI) analysis by repeating the analyses described above focusing on specific ROIs associated with PTSD: bilateral hippocampus, amygdala, ACC, vmPFC and insula. Hippocampus, amygdala, ACC and insula masks were extracted from the conservatively thresholded (maxprob 50%) Harvard-Oxford atlas (Desikan et al., 2006). The vmPFC masks was obtained from a previous study by Delgado and colleagues (https://neurovault.org/collections/5631/) (Delgado et al., 2016). These analyses were only performed with the GMV images.

RESULTS

Demographic and clinical characteristics

A summary of participant characteristics is presented in Table 1 and Table S1. Based on joint child (CAPS-CA) and caregiver (ADIS-P) reports 80% of participants met the full DSM-IV diagnostic criteria for PTSD at baseline, the remaining 20% met criteria for partial PTSD. The average baseline CAPS-CA score was M = 54.14 points, SD = 23.84, which is indicative of moderately severe PTSD. The most common index trauma was sexual abuse, followed by community violence and domestic violence. 54.3% of participants were exposed to multiple-event trauma and the remaining 45.7% was exposed to single-event trauma. Average age at trauma exposure was M = 9.62 years, SD = 4.09 (range 3-16) and average time since trauma was M = 3.32 years, SD = 3.02 (range 0-11).

Changes in psychopathology

Treatment completers and non-completers did not differ in baseline sociodemographic, trauma or clinical characteristics. Across the completer sample, we found significant reductions in CAPS-CA total score ($t(34) = 7.90$, $p < .001$, Cohen's effect size (d) = 1.35), re-experiencing ($t(34) = 7.92$, $p < .001$, $d = 1.39$), avoidance ($t(34) = 5.43$, $P < .001$, $d = 0.94$) and hyperarousal clusters ($t(34) = 3.39$, $p = .002$, $d = 0.59$.

Applying the 30% response criterion yielded 21 responders and 14 non-responders, applying the 50% response criterion yielded 13 responders and 22 non-responders. See Table 1 and Table S1 for the distribution of baseline sociodemographic, trauma or clinical characteristics between responders and non-responders using the 30% response criterion and 50% response criterion respectively. There were no significant group differences at baseline when using the 30% criterion. When applying the 50% response criterion, responders had a lower average time since trauma, CAPS-CA total score, CAPS-CA re-experiencing score and female to male ratio at baseline ($p < 0.05$).

Table 1 Subject characteristics.

	Responders (n= 21) ≥30% CAPS-CA	Non-responders (n= 14) <30% CAPS-CA	p-value[a]
Sociodemographic characteristics			
Female (%)	52.3	71.4	.260
Age (years; mean, SE)	12.4 (0.68)	13.6 (0.76)	.448
West European Ethnicity (%)	68.4	50.0	.305
Current educational level (%)			.189
Elementary school	57.1	28.6	
Middle/High school lower level	0.0	7.1	
Middle/High school middle level	28.6	35.7	
Middle/High school higher level	14.4	14.3	
Vocational school	0.0	14.3	
Household income (%)			.776
< €25.000	31.2	37.5	
€25.000-35.000	25.0	12.5	
> €35.000	43.8	50.0	
Weight (kg; mean, SE)	50.4 (3.43)	52.1 (2.52)	.144
Current psychotropic medication (%)	9.5	7.1	.805
Smoking (%)	5.5%	10.0%	.662
Alcohol >1 consumption/day (%)	0%	0%	N/A
Trauma characteristics			
Index trauma (%)			.358
Sexual abuse	28.6	28.6	
Domestic violence	23.4	21.4	
Community violence	14.3	35.8	
Accidents/Medical	14.3	14.3	
Other	19.0	0.0	
Multiple-event trauma exposure (%)	61.2	42.3	.268
Age at index trauma (years; mean, SE)	9.5 (0.82)	9.9 (1.27)	.126
Time since index trauma (years; mean, SE)	3.1 (0.58)	3.7 (0.95)	.053
Clinical characteristics			
CAPS-CA study entry (mean, SE)[b]			
Total	51.8 (4.90)	57.7 (7.01)	.231
Re-experiencing	15.1 (1.92)	19.8 (2.82)	.430
Avoidance	21.7 (2.32)	20.6 (3.23)	.912
Hyperarousal	15.4 (2.05)	18.7 (2.67)	.641
Full PTSD diagnosis (%)	76.2	85.7	.490
RCADS study entry (mean, SE)[b]			
MDD	10.5 (1.58)	12.4 (2.05)	.710
GAD	7.8 (1.17)	5.9 (0.97)	.209
OCD	6.2 (1.02)	5.5 (0.71)	.076
PD	8.0 (1.59)	7.9 (1.77)	.541
SAD	6.8 (0.96)	4.6 (1.34)	.883
SP	12.1 (1.89)	11.1 (1.83)	.225

Abbreviations: CAPS-CA, Clinician-Administered PTSD Scale for Children and Adolescents; RCADS, Revised Child Anxiety and Depression Scale; MDD, major depressive disorder; GAD, general anxiety disorder; OCD, obsessive compulsive disorder; PD, panic disorder; SAD, separation anxiety disorder; SP, social phobia; SE, standard error.

[a] Independent samples t-test for continuous and x^2 tests for categorical variables.

[b] Ranges: CAPS-CA total, 0-139; RCADS MDD, 0-30; RCADS GAD, 0-18; RCADS OCD, 0-18; RCADS PD, 0-27; RCADS SAD, 0-21; RCADS SP, 0-27.

Changes in brain morphometry

When applying the 30% response criterion, a significant group by time interaction effect in the GMV was observed (Fig. 1 and Table 2). Four clusters involving the right posterior insula/rolandic operculum, the left posterior insula/rolandic operculum, the left insula and the right anterior insula/superior temporal pole showed a significant interaction effect after FWE correction for multiple comparisons. These results were related to a decrease of GMV over time in non-responders compared to responders (fig. 2). Post-hoc tests investigating time-effects per group and difference between groups at each time point showed no significant difference after further correction for the number of post-hoc tests. When applying the 50% criterion, no group by time interaction effects in GMV or WMV were observed. The additional ROI analysis revealed no significant interaction in additional brain regions for both criteria.

Post-hoc analyses

To facilitate further understanding of the main findings we conducted exploratory post-hoc analyses investigating gender-by-group, gender-by-group-by-time, age-by-time and age-by-time-by-group interactions as well the continuous association between pre- to posttreatment change in total CAPS-CA scores and delta GMV/WMV. As post-hoc tests were exploratory and were not further corrected for the additional number of tests performed, results have to be interpreted cautiously. All post-hoc analyses are fully described in the Supplementary material and are only briefly summarized here. There was no significant effect of the gender by group or gender by group by time interaction. Also, no significant effect was found for the age by time or age by group by time interaction. However, when investigating the age by time interaction within the responders and non-responders groups separately, we found a significant positive effect of age and delta GMV within the left insula for the responders group only (nvoxel = 563, peak –log10(pFWE) = 2.24, peak MNI coordinates = -36, 3, 4.5 [mm], Fig. S2). Finally, we did not find any significant association between pre- to posttreatment change in total CAPS-CA scores and delta GMV/WMV.

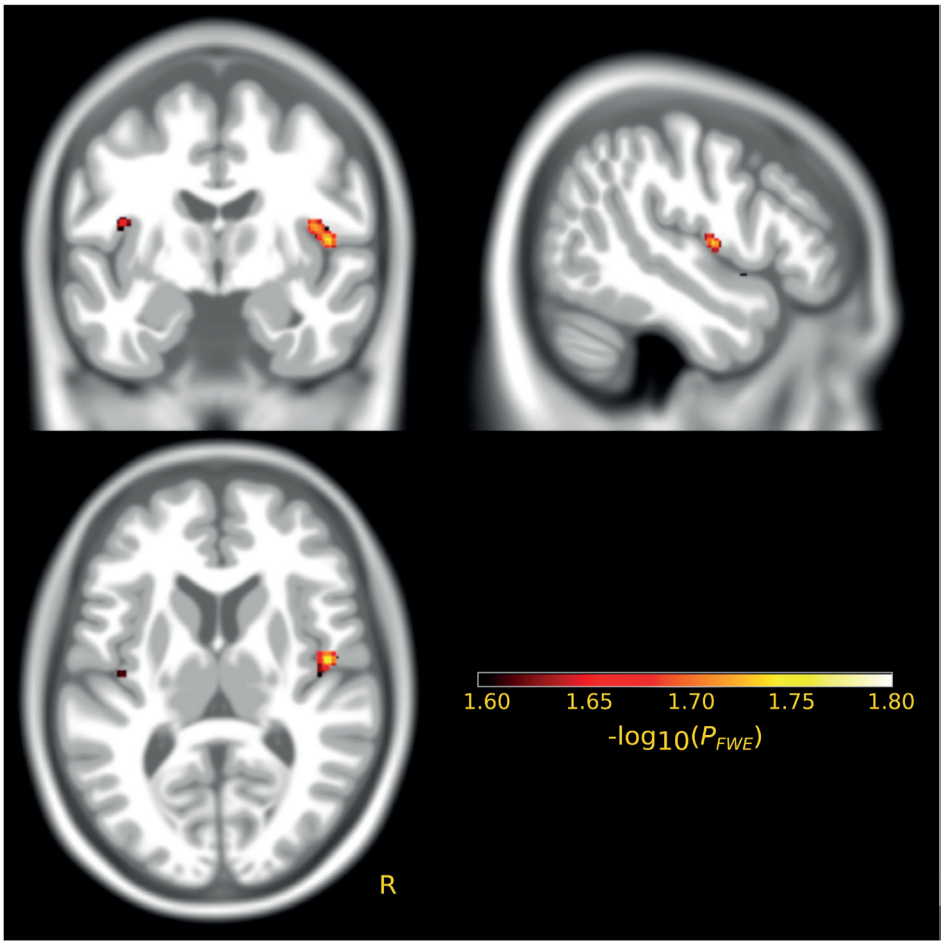

Fig. 1. Results of the group by time interaction of the GMV data for the 30% response criterion. Figure shows -log10 transformed FWE-corrected p-values thresholded at alpha = 0.025. Significant clusters include the right and left insula. Results are visualized on the average T1 image transformed to MNI-space build on 555 subjects of the IXI data base distributed with the CAT12 toolbox. MNI-coordinates: 49, −7, 9

Table 2. Significant clusters of the group by time interaction analysis for the GMV

Region (according to AAL atlas)	MNI-coordinates (peak) [x, y, z]	Peak – $\log_{10}(P_{FWE})$	Number of voxels
Right Rolandic Operculum, Right Insula	48, -6, 7.5	1.762	223
Left Rolandic Operculum, Left Insula	-39, -7.5, 16.5	1.658	29
Left Insula	-40.5, -13.5, 9	1.618	11
Right Superior Temporal Pole, Right insula	49.5, 7.5, -6	1.604	2

GMV: Grey-matter volume; AAL: Automated Anatomical Labeling atlas.

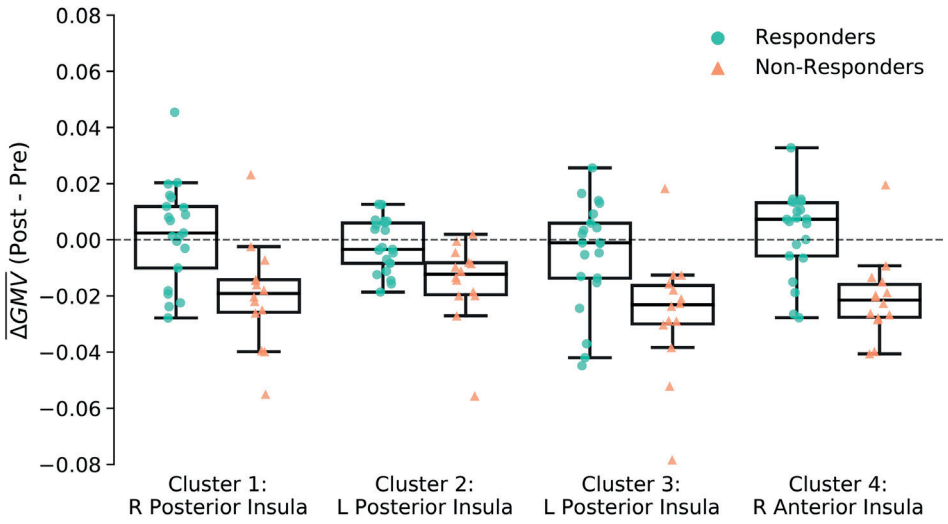

Fig. 2. Average delta GMV values extracted from the four significant clusters of the group by time interaction analysis using the 30% criterion. Boxplots summarize the data of individual responders (circles) and non-responders (triangles).

DISCUSSION

We investigated trauma-focused psychotherapy-related changes in structural MRI measurements in youth with PTSD. Our results show that treatment non-responders, relative to responders are characterized by a decrease over time of GMV in the bilateral anterior and posterior insula. These differences were only observed when applying a lenient response criterion (≥30% CAPS-CA reduction) and not when applying a stricter response criterion (≥50% CAPS-CA reduction). Apart from the insula we did not find evidence for differential volume change in other brain areas associated with PTSD.

The insula can be subdivided in distinct regions, each with their own set of functions: the posterior insula is involved in the detection, interoceptive awareness and interpretation of somatosensory and autonomic stimuli. By integration with attentional systems the anterior insula initiates emotional experience and appropriate autonomic and behavioral responses to stimuli that are important to an individual (Menon and Uddin, 2010; Paulus and Stein, 2006; Uddin, 2015). In typically developing youth, developmental change of the anterior insula is characterized by linear volume increase throughout childhood and adolescence while volume increase of the posterior insula shows a quadratic trajectory in which volume increase flattens during late adolescence (Shaw et al., 2008; Tamnes et al., 2017). Our results show that in youth with PTSD, non-response to trauma-focused psychotherapy in comparison to treatment response is characterized by longitudinal bilateral volume decrease in both the posterior and anterior insula. Exploratory moderation analyses, indicated that while the responder group showed age-related volume increase over time of the left (but not right) insula, the non-responders group failed to show age-related increase over time (Fig S2.). This suggests that difference in insula volume change over time, reflects an absence of normal developmental volume increase of the insula in non-responders. Surprisingly, differential insula volume change occurred within three months, which is a short period, as developmental change typically progresses gradually over years. In addition, the absence of volume changes in treatment responders, suggests that successful trauma-focused psychotherapy may not directly normalize differences on MRI measurements associated with PTSD, which may take longer than the timespan of the current study. From another neurodevelopmental perspective, successful trauma-focused psychotherapy may cease adverse neurodevelopmental effects, at least for the duration of treatment.

The anterior insula is an integral hub in the 'salience network' and the posterior insula has been identified as a multimodal convergence zone for sensory information and body condition; both the anterior and posterior insula have been implicated in PTSD (Herringa, Phillips, Almeida, Insana, & Germain, 2012; Menon & Uddin, 2010;

Nicholson et al., 2016). Functional neuroimaging studies of the insula as a whole and insula subregions have shown greater activation and increased functional connectivity with the amygdala in response to salient stimuli in adults with PTSD (Etkin & Wager, 2007). Sustained enhanced insular activation and connectivity in response to salient stimuli might initially represent an adaptive response to prolonged threat, which becomes maladaptive and increases PTSD risk if it persists when threat ceases (Van Wingen, Geuze, Vermetten, & Fernández, 2011). Interestingly, successful trauma-focused psychotherapy has been associated with decrease in (anterior) insula activity and connectivity with the amygdala in both adults and youth with PTSD (Cisler et al., 2016; Thomaes et al., 2012; S. J. Van Rooij, Kennis, Vink, & Geuze, 2016).

Whereas PTSD patients show increased insula function, some but not all prior structural neuroimaging studies show decreased insula volumes in both adults and adolescents with PTSD (Bromis et al., 2018; Herringa et al., 2012; Klabunde, Weems, Raman, & Carrion, 2017; Kühn & Gallinat, 2013; Meng et al., 2014). These findings suggest decreased insula volume as either a developmental risk factor for PTSD or as a plastic response to illness. The former possibility suggests that heightened insula activation represents a compensation for reduced insula volume in predisposed individuals, while the latter possibility suggests that reduced insula volume is a consequence of heightened insula activation in PTSD patients. Furthermore, previous studies in adults have shown that PTSD related brain abnormalities are more pronounced in patients with chronic PTSD compared to patients with new onset PTSD (Chao, Yaffe, Samuelson, & Neylan, 2014). Our study provides supportive evidence for a longitudinal association between PTSD persistence and failure of both anterior and posterior insula volume increase over time in youth. This poses a potential explanation for the reduced insula volumes found in some cross-sectional studies comparing adult PTSD patients and healthy controls (Bromis et al., 2018; Kühn & Gallinat, 2013; Meng et al., 2014). In our study differential insula volume change occurred over a surprisingly short treatment period of eight weeks. If abnormal insula development would be a continuous process, this would consistently result in distinctively smaller insula volumes in patients in which PTSD persists over a period of years. However, prior cross-sectional studies in patients with persistent PTSD have not found evidence for consistent or pronounced insula volume differences. This further emphasizes that interpretation of the nature of structural MRI changes should be done with caution. Weinberger and Radulescu have for instance pointed out that variations measured with MRI can be associated with variation in water content, tissue perfusion, body weight, cholesterol levels, imperceptible head motion, endogenous steroid levels, time of day, and exercise and mental activities, rather than represent only gain or loss of regional brain tissue (Weinberger & Radulescu, 2020). Longitudinal neuroimaging studies with high resolution (7T)

MRI scans, which examine neurodevelopmental trajectories of children with PTSD when they develop into adolescence and adulthood could help to further clarify the underlying mechanisms of structural MRI changes.

Interestingly, we only found evidence for differential longitudinal MRI changes when applying a lenient response criterion (30% CAPS-CA reduction) to divide responders and non-responders. With the 30% response criterion the non-responder group is smaller and more selective, representing true non-response. With the 50% response criterion this group is less selective as it also contains youth who are partial responders (30%-50% improvement). Including partial responders in the non-responder group could thus have obscured group by time differences related to non-response and could be an explanation for the differential findings employing the 30% or 50% criterion.

In contrast to some studies in adults with PTSD, we were unable to identify longitudinal changes in other brain areas, notably in areas which have been implicated in PTSD and undergo considerable developmental change throughout childhood and adolescence, such as the hippocampus and (vm-) PFC (Bromis et al., 2018; Giedd & Rapoport, 2010; Logue et al., 2018). Even with our ROI analysis in these regions, we were unable to detect longitudinal changes, reducing the chance of type II error. A recent naturalistic longitudinal study in youth with PTSD showed persistence of PTSD over a one-year period to be associated with sustained reduced PFC volume, abnormal PFC development and abnormal development of amygdala-hippocampus and amygdala (vm-)PFC connectivity (Heyn et al., 2019). Also, the few structural neuroimaging studies of trauma-focused psychotherapy in adults that found evidence for pre- to posttreatment changes, found longitudinal volume increase of the hippocampus and PFC (Boukezzi et al., 2017; Helpman et al., 2016; Levy-Gigi et al., 2013). One possible explanation could be that changes in hippocampus and (vm-)PFC are latent and only become apparent after a longer period or later in development (Lindauer et al., 2005). This is supported by the notion that time between scans is considerably shorter in the current study relative to both the naturalistic longitudinal study in youth with PTSD and treatment outcome studies in adults with PTSD. This emphasizes the need for studies with longer follow-up periods to establish if short term structural MRI changes persist over time and whether there are changes associated with psychotherapy response that do not become apparent directly after treatment but are expressed later on in development.

The present study details novel findings regarding the relationship between longitudinal insula changes and treatment response in youth with PTSD. It is not, however, without limitations. First, because it is considered unethical to withhold or delay a potentially effective treatment in youth with PTSD, we were unable to include

a waitlist control group in the current study. The absence of a waitlist control group impedes controlling for non-treatment related factors with potential effects on brain morphometry, for example exposure to ongoing stressful life events such as ongoing third custody cases and out-of-home placement during the course of treatment. Therefore, the question whether there is a causal link between insula volume change and treatment non-response remains inconclusive. Establishing causality would be important because evidence for morphometric change specific to treatment non-response would support the troubling possibility that treatment non-response represents an ongoing environmental stressor, above and beyond PTSD persistence alone, and might in turn accelerate abnormal neurodevelopment (Felmingham et al., 2009; Keding & Herringa, 2015). Furthermore, because we did not have long-term follow-up scans, we could not assess if changes in MRI measurements persist over time or if there are changes which are expressed later in development. Both stress the need for future treatment studies with long-term naturalistic follow up scans after trauma-focused psychotherapy. Second, although the majority (80%) of included youth had a full PTSD diagnosis, the remaining 20% had a partial PTSD diagnosis. Including youth with partial PTSD increased clinical heterogeneity and might have lowered overall treatment response due to a floor effect, however, by including youth with partial PTSD, our sample better reflects the real-life clinical setting, which adds to the ecological validity of our findings. Furthermore, the distribution between full and partial PTSD patients did not differ between responders and non-responders at baseline (table 1), rendering it unlikely that the inclusion of partial PTSD patients influenced our main MRI findings. Third, youth were randomized to receive either TF-CBT or EMDR, and both treatment conditions were collapsed for the current analysis. Due to limited power it was not feasible to examine differences between treatment responders and non-responders separately for both treatment conditions. Importantly, efficacy of both treatment conditions has been shown comparable in an RCT with considerable sample overlap with the current study (Diehle et al., 2015). Finally, our study had a substantial drop-out rate, as 34% of randomized patients were lost to follow-up. Although such dropout rates reflect routine clinical practice and treatment completers and non-completers did not differ on baseline demographic, clinical and trauma related variables, there is a possibility that drop-out could have influenced our main findings through attrition bias. Nevertheless, baseline variables were matched in the 30% responder and non-responder groups, suggesting that the MRI results are not related to clinical, demographic or trauma related differences at baseline.

To our knowledge, this is the first report of structural MRI changes related to trauma-focused treatment response in youth with PTSD. Our findings show that treatment non-responders are characterized by failure to show age-related volume increase

over time in both the anterior and posterior insula. This may suggest a relationship between PTSD persistence after treatment and ongoing atypical development within the salience network. On the other hand, the absence of MRI changes in treatment responders suggests that successful trauma-focused psychotherapy might be associated with a (temporary) delay or surcease of abnormal brain development but not with a direct normalization of PTSD related brain abnormalities. Future studies with a long-term follow-up period after treatment should first aim to replicate our findings and determine if changes in MRI measurements persist after treatment, secondly examine if these changes are specific to trauma-focused psychotherapy response or related to the course of PTSD in general, and finally assess potential effects related to treatment response which are expressed later in development.

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SUPPLEMENTARY MATERIAL

Acknowledgements

The authors are most grateful to all the study participants for their involvement. This study was supported by the Netherlands Organization for Scientific Research (NWO/ZonMW Vidi 016.156.318) and the AMC Research Council (150622).

Supplementary Methods

Imaging data acquisition

All scans were acquired using a 3.0T Philips Achieva scanner (Philips Healthcare, Best, The Netherlands) equipped with a SENSE eight-channel receiver head coil. For each participant, a T1-weighted structural MRI image was acquired with the following parameters: TE: 3.527 ms, TR: 9 ms, slice thickness: 1 mm, 170 slices, flip angle: 8˚ and image matrix 256 x 256 that covert the entire brain.

Imaging data preprocessing

MRI images were preprocessed with the CAT12 toolbox (r1446, http://www.neuro.uni-jena.de/cat/) and SPM12 (r7487, https://www.fil.ion.ucl.ac.uk/spm/software/spm12/) in MATLAB R2017a (The Mathworks, Natick, MA). Preprocessing was performed by running the longitudinal segmentation pipeline of CAT12 with default parameters. This pipeline involved intra-subject realignment and bias-field correction, followed by segmentation into grey-matter, white-matter and cerebrospinal fluid tissue types. The individual segmentations were then normalized to MNI space at $1.5mm^3$ resolution via an optimized Geodesic shooting procedure (Ashburner and Friston, 2011). The estimated deformation fields were averaged across the pre- and posttreatment scans per participant and the mean deformation field was applied to each individuals' estimated segmentations. For the normalization a template derived from 555 healthy controls of the IXI-database (http://www.brain-development.org/) included in the CAT12 toolbox was used. Normalized grey- and white-matter segments were modulated by linear and non-linear components of the Jacobian determinant yielding voxel-wise grey- and white-matter volume estimates (GMV/WMV). The final step of the preprocessing included spatial smoothing with an 8mm full-width-at-half-maximum kernel of the GMV and WMV images. GMV/WMV masks were created by thresholding each subjects' individual image at 0.15 and only including voxels which survived thresholding across all subjects. Each segmentation was checked visually and using the quality measures provided by the CAT12 toolbox.

Justification on the chosen covariates

Total intracranial volume was included as a covariate into the model because it is explicitly stated to be done so in the manual of the CAT12 toolbox (http://www.neuro.uni-jena.de/cat12/CAT12-Manual.pdf) which is based on recommendations by (Malone et al., 2015). Age and gender have a tremendous effect on the brain-volume development of adolescents in general (Giedd & Rapoport, 2010) and adolescents with PTSD specifically (especially for the insula) (Klabunde et al., 2017). In addition, the prevalence of PTSD is gender-dependent (Olff, Langeland, Draijer, & Gersons, 2007).Prior structural neuroimaging studies have also shown an association between PTSD symptom severity and development of (regional) brain-volume in children and adolescents (e.g. (Heyn et al., 2019)). Therefore we also corrected for baseline CAPS-CA total scores in our analysis.

Post-hoc analyses

To facilitate further understanding of the main findings we conducted further exploratory post-hoc analyses. These analyses were limited to the grouping according to the 30% criterion and the bilateral insula (extracted from the Harvard-Oxford atlas (maxprob 25%)) as this was the main analysis which showed a significant interaction. As these post-hoc tests were exploratory we did not further correct for the additional number of tests performed and kept the significance level the same as in the case of the main interaction analyses. Therefore, these results have to be interpreted cautiously.

The first exploratory post-hoc tests investigated the gender-by-group and gender-by-group-by-time interactions. No significant effect was found in the insula.

The second set of tests investigated the effect of age-by-time and age-by-time-by-group interactions. There was no significant effect of the age-by-time interaction or the formal test for interaction for age-by-group-by-time. However, when investigating the age-by-time interaction within the responders and non-responders groups separately, we found a significant positive effect of age and delta GMV within the left insula for the responders group only (nvoxel=563, peak –log10(pFWE) = 2.24, peak MNI coordinates = -36, 3, 4.5 [mm], Fig. S2). ‘

In addition, we also investigated if a continuous association between total CAPS-CA scores (posttreatment – pretreatment scores) was associated with delta GMV/WMV. This approach can be seen as a companion analysis to our main test which does not require the separation of patients into distinct groups. The same covariates, testing procedures and multiple-comparison corrections which were performed for the main analyses were applied here. We did not find any significant association between delta CAPS-CA scores and delta GMV/WMV.

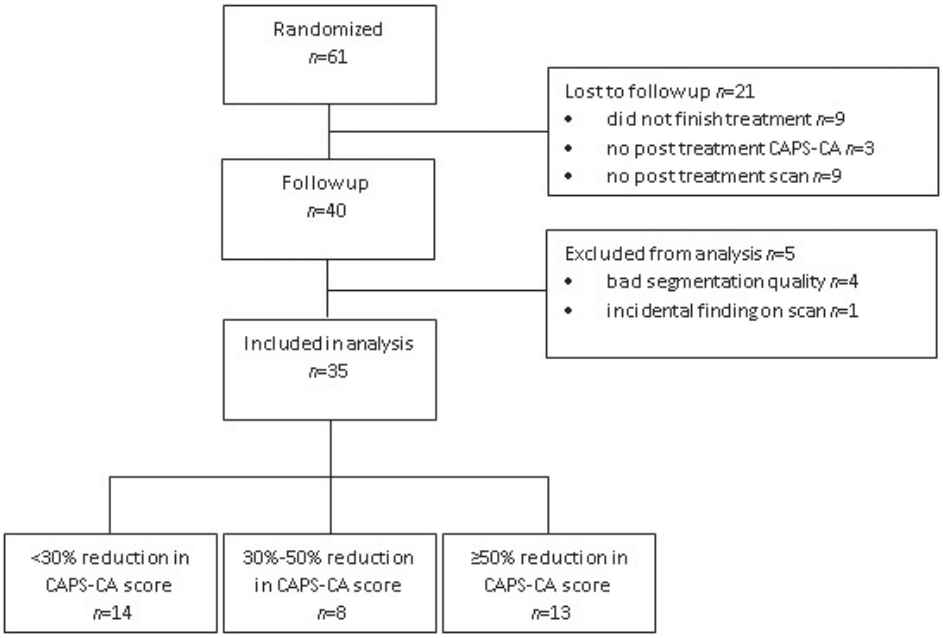

Figure S1. Flow diagram of included patients.

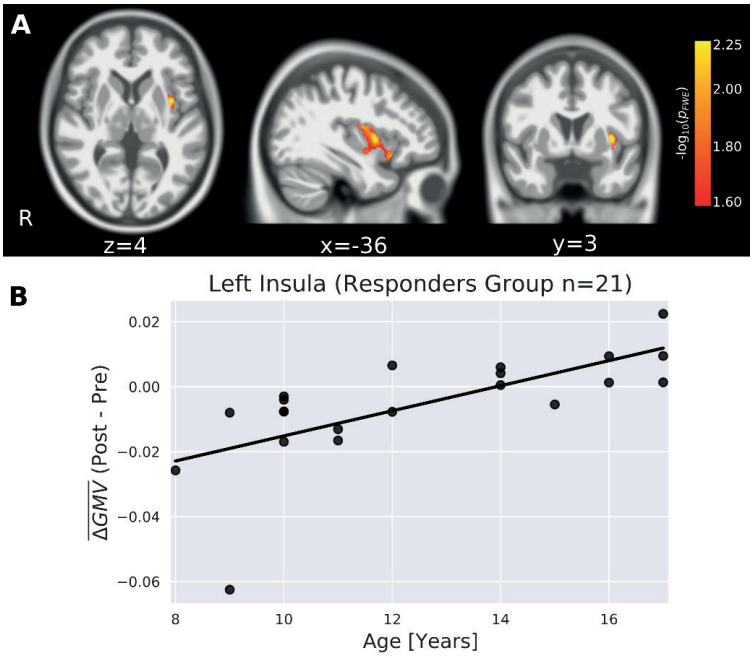

Figure S2. Exploratory age-by-time interaction analysis within responders (30% criterion)

Exploratory age-by-time interaction analysis within the responder group (30% criterion). Analysis was FWE-corrected according to the same level as described for the main analyses. The final results were only investigated within the bilateral insula (extracted from Harvard-Oxford atlas (25% threshold)) as this was the region showing a significant time-by-group interaction in the main analysis. *A.* Significant positive effect is observed within the left insula. Coloring corresponds to $-\log_{10}(p_{FWE})$ values FWE-corrected for whole-brain, two-sided contrasts, GMV/WMV images via synchronized permutations. Thresholding is done according to Bonferroni-correction of the alpha value at 0.025 (correcting for the two response criteria initially investigated) ($-\log_{10}(0.025) = 1.602$). *B.* Visualization of positive association between age and average delta GMV within the whole cluster shown in A.

Table S1. Subject characteristics.	**Responders (n= 13) ≥50% CAPS-CA**	**Non-responders (n= 22) <50% CAPS-CA**	***p*-value***
Sociodemographic characteristics			
Female (%)	30.7	77.2	**.007**
Age (years; mean, SE)	12.0 (0.83)	13.4 (0.64)	.778
West European Ethnicity (%)	75.0	52.6	.213
Current educational level (%)			.496
Elementary school	61.5	36.4	
Middle/High school lower level	0.0	4.5	
Middle/High school middle level	23.1	36.4	
Middle/High school higher level	15.4	13.6	
Vocational school	0.0	9.1	
Household income (%)			.283
< €25.000	40.0	21.4	
€25.000-35.000	10.0	28.6	
> €35.000	50.0	50.0	
Weight (kg; mean, SE)	49.6 (4.88)	51.9 (2.45)	.446
Current psychotropic medication (%)	15.4	4.5	.268
Smoking (%)	0%	11.8%	.238
Alcohol >1 consumption/day (%)	0%	0%	N/A
Trauma characteristics			
Index trauma (%)			.561
Sexual abuse	30.7	27.2	
Domestic violence	30.7	18.2	
Community violence	7.7	31.8	
Accidents/Medical	15.8	13.6	
Other	15.8	9.1	
Multiple-event trauma exposure (%)	46.1	59.1	.458
Age at index trauma (years; mean, SE)	9.8 (0.98)	9.6 (0.96)	.164
Time since index trauma (years; mean, SE)	2.4 (0.43)	3.8 (0.75)	**.003**
Clinical characteristics			
CAPS-CA study entry (mean, SE) †			
Total	41.8 (4.28)	61.5 (5.36)	**.006**
Re-experiencing	10.3 (1.20)	20.8 (2.06)	**.005**
Avoidance	18.6 (2.43)	22.8 (2.54)	.163
Hyperarousal	11.2 (2.32)	19.2 (2.02)	.217
Full PTSD diagnosis (%)	69.2	86.4	.221
RCADS study entry (mean, SE) †			
MDD	9.0 (2.00)	12.8 (1.51)	.627
GAD	7.6 (1.52)	6.6 (0.93)	.222
OCD	5.7 (1.27)	6.0 (0.73)	.247
PD	6.6 (1.12)	8.9 (1.36)	.349
SAD	5.6 (1.79)	6.1 (1.12)	.649
SP	9.2 (2.15)	13.5 (1.61)	.839

Abbreviations: CAPS-CA, Clinician-Administered PTSD Scale for Children and Adolescents; RCADS, Revised Child Anxiety and Depression Scale; MDD, major depressive disorder; GAD, general anxiety disorder; OCD, obsessive compulsive disorder; PD, panic disorder; SAD, separation anxiety disorder; SP, social phobia; SE, standard error.

* p-values <0.05 shown in bold. Independent samples t-test for continuous and x^2 tests for categorical variables.

† Ranges: CAPS-CA total, 0-139; RCADS MDD, 0-30; RCADS GAD, 0-18; RCADS OCD, 0-18; RCADS PD, 0-27; RCADS SAD, 0-21; RCADS SP, 0-27.

Chapter 6

Autonomic nervous system function before and after trauma-focused psychotherapy in youth with posttraumatic stress disorder

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Under review: Psychoneuroendocrinology

ABSTRACT

While trauma-focused psychotherapies have been shown effective in youth with PTSD, the relationship between treatment response and alternations in autonomic nervous system (ANS) associated with PTSD, remains incompletely understood. During neutral and personalized trauma script imagery heart rate (HR), pre-ejection period (PEP) and respiratory sinus arrhythmia (RSA) were recorded in youth aged 8-18 with (partial) PTSD (*n*= 76) and trauma exposed controls (TEC) (*n*= 27) to determine ANS activity and stress reactivity. Youth with PTSD were subsequently treated with eight sessions of trauma-focused psychotherapy. PTSD severity was assessed using the Clinician-Administered PTSD scale for Children and Adolescents to divide patients into responders and non-responders. Youth with PTSD relative to TEC had higher overall HR and lower PEP during both neutral and trauma imagery. Youth with PTSD showed RSA decrease during trauma imagery relative to neutral imagery, the reverse of TEC. Relative to non-responders, responders demonstrated a significant baseline to posttreatment increase of RSA response to stress only when employing a ≥50% response criterion and not with the primary ≥30% criterion. Our results suggest overall higher HR and sympathetic nervous system activity as well as vagal withdrawal in response to stress in youth with PTSD, and only provide partial support for normalization of the latter with successful trauma-focused psychotherapy.

INTRODUCTION

Posttraumatic stress disorder (PTSD) is a common mental health disorder that develops in approximately 16% of youth exposed to traumatic events (Alisic et al., 2014). Youth with PTSD are troubled by frequent re-experiencing of the traumatic event, persistent avoidance, hyperarousal and negative alterations in cognition and mood (American Psychiatric Association, 2013). These symptoms can interfere with social functioning and school performance, have a negative effect on the quality of life of the affected youth and their families (Carrion, Weems, Ray, & Reiss, 2002) and are a crucial factor in shaping the vulnerability to depression, suicidality and cardiovascular disease later in life (Molnar, Berkman, & Buka, 2001).

Randomized controlled trials have demonstrated efficacy of trauma-focused cognitive behavioral therapy (TF-CBT) and eye movement desensitization and reprocessing (EMDR) in youth with PTSD (Morina, Koerssen, & Pollet, 2016). However, less is known about the association between these trauma-focused psychotherapies response and changes in autonomic nervous system (ANS) function in youth with PTSD (J. B. Zantvoord, Diehle, & Lindauer, 2013). Examining ANS changes associated with treatment response can provide a better understanding of why some youth recover while in some PTSD persists (J. B. Zantvoord et al., 2013). This is important because, response varies considerable among individuals, with 30-50% of youth not benefiting sufficiently (Diehle, Opmeer, Boer, Mannarino, & Lindauer, 2015; Morina et al., 2016), leading to persistent symptoms and longer treatment trajectories.

A mounting body of literature emphasizes disturbances in both the sympathetic nervous system (SNS) and parasympathetic nervous system (PNS) in adults with PTSD (Pole, 2007). Increased heart rate (HR) during trauma-related stimuli has been one of the most consistent findings in studies of ANS function in adults with PTSD (Pole, 2007). HR is under joint control of the SNS and PNS and increased HR during stress could be considered as the result of SNS and PNS disbalance in individuals with PTSD (Hopper, Spinazzola, Simpson, & van der Kolk, 2006). The parasympathetic control over HR is exerted through the action of the vagal nerve, in which higher vagal tone produces a lowered HR compared with the basal firing rate (Porges, 2007). Vagal tone can be measured noninvasively via respiratory sinus arrhythmia (RSA). RSA reflects the rhythmic fluctuation of HR, and is quantified as the variability of HR increases with inspiration and decreases with expiration (Grossman & Taylor, 2007). Studies using RSA to examine vagal control in PTSD have rendered more mixed findings, but together indicate an association between PTSD and vagal withdrawal (i.e. reduced RSA) during both rest and stress in adults with PTSD (Campbell, Wisco, Silvia, & Gay, 2019), suggesting an overall lower parasympathetic activity in adults with PTSD (Schneider & Schwerdtfeger, 2020). Studies specifically measuring SNS

parameters in PTSD have mainly employed skin conductance levels and the pre-ejection period (PEP, the period between the electrical initiation of the heart beat and the time that blood is ejected into the aorta) as sympathetic indices and suggest heightened SNS activity in adults with PTSD relative to controls (Michopoulos, Norrholm, & Jovanovic, 2015).

Importantly, the ANS undergoes substantial development throughout childhood and adolescence. PNS-activity follows a cubic trend, with an exponential increase from infancy, a plateau phase during middle childhood, followed by a decrease to adolescence (Harteveld et al., 2021). SNS-activity shows a more linear trend, with a gradual decrease from infancy to adolescence (Harteveld et al., 2021). A recent meta-analysis in youth with PTSD replicated the finding of overall heightened ANS activity during stress tasks found in adults (Siciliano, Anderson, & Compas, 2022). However, contrary to results in adults, decreased SNS activity at rest corresponded to increased posttraumatic stress symptoms and the correlation between PNS measures and PTSD was non-significant (Siciliano et al., 2022). Divergent ANS findings between youth and adults with PTSD might indicate developmental effects that are not yet apparent until adulthood as well as plastic response to illness over time (Mikolajewski & Scheeringa, 2018; Weems, Russell, Neill, & McCurdy, 2019). Together, this highlights that ANS results obtained in adult PTSD population cannot be readily translated to youth and emphasize the need for specific studies examining ANS function which take potential age-related effects into account.

There remains a debate as to whether successful trauma-focused psychotherapy may reverse ANS abnormalities associated with PTSD. Results from longitudinal ANS studies in adults with PTSD have been mixed (J. B. Zantvoord et al., 2013). The majority of these studies have focused on HR, with most suggesting that HR (reactivity) decreases during trauma-focused psychotherapy (Blanchard et al., 2002; Lindauer et al., 2006), while some did not find change of HR during trauma-focused psychotherapy (Wangelin & Tuerk, 2015). To date, fewer studies have examined the association between trauma-focused psychotherapy and specific measures of PNS or SNS activity. Most, but not all of these studies, did not find an association between trauma-focused psychotherapy outcome and PNS or SNS activity (Blanchard et al., 2002; D'Andrea & Pole, 2012; M. Sack, Lempa, Steinmetz, Lamprecht, & Hofmann, 2008). However, studies had limited sample sizes or used crude measures of PNS/SNS activity, precluding a strong synthesis of the current evidence (Bourassa, Hendrickson, Reger, & Norr, 2021). To the best of our knowledge, only two studies have been published that examine ANS changes associated with trauma-focused psychotherapy response in youth with PTSD. Lipschutz et al. (2017) did not find an association between RSA change and trauma-focused cognitive behavioral therapy (TF-CBT) outcome (Lipschutz, Gray, Weems, & Scheeringa, 2017), while Shenk et al.

(2022) did demonstrate a significant association between increased RSA withdrawal during the course of treatment and symptom improvement (Shenk et al., 2022). The limited amount of studies with mixed findings so far indicate that the question whether successful trauma-focused psychotherapy in youth may reverse ANS abnormalities associated with PTSD, remains to be answered.

In the present study, we first compared ANS activity and stress reactivity in youth (aged 8-18 years) with PTSD relative to trauma exposed healthy youth (TEC) and examined potential moderating effects of age and gender. We then investigated longitudinal changes in ANS activity and stress reactivity in youth with PTSD treated with trauma-focused psychotherapy. We used HR, RSA and PEP during a script driven imagery (SDI) paradigm to compare youth with PTSD and TEC as well as compare baseline to posttreatment changes in treatment responders relative to non-responders. We hypothesized that youth with PTSD relative to TEC would show an overall increased ANS stress reactivity as indicated by greater increase of SNS activity (PEP reduction) together with a greater vagal withdrawal (RSA reduction) resulting in a greater HR increase during SDI at baseline. Given ANS developmental change and previous results on gender differences in posttraumatic autonomic functioning we further hypothesized moderating effects of age and gender on the relationship between ANS stress reactivity and PTSD diagnosis. Last, we hypothesized that trauma-focused psychotherapy response would be associated with an increase of vagal control during stress and a reduction of HR and SNS stress reactivity.

MATERIALS AND METHODS

Study design

A graphical overview of the study cohort is depicted in figure S1. First, we compared indicators of ANS activity (HR, RSA and PEP) in response to SDI at baseline between 76 PTSD patients and 27 TEC and investigated potential moderating effects of age and gender. Second, we compared the same parameters between treatment responders (*n*= 32) and non-responders (*n*= 16) from baseline to post-treatment.

Participants

All participants were Dutch speaking, and 8-18 years old. Gender categories were based on the personal identification of participants' own gender. Patients were recruited between June 2011 and September 2018 at the outpatient child psycho-trauma center of the department of child and adolescent psychiatry, Levvel in Amsterdam, The Netherlands as part of a RCT comparing TF-CBT and eye movement desensitization and reprocessing (EMDR) (Diehle et al., 2015). Patients were referred

by child welfare services, physicians or general practitioners. Diagnoses for PTSD or partial PTSD were established clinically by an experienced child and adolescent psychiatrist or psychologists according to the DSM-IV-TR criteria using joint child reports on individual symptoms on the Clinician-Administered PTSD Scale for Children and Adolescents (CAPS-CA) semi-structured interview (Diehle, de Roos, Boer, & Lindauer, 2013; Nader et al., 1996) and the caregiver reports from the PTSD scale of the Anxiety Disorders Interview Schedule – Parent Version (ADIS-P) (Verlinden et al., 2014). A symptom was established as present, if either child or caregiver reported its presence. Partial PTSD was defined as either fulfilling two of the three PTSD symptom clusters or having one symptom present in each of the three symptom clusters (Stein, Walker, Hazen, & Forde, 1997). Furthermore, participants were required to have a CAPS-CA total score indicating at least mild PTSD symptom severity (>20 points). Exclusion criteria were: acute suicidality, IQ<70, pregnancy, neurological disorders or serious medical illnesses or meeting the criteria of the following diagnosis: psychotic disorders, substance-use disorder or pervasive developmental disorder. Psychotropic medication was required to be stable for at least three weeks before and during trauma-focused psychotherapy. In our sample one participant was taking sertraline at a stable dose. TEC were recruited through local elementary - and high schools. Exposure to traumatic events was validated according to A1 and A2 criteria of DSM-IV-TR using the life-events checklist of the CAPS-CA. TEC were excluded if they met current or lifetime PTSD or partial PTSD diagnosis using both the CAPS-CA and caregiver reports from the PTSD scale of the ADIS-P or had a current CAPS-CA total score of > 20 points. In accordance with procedures approved by the Institutional Review Board of the Amsterdam University Medical Centers and the declaration of Helsinki, written informed consent was obtained from all parents or legal guardians. Written informed consent from youth aged 12 years and older and assent from youth aged 11 and younger, was also obtained from the youth themselves. All participants received a monetary incentive for participation (€5 for each assessment).

Trauma-focused psychotherapy

Patients were randomly assigned to weekly protocolized sessions for a total of 8 weeks of either TF-CBT or EMDR. The data reported here were obtained as part of a larger study on the long-term efficacy of TF-CBT and EMDR. Treatment was delivered by experienced trauma therapists who were trained in TF-CBT and EMDR before study initiation. Supervision by TF-CBT and EMDR experts was provided throughout the study. Treatment protocols, training and supervision of therapists, as well as treatment fidelity have been described in detail previously (Jasper B Zantvoord et al., 2019).

Trained psychologists administered the CAPS-CA and the PTSD scale of the ADIS-P to measure PTSD symptoms at baseline and after treatment. The Dutch Revised Child Anxiety and Depression Scale (RCADS(-P)) questionnaires was administered to assess depressive and anxiety symptoms (Chorpita, Yim, Moffitt, Umemoto, & Francis, 2000). Symptom change was calculated by subtracting the baseline from the post-treatment CAPS-CA total score. There is no established definition of response criterion or a consensus definition of response terms in the child trauma field. Based on the psychometric properties of the CAPS (-CA) and previous treatment outcome studies using the CAPS-CA we used ≥30% reduction of CAPS-CA total score as our primary response criterion for clinically meaningful improvement. A ≥50% reduction of CAPS-CA total score was selected as a secondary response criterion entailing substantial clinical improvement (J. B. Zantvoord et al., 2021; Zhutovsky et al., 2021).

Script driven imagery (SDI)

All study participants performed a standardized protocol for script driven imagery (SDI) (Shalev, Orr, & Pitman, 1992). The SDI protocol and procedure have been described in detail previously (Jasper B Zantvoord et al., 2019). In summary, patients performed SDI twice, first at baseline within two weeks before the start of trauma-focused psychotherapy and second two weeks after the 8th trauma-focused psychotherapy session. TEC were only assessed once at baseline. A 30 sec (range 29-31 sec) personalized trauma audio script describing the participants most disturbing traumatic event and a general 30 sec neutral audio script describing tooth brushing were recorded. During the SDI procedure the neutral script always preceded the trauma script, both scripts were preceded by a 3 min movie clip depicting neutral landscapes. Participants were instructed to vividly imagine the events described in the scripts with their eyes closed during and for 30 seconds after the scripts.

Psychophysiological measures

Physiological data were measured by the "Vrije Universiteit Ambulatory Monitoring System" (VU-AMS), a lightweight portable device that records ECGs and changes in thorax impedance (impedance cardiography [ICG]) from a six-electrode configuration (VU University, Amsterdam, The Netherlands, www.vu-ams.nl). Participants wore the VU-AMS device during the SDI procedure.

From the VU-AMS recordings, the following outcome variables were computed: HR, RSA, and PEP during both the 30-second neutral and trauma imagery period. HR was directly derived from the inter-beat-interval time series (De Geus & Van Doornen, 1996). RSA was obtained by peak-valley estimation that combined the ECG with the respiration signal assessed with the thorax impedance (De Geus, Willemsen, Klaver,

& van Doornen, 1995). PEP was extracted from the interval between the Q-onset in the ECG, indicating onset of left ventricular electrical activity, and the upstroke (B-point) of the ICG signal, indicating the beginning of left ventricular ejection (van Lien, Neijts, Willemsen, & de Geus, 2015).

During automated and visual data cleaning, suspicious IBIs and breathing cycles were corrected or discarded when displaying irregularities. An automated scoring algorithm was also used to detect crucial landmarks in the ICG, which were then visually inspected and manually corrected.

RSA data was positively skewed and therefore log-transformed to approximate a normal distribution.

Covariates

Adjustments were made for a-priori selected putative demographic characteristics including age, gender, educational level as well as trauma related and health indicators associated with PTSD and ANS activity, including smoking status (yes/no), alcohol use (>1 unit per day, yes/no), index trauma type and time since trauma exposure (Harteveld et al., 2021; Iffland et al., 2020). Because it has been linked to HR variability, respiration rate was also included as a covariate (Harteveld et al., 2021). In the baseline to posttreatment analysis (responders vs. non-responders) baseline total CAPS-CA score was added as a covariate.

Statistical analyses

The distribution of baseline clinical, trauma and demographic characteristics across TEC and youth with PTSD as well as treatment responders and non-responders were examined using X^2-tests, independent sample t-tests or Mann-Whitney tests as appropriate. For descriptive purposes, independent sample t-tests or Mann-Whitney tests were also used to outline the differences at baseline between ANS values during neutral and trauma SDI, and ANS stress reactivity values across youth with PTSD and TEC as well as treatment responders and non-responders respectively. Absolute ANS stress reactivity values were calculated by subtraction of averaged ANS values during neutral SDI from averaged ANS values during trauma SDI.

To formally examine the differences in HR, RSA, and PEP between youth with PTSD and TEC at baseline, we conducted linear mixed model analyses. We used linear mixed models, because this method accounts for the dependency of repeated measurements obtained from the same participant over time. In addition, mixed models also have the advantage of allowing missing measurements without discarding data (Gueorguieva & Krystal, 2004). Two models were used: one to estimate the main effects of group (youth with PTSD vs TEC) and condition (trauma

versus neutral SDI), and the other model to additionally estimate the interaction effect of group-by-condition (ANS stress reactivity differences across the groups at baseline). The mixed models were then repeated with adjustments for covariates.

To examine a moderating effect of gender and age at baseline we performed linear mixed models with 3-way-interaction between group (youth with PTSD vs TEC)-by-condition (trauma vs. neutral SDI)-by-age as well as group-by-condition-by-gender interaction. In case the higher order interaction was non-significant, we removed this term and used the remaining more parsimonious model. Furthermore, LLM with condition-by-age as well as the condition-by-gender interactions were performed in the PTSD and TEC groups separately. Mixed models were performed with and without covariates.

To assess the (baseline to posttreatment) effects of trauma-focused psychotherapy response in patients on HR, RSA and PEP, we first tested whether responders and non-responders were comparable at baseline using both the primary ≥30% and secondary ≥50% response criterion. We then performed LMM with a 3-way-interaction between time (baseline versus posttreatment), condition (trauma versus neutral SDI) and response status (responder versus non-responder) separately for both response criteria. When a higher order interaction did not contribute significantly to the model, we removed this term and used the more parsimonious model. LLM were additionally performed in the PTSD and TEC groups separately. We repeated the mixed models with incorporation of covariates in the model.

Statistical analyses were performed using SPSS version 26.0 (SPSS Inc., Chicago IL, USA). The criterion for statistical significance was $p < .05$.

RESULTS

Participant characteristics

Table 1 shows that relative to TEC, youth with PTSD had lower educational levels and were more likely to be of non-western ethnicity. In addition, youth with PTSD were more often exposed to interpersonal and multi-event trauma as well as exposed to trauma at an earlier age. The most common index trauma in patients was sexual abuse, followed by community violence and witnessing domestic violence. In TEC the most common index trauma were accidents followed by the category 'other trauma' from the life-events checklist of the CAPS-CA. 52.6% of patients and 29.6% of TEC were exposed to multiple-event trauma (table 1.). Within the patient group 77.6 % of patients met the full DSM-IV diagnostic criteria for PTSD at baseline, the remaining 22.4 % met criteria for partial PTSD. The average baseline CAPS-CA score in patients was *M* = 52.9 points, *SD* =23.1, which is indicative of moderately severe PTSD.

Table 1 Subject characteristics.

	Trauma exposed healthy controls (n= 27)	PTSD (n= 76)	*p*-value[a]
Sociodemographic characteristics			
Girls (%)	55.5	60.5	.652
Age (years; mean, SD)	13.6 (3.33)	12.6 (3.09)	.170
West European Ethnicity (%)	80.0	45.2	**.002**
Current educational level (%)			**.004**
Elementary school	34.6	46.1	
Middle/High school lower level	0.0	6.6	
Middle/High school middle level	11.5	30.3	
Middle/High school higher level	30.8	10.5	
Vocational school	23.1	6.6	
Household income (€, %)			.942
< 25.000	46.1	42.3	
25.000-35.000	15.3	19.2	
> 35.000	38.5	38.5	
Weight (kg; mean, SD)	48.3 (25.7)	49.1 (15.1)	.195
Current psychotropic medication (%)	0.0	2.6	.662
Smoking (%)	4	10	.284
Alcohol >1 consumption/day (%)	4	13.3	.173
Trauma characteristics			
Index trauma (%)			**<.001**
Sexual abuse	0.0	28.9	
Domestic violence	7.4	22.4	
Community violence	7.4	25.0	
Accidents/Medical	44.4	9.2	
Other	40.7	14.5	
Multiple-event trauma exposure (%)	29.6	54.0	**.030**
Age at index trauma (years; mean, SD)	11.2 (3.83)	8.9 (4.22)	**.020**
Time since index trauma (years; mean, SD)	2.6 (2.57)	3.8 (3.44)	.095
Clinical characteristics			
CAPS-CA study entry (mean, SD)[b]			
Total	11.6 (8.77)	53.3 (22.99)	**<.001**
Re-experiencing	3.8 (4.12)	16.9 (9.87)	**<.001**
Avoidance	3.0 (5.52)	19.8 (9.16)	**.001**
Hyperarousal	4.2 (3.63)	16.9 (9.40)	**<.001**
RCADS study entry (mean, SD)[b]			
MDD	3.5 (2.61)	11.7 (6.42)	**<.001**
GAD	2.3 (2.11)	7.2 (4.44)	**<.001**
OCD	1.2 (1.48)	6.4 (3.53)	**<.001**
PD	1.5 (1.78)	7.7 (5.58)	**<.001**
SAD	1.6 (1.43)	5.7 (4.15)	**<.001**
SP	4.7 (4.37)	11.9 (7.01)	**<.001**

Abbreviations: CAPS-CA, Clinician-Administered PTSD Scale for Children and Adolescents; RCADS, Revised Child Anxiety and Depression Scale; MDD, major depressive disorder; GAD, general anxiety disorder; OCD, obsessive compulsive disorder; PD, panic disorder; SAD, separation anxiety disorder; SP, social phobia; SD, standard deviation.

[a] p-values <0.05 shown in bold. Independent samples t-test or Mann-Whitney U test for continuous and x^2 tests for categorical variables.

[b] Ranges: CAPS-CA total, 0-139; RCADS MDD, 0-30; RCADS GAD, 0-18; RCADS OCD, 0-18; RCADS PD, 0-27; RCADS SAD, 0-21; RCADS SP, 0-27.

Treatment outcome

Figure S1 shows a flow diagram of patients. Of 64 patients randomized to EMDR or TF-CBT, 16 (25 %) were lost to follow-up (reasons described in figure S1). Treatment completers and non-completers did not differ in baseline sociodemographic, trauma or clinical characteristics. Across the completer sample, we found significant reductions in CAPS-CA total score (t(47)= 7.25, p<.001, Cohen's effect size (d)= 1.05), re-experiencing (t(45) = 6.33, p <0.001, d= 0.93), avoidance (t(45) = 6.62, p<0.001, d= 0.98) and hyperarousal clusters (t(45) = 4.55, p <.001, d= 0.67).

Applying the ≥30% response criterion yielded 32 responders and 16 non-responders, applying the ≥50% response criterion yielded 23 responders and 25 non-responders. Table S1a indicates that there were no significant group differences at baseline for the distribution of sociodemographic, trauma or clinical characteristics between responders and non-responders when using the primary ≥30% response criterion. Table S1b shows when applying the ≥50% response criterion, responders had a lower CAPS-CA re-experiencing score and female to male ratio at baseline (p< .05).

Autonomic nervous system activity during script driven imagery in youth with PTSD vs. TEC at baseline

Table 2 describes the unadjusted means of ANS data during neutral and trauma SDI and the stress reactivity values for TEC and youth with PTSD at baseline. Results of the unadjusted and adjusted main and interaction effects of group and condition at baseline are shown in Table 3. Table 3 shows that mixed-model analyses resulted in a significant main condition effect for HR but not RSA and PEP for neutral SDI compared with trauma SDI, indicating that trauma SDI at baseline yielded the expected stress activation as measured with HR but not with PEP and RSA. Significant main group effects at baseline (PTSD vs. TEC) were seen for HR, which was higher in PTSD group as compared to TEC (table 3). In addition, PEP was lower in the unadjusted but not adjusted analyses for the PTSD group as compared to TEC. No main group effect was found for RSA. Illustrating that there were general differences in HR and PEP (only in the unadjusted model) but not RSA across youth with PTSD and TEC. Importantly, a significant result was found for the group-by-condition interaction for RSA (i.e. differences in RSA stress reactivity across youth with PTSD and TEC), related to RSA decrease during trauma SDI in youth PTSD and RSA increase during trauma SDI in TEC (tables 2 and 3). There were no significant interactions for HR and PEP. When excluding the patient using sertraline from the analyses, no changes occurred in significance or magnitude of findings.

Table 2. ANS values during script driven imagery with their respective imagery conditions and reactivity values at baseline in youth with PTSD and trauma exposed healthy controls

	Participants		
	Trauma exposed healthy controls (*n*= 27)	PTSD (*n*= 76)	*p*[a]
HR, beats/min			
Neutral imagery	75.0 (14.0)	80.8 (12.5)	.048
Trauma imagery	76.1 (13.3)	81.9 (12.6)	.043
Trauma Reactivity	1.1 (4.4)	1.2 (6.1)	.958
RSA, ms[b]			
Neutral imagery	98.7 (61.1)	90.9 (48.0)	.507
Trauma imagery	106.6 (59.3)	82.3 (42.1)	.027
Trauma Reactivity	8.0 (38.1)	-7.1 (40.0)	.014
PEP, ms			
Neutral imagery	107.5 (17.2)	98.8 (18.2)	.036
Trauma imagery	107.8 (18.1)	98.1 (18.4)	.025
Trauma Reactivity	0.3 (9.6)	-1.1 (10.8)	.566

Values represent mean (standard deviation).
ANS = autonomic nervous system; HR = heart rate; RSA = respiratory sinus arrhythmia; PEP = pre-ejection period.
Trauma reactivity values represent averaged ANS values during trauma imagery minus their respective averaged values during neutral imagery.
[a] Comparison using independent sample t-tests or Mann-Whitney U tests as appropriate
[b] Values were ln-transformed for analyses and back-transformed for representation of the means.

Moderation by age and gender of ANS activity during SDI in youth with PTSD vs. TEC at baseline

All moderation analysis are fully described in the Supplementary material and are briefly summarized here. There was no significant effect of the age-by-group-by-condition or age-by-group interaction at baseline, indicating that ANS differences between PTSD and TEC were independent of age. The gender-by-group-by-condition and group-by-gender interactions indicated a moderating effect of gender on differences at baseline in HR, PEP and RSA during SDI between youth with PTSD and TEC. Post-hoc analysis stratified by gender showed a significant group-by-condition interactions for RSA in boys but not girls and vice versa for PEP.

Table 3. Main and interaction effects on ANS of group (youth with PTSD and trauma exposed healthy controls) and condition (neutral and trauma imagery) at baseline.

	Group Effect[a]		**Condition effect[a]**		**Group by Condition effect[a]**	
	PTSD versus Trauma exposed healthy controls		Trauma versus Neutral imagery		PTSD by Trauma imagery	
Variable	*F*	*p*	*F*	*p*	*F*	*p*
HR, beats/min						
Unadjusted	4.322	.040	4.172	.044	.003	.958
Adjusted[c]	3.844	.053	4.003	.048	.035	.852
RSA, ms[b]						
Unadjusted	1.968	.164	.005	.942	5.690	.019
Adjusted[c]	2.544	.115	.021	.885	6.383	.013
PEP, ms						
Unadjusted	5.502	.021	.351	.555	.298	.586
Adjusted[c]	1.304	.257	.129	.720	.018	.795

ANS = autonomic nervous system; HR = heart rate; RSA = respiratory sinus arrhythmia; PEP = pre-ejection period.
[a] Main effects of group and condition were analyzed in a mixed model separate from the interaction effect of group by condition.
[b] Values were ln-transformed for analyses
[c] Adjusted for age, gender, ethnicity, smoking status, alcohol consumption, index trauma and time since trauma as well as respiration rate for RSA

Baseline to posttreatment change in autonomic nervous system activity during script driven imagery in responders vs. treatment non-responders

At baseline the responder status-by-condition interaction and main effects of responder status indicated that HR, PEP and RSA (re)activity during SDI before treatment were comparable between trauma-focused psychotherapy responders and non-responders (see table s2a and s2b for ≥30% and ≥50% response criterion respectively in supplementary materials).

Table 4 reports that during trauma-focused psychotherapy, the 3-way-interaction between time (baseline, posttreatment), condition (neutral, trauma script) and responder status and subsequent 2-way responder status-by-time interaction using our primary response criterion (≥30% CAPS-CA reduction) were non-significant for HR, PEP and RSA, so both terms were omitted. In the subsequent parsimonious model, the interaction of responder status-by-condition reached significance for HR, but not PEP and RSA, indicating increased HR stress reactivity, but not RSA or PEP stress reactivity, in patients who responded to trauma-focused psychotherapy independent of time (baseline, posttreatment) (figure 1).

Table 4. Baseline- to posttreatment change in ANS during script driven imagery in responders vs. treatment non-responders with ≥30% response criterion.

	Responder status x Condition x Time		Responder status x Time		Responder status x Condition	
Variable	*F*	*p*	*F*	*p*	*F*	*p*
HR, beats/min						
Unadjusted	.154	.696	.101	.751	14.85	<.001
Adjusted[c]	.009	.925	.186	.667	10.81	.001
RSA, ms[b]						
Unadjusted	2.087	.151	.166	.685	.353	.554
Adjusted[c]	2.405	.124	.002	.964	.796	.374
PEP, ms						
Unadjusted	.255	.614	.655	.420	.035	.852
Adjusted[c]	1.304	.134	.326	.569	1.162	.284

ANS = autonomic nervous system; HR = heart rate; RSA = respiratory sinus arrhythmia; PEP = pre-ejection period.
[a] Values were ln-transformed for analyses
[b] Adjusted for age, gender, ethnicity, smoking status, alcohol consumption, index trauma, time since trauma baseline CAPS-CA as well as respiration rate for RSA

The same 3-way-interaction and subsequent analyses with the secondary response criterion (≥50% CAPS-CA reduction) are fully described in the Supplementary material and summarized here. We found a significant effect of the time-by-condition-by-responder status for RSA indicating differential baseline to posttreatment changes in RSA stress reactivity between trauma-focused psychotherapy responders and non-responders (figure S2. In supplementary materials). Post-hoc analysis stratified by response status showed a significant increase of RSA in response to trauma SDI from baseline to posttreatment in treatment responders, while non-responders did not show a significant increase of RSA in response to trauma SDI with treatment. We did not identify any significant 3-way or subsequent 2-way interactions with HR or PEP using our secondary criterion.

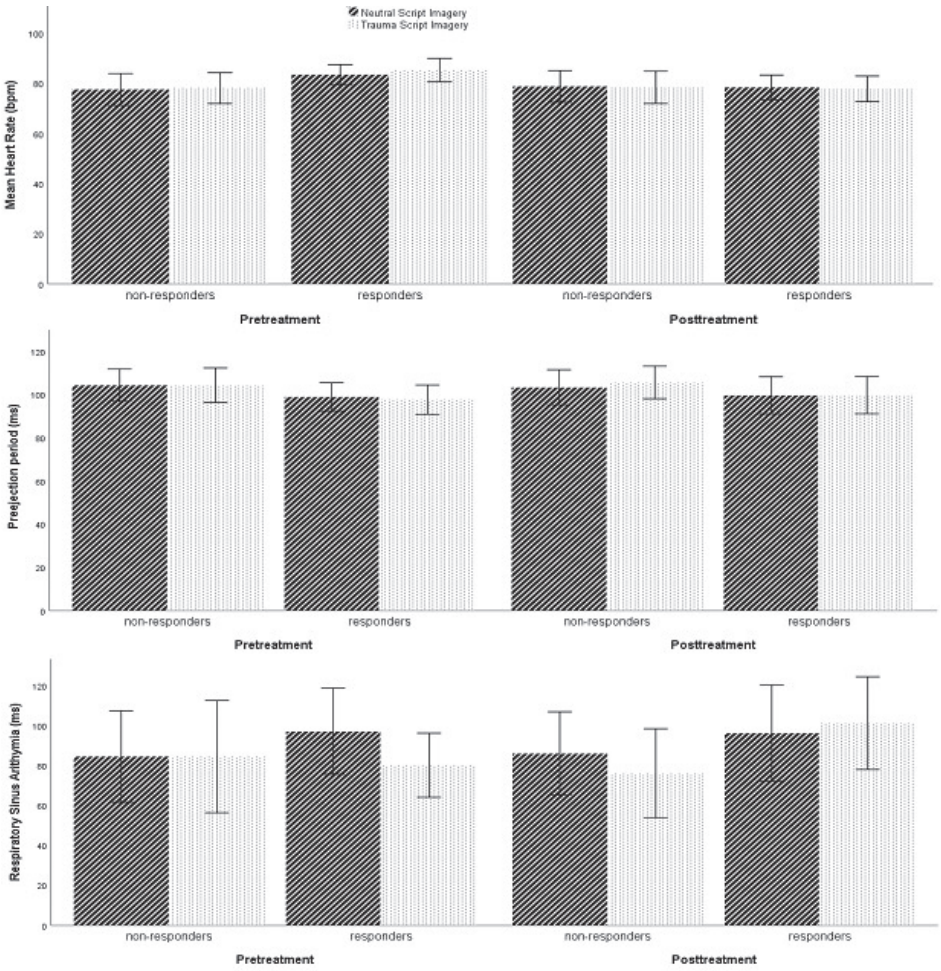

Figure 1 Autonomic nervous system activity in youth with PTSD who responded (>30% CAPS-CA reduction) during eight sessions of trauma-focused psychotherapy compared to those who did not respond. All results are adjusted for age, gender, ethnicity, smoking status (yes/no), alcohol consumption (yes/no), index trauma, time since trauma baseline CAPS-CA as well as respiration rate for RSA. Error bars indicate SD. Mixed model analyses results for response status: responder status x condition HR: F = 10.81, p = .001, PEP: F = 1.162, p = . 284 and RSA: F = .796, p = . 374.

DISCUSSION

In this study we investigated ANS stress reactivity in trauma-exposed youth and examined trauma-focused psychotherapy-related changes in ANS stress reactivity. Our results indicate that before treatment youth with PTSD are characterized by RSA decrease during trauma SDI while trauma exposed healthy controls show a RSA increase. This suggests withdrawal of vagal activity in response to stress in PTSD patients and contrasting increase of vagal activity in response to stress in TEC. Relative to TEC, youth with PTSD also showed an overall lower PEP and higher HR before treatment, the former suggests higher SNS activity in youth with PTSD and the latter may reflect an overall SNS and PNS disbalance in youth with PTSD. In contrast, we did not find evidence for differences in HR and PEP stress reactivity in response to trauma SDI. Our results provide preliminary evidence for a moderating role of gender on ANS differences between youth with PTSD and TEC, in which RSA differences were mainly found in boys while PEP differences occurred in girls. We did not find evidence for a moderating effect of age. We further found that, relative to non-responders, trauma-focused psychotherapy responders were characterized by an overall increased HR stress reactivity, which was independent of time (baseline and posttreatment). We did not observe change of HR, PEP and RSA after successful trauma-focused psychotherapy with our primary (>30% CAPS-CA reduction) response criterion. We did however, find change of RSA reactivity after successful trauma-focused psychotherapy employing our secondary (≥50% CAPS-CA reduction) response criterion, the latter may suggest an association between treatment response and an increase in vagal control during stress.

Our finding of RSA decrease during trauma SDI in youth with PTSD suggests lower vagal control during trauma related stressors through a withdrawal of vagal input. According to Porges, RSA serves as an index of stress reactivity and stress vulnerability (Porges, 2007). High vagal activity enhances cardiac control to environmental demands while vagal withdrawal is associated with a less adaptive capacity and associated with increased vulnerable to dysregulated and excessive responses to (traumatic) stressors (Porges, 2007; Martin Sack, Hopper, & Lamprecht, 2004). Dysfunctional vagal stress control could either be a pre-existing risk factor in the development of PTSD or a plastic response to PTSD over time. Although, we cannot differentiate between these possibilities in the current study, previous research by Mikolajewski et al. (2018) has provided evidence for the former, with RSA and RSA reactivity as pre-existing marker of stress sensitivity that predicts posttraumatic stress symptomatology after trauma exposure (Mikolajewski & Scheeringa, 2018).

Increased vagal withdrawal during stress has been a finding in some but not all studies in adult PTSD (Campbell et al., 2019). Interestingly, the few prior studies which measured RSA reactivity to investigated the relationship between vagal control during stress and PTSD in youth did not find significant differences in RSA stress reactivity between PTSD and TEC (Katz & Gurtovenko, 2015; Kirsch, Wilhelm, & Goldbeck, 2015; Scheeringa, Zeanah, Myers, & Putnam, 2004), which contrasts findings in the current study. Aside from methodological differences in RSA measurements, sample size and analysis, PTSD sample characteristics could be an explanation for divergent results between these studies. For example, the sample of Scheeringa et al (2004) and Katz and Gurtovenko (2015) consisted of children aged 2-6 years and 6-12 years respectively, which do not, or only partially overlap with the age range in the current study (8-18 years). Although, we did not find a moderating effect of age, this does not rule out that age might be a factor in explaining discrepancies between studies. Particularly, as previous research has reported a cubic developmental trajectory of RSA (Harteveld et al., 2021). Another possibility could be that the divergent findings might be related to differences in contextual factors. For example, parenting style and caregiver' PTSD symptomatology have been reported to influence the relationship between PTSD and RSA stress reactivity in the studies of Scheeringa and Katz (Katz & Gurtovenko, 2015; Scheeringa et al., 2004). This might be relevant as a considerable part of our sample was exposed to trauma in the domestic environment and were subject to out-of-house placement and foster care. Future research in older youth could therefore benefit from inclusion of measures of parental PTSD and parenting style to examine potential moderating effects on the relationship between RSA and PTSD.

In contrast to previous studies in adult PTSD (Pole, 2007), we did not find evidence for differences in HR stress reactivity at baseline in youth with PTSD relative to TEC. Importantly, only half of the included participants showed the expected HR stress response during SDI. However, even when we limited our analysis to those individuals who did show the expected stress response, we did not identify differences between youth with PTSD and TEC in HR stress reactivity. Interestingly, prior studies comparing youth with PTSD and TEC also did not find significant differences in HR stress reactivity during exposure to trauma related stimuli (Jones-Alexander, Blanchard, & Hickling, 2005; Kirsch et al., 2015; Scheeringa et al., 2004). Together, these studies suggest divergent findings regarding HR stress reactivity between youth and adults with PTSD. A possible explanation for this is the difference in type of trauma exposure of included patients and differences in control groups. The majority of studies in adults included patients who were either exposed to motor vehicle accident or were (male) veterans, while in studies in youth, domestic violence and sexual abuse were more prevalent. Moreover, studies in adults more

often compared PTSD patients with non-traumatized controls, which prior research has shown to increase the change of finding group differences (Meewisse, Reitsma, de Vries, Gersons, & Olff, 2007). Also, average baseline CAPS-CA scores in our patients sample indicated moderately severe PTSD. As previous research in youth has demonstrated a correlation between PTSD severity and ANS activity (Siciliano et al., 2022), including a patient sample with more severe PTSD symptoms could have increased chances of finding ANS differences between TEC and PTSD.

Similarly, we did not find evidence for differences in PEP stress reactivity at baseline in youth with PTSD relative to TEC. This corroborates results from a recent meta-analysis on SNS stress reactivity in youth with PTSD, which revealed that the relation between SNS activity during stress tasks and PTSD was non-significant (Siciliano et al., 2022). The only prior publication on PEP of youth with PTSD also found that PEP stress reactivity in isolation did not significantly differ between youth with and without PTSD (Cohen et al., 2020). Together, these findings provide preliminary evidence that isolated SNS stress reactivity does not seem to be able to discriminate between youth with PTSD and TEC.

A possible explanation for the absence of the expected HR and PEP stress response in half of the included participants, could be that effectiveness of a stressor paradigm to elicit a stress response is subjective to developmental change (Gunnar, Talge, & Herrera, 2009). Throughout childhood and adolescence, marked changes are found in the ability of the same stressor paradigm to provoke a stress response (Gunnar et al., 2009). These changes in stressor paradigm effectiveness may be related to ANS development and psychological changes during childhood and adolescence (Gunnar et al., 2009; Harteveld et al., 2021). Interestingly, however, we did not find a moderating effect of age on stress reactivity in our sample. Longitudinal studies measuring stress reactivity over the course of development from childhood and adolescence into adulthood will eventually be necessary to examine the role of developmental change as an explanation for the different stress reactivity findings in adults and youth with PTSD.

Our study did show generally higher HR and lower PEP during both neutral and trauma SDI at baseline in youth with PTSD relative to TEC. These findings suggest a heightened heart rate and SNS activity throughout the SDI procedure independent of script content. One possibility that could account for the increased HR and SNS activity during neutral SDI, is a generally increased HR and SNS activity in youth with PTSD. However, a previous meta-analysis did not find a general increased HR in youth and even demonstrated a small negative correlation between SNS activity during rest and PTSD, which contrasts with findings in the current study (Siciliano et al., 2022). Another possibility, is that the increased HR and SNS activity during

neutral script in youth with PTSD might reflect increased anticipatory anxiety relative to TEC or alternatively the lack of HR and SNS activity increase during trauma script might be related to avoidance of trauma script imagery. Importantly, the neutral script always preceded the trauma script and participants knew that they would be exposed to a trauma script. Unfortunately, we did not collect (ambulatory) ANS data outside of the SDI procedure. Further studies, with collection of ambulatory ANS data together with stress reactivity data, would be needed to conclude if higher HR and PEP in PTSD reflect general heightened ANS activity or were limited to the period of (anticipation of) the SDI paradigm.

Our exploratory moderation analysis provide preliminary evidence for a moderating role of gender on HR, RSA and PEP differences at baseline in youth with PTSD relative to TEC. Prior studies have shown clear gender differences in ANS development and have found notable gender differences in autonomic functioning among trauma-exposed individuals (Harteveld et al., 2021; Seligowski et al., 2021). Surprisingly, a recent meta-regression analysis, did not find a moderating role of gender on the association between posttraumatic stress symptoms and different ANS measures (Siciliano et al., 2022). To the best of our knowledge, prior cross-sectional studies comparing ANS measures between youth with PTSD and TEC have not reported on potential moderating effects of gender (Cohen et al., 2020; Jones-Alexander et al., 2005; Katz & Gurtovenko, 2015; Kirsch et al., 2015; Scheeringa et al., 2004). The exploratory nature of our moderation analysis and paucity of comparable studies precludes a strong synthesis of evidence at this time and emphasizes the need for additional ANS studies which examine gender differences throughout development.

In the baseline to posttreatment comparison between trauma-focused psychotherapy responders and non-responders, we did not observe a change of HR or PEP after successful treatment. The former contrast with the majority of studies in adults with PTSD (Wangelin & Tuerk, 2015; J. B. Zantvoord et al., 2013). To the best of our knowledge, this is the first reported study to longitudinally examine PEP in relation to trauma-focused psychotherapy response. Our results suggest that PEP, as a measure of SNS activity, may not directly change with successful treatment. However, to allow a reasonable interpretation of these findings, it should be taken into account that we only found overall lower PEP and higher HR in youth with PTSD relative to TEC at baseline but failed to observe differences in HR and PEP stress reactivity. Also, although the average total CAPS-CA reduction in the current sample could be considered relatively low (for in depth discussion of response rates, see Zantvoord et al 2019 (Jasper B Zantvoord et al., 2019)) and the number of patients in the non-responder group was small (n=16), which reduces power and raises the possibility of a type II error.

Interestingly, in analysis with the secondary response criterion (≥50% CAPS-CA reduction), we observed significant baseline to posttreatment increase in RSA in response to trauma SDI in responders, while in non-responders RSA stress reactivity did not change from pre- to posttreatment (figure S2). Based on this finding one could speculate that trauma-focused psychotherapy response might be associated with a increased of vagal control in response to stress. However, interpretation of this finding should be done with caution, as we only found a trend in the condition-by-response-by-treatment interaction with our primary ≥30% response criterion. This could be due to more pronounced group differences when employing a stricter response criterion or could be related to larger power with more balanced sample sizes in the ≥50% response criterion. Future longitudinal replication studies with larger samples are therefore warranted to determine the relationship between trauma-focused psychotherapy response and ANS activity over time.

Several limitations of this study should be acknowledged. First, youth with PTSD and TEC differed on several key demographic and trauma related variables, in particular type of trauma exposure. Relative to TEC, youth with PTSD were more often exposed to interpersonal and repeated traumatic events. Although, inclusion of trauma-exposure type did not change the overall direction and significance of results, it cannot be ruled out that group differences are also related to difference in trauma-exposure rather than PTSD diagnosis alone. Also, the sample size of the PTSD group (n= 76) exceeded that of the TEC (n=27), creating the risk for both false positive and false negative findings. To mitigate these limitations future studies should therefore aim at more balanced PTSD and TEC groups, both regarding sample size and trauma exposure. Also, although the majority (77.6%) of included patients had a full PTSD diagnosis, the remaining 22.4% had a partial PTSD diagnosis. Including youth with partial PTSD increased clinical heterogeneity and might have lowered overall group differences between patients and TEC, however, by including youth with partial PTSD, our sample better reflects the real-life clinical setting, which adds to the ecological validity of our findings. Second, ANS (re)activity is related to many state and trait factors, we have tried to account for most of these confounding factors in the current study. However, we omitted inquiry on menstrual status, (oral) contraceptive use and BMI, which are factors which influence the ANS . Third, youth with PTSD were randomized to receive either TF-CBT or EMDR, for the current analysis both treatment conditions were collapsed. Due to limited power, it was not feasible to examining differences between treatment responders and non-responders separately for both treatment conditions. Importantly, efficacy of both treatment conditions has been shown comparable in an RCT with considerable sample overlap with the current study (Diehle et al., 2015). Furthermore, because we did not have long-term follow-up assessment, we could not assess if there are

ANS changes which are expressed later in development. This emphasizes the need for future treatment studies with long-term naturalistic follow up of ANS function after trauma-focused psychotherapy. Finally, our study had a considerable drop-out rate, as 25% of randomized patients were lost to follow-up. Although, dropout in the current study reflects routine clinical practice and ANS measures did not differ between treatment completers and non-completers, there is a possibility that drop-out could have influenced our main findings through attrition bias.

CONCLUSIONS

Our findings provide new insights into the ANS function underlying PTSD in youth and trauma-focused psychotherapy response. We demonstrated overall higher HR and SNS activity as well as withdrawal of vagal control in response to stress in youth with PTSD, with some preliminary evidence that the latter might be restored with successful trauma-focused psychotherapy. Future research is merited to test the robustness and generalizability of these findings in larger independent cohorts. If replicated, the ANS differences identified here could serve as new targets for (pharmacological augmentation) interventions to improve treatment outcome in youth with PTSD (Brunet et al., 2018).

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SUPPLEMENTARY MATERIAL

Supplementary results

Moderation by age and gender of ANS activity during SDI in youth with PTSD vs. TEC

The 3-way-interaction between group (PTSD, TEC), condition (neutral, trauma script) and age was non-significant (group by condition by age interaction) for HR (unadjusted model: p = .199, adjusted model: p = .198), PEP (unadjusted model: p = .386, adjusted model: p = .485) and RSA (unadjusted model: p = .587, adjusted model: p = .805) so this term was omitted. Thereafter, the interaction of group by age was also non-significant for HR (unadjusted model: p = .189, adjusted model: p = .176), PEP (unadjusted model: p = .338, adjusted model: p = .422), as well as RSA (unadjusted model: p = .975, adjusted model: p = .983), and omitted. In the subsequent model, the interaction of age with condition did not reach significance for HR (unadjusted model: p =.495, adjusted model: p = .401) or PEP (unadjusted model: p = .657, adjusted model: p = .471) but was significant for RSA (unadjusted model: p <.001, adjusted model: p <.001), indicating decreased RSA reactivity, but not HR or PEP reactivity, with age.

The gender-by-group-by-condition interaction was significant for PEP (unadjusted model: p = .019, adjusted model: p = .026) and the adjusted model for RSA (unadjusted model: p = .147, adjusted model: p = .012) but not for HR. This indicates a moderating effect of gender on differences in PEP and RSA reactivity during script driven imagery between youth with PTSD and TEC. The subsequent parsimonious model for HR with a group-by-gender interaction, was significant (unadjusted model: p = .028, adjusted model: p = .006) indicating a moderating effect of gender on HR differences between youth with PTSD and TEC.

These findings were further supported when investigating the group-by-condition interaction within boys and girls separately (post hoc analysis). We found a significant group-by-condition interactions for RSA in boys (unadjusted model: p = .017, adjusted model: p = .001) but not girls (unadjusted model: p = .376, adjusted model: p = .709) and vice versa for PEP with (trend-level) significant group-by-condition interactions in girls (unadjusted model: p = .049, adjusted model: p = .085) but not boys (unadjusted model: p = .177, adjusted model: p = .172). The group-by-condition interaction for HR was non-significant in both boys and girls.

Longitudinal change in autonomic nervous system activity during script driven imagery in treatment responders vs. non-responders with a 50% CAPS-CA reduction response criterion

During trauma-focused psychotherapy (pre- and posttreatment), the 3-way-interaction between time (pretreatment, posttreatment), condition (neutral, trauma script) and responder status (responder, non-responder) using our secondary response criterion (>50% CAPS-CA reduction) was non-significant (responder status by time by condition interaction) for HR (unadjusted model: p = .995, adjusted model: p = .778) and PEP (unadjusted model: p = .726, adjusted model: p = .598), but significant for RSA (unadjusted model: p = .045, adjusted model: p = .052) indicating differential pre- to posttreatment changes in RSA stress reactivity between trauma-focused psychotherapy responders and non-responders. Post hoc analysis stratified by response status showed an significantly increased RSA in response to trauma script driven imagery from pre- to posttreatment in treatment responders, while non-responders did not show a significant increase of RSA stress reactivity with treatment. After omission of the 3 way interaction for HR and PEP, the interaction of responder status by time was also non-significant for HR (unadjusted model: p = .751, adjusted model: p = .854) and PEP (unadjusted model: p = .420, adjusted model: p = .609), and omitted. In the subsequent model, the interaction of responder status with condition was also non-significant for HR (unadjusted model: p = .135, adjusted model: p = .295) and PEP (unadjusted model: p = .548, adjusted model: p = .450). See also Figure S2.

Figure S1. Flow diagram of included patients. Response is defined as **≥30%** reduction in CAPS-CA total score from pre- to post-treatment.

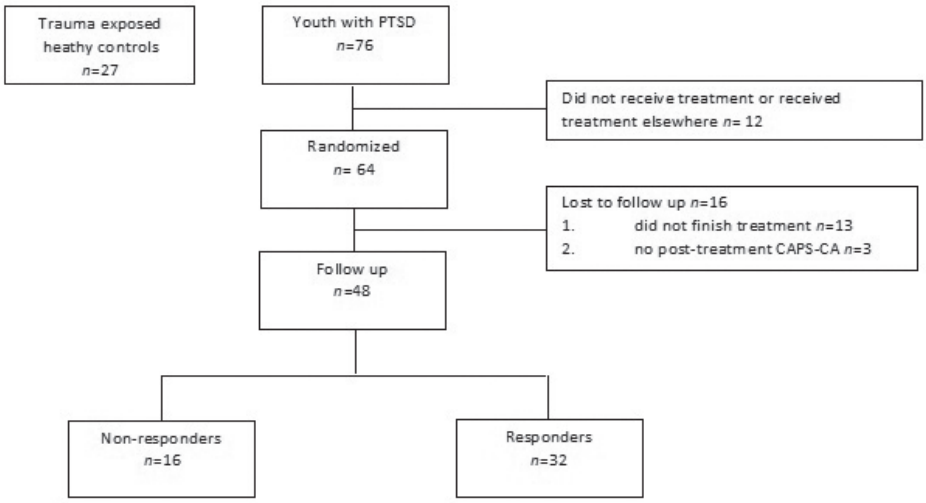

Table S1a Subject characteristics with **>30%** response criterion.

	Non-Responders (n= 16)	Responders (n= 32)	*p*-value[a]
Sociodemographic characteristics			
Girls (%)	68.8	46.8	.152
Age (years; mean, SD)	12.8 (2.96)	12.4 (3.00)	.709
West European Ethnicity (%)	40.0	48.4	.592
Current educational level (%)			.476
Elementary school	48.4	53.2	
Middle/High school lower level	6.3	6.3	
Middle/High school middle level	43.8	31.3	
Middle/High school higher level	0.0	6.3	
Vocational school	12.5	3.1	
Household income (€, %)			.959
< 25.000	40.0	45.5	
25.000-35.000	20.0	18.2	
> 35.000	40.0	36.4	
Weight (kg; mean, SD)	50.9 (8.6)	50.2 (15.8)	.881
Current psychotropic medication (%)	0.0	0.0	n/a
Smoking (%)	6.7	6.7	1.0
Alcohol >1 consumption/day (%)	13.3	13.3	1.0
Trauma characteristics			
Index trauma (%)			.794
Sexual abuse	31.3	31.3	
Domestic violence	12.5	18.8	
Community violence	37.5	21.9	
Accidents/Medical	6.3	12.5	
Other	12.5	15.6	
Multiple-event trauma exposure (%)	43.8	53.1	.540
Age at index trauma (years; mean, SD)	9.9 (4.67)	8.5 (4.27)	.303
Time since index trauma (years; mean, SD)	3.9 (3.63)	3.9 (3.63)	.333
Clinical characteristics			
CAPS-CA study entry (mean, SD)[b]			
Total	49.6 (22.08)	52.1 (23.93)	.735
Re-experiencing	17.9 (11.50)	16.1 (10.16)	.591
Avoidance	17.21 (9.52)	20.2 (9.34)	.324
Hyperarousal	16.07 (9.45)	16.9 (9.763)	.789
Full PTSD diagnosis (%)	75.0	81.3	.615
RCADS study entry (mean, SD)[b]			
MDD	12.4 (6.41)	11.1 (6.37)	.525
GAD	7.5 (4.56)	6.5 (3.12)	.250
OCD	6.5 (3.12)	6.1 (3.63)	.690
PD	7.8 (5.65)	7.8 (6.21)	.993
SAD	5.2 (4.67)	6.27 (4.18)	.469
SP	11.5 (6.49)	12.0 (7.28)	.825

Abbreviations: CAPS-CA, Clinician-Administered PTSD Scale for Children and Adolescents; RCADS, Revised Child Anxiety and Depression Scale; MDD, major depressive disorder; GAD, general anxiety disorder; OCD, obsessive compulsive disorder; PD, panic disorder; SAD, separation anxiety disorder; SP, social phobia; SD, standard deviation.

[a] Independent samples t-test or Mann-Whitney U test for continuous and x^2 tests for categorical variables.

[b] Ranges: CAPS-CA total, 0-139; RCADS MDD, 0-30; RCADS GAD, 0-18; RCADS OCD, 0-18; RCADS PD, 0-27; RCADS SAD, 0-21; RCADS SP, 0-27.

Table S1b Subject characteristics with **>50%** response criterion.

	Non-Responders (n= 25)	Responders (n= 23)	*p*-value[a]
Sociodemographic characteristics			
Girls (%)	68.0	39.1	**.045**
Age (years; mean, SD)	12.3 (2.92)	12.3 (3.05)	.565
West European Ethnicity (%)	37.5	54.5	.246
Current educational level (%)			.209
Elementary school	40.0	56.5	
Middle/High school lower level	4.0	8.7	
Middle/High school middle level	48.0	21.7	
Middle/High school higher level	0.0	8.7	
Vocational school	8.0	4.3	
Household income (€, %)			.149
< 25.000	31.3	56.3	
25.000-35.000	31.3	6.3	
> 35.000	37.5	37.5	
Weight (kg; mean, SD)	50.5 (10.1)	50.4 (17.8)	.981
Current psychotropic medication (%)	0.0	0.0	n/a
Smoking (%)	8.3	4.8	.632
Alcohol >1 consumption/day (%)	12.5	14.3	.860
Trauma characteristics			
Index trauma (%)			.096
Sexual abuse	24.0	39.1	
Domestic violence	12.0	21.7	
Community violence	40.0	13.0	
Accidents/Medical	4.0	17.4	
Other	20.0	15.6	
Multiple-event trauma exposure (%)	52.0	47.8	.773
Age at index trauma (years; mean, SD)	9.5 (4.23)	8.4 (4.62)	.380
Time since index trauma (years; mean, SD)	3.3 (3.32)	3.9 (3.83)	.543
Clinical characteristics			
CAPS-CA study entry (mean, SD)[b]			
Total	56.1 (24.75)	46.0 (20.43)	.129
Re-experiencing	19.7 (11.21)	13.7 (8.98)	**.051**
Avoidance	19.7 (11.21)	18.9 (9.03)	.757
Hyperarousal	18.2 (9.81)	15.1 (9.28)	.272
Full PTSD diagnosis (%)	80.0	78.3	.882
RCADS study entry (mean, SD)[b]			
MDD	12.4 (6.19)	10.7 (6.52)	.401
GAD	6.6 (3.58)	7.3 (4.75)	.636
OCD	6.8 (3.58)	5.6 (3.24)	.277
PD	8.4 (5.50)	7.2 (6.44)	.513
SAD	6.4 (4.68)	5.4 (3.99)	.474
SP	12.7 (6.89)	11.0 (7.05)	.458

Abbreviations: CAPS-CA, Clinician-Administered PTSD Scale for Children and Adolescents; RCADS, Revised Child Anxiety and Depression Scale; MDD, major depressive disorder; GAD, general anxiety disorder; OCD, obsessive compulsive disorder; PD, panic disorder; SAD, separation anxiety disorder; SP, social phobia; SD, standard deviation.

[a] Independent samples t-test or Mann-Whitney U test for continuous and x^2 tests for categorical variables.

[b] Ranges: CAPS-CA total, 0-139; RCADS MDD, 0-30; RCADS GAD, 0-18; RCADS OCD, 0-18; RCADS PD, 0-27; RCADS SAD, 0-21; RCADS SP, 0-27.

Table s2a. Main and interaction effects on ANS of group (treatment responders and non-responders) and condition (neutral and trauma imagery) with **>30%** response criterion **at baseline.**

	Group Effect[a]		**Condition effect[a]**		**Group by Condition effect[a]**	
	Responders versus Non-responders		Trauma versus Neutral imagery		PTSD by Trauma imagery	
Variable	*F*	*p*	*F*	*p*	*F*	*P*
HR, beats/min						
Unadjusted	3.326	.075	3.143	.083	.578	.451
Adjusted[c]	.949	.337	2.509	.121	.277	.601
RSA, ms[b]						
Unadjusted	.053	.819	1.836	.182	.460	.501
Adjusted[c]	.088	.769	3.578	.065	.694	.410
PEP, ms						
Unadjusted	1.778	.189	.318	.576	.191	.664
Adjusted[c]	1.648	.209	.015	.905	.145	.705

Values represent mean (standard deviation).
ANS = autonomic nervous system; HR = heart rate; RSA = respiratory sinus arrhythmia; PEP = pre-ejection period.
[a] Main effects of group and condition were analyzed in a mixed model separate from the interaction effect of group by condition.
[b] Values were ln-transformed for analyses
[c] Adjusted for age, gender, ethnicity, smoking status, alcohol consumption, index trauma and time since trauma as well as respiration rate for RSA

Table s2b. Main and interaction effects on ANS of group (treatment responders and non-responders) and condition (neutral and trauma imagery) with **>50%** response criterion **at baseline.**

	Group Effect[a]		**Condition effect[a]**		**Group by Condition effect[a]**	
	Responders versus Non-responders		Trauma versus Neutral imagery		PTSD by Trauma imagery	
Variable	*F*	*p*	*F*	*P*	*F*	*P*
HR, beats/min						
Unadjusted	.904	.347	3.143	.083	.044	.834
Adjusted[c]	.189	.667	2.509	.121	.225	.638
RSA, ms[b]						
Unadjusted	.708	.405	1.876	.177	1.776	.189
Adjusted[c]	.868	.358	3.571	.059	2.927	.095
PEP, ms						
Unadjusted	3.352	.074	.306	.583	.466	.499
Adjusted[c]	.490	.490	.014	.905	.035	.852

Values represent mean (standard deviation).
ANS = autonomic nervous system; HR = heart rate; RSA = respiratory sinus arrhythmia; PEP = pre-ejection period.
[a] Main effects of group and condition were analyzed in a mixed model separate from the interaction effect of group by condition.
[b] Values were ln-transformed for analyses
[c] Adjusted for age, gender, ethnicity, smoking status, alcohol consumption, index trauma and time since trauma as well as respiration rate for RSA

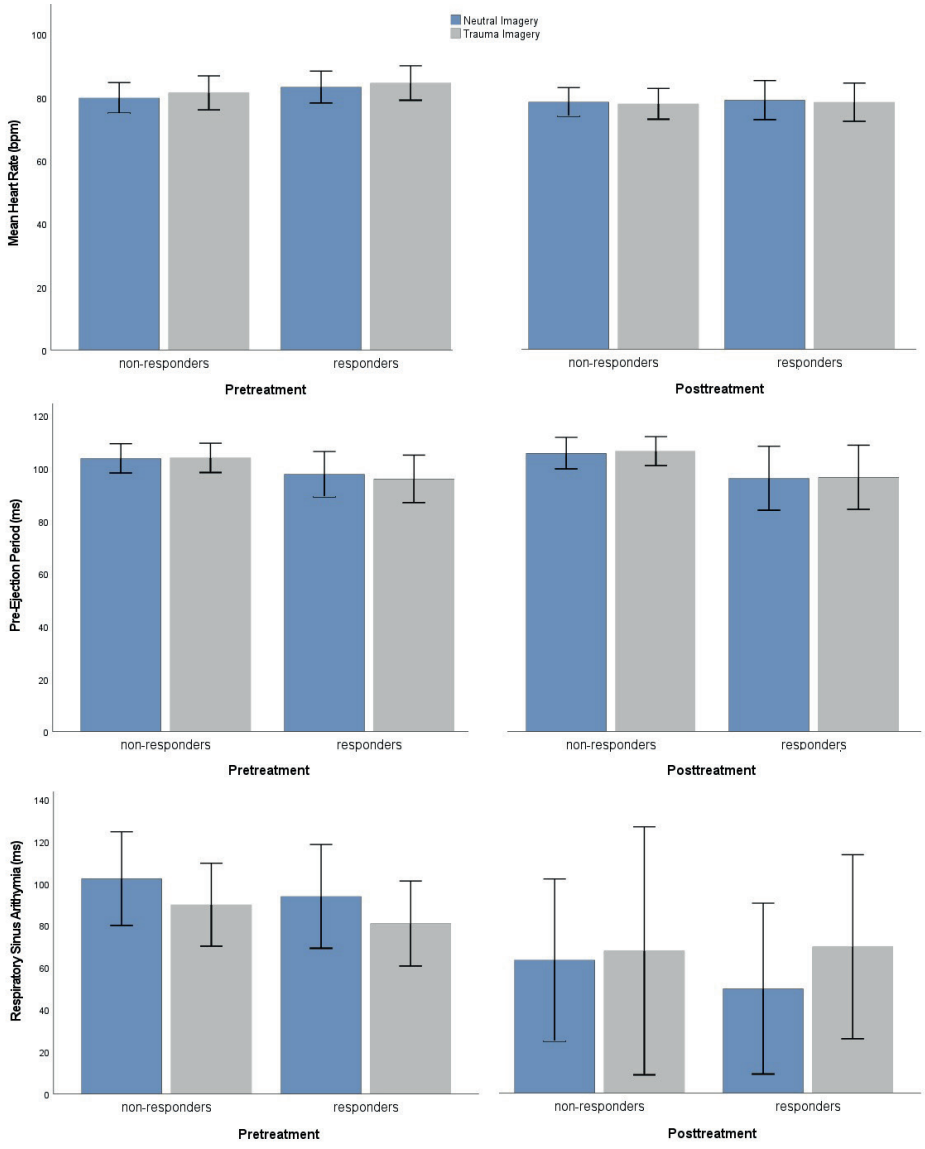

Figure S2. Autonomic nervous system activity in youth with PTSD who responded (**>50%** CAPS-CA reduction) during eight sessions of trauma-focused psychotherapy compared to those who did not respond. Error bars indicate SD. Mixed model analyses results for the 3-way-interaction between time (pretreatment, posttreatment), condition (neutral, trauma script) and responder status (responder, non-responder) for RSA *F*= 4,089, p = .045 in the unadjusted model and *F*= 3,857, p = .052 in the model adjusted for age, gender, ethnicity, smoking status (yes/no), alcohol consumption (yes/no), index trauma, time since trauma, baseline CAPS-CA and respiration rate.

Chapter 7

Summary of main findings

Posttraumatic stress disorder (PTSD) is a common mental health disorder which imposes a substantial burden on affected youth and their families as PTSD symptoms often interfere with social functioning as well as school performance and have a negative effect on quality of life of the affected youth and their families. While trauma-focused psychotherapies are effective in the majority of youth with PTSD a substantial part of youth does not benefit from current first line trauma-focused psychotherapies. The fundamental aim of this thesis was to improve understanding of neurobiological mechanisms of trauma-focused psychotherapies and to investigate whether these insights could eventually enhance treatment outcome in youth with PTSD. In part I of this thesis we addressed this aim by examining different predictive neurobiological measures (biomarkers) of trauma-focused psychotherapy response. Part II of this thesis focused on improving insight in the biological mechanisms underlying trauma-focused psychotherapy response in youth with PTSD by examining longitudinal neurobiological changes associated with trauma-focused psychotherapy response.

PART I: PREDICTING TRAUMA-FOCUSED PSYCHOTHERAPY RESPONSE IN YOUTH WITH PTSD USING NEUROBIOLOGICAL MEASURES

In **chapter 2**, we reported a relationship between pretreatment cortisol and trauma-focused psychotherapy response in 53 youth with (partial-) PTSD. Specifically, we showed higher basal cortisol secretion during script-driven imagery prior to eight sessions of trauma-focused psychotherapy in treatment responders relative to non-responders. We also found a positive association between basal cortisol secretion prior to treatment and clinical improvement during trauma-focused psychotherapy. Because the script driven imagery procedure failed to provoke a cortisol stress response, we could not test whether cortisol stress reactivity was related to treatment response. Although the amount of uniquely explained variance in clinical improvement by cortisol secretion was limited, the findings suggested that higher cortisol prior to treatment is associated with treatment success.

In **chapter 3**, we described whether resting state fMRI data could be used to distinguish between trauma-focused psychotherapy responders and non-responders on the group- and individual patient level. In 40 youth with PTSD, we indeed found that functional connectivity between the frontoparietal and sensorimotor network was stronger in non-responders on the group level, while we did not find evidence for within network connectivity differences. We then employed multivariate, cross-validated support vector machine learning analysis to identify networks which predicted treatment response for individual patients. A network centered on the

bilateral superior temporal gyrus predicted treatment response with 76% accuracy (87% sensitivity, 65% specificity and an area-under-receiver-operator-curve of 0.82). These findings provide proof-of-concept evidence for the feasibility of rs-fMRI combined with machine learning analysis to predict trauma-focused response in youth with PTSD. However, for clinical translation to commence, the robustness and generalizability of these findings have to be shown in larger independent cohorts.

PART II: LONGITUDINAL NEUROBIOLOGICAL CHANGES ASSOCIATED WITH TRAUMA-FOCUSED PSYCHOTHERAPY RESPONSE IN YOUTH WITH PTSD

In **chapter 4** we performed a systematic literature review of randomized controlled trials focused on longitudinal neurobiological changes associated with trauma-focused cognitive behavioral therapy (TF-CBT) and EMDR. We further reviewed trials with neurobiological markers as predictors of trauma-focused psychotherapy response. We included a total of 23 publications on 16 separate trials in which TF-CBT was compared to a waitlist condition in most trials while EMDR was compared to other active treatments in included trials. We found evidence for an association between TF-CBT and decrease of blood pressure and heart rate, indicating an association between TF-CBT and a reduction of autonomic nervous system reactivity. Moreover, we found preliminary evidence for an association between TF-CBT and changes in activity but not volume of frontal brain structures and the amygdala. The former suggests a relationship between TF-CBT and changes in activity in brain regions involved in fear condition, extinction learning and working memory. We found no evidence for differential neurobiological effects of EMDR relative to other treatments. Publications on neurobiological predictors of treatment response showed ambiguous results and were insufficient to be useful in clinical practice at that time. Importantly, all included trials were conducted in adults with PTSD and we identified a substantial knowledge gap regarding neurobiological treatment effects studies in youth of PTSD.

To fill this knowledge gap, we therefore subsequently performed longitudinal studies examining the association between trauma-focused psychotherapy response and pre-to-posttreatment changes in brain structure and autonomic nervous system which were described in chapter 5 and 6.

In **chapter 5** we examined the association between treatment response and longitudinal change in structural MRI scans in 35 youth with PTSD treated with eight sessions of trauma-focused psychotherapy (either EMDR or TF-CBT). With a voxel-based morphometry approach the group by time (pre- to posttreatment) interactions analysis showed significant differences in grey-matter volume in the bilateral

anterior and posterior insula due to volume reductions over time in trauma-focused psychotherapy non-responders relative to responders. Despite the significant group-by-time interaction, we did not find significant group differences at baseline or follow-up. These findings indicate that treatment non-responders are characterized by failure to show age-related volume increase over time in the (anterior and posterior) insula, which is an integral hub in the salience network and abnormal insula structure and function have been associated with PTSD. Although, we could not determine the direction of effect, these results may suggest a relationship between PTSD persistence after treatment and ongoing atypical development within the salience network. The absence of structural MRI changes in treatment responders suggest that successful trauma-focused psychotherapy may not directly normalize brain abnormalities associated with PTSD, however, this interpretation should be done with caution as the time between pre and posttreatment scans was short, and our sample size was limited.

Chapter 6 reported the results of our longitudinal study on the association between trauma-focused psychotherapy response and autonomic nervous system (ANS) stress reactivity. In this study we recorded heart rate (HR), pre-ejection period (PEP and respiratory sinus arrhythmia (RSA) during script driven imagery to determine sympathetic nervous system (SNS) and parasympathetic nervous system (PNS) activity and stress reactivity in 76 youth with PTSD and 27 trauma-exposed controls (TEC). Youth with PTSD were assessed twice, once at baseline and once after eight sessions of trauma-focused psychotherapy and were divided into treatment responders and non-responders. TEC were only assessed at baseline. A cross-sectional comparison at baseline showed higher overall HR and lower PEP during both neutral and trauma imagery in youth with PTSD relative to TEC. Moreover, youth with PTSD showed RSA decrease during trauma imagery relative to neutral imagery, with the reverse in TEC. These results provide evidence for an overall higher HR and SNS activity as well as withdrawal of vagal control in response to stress in youth with PTSD. Relative to non-responders, responders demonstrated a significant baseline to posttreatment increase of RSA response to stress only when employing a ≥50% response criterion and not with the primary ≥30% criterion. This only provides partial support for the hypothesis that vagal control in response to stress might increase with successful trauma-focused psychotherapy.

Chapter 8

General discussion

Chapter 8The aim of this thesis was to improve understanding of neurobiological mechanisms of trauma-focused psychotherapies and to investigate whether these insights can eventually improve treatment efficacy in youth with PTSD. In part I of this thesis we addressed this aim by examining different predictive neurobiological measures (biomarkers) of trauma-focused psychotherapy response. Part II of this thesis focused on improving insight in the biological mechanisms underlying trauma-focused psychotherapy response in youth with PTSD.

In this final chapter, we will first integrate the main findings of this thesis into a neurodevelopmental framework. We will subsequently discuss methodological considerations, describe implications for clinical practice and provide recommendations for future research.

Integration of findings

Neurodevelopmental changes as an explanation for discrepant findings between youth and adults.

The present thesis details novel findings regarding the relationship between neurobiological measures and trauma-focused psychotherapy response in youth with PTSD. Some of the results presented in this thesis show considerable overlap with results from (treatment outcome) studies using the same neurobiological measures in adults with PTSD. Studies in adults with PTSD, for example, also provide evidence that lower cortisol secretion is related to reduced treatment gains (Pacella, Feeny, Zoellner, & Delahanty, 2014; Rapcencu, Gorter, Kennis, van Rooij, & Geuze, 2017; Rauch et al., 2015; Yehuda et al., 2014) and indicate lower RSA stress reactivity in PTSD patients relative to controls (Campbell, Wisco, Silvia, & Gay, 2019). Furthermore, application of the same approach to treatment-response classification as reported in chapter 4, has been associated with significant classification accuracies using rs-fMRI data in adults with PTSD as well (Zhutovsky et al., 2019).

However, some of our findings show marked contrasts with findings in adults. First, in chapter 5 we were unable to identify longitudinal structural brain changes in the hippocampus and (vm-) PFC, while both areas have been implicated in PTSD, and have shown longitudinal volume increase in adults treated with trauma focused-psychotherapy (Bromis, Calem, Reinders, Williams, & Kempton, 2018; Giedd & Rapoport, 2010; Logue et al., 2018). Second, contrary to our findings in chapter 2, studies in adults suggest that lower cortisol secretion in treatment non-responders is mainly driven by lower cortisol reactivity and not lower basal cortisol secretion (Nijdam, van Amsterdam, Gersons, & Olff, 2015; Pacella et al., 2014; Rapcencu et al., 2017; Rauch et al., 2015). Third, in contrast to previous studies in adults with PTSD

we did not find evidence for group differences in HR and SNS stress reactivity and did not find an association between trauma-focused psychotherapy response and longitudinal change in HR and SNS (reactivity) (chapter 6) (Pole, 2007; Zantvoord, Diehle, & Lindauer, 2013). Fourth, compared with previous studies in adults, we observed different function connectivity patterns to differentiate between trauma-focused psychotherapy responders and non-responders (Etkin et al., 2019; Sheynin et al., 2020; Zhutovsky et al., 2019). Finally, studies utilizing machine learning together with rs-fMRI in adults have reported classification accuracies which mostly exceeded accuracy found in our study using the same approach in youth (Etkin et al., 2019).

One possible explanation for the difference between results from our sample and previous results from adult PTSD samples, is that differences are related to (neuro) developmental changes throughout childhood and adolescence. More specifically, our failure to identify longitudinal structural brain changes in the hippocampus and (vm-) PFC could be related to changes in hippocampus and (vm-)PFC being latent and only become apparent after a longer period or later in development (Ramón J. L. Lindauer et al., 2005). Importantly, the time between scans in our study was relatively short (8-12 weeks) while both hippocampus and vmPFC undergo considerable developmental change spanning the period from childhood into adolescence and young adulthood (Giedd & Rapoport, 2010).

Moreover, the absence of HPA and to lesser extend ANS stressor reactivity differences in our sample, could be related to developmental changes in effectiveness of a stressor paradigm to elicit a stress response (Gunnar, Talge, & Herrera, 2009). These changes in stressor paradigm effectiveness may be attributed to psychological changes during childhood and adolescence (Gunnar et al., 2009; Harteveld et al., 2021). Gunnar and colleagues (2009) showed that threat to the social self and not necessarily the traumatic event itself, is a critical psychological factor in determining the ability of a stressor paradigm to elicit a stress response in youth, especially in adolescents (Gunnar et al., 2009). Our stressor paradigm was based on the script driven imagery protocol, which was developed and validated in adults with PTSD. It contains a detailed contextual description of the patients' most disturbing traumatic event but does not necessarily contain a threat to the social self. Also, both the ANS and HPA axis undergo substantial intrinsic development throughout childhood and adolescence, which might account for differences in the ability of a stressor paradigm to elicit a physiological and endocrinological stress response and could potentially explain the divergent findings between adults and youth (Harteveld et al., 2021; Romeo, Lee, & McEwen, 2004).

Another possibility could be that the absence of ANS and HPA stressor reactivity differences and divergent findings between adults with PTSD with trauma exposure

in adulthood (e.g. veterans), are related to the adaptation of both stress response systems to early life (repeated) traumatic stress exposure (Teicher, Andersen, Polcari, Anderson, & Navalta, 2002). This evolutionary hypothesis proposes that early life adversities signal the stress response systems to develop along an alternative pathway, adapting itself to survive in a malevolent stress-filled environment (Teicher et al., 2003). That is, exposure to (repeated) trauma during a sensitive developmental period is associated sensitization of the stress response systems (Lupien, McEwen, Gunnar, & Heim, 2009). Previous findings from cross-sectional studies in adults with PTSD and borderline personality disorder who were exposed to early life trauma, have also indicated that stress sensitization is expressed in decreased stressor reactivity (Drews, Fertuck, Koenig, Kaess, & Arntz, 2019; Fries, Hesse, Hellhammer, & Hellhammer, 2005). Although, these early alternations in the stress response systems are adaptive and might be beneficial for survival on the short-term, they can become unnecessary and even maladaptive in a more benign environment later in development (Teicher, Samson, Anderson, & Ohashi, 2016) and more specifically might hinder trauma-focused psychotherapy success (Zantvoord et al., 2019).

Finally, differences with previous studies in adults regarding functional network connectivity and associated lower classification accuracy in our sample, could be related to reorganization of large-scale brain networks throughout childhood and adolescence (Weems, Russell, Neill, & McCurdy, 2019). Developmental change in large-scale brain organization is characterized by stronger within network connectivity and more efficient between-network connectivity (Menon, 2013). These neurodevelopmental trajectories add to the heterogeneity within our sample, which might in turn account for the overall lower classification accuracy and provide a potential explanation for the contrasting findings between youth and adults with PTSD. Moreover, heterogeneity in our sample was further increased by the broad range of types and timing of trauma-exposure.

Together, these findings suggest (neuro)developmental processes in general as a potential explanation for the divergent neurobiological findings between youth and adult PTSD. Whether (persistence of) PTSD in youth is also specifically associated with abnormal developmental trajectories of brain structure and organization as well as abnormal HPA and ANS development remains a question largely unanswered. Also due to the scarcity of longitudinal studies following traumatized youth through development, the question whether these developmental trajectories primarily serve and adaptive purpose or are pre-existing risk factor for the onset of psychopathology, remains open. In chapter 5, our results provide preliminary evidence that persistence of PTSD (i.e., in treatment non-responders) is characterized by a failure to show normal age-related volume increase over time in both the anterior and posterior insula. This may suggest a relationship between PTSD persistence after treatment

and ongoing atypical development within the salience network. Surprisingly, differential insula volume change occurred over a short treatment period of eight weeks, which puts the nature and sustainability of identified insula changes into question. Interestingly, a recent naturalistic longitudinal study in youth with PTSD showed an association between PTSD persistence over a one-year period and sustained reduced PFC volume, abnormal PFC development and delayed maturation of emotional circuits (Heyn et al., 2019; Keding et al., 2021), suggesting abnormal developmental trajectories specific to PTSD in youth.

Envisioning the future: if we would hypothesize that neurobiological differences between youth and adults with PTSD are indeed related with neurodevelopmental processes and if PTSD in youth would indeed be associated with abnormal developmental trajectories, this would support adopting a more developmentally informed approach in future neurobiological studies. This would entail a departure from cross-sectional case-control studies to identify neurobiological differences between youth with PTSD and controls at a single time point. Also, short-term longitudinal treatment outcome studies to evaluate short term pre- to posttreatment change in neurobiological measures would not be a logical choice from a developmental perspective. Instead, future studies should be designed to compare developmental trajectories between youth with PTSD and their healthy peers and treatment outcome studies should be aimed at associations between treatment response and developmental change. The latter would also mean that the traditional scope of longitudinal neurobiological treatment outcome studies, to test whether successful treatment is associated with normalization of PTSD related neurobiological abnormalities, should be broadened to examine whether treatment response might be associated with a (temporary) delay or surcease of abnormal development. Finally, adopting a neurodevelopmental framework to examine PTSD and effects of trauma-focused psychotherapy in youth, would also mean narrowing the age-range of included youth or improving power to reliably study specific developmental effects.

Positive prospective association between increased HPA and ANS activity and treatment success

In chapter 2 we demonstrated that lower basal cortisol secretion prior to trauma-focused psychotherapy is associated with higher PTSD symptoms after treatment. Cortisol, the end product of the hypothalamic-pituitary-adrenal (HPA) axis activation, plays a central role in an individual's response to threat and serves a variety of functions important to PTSD and psychotherapy (memory consolidation, retrieval of information from long-term memory, memory reconsolidation and extinction learning). Our findings suggest a relationship between HPA-axis function (i.e. reduced

cortisol secretion) and PTSD persistence after trauma-focused psychotherapy. Importantly, lower cortisol secretion was also previous identified in patients with PTSD relative to healthy controls in cross-sectional case-control studies (Morris, Compas, & Garber, 2012). These HPA axis differences were already apparent at a young age, as children aged 6 with PTSD showed lower cortisol secretion relative to their healthy peers. Furthermore, lower pre-deployment cortisol secretion in healthy active-duty soldiers was associated with greater increase in post-deployment posttraumatic stress symptoms (Steudte-Schmiedgen et al., 2015) and lower cortisol secretion in the direct aftermath of trauma in children was related to an increased risk of developing PTSD six month after trauma exposure (Pervanidou et al., 2007; Pfeffer, Altemus, Heo, & Jiang, 2007). A meta-analysis of longitudinal treatment outcome studies using repeated cortisol measures before and after treatment has failed to show normalization of cortisol secretion with successful treatment (Schumacher, Niemeyer, Engel, Cwik, & Knaevelsrud, 2018). Previously, Teicher and colleagues, have proposed that altered cortisol secretion might be a primarily adaptive response to a malevolent environment early in development which becomes maladaptive when it persist when threat ceases later in life (Teicher et al., 2003). Together, these findings suggest that lower cortisol secretion might be related to both PTSD development and PTSD persistence and HPA axis dysfunction might arguably constitute a trait rather than a state dependent feature of PTSD.

Results regarding autonomic nervous system activity in chapter 6 corroborate previous findings of higher sympathetic nervous system activity as well as vagal withdrawal during stress in patients with PTSD versus controls. Like the HPA axis, the ANS is central to an individuals' stress response and related to a variety of functions important to PTSD as well as psychotherapy (arousal, relaxation, concentration) (Gibbons, 2019). Our finding of vagal withdrawal during script driven imagery in youth with PTSD suggests reduced PNS control during trauma related stressors. High vagal activity enhances cardiac control to environmental demands while vagal withdrawal is associated with a less adaptive capacity and associated with increased vulnerability to dysregulated and excessive responses to (traumatic) stressors (Porges, 2007; Sack, Hopper, & Lamprecht, 2004). Our finding of heightened heart rate and SNS activity was independent of script content (i.e. during both neutral and trauma scripts) and could be related to a generally increased HR and SNS activity in youth with PTSD or might reflect increased anticipatory anxiety in PTSD patients relative to controls.

Although the design of our study impedes answering the question whether the above differences represent a vulnerability to develop PTSD or are a plastic response to PTSD over time, previous research has provided some evidence for the former with ANS dysfunction as pre-existing marker of stress sensitivity that predicts posttraumatic stress symptomatology after trauma exposure (Mikolajewski &

Scheeringa, 2018; Minassian et al., 2015). However, literature reviewed in chapter 4 provides evidence for normalization of dysfunctional ANS activity after successful trauma-focused psychotherapy. Which is partially supported by our finding in youth (chapter 6) with mixed evidence for increased vagal control in response to stress in trauma-focused psychotherapy responders. While previous longitudinal research in trauma exposed individuals has provided support for ANS dysfunction as a vulnerability factor for PTSD development, treatment outcome studies using repeated ANS measures indicate that ANS dysfunctions are changeable and might represent a state dependent feature of PTSD.

Group differences vs. individual prediction

Our systematic literature review (chapter 4) on neurobiological markers as predictors of trauma-focused psychotherapy response in PTSD, published in 2013, yielded only a limited number of trials with large heterogeneity in investigated biological markers. Moreover, all included studies utilized univariate analyses to detect group-differences between responders and non-responders. These univariate group-level analyses do not provide information for individual patients and may not generalize to new data (Arbabshirani, Plis, Sui, & Calhoun, 2017). Individual-level (machine-learning) analyses meanwhile, utilize multivariate data to assess generalization to new patients to determine a model which provides the highest prediction accuracy (Bzdok & Ioannidis, 2019). The latter is necessary to allow clinicians to inform patients and to assist in clinical decision making.

With the rapid development of machine learning (ML) analysis since publication of our systematic review, the field has shifted from univariate analysis to detect group-differences towards individual-level multivariate ML analysis to discover predictive markers for individual patients. Several studies have now utilized ML methods together with resting-state functional magnetic resonance imaging (rs-fMRI) to predict treatment-response in adults with PTSD, with accuracies ranging between 71 - 90% (Etkin et al., 2019; Korgaonkar et al., 2020; Zhutovsky et al., 2019). Importantly, the predictive markers identified in these individual-level multivariate ML analyses differed from markers identified in prior studies which employed univariate group-level analyses.

Chapter 3 describes the first study in youth with PTSD to investigate the utility of ML and rs-fMRI to predict treatment-response for individual patients and compare results from individual-level analyses with group-level analyses. A network centered on the bilateral superior temporal gyrus (STG) could distinguish between responders and non-responders on the individual-level, with an accuracy of 76.2% (87% sensitivity, 65% specificity). Previous studies have suggested that STG abnormalities

may be associated with re-experiencing as well as dissociative symptoms in PTSD patients (Engdahl et al., 2010; Lanius et al., 2002) and reported that STG activation may be related to trauma-focused psychotherapy response in adults with PTSD (R. J. Lindauer et al., 2008). The classification accuracies reported previously in adults with PTSD mostly exceeded the accuracy found in our pediatric sample. One possible explanation for the lower classification accuracy in youth is increased clinical heterogeneity related to the inclusion of both PTSD and partial PTSD patients as well as different types and timing of trauma exposure in our sample. Another possibility is that neurodevelopmental trajectories add to heterogeneity in network connectivity as we included patient from a wide age range.

In our group level analysis, we found increased connectivity between the left frontoparietal network (also known as the central executive network) and a sensorimotor network in non-responders relative to responders. The FPN is highly integrated with other brain networks and has a comprehensive role in attention, working memory and decision making by flexibly interacting with other brain networks (Menon, 2011). Abnormal recruitment of other brain networks into the FPN is linked with deficits in these cognitive processes. One could speculate that abnormal recruitment of the sensorimotor network into the FPN in non-responders might be related to deficient cognitive processes resulting in suboptimal engagement in trauma-focused psychotherapy and poor treatment response. The discrepant findings observed on the group- and on the individual-level emphasize the importance of performing individual-level prediction analyses, as these may lead to independent results from group-level analyses. In addition, our results provide first proof-of-concept evidence for the feasibility to perform individual-level prediction in youth treated with trauma-focused psychotherapy utilizing multivariate cross-validated machine learning classification together with rs-fMRI data.

Methodological reflections

Several methodological issues related to the chapters of this thesis have been comprehensively discussed in the limitation section of each chapter. Here, we will discuss methodological considerations spanning multiple chapters which are important when interpreting the thesis in general.

Recruitment difficulties and limited statistical power

At the outset of our study, we aimed to include 40 trauma exposed controls and 80 youth with PTSD treated with trauma-focused psychotherapy (40 treated with EMDR and 40 treated with TF-CBT). Despite considerable efforts, recruitment of both TEC and youth with PTSD progressed at a slower pace than anticipated and the drop-out rate was higher than expected, resulting in a lower final (completer) sample size

(i.e. 27 TEC and 40-48 youth with PTSD who completed treatment and longitudinal biological measures). The limited sample size is the main limitation of the current thesis. Low power might have obscured additional PTSD-TEC and responders-non-responder differences and inflated effect-sizes of observed differences. Moreover, the small sample size has an impact on the certainty of the estimated performance of the machine learning analysis in chapter 3, as cross-validation can lead to high variance in performance estimates when applied to studies with low sample sizes (Varoquaux, 2018). Although we followed best practices for the field to increase the confidence in the presented results (Poldrack, Huckins, & Varoquaux, 2019), only with larger sample sizes can these problems be fully addressed. Larger sample sizes at the same time may increase clinical heterogeneity, which might further limit classification performance (Arbabshirani et al., 2017). To retain power, we have included both full and partial PTSD patients and collapsed treatment groups in analysis, the implications of which will be discussed next.

We included both youth with full and a partial PTSD diagnosis, with the vast majority of patients (80%) fulfilling full PTSD diagnostic criteria. While this approach increased the overall number of eligible patients, and improved the ecological validity of findings by better reflecting a real-life clinical sample, it also increased heterogeneity within the sample. Moreover, including patients with partial PTSD in general and patients with partial PTSD reporting lower overall baseline PTSD symptoms severity in particular, could have lowered overall group differences between PTSD and TEC in chapter 6 and lowered overall treatment response due to a floor effect in all longitudinal analysis. Together, this emphasizes the tradeoff between increased statistical power with improved ecological validity and increased heterogeneity with lower prediction accuracy and possible floor effects.

Youth were randomly assigned to receive either TF-CBT or EMDR, which is a strength, as random treatment allocation enables that observed differences between treatments can be more confidently attributed to the treatment itself. However, due to limited power both treatment conditions had to be collapsed for analyses, making it unfeasible to examine differences between treatments or examine specific predictive markers for each treatment separately. TF-CBT and EMDR share some core treatment elements (e.g. psychoeducation, gradual controlled exposure to traumatic reminders, avoidance reduction and enhancement of future safety) and efficacy of both treatments has been shown comparable (Diehle, Opmeer, Boer, Mannarino, & Lindauer, 2015). However, there are also elements unique to each treatment (e.g. bodyscan, conjoint parent and child sessions, dual attention task) and some patients respond to EMDR while they do not respond to TF-CBT and vice versa. Therefore, longitudinal changes identified in brain structure and ANS could be associated with symptom change in general rather than with (each) specific treatment.

Specificity and sustainability of findings

We were unable to include a waitlist control group because it is considered unethical to withhold or delay a potentially effective treatment in youth with PTSD. Also blinding of both patients and therapists for treatment allocation was not possible, which is a common limitation in psychotherapy research. The absence of a waitlist control group and blinding impedes accounting for non-treatment related factors and non-specific treatment factors with potential effects on neurobiological findings and can only be partially mitigated by our utilization of blinded rates. Therefore, the question whether the observed changes in ANS and brain morphometry in chapters 5 and 6 are causally linked with trauma-focused psychotherapy response remains inconclusive.

In the cross-sectional comparisons between youth with PTSD and controls we included trauma exposed individuals (TEC) within our control group. This approach holds the advantage over inclusion of a non-exposed control group, as it enables identification of characteristics which are specifically related to PTSD as opposed to trauma-exposure in general. However, we were not able to match the PTSD group with TEC on key variables, in particular trauma-exposure and comorbid symptomatology. This introduces the risk that the identified group differences are (also) related to difference in trauma-exposure and comorbidity rather than specific to PTSD.

Our patient sample received less (eight) treatment sessions compared to most previous trials and had a relatively short follow-up period. Although the utilized treatment protocols have shown efficacy in previous trials (Diehle et al., 2015), youth had fewer sessions and less time to respond compared to most previous trials. Moreover, the absence of a long-term follow-up assessment, impeded inquiry on whether observed changes during the treatment period persist over time or whether there are changes which are expressed later in development.

Clinical implications

Individual prediction

In our rs-fMRI machine learning prediction study, we identified a network centered on the bilateral superior temporal gyrus to predict trauma-focused psychotherapy response for individual patients with 87% sensitivity and 65% specificity. The APA proposed a threshold for clinical applicability of biomarkers, in which the biomarker should have a sensitivity of at least 80% for detecting treatment responders and a specificity of at least 80% for distinguishing responders from non-responders (First et al., 2018). Moreover, data used to validate the biomarker should be confirmed

by at least two independent sets of qualified investigators in independent study samples (First et al., 2018). According to these standards, the prediction accuracy achieved in our rs-fMRI machine learning prediction study, does not support the clinical application of this predictive biomarker to positively distinguish trauma-focused psychotherapy responders and non-responders. However, our study was intended as a proof-of-concept study and cross-validated classification accuracy exceeded chance-level performance. Abandoning the search for reliable rs-fMRI based biomarkers to guide clinical decision making in youth with PTSD now, would therefore be premature. In this context it is important to consider that our sample included both youth with full and partial PTSD, included different types of trauma-exposure and consisted of youth from a wide age-range, which increased (clinical) heterogeneity and could thereby have lowered prediction accuracy. Also, due to limited power both treatment conditions were collapsed for the analysis, precluding examination of differences between treatment responders and non-responders separately for both treatments and examine specific predictors for each treatment separately. The latter is important because to be of true value in clinical decision making a predictor should be able to aid treatment selection (i.e. predict for an individual which treatment has the largest change of success) instead of predicting chances of trauma-focused psychotherapy response in general. This is because abstaining from trauma-focused psychotherapy, even when chances of response are small, would constitute an ethical dilemma as effective treatment alternatives are currently not available. The major hurdle lies in the fact that identifying treatment specific biomarkers requires acquiring a large amount of (new) longitudinal data, which has proven to be very challenging and requires additional time, commitment, and funding. Our systematic review on neurobiological treatment outcome predictors in PTSD (chapter 4), which was published a decade ago, concluded that trials with neurobiological measures as predictors of treatment outcome yield insufficient results to be useful in clinical practice. Moreover, no studies performed in youth with PTSD could be identified. Despite considerable progress in data analysis (e.g. application of machine learning analysis) and collaborative data sharing efforts which have been made since then, the conclusion remains that the current state of evidence does not support clinical application of neurobiological markers in youth with PTSD. Although our proof-of-concept study holds some promise for future development towards clinical application, eventual clinical implantation (if ever) is not something to be projected in the near future, as feasibility of necessary independent large-scale studies has proven challenging and their outcome uncertain.

Treatment targets

Results from chapters 2, 3 and 6 indicate that youth who are trauma-focused psychotherapy non-responders are characterized by lower basal cortisol secretion

prior to treatment, stronger functional connectivity between the frontoparietal and sensorimotor brain network and persistent vagal withdrawal in response to stress. These findings contribute to improve insight in the biological mechanisms related to trauma-focused psychotherapy response in youth with PTSD. They could offer directions for the development of novel treatment strategies or adjust existing treatments to directly target these mechanisms to improve treatment outcome. Studies in adults with PTSD have indeed yielded preliminary evidence for efficacy of glucocorticoid (i.e. cortisol) and propranolol (a noradrenergic beta blocker) augmentation in reducing PTSD symptoms. Also, repeated transcranial magnetic stimulation (rTMS) targeting dysfunctional PTSD related functional network connectivity added to trauma-focused psychotherapy has shown promising results. These results in adults together with the insight gained in the biological mechanisms of treatment response in youth with PTSD suggest testing efficacy and safety of these targeted (augmentation) treatments in youth with PTSD as a logical next step. However, it is important to consider that the amount of uniquely explained variance in clinical improvement by our neurobiological measures was limited. Moreover, only ANS differences were obtained during the course of treatment while differences in cortisol and functional connectivity were obtained from a cross-sectional comparison prior to treatment. Finally, our study design (i.e. no placebo controlled double blind randomized trial) disallowed establishing causal inference. Together, this emphasizes the necessity for further translational clinical trials in youth with PTSD to determine the clinical value (if any) of interventions directly targeting neurobiological mechanisms of treatment response.

Psychoeducation

Knowledge of the underlying biological disturbances in PTSD and mechanisms of treatment response could improve understanding of the clinical symptoms of the disorder and of effective treatment. Lindauer proposed that this knowledge could aid therapist in the psychoeducation of patients and their families (Ramón Joseph Lambert Lindauer, 2005). There is indeed some empirical evidence for the benefits of psychoeducation and (treatment) progress feedback to improve psychotherapy outcomes (de Jong et al., 2021). However, current evidence is mostly based on studies performed in adults (Bergman et al., 2018). Moreover, progress feedback has mainly consisted of feedback on symptom change and not changes in neurobiological measures. Although, the clinical value of psychoeducation and progress feedback using neurobiological measures thus remains unknown, daily clinical practice shows that there certainly is a demand among youth, families and therapist for information on how PTSD and psychotherapy might influence the brain. In answering this question, it is important not to overstate or be overconfident on what is currently known as the field is still in its infancy and continually developing.

Future research

Longitudinal PTSD developmental cohort

To assess (neuro)developmental trajectories in youth with PTSD and examine the relationship between trauma focused psychotherapy outcome and developmental change, a future prospective study with repeated integrated clinical and neurobiological long-term follow-up measures in youth with PTSD is a necessary next step. Such a study could be designed as a cohort study in which youth with PTSD are recruited, then treated with trauma-focused psychotherapy and followed-up at different stages of development from childhood into adolescence and eventually adulthood. This approach would enable to disentangle different state, trait and developmental factors associated with PTSD and examine their longitudinal relationship with trauma-focused psychotherapy response throughout development.

Individual prediction to personalized treatment allocation.

Although our proof-of-concept study in chapter 3 showed feasibility of rs-fMRI combined with machine learning analysis to predict trauma-focused response in youth with PTSD, prediction accuracy still falls below what is considered necessary for potential clinical applicability. A vital next step towards (potential) future clinical application, would be to progress from the current proof-of-concept study more extensively validated larger-scale studies. To limit heterogeneity, such studies should aim to include a less clinically heterogeneous patient sample (only patients with a full PTSD diagnosis and specific types of trauma exposure) and patients from a limited age-range. The downside of including a more narrowly defined patient sample would however be, that it poses additional challenges in acquiring the necessary amount of data and limits the generalizability of the predictor due to patient selection. One possibility to mitigate these problems is to combine multiple samples of previously collected neuroimaging data from youth treated with protocolized (trauma-focused) psychotherapies. Such an approach would also allow the required independent sample validation of identified markers and enable the search for specific predictors for specific treatments. We have recently shown the feasibility shared data analysis of multiple independent pediatric PTSD samples utilizing cross-sectional genetic and structural neuroimaging data (Ensink et al 2021). If such a combined samples analysis would indeed yield accurate treatment specific biomarkers, the ultimate test of efficacy of biomarker-guided treatment relative to treatment as usual (e.g stepped care) would require a prospective randomized comparison of both approaches in novel patients.

Novel treatment strategies targeting alternations associated with treatment response.

Our results indicate differences in basal cortisol secretion and between network functional connectivity prior to treatment in responders versus non-responders. Before proceeding to investigate if augmenting trauma-focused psychotherapy with cortisol or targeted non-invasive neuromodulation of functional connectivity patterns would improve treatment efficacy, a study using repeated biological measures examining the temporal relationship between cortisol secretion as well as functional connectivity and trauma-focused psychotherapy response over time is warranted. Only if this would show that cortisol secretion and functional connectivity patterns are also related to response during the course of treatment, testing whether augmenting trauma-focused psychotherapies with cortisol or changing function connectivity with rTMS would be opportune. Due to its non-invasive nature, rTMS in combination with trauma-focused psychotherapy might at first glance be the preferable option in developing youth. Considering the difficulties we have experienced in recruiting youth with PTSD in a RCT with neurobiological measures, we would recommend to first test the feasibility of any potential future RCT on neurobiological informed targeted augmentation strategies, in an pilot study.

Conclusion

In this thesis we examined different predictive neurobiological measures of trauma-focused psychotherapy response and investigated the biological mechanisms underlying trauma-focused psychotherapy response in youth with PTSD. Our results suggest that activity of the major neuroendocrine stress response systems and brain functional connectivity before treatment are indeed associated with trauma-focused treatment response. Moreover, trauma-focused psychotherapy response seems to be related to longitudinal changes in autonomic nervous system activity during stress and brain structure. Together, these findings improve our understanding of the relationship between neurobiological measures and trauma-focused psychotherapy response in youth with PTSD. However, these insights have currently limited to no clinical value because the current state of evidence does not support implementation of neurobiological biomarkers for treatment selection and necessary trials of (augmentation) treatments targeting neurobiological mechanisms related to treatment response have not been performed yet. The way forward now, is to perform individual prediction studies in less heterogeneous patient samples and to perform developmentally informed long-term studies examining (neuro) developmental trajectories related to PTSD and treatment response. These studies are necessary to address whether neurobiological measures can eventually improve treatment outcome and reduce the burden of PTSD in affected youth.

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APPENDICES

Nederlandse samenvatting

PhD portfolio

Author contributions

Dankwoord

NEDERLANDSE SAMENVATTING

Het meemaken van een traumatische gebeurtenis komt veel voor, ongeveer twee op de drie jongeren maakt een trauma mee voordat ze volwassen zijn. Een traumatische gebeurtenis wordt gedefinieerd als een directe of indirecte blootstelling aan een (dreigende) dood, ernstige verwonding of seksueel geweld. Voorbeelden van traumatische gebeurtenissen zijn huiselijk geweld, seksueel misbruik, ernstige verkeersongelukken en oorlogssituaties. De meeste jongeren die een traumatische gebeurtenis meemaken, ervaren in de eerste dagen tot weken klachten zoals angst, verminderde concentratie en slaapproblemen. Bij de meerderheid gaan deze klachten in de eerste weken ook weer vanzelf over. Toch houdt ongeveer 16% van de jongeren langere tijd last van aanzienlijke klachten en ontwikkeld een posttraumatische stress stoornis (PTSS) in de nasleep van een traumatische gebeurtenis. Het risico op het ontwikkelen van een PTSS na het meemaken van een traumatische gebeurtenis hangt af van het type en de ernst van de gebeurtenis maar ook factoren zoals coping en het ervaren van sociale steun spelen een rol.

Jongeren met PTSS kunnen last hebben van verschillende symptomen, zoals het steeds opnieuw ongewild herbeleven van de traumatische gebeurtenis met daarbij hevige psychologische en lichamelijke angstreacties. Ook vermijden jongeren met PTSS het praten over de traumatische gebeurtenis en situaties die hen aan de gebeurtenis doen herinneren. Andere kenmerkende PTSS symptomen zijn verhoogde prikkelbaarheid en aanhoudende veranderingen in stemming. Ook kan de blik waarmee een jongere naar zichzelf en zijn omgeving kijkt aanhoudend negatief veranderen. PTSS-symptomen kunnen zowel interfereren met het sociaal functioneren en schoolprestaties van de jongere zelf, maar kunnen daarnaast ook een negatief effect hebben op de kwaliteit van leven van het gezin en omgeving waarin de jongere opgroeit. Tezamen onderstrepen deze bevindingen het belang van effectieve behandelingen die de negatieve effecten van PTSS bij jongeren kunnen tegengaan.

De huidige behandelrichtlijnen adviseren om jongeren met PTSS te behandelen met een trauma-gerichte psychotherapie. Hoewel trauma-gerichte psychotherapieën zoals trauma-gerichte cognitieve gedragstherapie (TF-CGT) en EMDR effectief zijn bij de meerderheid van de jongeren met PTSS, profiteert een aanzienlijk deel van de jongeren niet of onvoldoende van deze behandelingen. Dit lijdt ertoe dat behandelingen langer duren en jongeren langer last houden van klachten en de psychosociale ontwikkeling van jongeren verstoord kan raken. Er is veel onderzoek gedaan naar klinische en demografische factoren die samenhangen met behandelsucces. Helaas heeft dit onderzoek er niet toe geleidt dat er betrouwbare voorspellers van behandeluitkomst zijn geïdentificeerd. Hierdoor is

het klinische behandelproces nog steeds een kwestie van vallen en opstaan. Er is dus een grote behoefte aan betrouwbare voorspellers van behandeluitkomst, die kunnen ondersteunen bij het maken van behandelkeuzes en nieuwe inzichten in de mechanisme van behandeling, zodat (bestaande) behandelingen kunnen worden verbeterd.

In de afgelopen decennia is daarom bij volwassen met PTSS veel onderzoek gedaan naar de neurobiologische mechanisme die betrokken zijn bij PTSS en succesvolle behandeling. De aanname die hieraan ten grondslag ligt, is dat een verbeterde kennis van de neurobiologische mechanismen die betrokken zijn bij PTSS en behandeling, uiteindelijk kan bijdragen aan het verbeteren van behandeluitkomst. Dit type onderzoek heeft zich in PTSS gefocust op het identificeren veranderingen in hersenstructuur en hersenfunctie die betrokken zijn bij het ontwikkelen van PTSS en effectiviteit van behandeling. Daarnaast hebben de stressregulatie systemen, met name hypothalamus-hypofyse-bijnier (HHB)-as en het autonome zenuwstelsel, traditioneel veel aandacht gekregen in PTSS onderzoek.

Volwassenen met PTSS worden gekarakteriseerd door volume afname van, met name, de hypocampus en ventromedicale prefrontale cortex. Beide zijn onderdeel van verschillende intrinsieke functionele hersennetwerken, waarbij functioneel hersenonderzoek met fMRI in volwassenen met PTSS ook afwijkingen in activiteit en connectiviteit in deze netwerken suggereert. Daarnaast lijken veranderingen in functionele hersennetwerken ook samen te hangen met behandelsucces. Tot slot zijn er veranderingen in het autonome zenuwstelsel en HHB-as gevonden die een rol lijken te spelen bij het ontwikkelen van PTSS enerzijds en anderzijds samenhangen met effectiviteit van behandeling.

Onderzoek naar neurobiologische mechanisme van behandeling en neurobiologische voorspellers van behandelresponse bij jongeren met PTSS zijn zeer schaars. Daarnaast blijkt uit de neurobiologische studies verricht in jongeren met PTSS dat de resultaten niet altijd overeenkomen met volwassen. Dit zou verklaard kunnen worden doordat er de hersenen zowel qua structuur als functie nog volop in ontwikkeling zijn bij jongeren. Ook in de HHB-as en autonome zenuwstelsel vinden nog aanzienlijke veranderingen plaatst tijdens de kindertijd en adolescentie. Het beperkte aantal neurobiologische behandelstudies in jongeren met PTSS in combinatie met de discrepantie in uitkomsten van neurobiologische studies in jongeren en volwassenen benadrukt de kennishiaten ten aanzien van neurobiologische mechanisme van trauma-gerichte psychotherapie bij jongeren met PTSS.

Het fundamentele doel bij aanvang van dit proefschrift was daarom om het begrip van neurobiologische mechanismen van trauma-gerichte psychotherapieën te verbeteren en om te onderzoeken of deze inzichten uiteindelijk de behandeling van

jongeren met PTSS zouden kunnen verbeteren. In Deel I van dit proefschrift hebben we daarom verschillende voorspellende neurobiologische maten (biomarkers) van de respons op trauma gerichte psychotherapie te onderzoekt. Deel II van dit proefschrift richtte zich op het verbeteren van het inzicht in de biologische mechanismen die ten grondslag liggen aan de respons op trauma-gerichte psychotherapie door longitudinale neurobiologische veranderingen bij jongeren met PTSS te onderzoeken die geassocieerd zijn met de respons op trauma gerichte psychotherapie.

Deel I: Voorspellen van de respons op trauma-gerichte psychotherapie bij jongeren met PTSS met behulp van neurobiologische metingen

In hoofdstuk 2 onderzochten we de relatie tussen cortisol afgifte voor behandeling en de respons op trauma-gerichte psychotherapie bij 53 jongeren met (partiele) PTSS. De resultaten van dit onderzoek suggereren dat de basale cortisol afgifte tijdens script-gedreven imaginatie vóór acht sessies trauma-gerichte psychotherapie bij jongeren die respondeerde op behandeling hoger was dan bij non-responders. We vonden daarnaast ook een positieve associatie tussen basale cortisol afgifte vóór behandeling en de mate van klinische verbetering tijdens een behandeling. Omdat de script-gedreven imaginatie procedure niet de verwachte cortisol-stressreactie kon uitlokken, hebben we niet kunnen testen of cortisol-stressreactiviteit gerelateerd is aan behandeluitkomst. Hoewel de hoeveelheid uniek verklaarde variantie in klinische verbetering door cortisol afgifte beperkt was, suggereren de bevindingen dat hogere cortisolafgifte voor de behandeling samenhangt met behandelsucces.

In hoofdstuk 3 hebben we onderzocht of fMRI-data tijdens een rusttoestand kon worden gebruikt om onderscheid te maken tussen jongeren die op behandeling respondeerde en non-responders. Dit hebben we onderzocht op zowel groepsniveau als bij individuele patiënten. Bij 40 jongeren met PTSS vonden we inderdaad dat op het groepsniveau de functionele connectiviteit tussen het frontopariëtale en het sensorimotorische netwerk sterker was bij jongeren die niet na behandeling waren verbeterd. We vonden echter geen bewijs voor groepsverschillen in connectiviteit binnen functionele connectiviteitsnetwerken. Vervolgens gebruikten we een multivariate, cross-gevalideerde support vector machine learning-analyse om netwerken te identificeren die de respons op de behandeling voorspelden bij individuele patiënten. Een netwerk met de bilaterale superieure temporale gyrus voorspelde de behandeluitkomst met 76% nauwkeurigheid (87% sensitiviteit, 65% specificiteit en een area-under-receiver-operatorcurve van 0,82). Deze bevindingen ondersteunen de haalbaarheid van het toepassen van rs-fMRI in combinatie met machine learning-analyse om de respons op trauma-gerichte behandeling bij jongeren met PTSS te voorspellen. Voor klinische toepassing is het echter nog te

vroeg en zal er eerst moeten worden aangetoond dat deze bevindingen robuust en generaliseerbaar zijn in grotere onafhankelijke cohorten.

Deel II: De relatie tussen longitudinale neurobiologische veranderingen en de respons op trauma-gerichte psychotherapie bij jongeren met PTSS

In hoofdstuk 4 wordt een systematisch literatuuronderzoek beschreven naar gerandomiseerde gecontroleerde studies die de relatie tussen longitudinale neurobiologische veranderingen en trauma-gerichte psychotherapie onderzoeken. Daarnaast hebben we onderzoeken met neurobiologische markers als voorspellers van de respons op trauma-gerichte psychotherapie geanalyseerd. We hebben in totaal 23 publicaties geïncludeerd over 16 afzonderlijke studies. In de meeste studies werd TF-CBT vergeleken met een wachtlijstconditie, EMDR werd daarentegen vooral vergeleken met andere actieve behandelingen. We vonden een verband tussen TF-CBT en een afname van bloeddruk en hartslag, dit wijst op een associatie tussen TF-CBT en een afname van activiteit van het autonome zenuwstelsel. Bovendien vonden we aanwijzingen voor een verband tussen TF-CBT en veranderingen in activiteit, maar niet in volume, van frontale hersenstructuren en de amygdala. Het eerste suggereert een relatie tussen TF-CBT en veranderingen in activiteit in hersengebieden die betrokken zijn bij angstconditionering, extinctieleren en werkgeheugen. We vonden geen bewijs voor differentiële neurobiologische effecten van EMDR ten opzichte van andere actieve behandelingen. Publicaties over neurobiologische voorspellers van de respons op behandeling vertoonden tegenstrijdige resultaten en ondersteunen toepassing in de klinische praktijk (nog) niet. Belangrijk is dat alle geïncludeerde studies werden uitgevoerd bij volwassenen met PTSS, waardoor er een kennislacune bestaat met betrekking tot onderzoeken naar neurobiologische effecten van behandeling bij jongeren met PTSS.

Om deze kennislacune op te vullen, voerden we vervolgens zelf longitudinale studies uit waarin we bij jongeren met PTSS de relatie tussen respons op trauma-gerichte psychotherapie en longitudinale veranderingen in hersenstructuur en het autonome zenuwstelsel onderzochten. De resultaten van deze studies zijn beschreven in hoofdstuk 5 en 6.

In hoofdstuk 5 onderzochten we de associatie tussen behandeluitkomst en de longitudinale verandering in structurele MRI-scans bij 35 jongeren met PTSS die werden behandeld met acht sessies trauma-gerichte psychotherapie (EMDR of TF-CBT). Met een voxel-based morphometry benadering toonde de interactieanalyse significante verschillen in verandering in grijze stof volume in de bilaterale anterieure en posterieure insula. Deze verschillen waren het gevolg van volumeverminderingen in de tijd bij non-responders ten opzichte van responders. Ondanks de significante interactie vonden we geen significante groepsverschillen bij de metingen voor

behandeling en bij follow-up scans direct na behandeling. Hoewel we de richting van het effect niet konden bepalen, suggereren deze resultaten een relatie tussen aanhoudende PTSS na de behandeling en voortdurende atypische ontwikkeling binnen het salience netwerk, specifiek in de (anterieure en posterieure) insula. Het ontbreken van structurele MRI-veranderingen bij jongeren die wel responderen op behandeling, suggereert dat succesvolle trauma-gerichte psychotherapie, de hersenafwijkingen die geassocieerd worden met PTSS, mogelijk niet direct normaliseert. Doordat de tijd tussen MRI-scans relatief kort was en het aantal geïncludeerde patiënten beperkt, moet bij de interpretatie van deze laatste bevinding echter terughoudendheid betracht worden.

Hoofdstuk 6 rapporteert de resultaten van ons longitudinale onderzoek naar de associatie tussen trauma-gerichte psychotherapie response en de stressreactiviteit van het autonome zenuwstelsel (AZS). In dit onderzoek hebben we hartslag (HR), pre-ejectieperiode (PEP) en respiratoire sinus aritmie (RSA) tijdens script-gedreven imaginatie gemeten om zo de activiteit en stressreactiviteit van het sympathische zenuwstelsel (SZS) en het parasympatische zenuwstelsel (PZS) in vorm van vagale controle te bepalen. Het onderzoek werd uitgevoerd bij 76 jongeren met PTSS en 27 aan trauma blootgestelde gezonde controles (TEC). Jongeren met PTSS werden tweemaal beoordeeld, eenmaal voor en eenmaal na acht sessies trauma-gerichte psychotherapie en vervolgens ingedeeld in responders en non-responders. TEC werden slechts eenmalig gemeten. Jongeren met PTSS hadden, in vergelijking met TEC, een hogere HR en lagere PEP zowel tijdens neutrale als trauma imaginatie. Bovendien vertoonden jongeren met PTSS een afname van RSA tijdens trauma imaginatie in vergelijking met neutrale imaginatie, met een omgekeerd patroon bij TEC. Deze resultaten ondersteunen de hypothese van een algemeen hogere HR en SZS-activiteit, evenals een afname van vagale controle (PSZ activiteit) in reactie op stress bij jongeren met PTSS. In vergelijking met non-responders, vertoonden responders een significante toename van voor tot na behandeling in vagale controle tijdens stress. Dit verschil werd alleen gevonden wanneer een ≥50% responscriterium werd gebruikt en niet bij het primaire ≥30% responsecriterium. Onze resultaten bieden dus slechts gedeeltelijke ondersteuning voor de hypothese dat de vagale controle in reactie op stress kan toenemen met succesvolle trauma-gerichte psychotherapie.

In hoofdstukken 7 en 8 worden de resultaten samengevat en in een geïnterpreteerd vanuit een ontwikkelingsperspectief. Er wordt geconcludeerd dat er inderdaad een relatie lijkt te bestaan tussen verschillende neurobiologische maten voor behandeling en behandeluitkomst en dat behandelresponse samenhangt met verandering in neurobiologische maten echter lijkt de klinische waarde van de resultaten op dit moment beperkt.

PHD PORTFOLIO

Name PhD student: Jasper Brian Zantvoord
PhD period: 2012-2023
PhD supervisor: Prof. Dr. Ramon Lindauer
Co-supervisors: Prof. Dr. Guido van Wingen. Dr. Anja Lok

1. PhD training

	Year	ECTS
General courses		
Clinical Epidemiology: Randomized Clinical Trials	2015	0.7
Data Analysis in MATLAB	2015	0.7
Practical Biostatistics	2016	1.1
Clinical Data Management	2016	0.7
Scientific Writing in English for Publication	2016	1.5
Project Management	2016	0.7
BROK ('Basiscursus Regelgeving Klinisch Onderzoek')	2016	1.5
Specific courses		
UCL SPM Course for fMRI and MRI/VBM	2016	0.7
Seminars, workshops and master classes		
Master class by Avshalom Caspi and Terrie Moffitt	2012	0.2
Presentations		
Oral presentation NVvP voorjaarscongres	2016	0.25
Oral presentation Amsterdamse School	2018	0.25
Oral presentation ISTSS New Orleans	2015	0.25
Oral presentation ISTSS Chicago	2017	0,25
Oral presentation ISTSS Chicago	2017	0,25
Oral presentation ESTSS Bologna	2013	0.25
Oral presentation ISTSS Los Angeles	2012	0.25
(Inter)national conferences		
32nd ECNP Congress in Copenhagen, Denmark	2019	0.7
33th ECNP Congress in Lisbon, Portugal	2021	0.7
APA Annual Meeting in New York, USA	2014	0.7
28th ISTSS Congress in Los Angeles, USA	2012	0.7
31st ISTSS Congress in New Orleans, USA	2015	0.7
33th ISTSS Congress in Chicago, USA	2017	0.7
13th ESTSS Congress in Bologna, IT	2013	0.7
ISAD Congress in Londen, UK	2018	0.7
NVvP congress in Maastricht NL	2016	0.7
NVvP congress in Maastricht NL	2018	0.7
NVvP congress in Maastricht NL	2022	0.7
NVvP congress in Maastricht NL	2023	0.7
Other		
Neuroimaging in Psychiatry research group meetings	2013-2016	
AMC	2013-2023	
Reviewer for a.o. psychotherapy and psychosomatics, psychoneuroendocrinology, British Journal of Psychiatry	2022-2023	
Editor Board of European Neuropsychopharmacology		

2. Teaching

	Year	ECTS
Lecturing		
WDD 3 bachelor Geneeskunde UvA, bipolar disorder	2018-2023	1,5
WDD 2 bachelor Geneeskunde UvA, Trauma and PTSD	2018-2023	1,5
Tutoring, Mentoring		
Bachelor and Master thesis Geneeskunde UvA >20 students	2013-2023	>10
Various bachelor and master thesis: o.a. Management, Policy Analysis and Entrepreneurship in the Health & Life Sciences VU, Master Biomedical Sciences, Neurobiology, Psychopharmacology and Pathophysiology. Bachelor psychobiologie UvA, Psychology UvA	2021	>5
Supervising		
PhD project Bram Storosum, AmsterdamUMC	2022-2023	
PhD project Sem Cohen, AmsterdamUMC	2022-2023	
PhD project Manouk den Toom, AmsterdamUMC	2023	
PhD project Caroline Heuschen, Amsterdam UMC	2020-2023	
PhD project Fabienne Willemen, UvA	2023	
Other		

List of Publications

Peer reviewed – in this thesis

Zhutovsky, P*., **Zantvoord, J. B*.**, Ensink, J. B. M., Op den Kelder, R., Lindauer, R. J. L., & van Wingen, G. A. (2021). Individual prediction of trauma-focused psychotherapy response in youth with posttraumatic stress disorder using resting-state functional connectivity. Neuroimage Clin, 32, 102898. doi:10.1016/j.nicl.2021.102898

Zantvoord, J. B., Ensink, J. B. M., Op den Kelder, R., Wessel, A. M. A., Lok, A., & Lindauer, R. J. L. (2019). Pretreatment cortisol predicts trauma-focused psychotherapy response in youth with (partial) posttraumatic stress disorder. Psychoneuroendocrinology, 109, 104380. doi:10.1016/j.psyneuen.2019.104380

Zantvoord, J. B., & Lindauer, R. J. (2013). Using neurobiological measures to predict and assess treatment outcome of psychotherapy in posttraumatic stress disorder: systematic review. Psychother Psychosom, 82(3), 142-151. doi:10.1159/000343258

Zantvoord, J. B. *, Zhutovsky, P. *, Ensink, J. B. M., Op den Kelder, R., van Wingen, G. A., & Lindauer, R. J. L. (2021). Trauma-focused psychotherapy response in youth with posttraumatic stress disorder is associated with changes in insula volume. J Psychiatr Res, 132, 207-214. doi:10.1016/j.jpsychires.2020.10.037

Under review – in this thesis

Zantvoord, J. B., Ensink, J. B. M., Op den Kelder, R., Diehle, J., Lok, A., & Lindauer, R. J. L. (2023). Autonomic nervous system function before and after trauma-focused psychotherapy in youth with posttraumatic stress disorder.

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Zantvoord, J. B., Vulink, N., & Denys, D. (2016). Cognitive Behavioral Therapy for Olfactory Reference Syndrome: A Case Report. J Clin Psychiatry, 77(9), e1144. doi:10.4088/JCP.15cr10451

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Cohen, S. E., **Zantvoord, J. B**., Wezenberg, B. N., Daams, J. G., Bockting, C. L. H., Denys, D., & van Wingen, G. A. (2023). Electroencephalography for predicting antidepressant treatment success: A systematic review and meta-analysis. J Affect Disord, 321, 201-207. doi:10.1016/j.jad.2022.10.042

Storosum, B. W. C., Mattila, T., Wohlfarth, T. D., Gispen-de Wied, C. C., Roes, K. C. B., den Brink, W. V., . . . **Zantvoord, J. B**. (2023). Gender differences in the response to antipsychotic medication in patients with schizophrenia: An individual patient data meta-analysis of placebo-controlled studies. Psychiatry Res, 320, 114997. doi:10.1016/j.psychres.2022.114997

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AUTHOR CONTRIBUTIONS

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

Chapter 2: Pretreatment cortisol predicts trauma-focused psychotherapy response in youth with (partial) posttraumatic stress disorder

Jasper B. Zantvoord: Conceptualization, Methodology, Investigation, Formal Analysis, Writing - Original Draft. **Judith B.M. Ensink**: Methodology, Investigation, Data Curation, Writing - Review & Editing. **Rosanne op den Kelder**: Methodology, Investigation, Data, Curation, Writing - Review & Editing. **Aimy M.A. Wessel**: Investigation, Data, Curation, Writing - Review & Editing **Anja Lok**: Methodology, Writing - Review & Editing, Supervision. **Ramon J.L. Lindauer**: Conceptualization, Methodology, Writing - Review & Editing, Supervision.

Chapter 3: Individual prediction of trauma-focused psychotherapy response in youth with posttraumatic stress disorder using resting-state functional connectivity

Paul Zhutovsky: Methodology, Software, Validation, Visualization, Writing – original draft. **Jasper B. Zantvoord**: Conceptualization, Methodology, Investigation, Writing – original draft. **Judith B.M. Ensink**: Investigation, Writing – review & editing. **Rosanne op den Kelder**: Investigation, Writing – review & editing. **Ramon J.L. Lindauer**: Conceptualization, Supervision, Funding acquisition, Writing – review & editing. **Guido A. van Wingen**: Methodology, Supervision, Funding acquisition, Writing – review & editing.

Chapter 4: Using neurobiological measures to predict and assess treatment outcome of psychotherapy in posttraumatic stress disorder: systematic review.

Jasper B. Zantvoord: Conceptualization, Methodology, Investigation, Formal Analysis, Writing - Original Draft. **Julia Diehle:** Methodology, Investigation, Writing - Review & Editing. **Ramon J.L. Lindauer**: Conceptualization, Methodology, Writing - Review & Editing, Supervision.

Chapter 5: Trauma-focused psychotherapy response in youth with posttraumatic stress disorder is associated with changes in insula volume

Jasper B. Zantvoord: Conceptualization, Methodology, Investigation, Writing – original draft. **Paul Zhutovsky**: Methodology, Software, Validation, Visualization, Writing – original draft. **Judith B.M. Ensink**: Investigation, Writing – review & editing. **Rosanne op den Kelder**: Investigation, Writing – review & editing. **Ramon J.L. Lindauer**: Conceptualization, Supervision, Funding acquisition, Writing – review

& editing. **Guido A. van Wingen**: Conceptualization, Methodology, Supervision, Funding acquisition, Writing – review & editing.

Chapter 6: Autonomic nervous system function before and after trauma-focused psychotherapy in youth with posttraumatic stress disorder

Jasper B. Zantvoord: Conceptualization, Methodology, Investigation, Formal Analysis, Writing - Original Draft. **Judith B.M. Ensink**: Methodology, Investigation, Data Curation, Writing - Review & Editing. **Rosanne op den Kelder**: Methodology, Investigation, Data, Curation, Writing - Review & Editing. **Julia Diehle:** Conceptualization, Investigation, Writing - Review & Editing. **Anja Lok:** Methodology, Writing - Review & Editing, Supervision. **Ramon J.L. Lindauer**: Conceptualization, Methodology, Writing - Review & Editing, Supervision.

DANKWOORD

Als eerste wil ik graag alle kinderen, jongeren en (pleeg)ouders bedanken die hebben deelgenomen aan dit onderzoek. Jullie deelname en inzet vormen de basis van dit proefschrift. Ik waardeer het enorm dat jullie, ondanks de soms moeilijke periode waarin jullie zaten, bereid waren om alle vragenlijsten, scans, speekselafnames en hartslagmetingen te ondergaan. Jullie inzet draagt bij aan het verder verbeteren van behandelingen voor jongeren met PTSS.

Professor Lindauer, beste Ramón, ondanks dat het al meer dan een decennium geleden is, kan ik onze eerste ontmoeting op je oude kantoor bij 'de Bascule' nog steeds heel goed herinneren. Je hebt me toen meteen het vertrouwen en de ruimte gegeven om als onderzoeker aan de slag te gaan. Wat ik enorm bewonder is hoe je, jaar in, jaar op zowel klinisch, wetenschappelijk en beleidsniveau je blijft inzetten om de zorg voor kinderen, jongeren en (pleeg)ouders die te maken hebben met trauma en stressor gerelateerde stoornissen. Ik ben je ontzettend dankbaar voor je inspiratie, begeleiding en geduld.

Professor van Wingen, beste Guido, jouw werk naar de gevolgen van stress bij veteranen was een grote inspiratiebron bij de start van mijn onderzoek. Ik was dan ook maar wat blij toen je vanuit het Donders overkwam naar het AMC. Dankzij jou is het gelukt om het (f)MRI onderzoek te laten slagen. Ik heb veel van je geleerd over de inzet van beeldvorming in de psychiatrie en in het bijzonder toepassen van (f)MRI om behandeleffecten te voorspellen. Ik heb het een eer gevonden om je opkomst tot dé lijdende figuur in individuele predictie in de psychiatrie van dichtbij mee te maken. Ik ben je erg dankbaar voor de inspirerende begeleiding en de tijd die je hebt gestoken in het uitleggen van (voor een clinicus) complexe imaging methodologie.

Dr. Lok, lieve Anja, bij onze eerste ontmoeting bij de workshop rond 'de anatomische les' van prof. Caspi en prof. Moffitt hadden we direct een klik. Sindsdien hebben we zoveel samengewerkt dat we er een apart boekje mee kunnen vullen. Door de jaren heen is ook een hele waardevolle vriendschap ontstaan die het werk overstijgt. Ik kijk op tegen je vermogen om mensen samen te brengen met positieve energie en optimisme, je originele en innovatieve ideeën en doorzettingskracht. Allen zijn essentieel geweest voor het (afronden) van dit proefschrift. Ik ben heel trots dat ik je eerste promovendus ben. Ik weet zeker dat we ook na mijn verdediging nog mooie projecten samen gaan doen en nog meer mooie wandelingen gaan maken in de duinen.

Daarnaast ook veel dank aan de leden van de promotiecommissie: prof. dr. A. Popma, prof dr. E.M.W.J. Utens, prof. dr. C.L.H. Bockting, prof. dr. mr. C.H. Vinkers, dr. I.M. Veer, prof. dr. L. Reneman. Veel dank dat u mijn proefschrift wilde lezen en beoordelen.

Beste Judith, ik ben je ontzettend dankbaar voor onze samenwerking. Als er één iemand is die ik moet bedanken voor het voltooien van dit project bij jij het. We hebben samen met Rosanne het onderzoek draaiende gehouden, jij met de focus op de (epi)genetica, Rosanne op het cognitieve functioneren en ik op de fysiologische en imaging. Samen vormden we een heel complementair team. Ik sluit nu als laatste van de drie de rij. Ik heb enorm veel bewondering hoe jij alle ballen in de lucht weet te houden, naast je onderzoek, klinische werk, management van jullie zorgboerderijen en je mooie gezin. Ik heb erg genoten van de gezellige momenten samen, tijdens de lunches en congressen. Ondanks dat dit project nu ten einde komt hoop ik in de toekomst toch samen te blijven werken met je.

Rosanne jij kwam als derde bij onze club maar was als eerste klaar. Dat zegt wel iets over je gedrevenheid, enthousiasme en talent. Ik vind het heel knap hoe jij je naast het onderzoek gepassioneerd klinisch inzet voor getraumatiseerde jongeren. Ik ben je heel dankbaar voor alle uren die je in de onderzoekmetingen en database opbouw hebt zitten. Ik kijk met veel plezier terug om onze gezellige samenwerking en op het gezamenlijk congres in Chicago.

Beste Julia en Katja jullie waren een onmisbare schakel bij het opzetten en start van dit project. Julia jouw RCT was het fundament waarop de biologische data verzameld kon worden. Ik denk met veel plezier terug aan ons gezamenlijk congres in LA en onze (mislukte) poging om extra subsidie binnen te halen bij ZonMw. Katja jij hebt enorm geholpen om de studie van een idee op papier te transformeren naar een daadwerkelijke studie waarin jongeren werden gediagnostiseerd, behandeld en bemeten. Ik ben je heel dankbaar voor hoe je dat hebt gedaan en de fijne samenwerking die daaraan te grondslag lag.

Professor Boer, beste Frits, jouw inspirerende colleges wakkerde bij mij de interesse voor het onderzoek in de kinder- en jeugdpsychiatrie aan. Dank dat je die interesse hebt gestimuleerd en mij toen hebt gekoppeld aan Ramón om de interesse uit te werken tot dit project.

Veel dank aan de collega's bij Levvel. Beate, Carlijn, Harriët, Nathalie, Maartje, Karen, Renée, Ria en Rianne bedankt voor jullie bevlogenheid bij de diagnostiek en behandeling van getraumatiseerde jongeren. Dank ook aan de mede-onderzoekers bij Levvel voor de samenwerking en jullie steun bij het opzetten en uitvoeren van het onderzoek: Marielle, Marthe, Caroline, Eva, Els, Maj, Esther, Irma, Sanne, Shelley, Lidewij, Chaya, Inger, Mara, Vionna, Lieke en Malindi. Suzan en Susan, dank voor jullie steun in afgelopen jaren voor mij als 'buiten' promovendus.

Dear Paul, our work on the neuroimaging chapters of this thesis feels like the apex of this project. I really enjoyed our collaboration and learned a whole lot of you

on neuroimaging analysis and individual (machine learning) prediction. Although getting through the review process was a frustrating effort, it generated a sense of camaraderie which I find quite unique. Our collaboration was a fine example of interdisciplinary research done right, in which we really complemented each other. I am still hoping that you will someday return to academia, if so, let's work some more 'magic' together.

Joost Daams dank voor de hulp met de vele zoekacties, jouw grondigheid geeft mij altijd een geruststellend gevoel dat we niks over het hoofd zien. Prof. Dr. de Geus en Cor Stoof, dank voor jullie hulp met het analyseren van de VU-AMS data. Paul Groot, bedankt voor de ondersteuning met het verwerken van de (f)MRI data.

Dr. Storosum, beste Jitschak, toen ik je als coassistent op de acute stoornissen voor het eerst aan het werk zag, heeft dit diepe indruk op mij gemaakt. Ik heb het tijdens dat coschap zo naar mijn zin gehad dat mijn keuze om psychiater te worden toen genomen is. Tijdens de opleiding tot psychiater werd je voor mij het voorbeeld van hoe ik het vak wil uitoefenen. Ik bewonder je scherpzinnigheid, je vermogen om wetenschappelijke kennis te vertalen naar klinische situaties en de hoge standaard van zorg voor patiënten die je constant weet te behouden. Ik ben daarom enorm trots dat ik je heb mogen opvolgen als psychiater bij de acute. Ik ben onder andere dankbaar voor de vele leerzame gesprekken over wetenschap en het reilen en zijlen in de (academische) psychiatrie, je introductie bij het CBG en het waarnemen bij de stemmingsstoornissen waardoor ik meters heb kunnen maken met het afronden van mijn boekje.

Professor Denys, beste Damiaan, ik wil je bedanken dat je, als afdelingshoofd bij de volwassen psychiatrie, mij de ruimte en tijd hebt gegeven om mijn promotieonderzoek te doen bij de kinder- en jeugdpsychiatrie. Ik bewonder je eloquentie en eruditie. Je hebt als afdelingshoofd een sfeer gecreëerd waarin originaliteit, spontaniteit en innovatie kunnen floreren; ik heb daar in de afgelopen jaren de vruchten van kunnen plukken. Ik ben erg dankbaar voor de kansen die je me geeft en de ondersteuning bij het maken van mijn carrière keuzes. Ik hoop onze samenwerking de komende jaren voort te kunnen zetten.

Beste Karel, bedankt dat je als paranimf mij wil bijstaan op deze bijzondere dag. Samenwerken met jou voelt voor mij altijd heel vertrouwd. Ik bewonder je inventiviteit en je vermogen om zelf projecten op te zetten, zoals de rTMS studies en de samenwerking met de hersenbank. Ik kijk uit naar de verschillende projecten die we de komende jaren samen gaan doen. Marise, als AIOS hadden wij een gezamenlijk doel, namelijk samen psychiater worden op de acute stoornissen van het AMC. Na wat omwegen is dat ook gelukt. Ik koester onze samenwerking en denk dat we elkaar als 'aarde en lucht' goed aanvullen. Met mijn promotie achter de rug, kijk ik er erg

naar uit om samen met jou en Arjen verder te werken aan ontwikkeling van de HIC en MIC. Arjen, ik ben heel erg blij met zo'n ervaren en goede allround psychiater aan mijn zijde op de HIC/MIC (en als GD), dat geeft mij veel rust en vertrouwen. Ik ben er erg dankbaar voor dat we voor elkaar klaarstaan om de continuïteit op de HIC/MIC te waarborgen, daardoor heb ik ook de afgelopen jaren aan mijn onderzoek kunnen werken. Hetzelfde geld voor Hiske, eerder op de acute en Dominique en Anja op de stemming. Verder wil ik alle (ex-)collega psychiaters: Pelle, Nienke, Yasmin, Roel (ook voor het sparren over statistiek en methodologie), Lieuwe, Marloes, Miranda, Renate, Ellen, Hiske, Herman, Jan, Martijn, Geeske en Ati bedanken voor de prettige samenwerking.

Verder gaat mijn dank uit naar alle verpleegkundige en paramedischi waar ik afgelopen jaren mee heb samengewerkt eerst bij de stemmingsstoornissen, later bij de acute stoornissen en esketamine poli. Marlies ik hoop dat je binnenkort weer helemaal terugkeert naar de MIC want we hebben samen met Denise, Paul, Anneberty en alle verpleegkundige van de MIC nog unfinished business wat betreft de verdere transformatie naar een academische MIC.

Beste Bram, Caroline, Sem, Manouk en Fabienne het is een voorrecht om met zoveel getalenteerde jonge collega's te mogen samenwerken. Het is heel bijzonder om jullie allen op jullie eigen manier te zien ontwikkelen, ik hoop daar de komende jaren nog een bijdrage aan te kunnen leveren.

Inga Aerts, Ellen Aerts, Aimy Wessel, Chenar Faraj, Yara Toenders, Oscar Wohlgemuth Kitslaar, Jacintha Tieskens, Cedrine Steinz, Daan Neutenboom, Jeffrey Stavenuiter, Charlotte Damstra, Rutger Poelakker, Judith Scherpenisse, Femke Brouwer, Ronja Berendse, Ward Keijsers, Nathan Frankel, Sam Hofhuis, Lisa Urlings, Dlawar Jabbari, Hee Beek, Pieter Taselaar, Manuela van den Brink, Adinda Lapre, Roos van Geffen, Karim Bouchnafi, Eva Bramer dank voor jullie hulp bij dit onderzoek of scriptie over andere (gerelateerde) onderwerpen.

Beste Bastiaan ik vind het heel erg leuk dat je mij gaat bijstaand als paranimf, al sinds de middelbare school vormen we met Joris, Guus en Douwe een hechte vriendengroep. De vele uren die we samen in de Ruk en Pluk (ook met Sjors, Moesa en Hannah) hebben doorgebracht vormen enerzijds een welkome afleiding en anderzijds een niet aflatende frustratie bij het uitblijven van Europees succes van Ajax of Nederlands tour succes. Joris en Guus ik wil jullie bedanken voor hele duurzame en waardevolle vriendschap en voor de mooie gezamenlijk avonturen op de fiets en daarbuiten.

Jurriaan, wij zijn samen met Sanne gestart met de opleiding tot psychiater, aan dat cohort heb ik mijn echtgenote overgehouden en met jou een hele dierbare vriend.

Ik wil je bedanken voor de momenten waarop de boog even niet gespannen hoefde te staan, op werk en daarbuiten. Je authenticiteit, veelzijdigheid en loyaliteit maken het altijd een genoegen om samen met je te zijn. Ook dank aan Oscar, Elias, Jorrit, Thijs, Eline, Emma en Nienke dat ik via Sanne en Jurriaan af en toe mijn karretje bij jullie Summa trein heb mogen aanhaken.

Lieve oma Stein, ik ben heel erg blij dat u bij mijn promotie bent, ik koester de herinneringen aan onze gezamenlijk rijzen en ander momenten samen. Uw veerkracht is een groot voorbeeld voor mijn. Wim, Annie, Stef, Pim, Frank, Claudia, Ron, Syl, Michele, Jasper, Pascal, Cynthia en alle kids dank voor jullie familiare gezelligheid, interesse en steun. Hanneke, Ton, Vonne en Marco dank voor warmte en steun. Ik prijs me gelukkig met zo'n lieve schoonfamilie.

Lieve Irma en Frans, lieve papa en mama, ik wil jullie bedanken voor de onvoorwaardelijke steun die jullie mij altijd hebben gegeven. Bij jullie thuis vind ik altijd een luisterend oor en een warm hart. Ik heb veel aan jullie opvoeding te danken ook bij het maken van dit proefschrift. Dingen komen niet aanwaaien in het leven en als je iets wil bereiken moet je er hard voor werken, doorzetten als het tegenzit. Dat zijn lessen die voor mij onmisbaar zijn geweest om dit te voltooien.

Lieve Sanne, ik wil je bedanken voor de relativering die je in mijn leven hebt gebracht. Hierdoor is werk, onderzoek en promoveren minder belangrijk geworden maar het leven des te rijker en mooier. Je helpt mij om in balans te blijven en te zien wat echt belangrijk is, want als ik later oud ben en terugkijk op het leven dan zijn onze momenten samen hetgeen wat het de moeite waard heeft gemaakt. Ik wil je bedanken voor je liefde voor mij, Robin en Resa.

Lieve Robin en Resa, ik ben zo blij en trots dat jullie in mijn leven zijn. Alles valt in het niet bij mijn liefde voor jullie en mama.