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Exposure-related cortisol predicts outcome of psychotherapy in veterans with treatment-resistant posttraumatic stress disorder

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ABSTRACT

Background: Hypothalamic-pituitary-adrenal axis functioning has been related to treatment outcome in posttraumatic stress disorder (PTSD). Previous studies have primarily focused on cortisol levels before and after a course of therapy and findings have not been fully consistent. This study investigated session-related cortisol levels in veterans with treatment-resistant PTSD over the course of a novel motion-assisted virtual reality exposure therapy and aimed to determine whether cortisol levels were related to changes in PTSD symptom severity.

Methods: Veterans (N = 22) received six exposure sessions during which salivary cortisol samples were collected pre-session, post-session and in the late afternoon following sessions. PTSD symptom severity was assessed by structured clinical interviews at pre- and post-treatment. Average cortisol levels were compared between responders and non-responders. Linear regression analyses were conducted with PTSD symptom change as criterion variable, average cortisol levels as predictor, and timing of sampling and baseline PTSD symptoms as covariates.

Results: Responders to treatment tended to have higher average cortisol levels at pre-session ($p = 0.064$) and post-session ($p = 0.050$) compared to non-responders. Higher average pre-session and post-session cortisol levels predicted greater PTSD symptom improvement (pre: b = −1.83, $p = 0.009$; post: b = −3.57, $p = 0.004$).

Conclusion: This study provides preliminary evidence for session-related cortisol as biomarker of response to exposure-based therapies for PTSD. Higher cortisol levels may have facilitated fear extinction and reconsolidation, and may indicate increased physiological stress activation necessary for appropriate treatment engagement. Further work involving comparable methodology is encouraged to establish session-related cortisol as biomarker and to determine the mechanisms through which it interacts with treatment outcome.

1. Introduction

Military personnel are at risk of exposure to potentially traumatic events during deployment. While most veterans recover naturally from such impactful events, a substantial portion develops posttraumatic stress disorder (PTSD), with prevalence rates varying between 4 and 17% in the US and 5–8% in the Netherlands (Reijnen et al., 2015; Richardson et al., 2010). This disorder is characterized by a persisting dysfunctional stress response resulting in symptoms of reexperiencing, avoidance, changes in mood and cognitions and hyperarousal. Evidence-based treatment for PTSD consists of psychotherapy, with most evidence for trauma-focused cognitive behavioral therapy (TF-CBT), and eye movement desensitization and reprocessing (EMDR) (Lewis et al., 2020). The hypothalamic-pituitary-adrenal (HPA) axis plays an important part in the neuroendocrine stress response and as such has received much interest in PTSD research. PTSD has most consistently been associated with attenuated resting cortisol levels (Mason et al., 1986; Yehuda et al., 2015a), increased cortisol levels in

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response to stressor-tasks such as reading a trauma script (Bremner et al., 2003; De Kloet et al., 2006; Elzinga et al., 2003), and increased HPA-axis negative feedback inhibition (Daskalakis et al., 2013; Resnick et al., 1995; Yehuda et al., 2015a).

A few studies have investigated whether pre-treatment HPA-axis functioning predicts successful PTSD treatment. These have demonstrated that greater changes in PTSD symptom severity in response to treatment is predicted by a higher cortisol awakening response (Rapcencu et al., 2017), higher bedtime cortisol (Yehuda et al., 2014), higher cortisol reactivity to a trauma script (Rauch et al., 2015), and greater cortisol suppression after dexamethasone administration (Nijdam et al., 2015). However, not all findings from single studies have been confirmed by others (Schumacher et al., 2018). Research has also compared responders and non-responders to PTSD treatment and found basal morning plasma cortisol and 24 h urinary free cortisol to increase from pre-to post-treatment in responders and to decrease in non-responders (Olff et al., 2007; Yehuda et al., 2014). However, Gerardi et al. (2010) demonstrated an opposite effect with decreased cortisol levels in responders as compared to non-responders from before treatment initiation to after the last exposure session. The discrepancies in findings could be due to heterogeneity in methodology across studies. In addition to the low number of studies conducted, this limits conclusions that can be drawn on the relation between cortisol and treatment outcome (Schumacher et al., 2018). Moreover, most studies have focused on pre- and post-treatment cortisol, and some have included cortisol halfway of treatment. However, none have followed the full course of treatment sessions, thereby missing potentially valuable information.

An explanatory model for the potential role of cortisol in treatment stems from animal studies which demonstrated that glucocorticoid administration enhanced fear extinction learning and (re)consolidation of memories (de Quervain et al., 2017). These processes have been theorized to be at the foundation of successful trauma-focused treatment (Foa et al., 2006; van Gelderen et al., 2018). Several clinical studies in PTSD patients have reported greater fear extinction and improved retention in treatment after glucocorticoid administration (Aerni et al., 2004; de Quervain et al., 2017; Yehuda et al., 2010, 2015b). Moreover, previous studies have suggested that higher endogenous cortisol levels during treatment improved symptom reduction through this mechanism as well (Rauch et al., 2015; Siegmund et al., 2011). Rauch et al. (2015) compared prolonged exposure (PE) to present centered therapy, a treatment without trauma-focused elements during which fear extinction and (re)consolidation were not expected to occur. This study found that higher pre-treatment cortisol reactivity to a trauma script predicted greater PTSD symptom reduction in veterans following PE, but not following present centered therapy, emphasizing the importance of heightened cortisol levels and reactivity in trauma-focused treatment and not in other forms of treatment.

Veterans tend to benefit less from PTSD treatment as compared to other populations (Bisson et al., 2013; Kitchiner et al., 2019), which has been related to difficulties with engaging in treatment (Hundt et al., 2018). A novel intervention, called multi-modular motion-assisted memory desensitization and reconsolidation (3MDR) was developed to address avoidance and optimize engagement and result in improved treatment outcomes (van Gelderen et al., 2018). To this end, 3MDR exposure therapy is provided in an immersive, activating and personalized context: patients walk on a treadmill in a virtual environment and interact with trauma-related pictures and music. In addition, a dual-attention task, similar to that in EMDR, was applied and expected to enhance reconsolidation (James et al., 2015). Two randomized controlled trial trials demonstrated that 3MDR as compared to control conditions significantly reduced PTSD symptoms in veterans with treatment-resistant PTSD, with medium to large effect sizes (Bisson et al., 2020; van Gelderen et al., 2020). Although patients had an average of four prior unsuccessful treatments for PTSD before entering the trial by van Gelderen et al. (2020), almost half of the veterans responded to 3MDR treatment.

The aim of the current study was to explore session-related cortisol levels in 3MDR and investigate whether these levels predicted treatment success for veterans with treatment-resistant PTSD. This study was part of a larger randomized controlled trial comparing six sessions of 3MDR to a non-specific treatment component control group for veterans with treatment-resistant PTSD (van Gelderen et al., 2020). The current sub-study in the 3MDR group included saliva collection before each 3MDR session, after each 3MDR session and in the late afternoon following each session. Our first research question was whether responders and non-responders to 3MDR treatment had different cortisol levels over the course of treatment. In addition, we were interested in the difference from pre-session to post-session. Given that the literature reviewed above was inconsistent, and that this was the first study to investigate cortisol in this way, our analyses were explorative. We hypothesized that cortisol levels would be higher in treatment responders and that cortisol from before to after each session would increase more in responders. Our second research question was whether cortisol levels predicted PTSD symptom change. We hypothesized that higher cortisol levels and greater change in cortisol levels from pre-to post-session would predict greater PTSD symptom change.

2. Material and methods

2.1. Study population and procedure

Participants (N = 22) were recruited between 2015 and 2018 at two tertiary mental health care institutes in the Netherlands (ARQ Centrum'45 and Mental Health Center Beilen). They had to be aged between 18 and 70, diagnosed with PTSD as according to DSM-5 criteria, and treatment-resistant for trauma-focused PTSD treatment prior to 3MDR treatment, defined as persisting PTSD diagnosis and lack of improvement in PTSD symptom severity following a full course of evidence-based trauma-focused therapy or repeated failed trials of evidence-based trauma-focused therapy and treatment duration of at least six months. Participants were excluded in case of acute suicidality, severe walking difficulties, current severe alcohol and/or substance dependence according to DSM-IV, acute psychosis, and use of oral contraception. Participants were required to be on a stabilized dose of psychotropic medication for four weeks before entering the study and had to agree to not change psychotropic medication during the study period.

After referral to the study, participants received written information and were contacted by telephone. Interested participants were invited for a baseline assessment, at the start of which they provided written informed consent. If participants met eligibility criteria and were randomized into the 3MDR condition of the study, they received 6 weekly sessions of 3MDR followed by ten weeks of treatment-as-usual. Participants who were randomized to the control condition in the main RCT were not included in this sub-study. Saliva was collected on the day of each 3MDR session at three time points: before each session (pre-session: participants had just entered the 3MDR room), after each session (post-session: participants were off the platform and seated comfortably), and following a session between 16.00 and 18.00 (late afternoon). Timing of the pre-session and post-session samples varied as treatment sessions could not be scheduled at similar times. A post-treatment assessment was conducted 16 weeks post-baseline during which the clinical interview for PTSD symptom severity was repeated. The study was approved by the Medical-Ethical Review Committee of Leiden University Medical Center (approval number: P14.325). All procedure were conducted in accordance with the Helsinki Declaration of 1975, as revised in 2008.

3. Measures

3.1. PTSD symptoms severity

The Clinician Administered PTSD Scale for DSM-5 (CAPS-5) was used to assess PTSD symptoms severity and PTSD diagnosis at baseline and post-treatment. This interview measured presence and severity of the PTSD symptom clusters 'intrusions', 'avoidance', 'alterations in mood/cognitions' and 'hyperarousal'. Twenty questions were rated on a 5-point scale resulting in a total symptom severity score of between 0 and 80 points. For a diagnosis of PTSD at least one intrusive symptom, one avoidance symptom, two mood and cognitive alteration symptoms, two hyperarousal symptoms and impairment needed to be present (score≥2).

3.2. Cortisol

Participants were instructed to not eat, brush their teeth of drink anything but water the hour before saliva collection and not to smoke in the two hours before saliva collection. Saliva was collected in Salivette tubes (Sarstedt, Germany). On each day of saliva collection, participants completed a questionnaire to determine any protocol violations. Samples were frozen at −20° and analyzed in one batch. Cortisol analyses were performed at the Leiden University Medical Center lab. Before analysis, salivary samples were first treated with Elecsys Cortisol II reagent. Cortisol was measured in duplo using electrochemiluminescent immunoassay (ECLIA) on a Cobas 8000 module e602 (Roche Diagnostics, Mannheim, Germany).

3.3. Treatment

3MDR is a manualized trauma-focused therapy for which the theoretical rationale and protocol are available online (van Gelderen et al., 2018). 3MDR hardware consisted of a dual-belt treadmill, a 180° projection on three screens by three projectors and a surround sound system. The software contained a purpose-built virtual environment to walk in, personalized for each patient with pictures and music selected by patients. A junior psychologist operated hardware and software. Participants received 6 weekly 70–90 min 3MDR sessions. Prior to 3MDR, patients selected 10–20 pictures that strongly reminded them of (details) of their deployment-related traumatic events. They were asked to choose music that reminded them of their time of deployment, as well as contemporary music that reminded them of the here and now. Throughout each 3MDR session, veterans walked on the treadmill in the virtual environment at their normal walking speed with the therapist standing beside them. A session started with a mental and physical warm-up during which the music from the time of deployment was played. Next, patients were exposed to a structured sequence of seven pre-selected pictures. Upon approaching each picture, the therapist asked about what could be seen on the picture, the memory related to the picture, and associated emotions and cognitions related to the memory. Whilst focusing on the picture and associations, patients were asked to track a ball moving from left to right over the picture with numbers appearing in the ball at edge of the picture. Patients were required to call these numbers aloud. After exposure to seven pictures, patients listened to contemporary music on the treadmill to return to the here and now. Once off the treadmill, therapist and patients took time to reflect on the content of the session.

3.4. Statistical analysis

Participants were qualified as responders or non-responders based on individual change in PTSD symptom severity as measured by CAPS-5 score from baseline to post-treatment. A reliable change index (RCI) was calculated to serve as margin for clinically relevant change. Using the pooled variance at baseline (SD = 7.26) and test-retest reliability (r = 0.78), a change of 10 or more points on the CAPS-5 was deemed clinically relevant and served as a margin for treatment response (≥10 positive change = responder, <10 positive change = non-responder).

Statistical analysis was performed using IBM SPSS statistics 25 (IBM Corp. Armonk, NY, USA). Data was checked for outliers using a scatter plot. Samples with high cortisol values were checked for violations of the salivary sampling protocol (e.g. smoking within two hours before sampling or eating within one hour before sampling). An average cortisol level over the 6 sessions was calculated for each patient for every sampling time separately (pre-session, post-session and late afternoon). When a single measurement was missing for the calculation of an average cortisol level, pairwise deletion was used. When a patient had two or more missing samples for the calculation of an average cortisol, listwise deletion of this patient's average cortisol followed. When assessing single measurement moments, pairwise deletion was used for missing values.

Descriptive statistics were calculated and differences between the two outcome groups were analyzed with an independent samples *t*-test or chi-square test, where appropriate. When comparing single measurement moments between the outcome groups, correction for multiple testing was applied via the Holm-Bonferroni method. Correlations were analyzed using the Pearson correlation statistic. To analyse the strength of the influence of factors on continuous variables, multiple linear regression was used. Influence of timing of sampling on cortisol levels was tested with correlation analysis for each separate measurement moment. In the regression models corrections for mean time of sampling, pre-treatment CAPS-5 score, and age were included.

4. Results

4.1. Demographic and clinical information

Twenty-two patients were recruited for this sub-study. One patient was excluded from analysis because he did not adhere to the protocol of the salivary sampling and two other patients were excluded because they dropped out of the study before starting or completing the 3MDR sessions and missed the final post-assessment, resulting in lack of cortisol data and CAPS-5 data for those patients. The only female participant was excluded as she took oral contraception, which can influence cortisol metabolism (Barel et al., 2018). Therefore, the results of a total of 18 patients were analyzed. Demographic and clinical characteristics of these participants are displayed in Table 1. Based on change in CAPS-5 score from baseline to post-treatment, eight participants were qualified as responders and ten as non-responders. Mean CAPS-5 score at baseline was 43.67 ± 6.80, with a mean score of 41.40 ± 7.25 in the non-responders group and a mean score of 46.50 ± 5.32 in the responders group (ns). Mean CAPS-5 score after treatment was 36.00 ± 9.41 in all patients, 40.90 ± 6.28 in the non-responders group, and 29.88 ± 9.33 in the responders group ($p = 0.009$). The reliable change index (RCI) was −1.69 ± 2.21 for all patients, −0.10 ± 1.60 for the non-responders, and −3.46 ± 1.19 for the responders ($p < 0.001$).

4.2. Salivary cortisol

Results of the between-group comparisons of salivary cortisol are displayed in Fig. 1 and Table 2. Time of sampling did not differ significantly between responders and non-responders at any of the sampling moments. Time of sampling did not correlate with treatment response (CAPS-5 change). Pre-session sampling time correlated negatively with average pre-treatment cortisol levels ($r = -0.286$, $p = 0.005$), whereas post-session and late afternoon sampling time did not correlate with average post-session and late afternoon cortisol levels ($r = -0.057$, $p = 0.586$ and $r = 0.022$, $p = 0.861$, respectively).

The average level of pre-session salivary cortisol showed a trend towards higher levels for responders group as compared to non-responders (7.1 ± 3.6 *vs.* 4.2 ± 1.6 nmol/L, t (9.14) = 2.11, p =

Table 1
Demographic and clinical characteristics at baseline.

Demographic characteristics	Total sample (N = 18)		Responders (n = 8)		Non-responders (n = 10)	
	N	%	n	%	n	%
Male	18	100.0	8	100.0	10	100.0
Education						
High school or less	7	38.9	3	37.5	4	40.0
Some college	9	50.0	3	37.5	6	60.0
Bachelor degree or higher	2	11.1	2	25.0	0	0.0
Married or partnered	15	83.3	8	100.0	7	70.0
Employed	4	22.2	2	25.0	2	20.0
Comorbidity	15	83.3	6	75.0	9	90.0
Mood disorder	12	66.7	4	50.0	8	80.0
Anxiety Disorder	9	50.0	4	50.0	5	50.0
Alcohol dependence (mild- moderate)	3	16.7	3	37.5	0	0.0
Current psychotropic medication for PTSD	14	77.8	6	75.0	8	80.0
SSRIs	8	44.4	2	25.0	6	60.0
Benzodiazepines	5	27.8	2	25.0	3	30.0
Atypical antipsychotics	5	27.8	3	37.5	2	20.0
Other anti-depressants	2	11.1	1	12.5	1	10.0
SNRIs	2	11.1	0	0.0	2	20.0
	M	**SD**	**M**	**SD**	**M**	**SD**
Age	43.75	10.09	45.06	9.94	42.70	10.63
Number of deployments	1.72	1.02	1.88	1.13	1.60	0.97
Number of prior psychological treatments for PTSD	4.11	1.91	4.25	1.75	4.00	2.12
Number of prior pharmacological treatments for PTSD	3.83	2.79	3.63	2.62	4.00	3.10

Note. No statistically significant differences between treatment groups on any variable.

0.064). The average pre-session cortisol level showed a significant negative correlation with change of CAPS-5 score from baseline to 16 weeks after baseline (Fig. 2) (r = −0.542, $p = 0.020$). Decrease in CAPS-5 score over treatment was 1.83 points greater for every nmol/L of average pre-session cortisol (b = −1.83 [-3.19 to −0.46], $p = 0.013$), when corrected for age, timing of sampling, and pre-treatment CAPS-5 score (F (4,13) = 5.09, $r^2 = 0.610$, $p = 0.011$). Pre-treatment CAPS-5 score predicted change in CAPS-5 score in this model (b = −0.77 [-1.38 to −0.15], $p = 0.019$). Time of saliva sampling and age were unrelated to change in CAPS-5 score over treatment in the univariate and multivariate analyses. Before session two and session five, salivary cortisol appeared to be higher in the responders group (7.0 ± 3.1 and 7.1 ± 3.4) than in the non-responders group (3.8 ± 1.5 nmol/L and 3.5 ± 1.5 nmol/L), however, statistical significance was lost after correction for multiple testing.

The average level of post-session salivary cortisol showed a trend towards higher levels for responders (4.3 ± 2.0 nmol/L) than non-responders (2.6 ± 0.9 nmol/L, t (9.22) = 2.24, $p = 0.050$). The average post-session cortisol level showed a significant negative correlation with change of CAPS-5 score from baseline to 16 weeks following baseline (Fig. 3) (r = −0.499, $p = 0.035$). Decrease in CAPS-5 score over treatment was 3.56 points greater for every nmol/L of average post-session cortisol (b = −3.56 [-5.91 to −1.21], $p = 0.006$), when corrected for age, timing of sampling, and pre-treatment CAPS-5 score (F (4,13) = 5.67, $r^2 = 0.636$, $p = 0.007$). Pre-treatment CAPS-5 score predicted change in CAPS-5 score in this model (b = −0.89 [-1.50 to −0.28], $p = 0.007$). Time of saliva sampling and age were unrelated to change in CAPS-5 score over treatment in the univariate and multivariate analyses. After session two and session three, salivary cortisol appeared to be higher in the responders group (5.6 ± 2.7 and 4.9 ± 2.8) than in the non-responders group (2.5 ± 0.8 nmol/L and 2.4 ± 0.9 nmol/L), however, statistical significance was again lost after correction for multiple testing.

The average levels for salivary cortisol in the late afternoon showed no differences between the two outcome groups (t (11.61) = -0.05, $p = 0.950$). No differences in late afternoon cortisol levels where found when assessing single measurement moments. There was no significant correlation between change of CAPS-5 score over treatment and late afternoon cortisol (r = −0.091, $p = 0.756$).

When assessing the average difference in cortisol levels from pre-session to post-session, there was a decrease in cortisol over the sessions with an average of 2.08 nmol/L (28%) (t (17) = 4.85, $p < 0.001$), with no significant differences between sessions or outcome groups (t (16) = 1.54, $p = 0.143$). There was no significant correlation between change of CAPS-5 score over treatment and average decrease in cortisol over the 3MDR-sessions (r = 0.438, $p = 0.069$). The change in CAPS-5 score over treatment showed a trend towards being 2.25 points greater for every nmol/L average decrease of cortisol levels from pre-session to post-session (b = 2.25 [-0.32 to 4.83], $p = 0.082$), when corrected for age, timing of sampling, and pre-treatment CAPS-5 score (F (3,14) = 3.12, $r^2 = 0.400$, $p = 0.060$). Pre-treatment CAPS-5 score showed a trend towards predicting change in CAPS-5 score in this model (b = −0.71 [-2.08 to 0.06], $p = 0.056$). Time of saliva sampling and age were unrelated to change in CAPS-5 score over treatment in the univariate and multivariate analyses.

5. Discussion

This exploratory study, conducted in the context of a trial of 3MDR for veterans with treatment-resistant PTSD, demonstrated that higher average levels of cortisol both directly before and after 3MDR sessions predicted greater PTSD symptom decrease. Responders to treatment tended to have higher average cortisol levels at pre-session and post-session than non-responders. The linear regression models accounted for 61–64% of the variance in PTSD symptom change, implying that exposure-related salivary cortisol levels may be candidate biomarkers for change in PTSD symptom severity following treatment. Average cortisol levels in the late afternoon following sessions did not differ between groups, nor did they relate to treatment outcome. This could indicate that cortisol levels in treatment responders within this study were not chronically elevated, but rather that responders showed stronger anticipatory cortisol output before sessions. Unexpectedly, but supporting the notion of an anticipatory response, we found average cortisol levels to decrease from before to after sessions for the total group, with a larger decrease associated with greater change in PTSD symptom severity at a trend level. The average decrease in cortisol levels

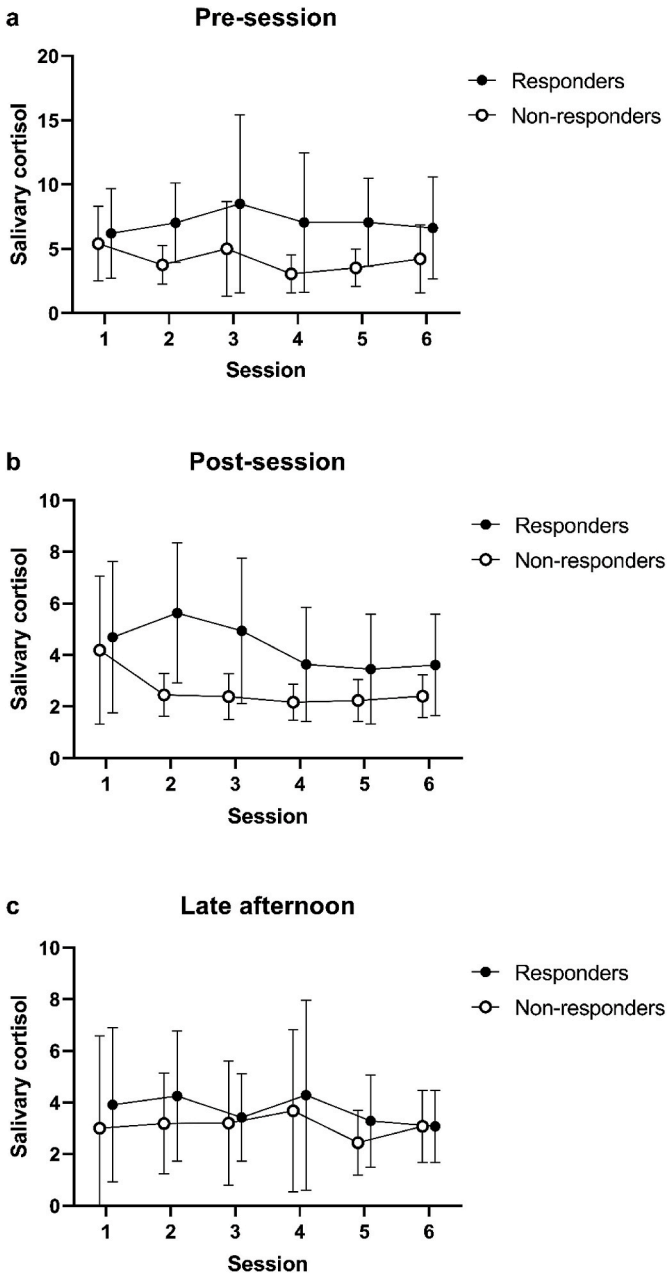

Fig. 1. Average salivary cortisol levels (nmol/L) over the course of 3MDR treatment at pre-session (a), post-session (b) and late afternoon (c) for responders and non-responders to 3MDR treatment.

from pre-session to post-session did not differ between groups. To the best of our knowledge this was the first study to investigate cortisol related to a full course of treatment sessions. The current results provide preliminary evidence that exposure-related cortisol could be a biomarker of exposure-based PTSD treatment response.

The relation between elevated session-related cortisol levels and greater PTSD symptom reduction were in line with several studies demonstrating that higher cortisol before or during exposure to a trauma script predicted positive symptom change (Rauch et al., 2015; Siegmund et al., 2011). Other studies have also indicated a relationship between higher cortisol levels and PTSD symptom improvement, but these concerned levels at awakening and bedtime before initiating treatment (Olff et al., 2007; Rapcencu et al., 2017; Yehuda et al., 2014). In contrast, we did not find late afternoon cortisol levels to be related to treatment outcome. This could be the result of differences in methods: the current study did not include baseline or post-treatment cortisol samples,

Table 2
Average cortisol levels in saliva pre-session, post-session and late afternoon for non-responders and responders.

Timing	Session	Non-responders (n = 10)		Responders (n = 8)	
		M	SD	M	SD
Pre-session	1	5.4	2.9	6.2	3.5
	2	3.8	1.5	7.0	3.1
	3	5.0	3.7	8.5	6.9
	4	3.0	1.5	7.0	5.4
	5	3.5	1.5	7.0	3.4
	6	4.2	2.7	6.6	3.4
Post-session	1	4.2	2.9	4.7	2.9
	2	2.5	0.8	5.6	5.6
	3	2.4	0.9	4.9	2.8
	4	2.2	0.7	3.6	2.2
	5	2.2	0.8	3.5	2.1
	6	2.4	0.8	3.6	2.0
Late afternoon	1	3.0	3.6	3.6	2.0
	2	3.2	2.0	4.3	2.5
	3	3.2	2.4	3.4	1.7
	4	3.7	3.1	4.3	3.7
	5	2.4	1.3	3.3	1.8
	6	3.7	3.7	3.1	1.4

Note. Levels are presented in nmol/L.

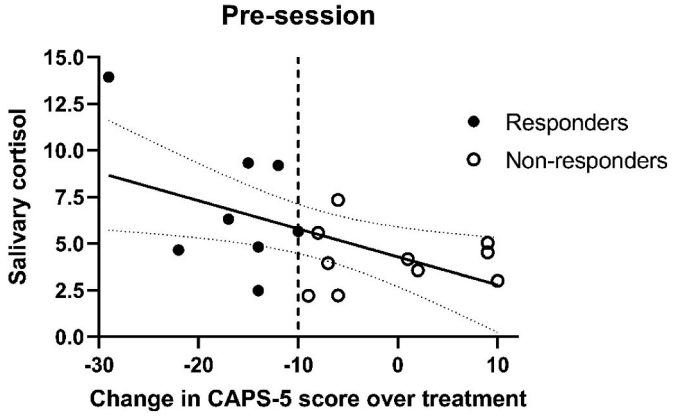

Fig. 2. Relation between 3MDR associated clinical improvement and average salivary cortisol levels pre-session (nmol/L).

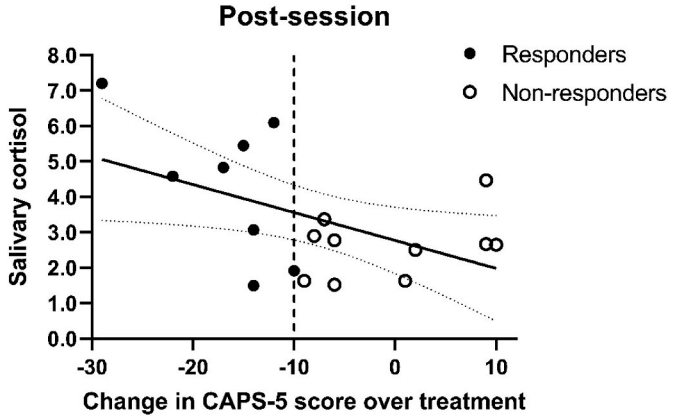

Fig. 3. Relation between 3MDR associated clinical improvement and average salivary cortisol levels post-session (nmol/L).

whereas the abovementioned studies did not assess cortisol tied to treatment sessions. Future research on HPA-axis functioning in PTSD treatment should aim for comparability across studies (Schumacher et al., 2018). Given that our study found average pre- and post-session cortisol to predict PTSD symptom improvement, we recommend studies to apply a similar approach in addition to baseline and post-treatment cortisol sampling.

Our findings suggest an important role of cortisol in PTSD treatment and indicated HPA-axis mediated mechanisms involved in PTSD treatment outcome. The higher cortisol levels in the current study may have primarily been a marker of increased stress activation. Stress activation has been interpreted as readiness to engage with the traumatic memory and associated emotions (emotional engagement), and as such may be necessary for beneficial trauma-focused treatment outcomes (Foa et al., 2006; Jaycox et al., 1998). This is consistent with research which demonstrated that higher distress predicted greater PTSD symptom reduction (Jaycox et al., 1998; Reger et al., 2019; Sripada and Rauch, 2015). Another potential mechanism, as discussed in the Introduction, is based on accumulating evidence that glucocorticoid administration enhances fear extinction and (re)consolidation of memories (Aerni et al., 2004; de Quervain et al., 2017; Schelling et al., 2006; Yehuda et al., 2010), processes on which PTSD symptom reduction following trauma-focused treatment has been theorized to rely (Foa et al., 2006; van Gelderen et al., 2018). The higher endogenous cortisol levels in the current study may have facilitated these memory processes, resulting in greater PTSD symptom reduction. If this is the case, it is in line with research that found higher salivary cortisol to relate to outcome of exposure treatment, but not to outcome of non-exposure treatment for PTSD (Rauch et al., 2015). An interesting follow-up study would be a trial in which treatment-resistant patients with low session-related cortisol levels are randomized to receive placebo or glucocorticoid augmentation of exposure treatment for PTSD. We should note, however, that the exact influence of cortisol on memory processes is not fully understood. Depending on timing and other circumstances, the effect of cortisol levels on memory has demonstrated to be an important factor on the contextualization of emotional memories (van Ast et al., 2013) and, therefore, is an avenue to be addressed in clinical research (Vermetten et al., 2014). It is unclear whether lower cortisol levels in the current study reflected dysfunctional HPA-axis functioning or poorer stress activation. Future studies should therefore conduct a thorough evaluation of HPA-axis functioning in non-responders to PTSD treatment.

The finding that average cortisol levels decreased from pre-to post-sessions, was in contrast to the literature which demonstrated an increase in cortisol levels in response to trauma-related stressors in PTSD patients (Elzinga et al., 2003; Gerardi et al., 2010; Rauch et al., 2015). This is likely due to the set-up of the current study. Cortisol levels have been found to increase in anticipation of a trauma-related stressor (Elzinga et al., 2003). Thus, anticipatory anxiety will likely have led to increased cortisol before a 3MDR session, at the time when the pre-session saliva sample was collected. Because the post-session sample was taken approximately 15–20 min after the end of exposure, the expected peak during exposure would not have been detected in this measurement either. Future studies should, therefore, measure cortisol in rest and during exposure when aiming to assess cortisol reactivity. Moreover, at the end of a 3MDR session, relaxation is encouraged by the therapist and facilitated with music. This may have attenuated cortisol levels at post-session. Such a decrease would not be seen in response to trauma-related scripts, as relaxation techniques are not part of such a research paradigm. Finally, the 3MDR intervention differs from other trauma-focused therapies in that it includes walking during the entire treatment session. This constant movement may have influenced HPA-axis functioning (Keyan and Bryant, 2019), and cortisol levels may have behaved differently during 3MDR as compared to sedentary trauma-focused treatments. Because the direction of effect of physical activity on cortisol levels depends on the intensity of activity (Hill et al., 2008), future studies of HPA-axis functioning during 3MDR may want to include measures of physical activity intensity.

There are some important limitations to this study. The time of day at which 3MDR sessions were provided, and thus cortisol samples were collected, differed between and within individuals. Although corrections were applied for this in the analyses, cortisol levels are known to change throughout the day, and a set time for all sessions would have increased validity of the results. Therefore, future studies should apply a methodology with consistent saliva sampling times. Over half of the sample met criteria for a comorbid mood disorder. Depression is known to influence HPA-axis functioning and as such could have impacted cortisol levels. Moreover, participants were allowed to stay on (psychotropic) medication during the trial, which could have affected cortisol levels as well. However, since medication use did not differ between the treatment outcome groups, we expect this to be of relatively little influence on the current study outcomes. We chose to use the reliable change index to define responder status. Given the treatment-resistant sample, we thought this to be a better reflection of response status as compared to loss of diagnosis. However, it is important to consider that not all responders showed symptom remission and as such results might differ from studies that chose a loss of diagnosis as criterion for treatment response. In addition, by categorizing patients based on response status, the variance in outcome within the subgroups became smaller and as such the power for the analyses was relatively small. This may account for the between-group findings being just below statistical significance. However, we included prediction models with a continuous outcome variable as well. The fact that these were significant strengthen our between-group findings. Finally, the small sample size needs to be acknowledged. The nature of the current study was, therefore, exploratory, and future replication studies are recommended.

Future research should be focused on applying comparable methods, studying the mechanisms through which cortisol interacts with treatment outcomes, and investigating HPA-axis functioning in patients who insufficiently benefitted from treatment (Vermetten et al., 2014). Moreover, despite growing evidence on the important role of cortisol in memory processes necessary for successful exposure treatment, few studies have investigated glucocorticoid augmentation to PTSD treatment (Dunlop and Wong, 2019; Yehuda and Golier, 2009). Those that have found encouraging results (Aerni et al., 2004; de Quervain et al., 2017; Yehuda et al., 2010, 2015b). The current study results indicated that glucocorticoid augmentation of treatment might be especially beneficial for those patients with low exposure-related cortisol levels. In addition, low exposure-related cortisol levels may be a contributing factor to developing treatment-resistant PTSD. If studies were to replicate that higher exposure-related cortisol levels predict positive treatment outcomes, early session cortisol levels could be used as biomarker for treatment response, and glucocorticoid augmentation may prove to be a fruitful strategy to advance treatment outcomes for veterans with PTSD.

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CRediT authorship contribution statement

Marieke J. van Gelderen: Conceptualization, Methodology, Investigation, Writing - original draft, Visualization, Project administration, Funding acquisition. **Mirjam J. Nijdam:** Conceptualization, Methodology, Investigation, Writing - review & editing, Supervision, Funding acquisition. **Friso de Vries:** Conceptualization, Formal analysis, Writing - review & editing, Visualization. **Onno C. Meijer:** Conceptualization, Writing - review & editing. **Eric Vermetten:** Conceptualization, Methodology, Investigation, Writing - review & editing, Supervision, Funding acquisition.

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