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Pre-treatment cortisol awakening response predicts symptom reduction in posttraumatic stress disorder after treatment

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ABSTRACT

Dysfunction of the HPA-axis has frequently been found in the aftermath of trauma exposure with or without PTSD. Decreasing HPA-axis reactivity to different stress cues has been reported during PTSD treatment. The cortisol awakening response (CAR_i) is a well-validated, standardized measure of HPA-axis reactivity which can be easily acquired in the clinical setting. Whether CAR_i changes over time in traumatized individuals are specific to PTSD treatment is unknown. Furthermore, a possible role for the baseline CAR_i in predicting symptom reduction after treatment in PTSD has not been examined before. To answer these questions, a cohort study was conducted in which the awakening cortisol was measured in both PTSD (N = 41) and non-PTSD (N = 25) combat-exposed male subjects. Measurements took place at inclusion and 6–8 months after inclusion for both the PTSD and the non-PTSD group. During the 6–8 months interval, PTSD patients received trauma-focused focused psychotherapy, whereas non-PTSD patients received no treatment. We found a decrease in the CAR_i over time in both groups, suggesting it was not specific to PTSD or the effect of treatment. Therefore, caution is warranted when attributing diminished HPA-axis reactivity over time to effects of PTSD treatment. Second, CAR_i prior to treatment predicted PTSD symptom reduction (CAPS score change) after treatment, and accounted for 10% of the variance, even when adjusted for changes in depressive symptoms and medication use during the study period. A putative role emerges for CAR_i as a predictive biomarker of symptom reduction in male individuals with combat-related PTSD.

1. Introduction

Following trauma exposure vulnerable individuals develop posttraumatic stress disorder (PTSD), a trauma and stress related disorder with intrusive and re-experiencing symptoms, avoidance, and negative changes in cognition (American Psychiatric Association, 2013) characterized by a broad range of abnormal stress reactions (e.g.,intrusive and re-experiencing symptoms, avoidance, physiological hyperarousal, and negative changes in cognition) (American Psychiatric Association, 2013). The hypothalamic-pituitary-adrenal axis (HPA-axis) has received particular attention in PTSD research, because it represents the organism's major neuroendocrine stress response system (Heim and Nemeroff, 2009). Although not thoroughly consistent, some HPA-axis alterations have been reproduced in several studies in individuals with PTSD, such as: low 24-h urinary cortisol, low daily cortisol secretion during early morning, increased glucocorticoid receptors on lymphocyte cell membranes and enhanced HPA-axis sensitivity to feedback inhibition. Moreover, some studies found that baseline urinary (Baker et al., 1999), plasma (Goenjian et al., 2003) and salivary cortisol concentrations (Wahbeh and Oken, 2013) were related to the severity of PTSD symptoms. A recent review of prospective studies focusing on psychobiological predictors of PTSD in the acute aftermath of traumatic stress showed that lower peritraumatic cortisol levels were associated with increased risk for PTSD (Morris and Rao, 2013). Other meta-analytic evidence for HPA-axis alterations have been described by Morris et al. (2012), as well as by Meewisse et al. (2007). The HPA axis shows a dynamic ultraradian rhythm that is manifested by fluctuating levels of ACTH and glucocorticoids (Walker et al., 2012). Fluctuations in glucocorticoid levels are necessary to maintain homeostasis, and may thus be indicative of treatment response.

Only a few studies have recently addressed the question whether treatment success in PTSD may be associated with changes in cortisol

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secretion, and they mainly assessed the HPA-axis function before and after trauma-focused therapy. According to international guidelines (National Institute for Health and Care Excellence, 2005), trauma-focused therapy is the most effective treatment available in PTSD. Trauma-focused therapy includes trauma-focused cognitive behavioral therapy (tf-CBT), eye movement desensitization and reprocessing (EMDR) and other related psychological treatments with the following common components: psychoeducation, imaginal exposure, cognitive processing and cognitive restructuring. Rothbaum et al. (2014) found a decrease in the salivary cortisol response to virtual exposure therapy over time, which was more evident when D-cycloserine was administered during the session. This finding has been recently reproduced by Norrholm et al. (2016), although the type of medication administered during the therapy sessions was no longer of influence. Others (Gerardi et al., 2010) found diminished cortisol reactivity after successful exposure therapy. It has been suggested that these results may reflect decreased HPA-reactivity to reminders of the stressor in response to treatment. In addition, Olff et al. (2007) reported an increase in the basal morning cortisol at the end of treatment in individuals with PTSD remission, as opposed to decrease in non-responders. This discrepancy may be explained by the fact that the latter study employed only one single morning sample as an estimate of the basal cortisol output, while the previous studies measured cortisol response to trauma cues as estimate of HPA-axis responsivity.

While paradigms of trauma cue exposure are difficult to standardize, single cortisol sample measurements have poor ecological validity and are prone to intra-individual variability (Stalder et al., 2015). These problems can be avoided by using the cortisol awakening response (CAR) as a well-validated measure of HPA-axis function (Stalder et al., 2015). The CAR captures information on both basal activity and reactivity of the HPA-axis without introducing an experimental stressor (Fekedulegn et al., 2015). The basal ascending morning cortisol output can be estimated by the area under the ground with respect to 0 (CAR_g, g = ground) while the superimposed response to awakening is estimated as the area under the curve relative to waking values (CAR_i, i = increase). To our knowledge, only one study has examined changes in both CAR indices following successful treatment in PTSD, up to now (Pacella et al., 2014). In a community sample with chronic PTSD, the remission or persistence of PTSD was not significantly related to CAR_i at 10 weeks of psychotherapeutic or pharmacological treatment.

Furthermore, CAR_i did not change between the pre- to post-treatment assessment in male responders, whereas the female responders displayed lower CAR_i post-treatment than female non-responders. The specificity of the findings for PTSD and for the PTSD treatment has not yet been tested. Earlier literature showed that trauma exposure per se, in the absence of a PTSD diagnosis (de Kloet et al., 2007; Meewisse et al., 2007), as well as time passed since trauma, regardless of treatment (Morris et al., 2012), can also induce detectable changes in the CAR. More specifically, a blunting of the HPA-axis responsivity, and thus CAR_i, can be expected in the aftermath of traumatic exposure, regardless of the PTSD diagnosis and PTSD treatment status.

Objective biomarkers of treatment efficacy are highly needed in guiding treatment choice in PTSD, considering the high rates of symptom persistence after treatment (Bradley et al., 2005). CAR_g was proved not to be predictive of treatment effect in two recent clinical trials including trauma-focused therapy (Rauch et al., 2015; Nijdam et al., 2015). On the other hand, a positive relationship existed between symptom reduction and the HPA-axis responsivity in the form of suppression of the CAR by dexamethasone challenge (Nijdam et al., 2015) or activation by script-driven imagery (Rauch et al., 2015). Similarly, Pacella et al. (2014) observed in their preliminary analysis a higher cortisol response to awakening (CAR_i) at baseline that was associated with lower PTSD symptoms post-intervention (Pearson's $r = -0.36$, $p < 0.10$). However, no measure of pre- to post-treatment improvement in PTSD symptomatology was assessed.

We present here a prospective, observational study exploring the cortisol awakening response and its association with clinical outcome during trauma-focused therapy in men with combat-related PTSD, while accounting for trauma exposure among other confounds. To this purpose, patients with combat-related PTSD, and combat-exposed, healthy individuals, were assessed at a fixed time interval of six months. This study is part of a larger cohort study on biological and psychological aspects of recovery following PTSD treatment in the Dutch Armed Forces. The first research question was whether there were changes in CAR after 6 months of trauma-focused psychotherapy. We expected to find lower CAR after 6 months in both combat exposed groups (with and without PTSD) and that this effect would be stronger for patients with PTSD that received the trauma focused psychotherapy. The second research question that was investigated was whether CAR at the beginning of the treatment can serve as a predictor of symptom change during treatment for PTSD. In the present study, we specifically tested the hypothesis that CAR_i, as a well validated estimate of HPA-axis responsivity, would positively predict the extent of clinical improvement in PTSD.

2. Methods

2.1. Participants

Study subjects were participants of an observational longitudinal study, which was conducted between September 2010 and September 2013. Cortisol data were available in 42 male subjects with combat-related PTSD (patients) and 25 male veterans without PTSD (combat controls). PTSD patients were veterans as well as active duty personnel recruited from one of four outpatient clinics of the Military Mental Healthcare Organization. PTSD was diagnosed by a clinician according to the DSM-IV criteria (American Psychiatric Association, 2000). Control participants were recruited via advertisements. At the time of inclusion, all PTSD patients had current PTSD, no current alcohol or substance dependence, and no neurological disorder. Combat controls had no clinical PTSD symptoms (CAPS < 15, see Section 2.2), no current psychiatric disorder, no alcohol or substance dependency, no neurological disorder, and no lifetime PTSD. All participants had been deployed at least once for a period of at least 4 months.

Participants received monetary compensation for participation (€12.50 per hour). Written informed consent was obtained from all participants after they had received a complete written and verbal explanation of the study, in accordance with procedures approved by the University Medical Center Utrecht ethics committee and the Declaration of Helsinki.

2.2. Psychological instruments

At study inclusion (T_0) and 6–8 months later (T_1), all participants were repeatedly assessed for psychiatric symptoms with several clinical interviews and self-report scales.

The PTSD diagnosis and severity were confirmed by a clinician or trained researcher using the Clinician-Administered PTSD Scale-IV (CAPS; Blake et al., 1995). This semi-structured interview, designed to assess DSM-IV symptoms of PTSD, is the gold standard PTSD instrument in the field and has good psychometric properties (Weathers et al., 2001). A PTSD case was identified if subjects endorsed the requisite DSM-IV symptoms at least at a frequency of 1 and at an intensity of 2 (Weathers et al., 2001). The threshold for the PTSD diagnosis was a minimal CAPS score of 45. Participants were included in the combat control group when the total CAPS score was 15 or lower. The *Structured Clinical Interview for DSM-IV* (SCID; First et al., 1996) was administered to assess co-existing psychopathology.

Besides PTSD, depression has been related to an excessive daily cortisol secretion and a blunted sensitivity of the HPA-axis (Wingenfeld and Wolf, 2015). *The Mood and Anxiety Symptoms Questionnaire* (MASQ; de Beurs et al., 2007) is a self-report inventory that is particularly useful

when measuring co-occurring depression and anxiety symptoms. The anhedonic depression subscale of the MASQ (MASQ-AD) has proved useful in discriminating depressive from anxiety symptoms both in the general population (Watson et al., 1995) and in clinical samples (Buckby et al., 2007). MASQ-AD was therefore selected to control for depressive symptoms.

Additional inventories were assessed at study inclusion in order to control for possible confounds. The *Early Trauma Inventory – Short Form* (ETI-SF; Bremner et al., 2007) contains 27 "true" or "false" items designed to assess whether the patient has been exposed to potential traumatic experiences before the age of 18 years. The questionnaire consists of 4 subscales measuring: General Trauma (11 items; eg. exposure to violence, natural disasters, and deaths in the family), Physical Punishment (five items; eg. being slapped in the face), Emotional abuse (five items; eg. 'often being put down' or 'ridiculed'), and Sexual Abuse (six items). The scores on each scale represent the number of items that have been answered with "Yes". The ETI-SF was used to account for the types and frequency of childhood trauma, given that childhood trauma per se has been shown to impact HPA-axis functioning (Meinlschmidt and Heim, 2005; Carpenter et al., 2007; Elzinga et al., 2008; Klaassens et al., 2009).

2.3. CAR assessment

Salivettes (*Sarstedt Inc. Newton, NC, USA*) were used for sampling of salivary free cortisol. In order to improve test-retest reliability, the CAR was sampled on two consecutive weekdays (Day 1 and Day 2) at each clinical evaluation (T_0 and T_1, respectively). Each participant received a set of salivettes along with verbal and written instructions about the sampling procedure. They were instructed to collect the first sample immediately upon awakening ("The moment you can open your eyes"), and the remaining samples at 15-, 30- and 60-min post-awakening. Participants were free to follow their normal routines, but were instructed not to eat, drink, smoke, or brush their teeth during the sampling period. Self-report information was collected on time of awakening, caffeine (coffee and tea) and nicotine consumption during the sampling procedure, and alcohol consumption during the previous day. The participants were asked to return their samples directly by mail. Returned samples were encoded and frozen at −80 °C until analysis. The samples were analyzed in Utrecht using a luminescence immunoassay. Intra- and inter-assay variations are < 4.5% and < 4.3% respectively. Physiologically unlikely high values of cortisol (i.e., > 60 nmol/L) were per subject excluded from further analyses (Patel et al., 2004). Data for missing sample concentrations on both days was interpolated by using the mean value of the preceding and following sample concentrations (Yehuda et al., 2005). Data from the other sampling day was used for missing sample values on one of the days. When more than one consecutive time-point was missing on one of the days, only one of the two sampling days was used in the analysis. In the case that the first or last measurement was missing, the second and third measurement were used respectively. The quality of the collected CAR data was evaluated by assessing the test-retest reliability between Day 1 and Day 2. According to Wust et al. (2000), intra-individual stability was expected to be high (Pearson's $r > 0.60$).

2.4. Treatment

All patients were about to start trauma-focused therapy at the time of inclusion. Trauma-focused therapy represents a broad class of psychotherapeutic interventions that include tf-CBT and EMDR. Patients received treatment as usual, consisting of tfCBT and/or EMDR, previously demonstrated to be equally effective in treating PTSD (Bisson and Andrew, 2009). In addition, psychotropic medication for PTSD or for comorbid conditions was occasionally part of the treatment. Symptom reduction was quantified by subtracting the baseline from the follow-up CAPS total score (ΔCAPS) for each participant. Improvement in different PTSD symptom clusters was quantified similarly for each of the corresponding CAPS subscales (ΔCAPS B, ΔCAPS C and ΔCAPS D for the re-experiencing, avoidance and hyperarousal clusters, respectively). Remission of PTSD was defined by CAPS score < 45 at T_1 while SCID diagnostic thresholds at T_1 were used to define remission of other comorbid disorders which were present at study inclusion in the patient group.

2.5. Statistical analysis

Statistical analyses were performed using the Statistics Package for the Social Sciences, Version 22 (IBM Corp, 2013) and R (R Core Team, 2016). An alpha level of 0.05 (two-tailed) was used to determine statistical significance in all analyses.

In order to detect potential confounds, differences between group characteristics at study inclusion (e.g., age, number of deployments, years since last deployment, depressive symptoms, and childhood trauma) were tested using independent *t*-tests for normally distributed, continuous variables, and the Mann-Whitney test for non-normally distributed variables. The non-parametric Kruskal-Wallis test was additionally performed to test group differences in education levels (ordinal variable). Pearson χ^2- tests were used to test group (e.g., lifetime comorbidity, psychotropic medication use, substance use during cortisol sampling) and time differences in proportions (e.g., treatment induced remission in PTSD and in other comorbid disorders). Paired-sample *t*-tests were performed in the PTSD group to detect changes from baseline in clinical outcome of PTSD (CAPS scores) and depression (MASQ-AD scores).

Average cortisol concentrations were computed per measurement over the two sampling days at T_0 and T_1, respectively. Using the trapezoidal formulas for area under the curve (Pruessner et al., 1997), two composite measures for salivary cortisol were derived: CAR_g as a measure of the total salivary cortisol output during the first hour after awakening, and CAR_i as a measure of salivary cortisol response to awakening only. All variables were examined for normality of distribution within each group. Outliers were detected using the outliers labeling rule as described by Hoaglin and Iglewicz (1987). A logarithmic transformation was applied to variables that violated the assumption of a normal distribution ($p > 0.05$, Kolmogorov-Smirnov test).

To test the first hypothesis, CAR changes across time and group differences in CAR_g and CAR_i were calculated by two separate repeated measures analyses of variance (ANOVAs) with evaluation time (T_0, T_1) as within-subjects variable and group (PTSD, no PTSD) as between-subjects factor. When a significant main effect of or interaction with evaluation time was found, Bonferroni corrected paired *t*-tests followed. A sensitivity analysis was carried out for the CAR data collected not later than 10 a.m., as late awakening times can highly impact results (Stalder et al., 2015).

In order to compare the change in CAR between patients that responded to treatment and patients that still were eligible for the PTSD diagnosis according to the DSM-IV criteria, Cliff's robust rank-based method for comparing two groups (Cliff, 1996) was used, since the groups showed different distributional properties and non-normality. Cliff's test is a heteroscedastic analog of the Wilcoxon-Mann-Whitney test. Cliff's Delta approaches 0 when a non-significant p-value is found and the two groups are overlapping. The analysis was carried out using the function "cid" (Wilcox, 2016) in the R-statistical software (R Core Team, 2016).

Finally, a sequential multiple regression analysis was conducted in the patient group in order to test the second hypothesis that the CAR can uniquely predict symptom reduction. First, we examined zero order correlations of treatment outcome variables (ΔCAPS, ΔCAPS B, ΔCAPS C and ΔCAPS D) with the CAR indices (CAR_g and CAR_i) as well as with potential confounds of treatment outcome (number of therapy sessions, baseline CAPS scores, baseline MASQ-AD scores, improvement of MASQ-AD scores at follow-up). The p-values for the Pearson's correla-

tions were adjusted to correct for multiple testing with the correction as suggested by Benajmini and Hochberg (1995) using the "p.adjust" function in R (R Core Team, 2016).

Because of the limited sample size (complete data for all predictors in $N = 31$), we opted to include a maximum of three predictors in our regression models (Green, 1991). In our patient sample, there was a strong intercorrelation between the two CAR measures at baseline, CAR_g and log transformed CAR_i, (Pearson's $r(39) = 0.631$, adjusted-$p < 0.01$). The log transformed CAR_i was used as a predictor, since CAR_i has previously been found to be a more appropriate measure of HPA-axis activation following awakening than CAR_g (Chida and Steptoe, 2009; Wilhelm et al., 2007). Among the possible confounds, improvement of anhedonic depression during treatment (ΔMASQ-AD) and antidepressant medication use during the follow-up period were selected as most relevant, based on the high rates of depression comorbidity and remission at study inclusion at follow-up, respectively. A sequential multiple regression model was constructed with ΔCAPS as outcome measure, with ΔMASQ-AD and antidepressant medication use as predictors at the initial step, and with log transformed baseline CAR_i as predictor at the final step.

3. Results

3.1. Subjects

CAR data and clinical interviews were available in 42 patients with combat-related PTSD and 25 combat controls aged 23–57 years. One patient displayed improbable high values of salivary cortisol in two of the samples (125–155 nmol/L) (Patel et al., 2004), and was therefore excluded from the analyses. In total, 41 patients with combat-related PTSD and 25 combat controls were included in the study.

There were no significant differences between groups in demographic characteristics (see Table 1). There were significantly more depressive symptoms in the patient group than in the control group, as assessed with the MASQ-AD ($M = 30$ points, $t(63) = 10.136$, $p < 0.001$). As such, the MASQ-AD was considered a possible covariate in further analyses. Patients also showed significantly more current and lifetime psychiatric comorbidity than controls. The lifetime comorbidity consisted mainly of a combination of diagnoses (43.9%) with comorbid mood and anxiety disorder as most prevalent. At study inclusion (T_0), 56.1% (23/41) of PTSD patients met the criteria of a major depression episode (MD) and 34.1% (13/41) the criteria of a comorbid anxiety disorder, while only 7.3% (3/41) had a comorbid somatoform disorder. Most of the patients received EMDR ($N = 30$), some received another form of trauma focused-CBT ($N = 11$), and seven received both. Antidepressant medication was received by 31.7% of the patients ($N = 13$) at study inclusion and continued throughout the treatment period, except for one patient.

For a detailed description of the CAR data, see Table 4, Appendix A. Fifteen cortisol samples (1.39%) had to be interpolated the other sampling day due to a lost or altered salivette or insufficient saliva. There were no significant differences between groups in awakening time at each CAR assessment, alcohol intake the day before each CAR assessment and cigarettes, coffee or tea intake during saliva sampling. Seven participants (of whom 6 patients) who did not register their awakening time on both days at one of the evaluation moments (T_0 or T_1, respectively) were excluded from the sensitivity analysis. Finally, 36 patients and 24 controls were included in the sensitivity analysis. However, data from only one of the two sampling days (Day 1 or Day 2, respectively) was available in seven of the included participants (of whom 6 patients) based on lack of registration of their awakening time or waking later than 10 o'clock on the other day.

3.2. Change in clinical features in the PTSD group

The treatment was effective in inducing remission in PTSD and comorbid major depression disorder, but not in comorbid anxiety disorders and somatoform disorders (see Table 2). At T_1, the mean total CAPS scores significantly improved in the PTSD group ($M = 22.8$ points, $SD = 26.4$, $t(40) = 5.545$, $p < 0.001$). The MASQ-AD had also significantly improved over time ($M = 11.4$ points, $SD = 17.5$, $t(36) = 3.952$, $p < 0.001$).

3.3. Change in cortisol levels

The results from the repeated measures ANOVA examining CAR_g showed no main effect or interaction between evaluation time and group. Log transformed CAR_i was used in all the analyses to approach a normal distribution with each group. There was a main effect of time ($F(1,64) = 3.223$, $p = 0.077$) indicating a decrease in CAR_i ($T_0 > T_1$), which was statistically significant when cortisol data collected later than 10 a.m. was excluded ($F(1,57) = 4.038$, $p = 0.049$). No group × -time interaction was found in this analysis. No covariate was added to

Table 1
Demographics.

	No-PTSD (N = 25)		PTSD (N = 41)	
Education Level	%	Count	%	Count
Low	20	5	17.1	7
Moderate	48	12	65,9	27
High	32	8	17.1	7
Lifetime Comorbidity**	20	5	97.6	39
Mood disorders	12	3	34.1	14
Anxiety disorders	–	–	12.2	5
Addiction	8	2	2.4	1
Somatic disorders	–	–	2.4	1
> 2 comorbid disorders	–	–	43.9	18
On Antidepressant Medication**	0	0	31.7	13
	M	SD	M	SD
Age	37.3	10.2	38.1	9.9
Years since Last Deployment	5.3	5.9	8.3	8.9
Number of Deployments	2.7	1.5	2.9	3.3
Number of Therapy sessions	–	–	9.5	5.3
ETI-SF				
General trauma	3.6	3.6	5.4	5.4
Physical abuse	0.6	1.1	1	1.4
Emotional abuse	0.6	1.1	1	1.6
Sexual abuse	0.1	0.3	0.4	0.9
MASQ-AD**	45.3	9.9	75.3	12.5

M, mean for all variables except for educational level (ordinal variable) where the median was computed; *SD*, standard deviation; *Education level* Low = equivalent to some years of high school, Moderate = equivalent to finished high school, High = equivalent to some years of college or university education or more; *MASQ-AD*, Anhedonic Depression subscale of the MASQ inventory; T_0, study inclusion; * and ** flag significant group differences at $p < 0.05$ and $p < 0.01$, respectively.

Table 2
Psychopathology in the PTSD group ($N = 41$) before and after treatment.

	T_0		T_1			
CAPS	M	SD	M	SD	*t*	*p*
Total	72.3	14.1	49.4	27.8	5.545	< 0.001
Re-experiencing	23.5	5.3	15.5	10.1	5.045	< 0.001
Avoidance	24.2	9.5	15	10.7	5.194	< 0.001
Hyperarousal	24.5	4.6	18.8	9.2	4.130	< 0.001
MASQ-AD	75.2	12.2	63.9	22.2	3.952	< 0.001
SCID diagnosis	%	Count	%	Count	χ^2	*p*
PTSD	100	41	78	32	10.110	0.002
MD	56.1	23	17.1	7	12.935	< 0.001
Anxiety disorder	34.1	14	21.9	9	1.351	0.326
Somatoform disorder	7.3	3	2.4	1	1.001	0.616

T_0, study inclusion; T_6, follow-up 6–8 months after study inclusion; *MASQ-AD*, Anhedonic Depression subscale of the MASQ inventoryT_0, study inclusion; T_6, follow-up 6–8 months after study inclusion; *MASQ-AD*, Anhedonic Depression subscale of the MASQ inventory.

the ANOVAs as baseline MASQ-AD scores did not hold a linear relationship with any of the CAR indices (Pearson's $r < 0.3$, $p > 0.05$). No baseline correction was applied since there were no significant differences between the PTSD and the non-PTSD group for the CAR_g as well as for the CAR_i ($M = 0.145$ points, $SD = 1.258$, $t(64) = 0.115$, $p > 0.05$) and ($M = 1.547$ points, $SD = 1.404$, $t(64) = 1.102$, $p > 0.05$) respectively. These findings are in line with the meta-analysis by Klaassens et al. (2012) who did not find a difference in cortisol levels between trauma exposed controls and patients with PTSD.

Furthermore, the difference between the CAR measurements on T_0 and T_1 were compared between patients with PTSD in remission (N = 9) and patients with PTSD at T_1 (N = 32). For the CAR_g as well as for the CAR_i, the difference between the two groups was not significant (Cliffs Delta $CAR_g = -0.031$, 95% CI = −0.434;.382, p = 0.9 and Cliffs Delta $CAR_i = -0.083$, 95% CI = −0.347;.484, p = 0.73).

3.4. Regression analysis

Prior to analysis, the predictor variable number of therapy sessions during the study period was logarithmically transformed in order to reduce the extreme skewness. Upon inspection of Pearson's correlations coefficients between ΔCAPS, ΔCAPS B, ΔCAPS C, ΔCAPS D, and log CAR_i, CAR_g at baseline and potential confounds (number of therapy sessions, baseline CAPS scores, baseline MASQ-AD scores, improvement of MASQ-AD scores at follow-up), no multicolinearity was found. After correcting the p-values for multiple testing, a significant correlation was found between CAPS baseline and ΔCAPS D (Pearsons r (n = 41) = 0.414, p-adjusted < 0.05). A significant correlation was found between baseline MASQ-AD and baseline CAPS total (Pearons r (n = 40) = 0.525, p-adjusted < 0.01). Significant correlations were found between improvement in MASQ-AD and ΔCAPS total, and improvement in MASQ-AD and ΔCAPS B (Pearsons r (n = 37) = 0.570, p-adjusted < 0.01; and Pearsons r (n = 37) = 0.606, p-adjusted < 0.001).

In Fig. 1, a scatterplot is given to show that the log CAR_i at baseline was linearly related to changes in overall PTSD severity and separate symptom clusters during treatment.

In Table 3, the results of the sequential multiple regression analysis are presented. At the first step of the model, changes in MASQ-AD together with medication use during the treatment period explained 37% of the variance in symptom reduction (ΔCAPS) (Adjusted R squared = 0.333, $F(2, 34) = 9.991$, p < 0.001). At the second step, there was a positive significant contribution of the logarithmically transformed CAR_i to explaining variance in overall PTSD symptom reduction. The cortisol response to awakening at the beginning of the treatment significantly explained 10.7% of the variance in change in PTSD severity above and beyond the improvement in depressive symptoms and the effect of antidepressant medication (R squared change = 0.107, F change(1, 33) = 6.735, Sig. F change = 0.014).

Table 3
Results of regression analysis ($N = 37$) with outcome variable ΔCAPS.

Step	R^2	Adjusted R^2	R^2 Change	F Change	Predictor variable	Beta
1	0.370	0.333		9.991**	ΔMASQ-AD	0.544**
					Antidepressants (T_0-T_1)	−0.214
2	0.477	0.429	0.107	10.029**	ΔMASQ-AD	0.536**
					Antidepressants (T_0-T_1)	−0.246
					Log CAR_i (T_0)	0.328*

Δ, difference in interview/inventory score at T_1 relative to T_0; * and ** flag significant group differences at $p < 0.05$ and $p < 0.01$, respectively.

Table 4
Test retest reliability coefficients of the individual cortisol concentrations and CAR indices in the pooled group ($n = 66$).

		Mean	SD	Min	Max	r Day 1x Day 2
$CC_{0.}$	T_0	14	3.9	5.8	22	*0.14 N.S.*
	T_6	14.1	5	3.8	27	0.34**
CC_{15}	T_0	18.7	5.5	7.6	32.5	0.36**
	T_6	17.1	6.3	5.7	39	0.41**
CC_{30}	T_0	20.2	7.3	6.2	45	0.51**
	T_6	19	7.3	7.3	50	0.66**
CC_{60}	T_0	18	7	7	41	0.59**
	T_6	16.8	5.9	7.1	31.5	0.40**
CAR_g	T_0	18.5	5.5	7.2	36.3	0.53**
	T_6	17.4	5.7	7.2	36.3	0.59**
CAR_i	T_0	4.5	4.9	−5	19.9	0.35**
	T_6	3.3	4.6	−9.1	14.4	0.48**

The final model explained 42.9% of the variance in improvement in total CAPS scores (Adjusted R squared = 0.429, $F(3, 33) = 10.029$, p < 001). Only the CAR ($beta = 0.328$, $p = 0.014$) and the improvement in anhedonic depression scores ($beta = 0.536$, $p < 0.001$) showed a positive independent contribution to the final model. There were no violations of regression assumptions in this analysis.

4. Discussion

Our first finding was a decreasing CAR (CAR_i) at 6 months after study inclusion that was not specific to PTSD and its treatment, as it was similarly present in healthy, trauma exposed individuals. Interestingly, this effect of an attenuation of the cortisol response in all trauma-exposed subjects fits nicely with the two-stage model of endocrine changes after traumatization (Steudte-Schmiedgen et al., 2016). The two-stage model proposes that after traumatization cortisol levels increase initially, leading to hypersensitivity of the HPA-axis, and eventually leading to reduced long-term basal cortisol secretion. The non-specific CAR decrease across time seen in our study was not due to delay in awakening, since an even stronger effect of time occurred

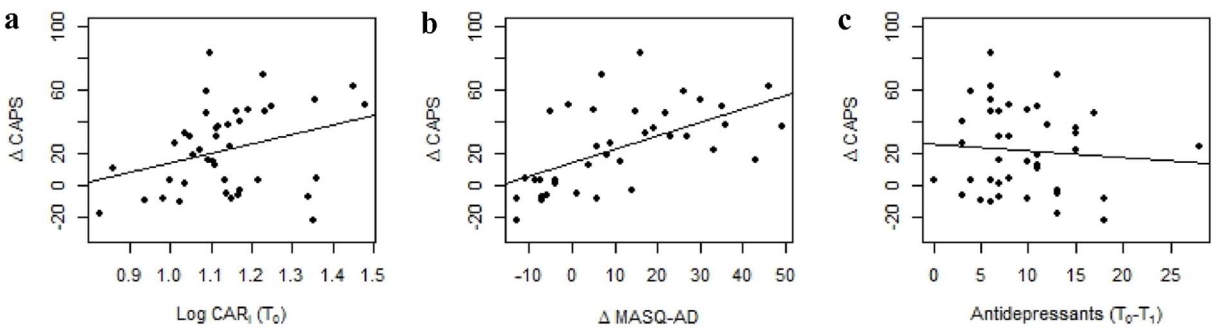

Fig. 1. Association of changes in PTSD symptoms with (a) log Cortisol awakening response at baseline, (b) delta MASQ-AD, and (c) with the change in use of antidepressants and changes in PTSD symptom at follow-up. ΔCAPS, improvement in total CAPS scoresT_0, study inclusion.

when retaining strictly the data collected early. Diminishing HPA-axis reactivity to stress challenge in PTSD has been reported at 12 months of therapy with paroxetine (Vermetten et al., 2006). This was also the case at follow-up after six weeks' exposure therapy (Rothbaum et al., 2014; Norrholm et al., 2016). Remarkably, this decrease was not already statistically significant at 6 weeks, immediately after treatment completion, but only later, at 6 months' follow-up. This is suggestive of a highly time-dependent observation that did not solely reflect the effect of treatment. The decrease of CAR over time in our study may be also due to time dependent effect of trauma on the cortisol secretion. While cortisol levels rise immediately following a stressful event, they may progressively diminish in the aftermath of traumatic stress and eventually rebound to below normal levels (Miller et al., 2007). The mean time since the last traumatic event was approximately five years in both groups in the current study, which implies that participants were confronted with long-term effects of trauma exposure on the HPA-axis. However, there was no linear relationship between the CAR and the number of years after the last deployment in our study. A possible explanation may be that our statistical model did not account for the fact that traumatic events are often repeated and cumulative in military trauma with multiple deployments. A more complex relationship should be assumed when assessing the impact of trauma on HPA-axis function in future studies. Earlier studies offer a mixed picture on this topic. Although Meewisse et al. (2007) found no impact of years since trauma on cortisol secretion in PTSD, a later meta-analysis (Morris et al., 2012) reported diminishing daily basal cortisol output and strengthening of the HPA-feedback function over time which were specific for PTSD and absent in trauma-exposed healthy participants. More longitudinal studies are needed to clarify these discrepancies. In the light of the present findings, caution is warranted when attributing a diminishing HPA-axis response over time exclusively to the effect of treatment in PTSD.

It seems counterintuitive that a high baseline CAR predicted symptom reduction in patients while we found a decrease in CAR over time in both patients and controls. The decrease in the CAR over six months' time can be perceived as habituation to the experimental assessment, and is thus independent of the PTSD diagnosis. On the other hand, in patients with PTSD, patients with a relatively low CAR showed lower symptom reduction after six months. Thus the lower CAR in patients with PTSD could indicate more severe disturbance of the physiological stress system in these patients, who will therefore respond less well to treatment and show less symptom reduction.

Upon inspecting the difference in CAR_i response, no difference was found between patients with a PTSD diagnosis at T_1 and patients where PTSD was in remission at T_1. These findings are not in concordance with the findings of Olff et al. (2007), who found that treatment responders (n = 15) showed an increase and treatment non-responders (n = 6) showed a decrease in HPA-axis activity. In Olff et al. (2007) the majority of participants were female and comorbidity was excluded, while in the current study only male participants were included and the majority of patients had comorbid MDD. Given that the HPG and HPA axis strongly influence each other (Reijnen et al., 2015) and depression also majorly affects the HPA axis (Wingenfeld and Wolf, 2015), these factors may explain the difference in results.

The results from the regression analysis might point into the direction on an emerging putative role for the cortisol awakening response as a predictive biomarker of symptom reduction in male individuals with combat-related PTSD. A robust HPA-axis responsivity as estimated by CAR_i at the beginning of the treatment is associated with higher symptom alleviation after six months of trauma-focused therapy, even after controlling for improvement of depressive symptoms and concomitant use of antidepressant medication. This finding has not been detected in earlier studies on the subject. A similar association between CAR_i and post-treatment PTSD severity has been not been detected in a sample with mostly female participants (N = 29) (Pacella et al., 2014). Pacella et al. did find a significant gender by treatment response interaction where female treatment responders displayed higher cortisol reactivity following treatment thanthan female non-responders.

In the present study, CAR_i explained 10.7% of the variance in overall improvement in PTSD severity. This effect size can be considered moderate compared to the predictive value of other glucocorticoid biomarkers. The reported effect sizes of the glucocorticoid biomarkers as mentioned in the systematic review by Colvonen et al. (2017) vary between 12 and 25% (urinary cortisol excretion predicted CAPS scores post-treatment (r = 0.35) (Yehuda et al., 2014)) and 35% (CAR response following dexamethasone administration were associated with PTSD symptom reduction (Nijdam et al., 2015)). It must be noted that quite a substantial number of studies in this structural review did not report effect sizes (Colvonen et al., 2017).

Robust responsivity of the HPA-axis may be required during trauma-focused therapy. Adaptation to subjectively novel situations elicited in therapy, such as re-evaluation of traumatic event and trauma-related cues, may take place efficiently in subjects who show strong cortisol oscillations indicative of a dynamic and versatile transcriptional system (Walker et al., 2012). The HPA axis shows a dynamic ultraradian rhythm that is manifested by oscillating levels of ACTH and glucocorticoids. At the cellular level, these oscillations in glucocorticoids may activate glucocorticoid-responsive genes by binding to glucocorticoid receptors associated with these genes. Thus oscillations in glucocorticoid levels are necessary to maintain homeostasis. When these oscillations do not occur, but are replaced by a constant level of glucocorticoids, abnormal gene expression and detrimental response to stress result. Thus, a high CARi may be indicative of strong cortisol oscillations and thus a versatile and dynamic transcriptional system. An alternative explanation can be that the CAR (CAR_i) is a proxy for some other predictor of symptom reduction.

However, among the possible predictors of symptom reduction that we included, CAR_i showed moderate correlation with the basal cortisol measure CAR_g. Consistent with earlier findings (Pacella et al., 2014), CAR_g showed no significant association with symptom reduction in our study. Other indices of HPA-axis reactivity, such as the dexamethasone-suppressed CAR_g (Nijdam et al., 2015) and the cortisol response to trauma cues (Rauch et al., 2015) at entry into treatment have been demonstrated to positively influence treatment outcome in PTSD. Moreover, higher cortisol responses in the acute aftermath of trauma exposure may lower the risk of PTSD at long term follow-up (Walsh et al., 2013). Preliminary research also suggests lower risk for PTSD symptoms in those who received cortisol during cardiac surgery comparing to those who received placebo (Schelling et al., 2004). These data are convergent with the present results, suggesting that a strong HPA-axis reactivity to different stress cues may facilitate recovery from trauma, as well as recovery from PTSD during treatment. The question arises whether combining the CAR_i with other objective biomarkers, such as the startle response to trauma cues (Norrholm et al., 2016) or specific neuroimaging findings (Kennis et al., 2015; van Rooij et al., 2015a, 2015b) could provide a more accurate prediction of treatment outcome. This possibility should be explored in future research. There are several limitations in the present study. To be noted, the global severity of PTSD before treatment and the number of attended trauma-focused therapy sessions did not relate to treatment outcome in our patient sample, as expected based on previous results (Haagen et al., 2015; Karatzias et al., 2007). These discrepancies could be explained by low variance in PTSD severity at baseline where severe symptomatology prevailed, and by the contribution of adjuvant interventions other than psychotherapy (e.g., medication monitoring sessions) to the treatment outcome. Another limitation is that no objective monitoring of awakening time has been employed and sampling could have been delayed. It is therefore possible that higher CAR_i values are also indicative of slower recovery of the cortisol response rather than of robust HPA-reactivity. To account for this possibility, the time dependent variation of individual sample concentrations should be analyzed

Table A1
Test-retest reliability coefficients of the individual cortisol concentrations and CAR indices in the pooled group ($N = 66$).

		M	SD	Min	Max	r Day 1 × day 2
$CC_{0.}$	T_0	14	3.9	5.8	22	*0.14 N.S.*
	T_6	14.1	5	3.8	27	0.34**
CC_{15}	T_0	18.7	5.5	7.6	32.5	0.36**
	T_6	17.1	6.3	5.7	39	0.41**
CC_{30}	T_0	20.2	7.3	6.2	45	0.51**
	T_6	19	7.3	7.3	50	0.66**
CC_{60}	T_0	18	7	7	41	0.59**
	T_6	16.8	5.9	7.1	31.5	0.40**
CAR_g	T_0	18.5	5.5	7.2	36.3	0.53**
	T_6	17.4	5.7	7.2	36.3	0.59**
CAR_i	T_0	4.5	4.9	−5	19.9	0.35**
	T_6	3.3	4.6	−9.1	14.4	0.48**

CC, mean cortisol concentration (nmol/L) across Day 1 and Day 2 at each sampling point (0, 15, 30 and 60 min after awakening); CAR_g, area under the curve with respect to the ground; CAR_i, area under the ground with respect to increase; $CAR_g = (CC_0 + CC_{15}) \times 1/2 \times 1/4 + (CC_{15} + CC_{30}) \times 1/2 \times 1/4 + (CC_{30} + CC_{60}) \times 1/2 \times 1/2$; $CAR_i = CAR_g - CC_0$; * and ** flag significant correlations at $p < 0.05$ and at $p < 0.005$, respectively; *N.S.* = not significant, $p > 0.05$.

in addition to area under the curve estimates in future studies. Inherent to the cohort design, treatment interventions in the present study were not standardized. Importantly, however, observing effects of 'treatment as usual' has the advantage of being representative of the clinical practice. Clinical trials with a placebo intervention are needed to disentangle treatment and other time dependent effects. Because our participants scarcely reported childhood sexual abuse, the present results cannot account for the whole spectrum of sexual trauma in PTSD. Some other possible CAR confounds (*e.g.,* job stress, fatigue and general life stress; Chida and Steptoe, 2009) should also be controlled for in future studies. Another limitation of this study is the small sample size. Due to the limited number of participants we were only able to investigate the effect of three covariates in the regression analysis. It would be interesting to study the effect of other covariates in a larger study sample to find other noteworthy relations that could explain the variance in the CAPS change scores. Possible influences of intensity of the traumatic event, age at trauma, early life trauma or other biomarkers should thus be examined in future studies. Furthermore, it is possible that the participants in the current study have been exposed to traumatic events after combat exposure and before inclusion in the study. It would be interesting to investigate the influence of traumatic events after combat exposure and before treatment on the PTSD diagnosis in future studies. It is possible that these events explain some of the variance in PTSD symptoms and symptom reduction between patients.

All patients received trauma-focused psychotherapy (treatment-as-usual) which consisted of EMDR and/or trauma-focused CBT. Our design cannot separate effects of different types of treatment, since this was an observational study and we did not randomize participants into specific treatment settings. Although treatment was tailored to each patients' needs, all patients received protocolized trauma-focused therapy. In the current study we investigated symptom reduction, which has more variance than the dichotomized variable. where patients in remission are compared to patients who still have PTSD. This explains why no differences in CAR response were found between treatment responders and treatment non-responders while the CAR did explain 10.7% of the variance in symptom reduction in the current study.

5. Conclusion

Here we report longitudinal data supporting pre-treatment cortisol awakening response as a potential predictor of symptom reduction in male individuals with combat-related PTSD. As the CAR indices are well-validated and easily acquired in the clinical practice, this finding, when replicated, can be of clinical significance. More objective biomarkers need to be included in future prediction models in order to achieve accurate estimates of treatment success.

Declaration of interest

This research was funded by the Dutch Ministry of Defence.

Appendix A

A positive awakening response was seen in 77.6% of the CAR_i computed on the four sampling days. Only 43.2% of the total CAR assessments (4 × 66 assessments) showed a robust increase of cortisol concentration of 50% or more at 30 min after awakening. The intra-individual stability of individual cortisol concentrations varied largely across the two consecutive sampling days (see Table A1).

References

American Psychiatric Association, 2000. Diagnostic and Statistical Manual, 4th Edn, Text Revision (DSM-IV-TR). Am Psychiatr Assoc Washingt.

American Psychiatric Association. AP, Association. AP, Force. D–5 T (2013). Diagnostic and statistical manual of mental disorders: DSM-5. at http://dsm.psychiatryonline.org/book.aspx?bookid=556.

Baker, D.G., West, S.A., Nicholson, W.E., Ekhator, N.N., Kasckow, J.W., Hill, K.K., et al., 1999. Serial CSF corticotropin-releasing hormone levels and adrenocortical activity in combat veterans with posttraumatic stress disorder. Am. J. Psychiatry 156, 585–588.

Benajmini, Y., Hochberg, Y., 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J. R. Stat. Soc. B 57, 289–300.

de Beurs, E., den Hollander-Gijsman, M.E., Helmich, S., Zitman, F.G., 2007. The tripartite model for assessing symptoms of anxiety and depression: psychometrics of the Dutch version of the mood and anxiety symptoms questionnaire. Behav. Res. Ther. 45, 1609–1617.

Bisson, J., Andrew, M., 2009. Psychological treatment of post-traumatic stress disorder (PTSD) (review), Cochrane database Syst Rev CD00338810.1002/14651858.CD003388. pub2.

Blake, D.D., Weathers, F.W., Nagy, L.M., Kaloupek, D.G., Gusman, F.D., Charney, D.S., et al., 1995. The development of a clinician-administered PTSD scale. J. Traum. Stress 8, 75–90.

Bradley, R., Greene, J., Russ, E., Dutra, L., Westen, D., 2005. A multidimensional meta-analysis of psychotherapy for PTSD. Am. J. Psychiatry 162, 214–227.

Bremner, J.D., Bolus, R., Mayer, E.A., 2007. Psychometric properties of the early trauma inventory-self report. J. Nerv. Ment. Dis. 195, 211–218.

Buckby, J., Yung, A.R., Cosgrave, E.M., Killackey, E.J., 2007. Clinical utility of the Mood and Anxiety Symptom Questionnaire (MASQ) in a sample of young help-seekers. BMC Psychiatry 7, 50.

Carpenter, L.L., Carvalho, J.P., Tyrka, A.R., Wier, L.M., Mello, A.F., Mello, M.F., et al., 2007. Decreased adrenocorticotropic hormone and cortisol responses to stress in healthy adults reporting significant childhood maltreatment. Biol. Psychiatry 62,

1080–1087.
Chida, Y., Steptoe, A., 2009. Cortisol awakening response and psychosocial factors: a systematic review and meta-analysis. Biol. Psychol. 80, 265–278.
Cliff, N., 1996. Ordinal Methods for Behavioral Data Analysis. Psychology Press.
Colvonen, P., Glassman, L., Crocker, L., Buttner, M., Orff, H., Schiehser, D., et al., 2017. Pretreatment biomarkers predicting PTSD psychotherapy outcomes: a systematic review. Neurosci. Biobehav. Rev.
Corp, I., 2013. IBM SPSS Statistics for Windows, Version 22.0.
Elzinga, B.M., Roelofs, K., Tollenaar, M.S., Bakvis, P., van Pelt, J., Spinhoven, P., 2008. Diminished cortisol responses to psychosocial stress associated with lifetime adverse events. A study among healthy young subjects. Psychoneuroendocrinology 33, 227–237.
Fekedulegn, D.B., Andrew, M.E., Burchfiel, C.M., Violanti, J.M., Hartley, T.A., Charles, L.E., et al., 2015. Area under the curve and other summary indicators of repeated waking cortisol measurements. Psychosom. Med. 69, 651–659.
First, M.B., Spitzer, R.L., Gibbon, M., Williams, J.B.W., 1996. Structured Clinical Interview for DSM-IV Axis I Disorders, Clinician Version (SCID-CV). American Psychiatric Press, Inc., Washington, D.C.
Gerardi, M., Rothbaum, B.O., Astin, M.C., Kelley, M., 2010. Cortisol response following exposure treatment for PTSD in rape victims. J. Aggress. Maltreat. Trauma 19, 349–356.
Goenjian, A.K., Pynoos, R.S., Steinberg, A.M., Endres, D., Abraham, K., Geffner, M.E., et al., 2003. Hypothalamic-pituitary-adrenal activity among Armenian adolescents with PTSD symptoms. J. Traum. Stress 16, 319–323.
Green, S.B., 1991. How many subjects does it take to do a regression analysis. Multivariate Behav. Res. 26, 499–510.
Haagen, J., Smid, G., Knipscheer, J., Kleber, R., 2015. The efficacy of recommended treatments for veterans with PTSD: a metaregression analysis. Clin. Psychol. Rev. 40, 184–194.
Heim, C., Nemeroff, C.B., 2009. Neurobiology of posttraumatic stress disorder. CNS Spectr. 14, 13–24.
Hoaglin, D.C., Iglewicz, B., 1987. Fine-tuning some resistant rules for outlier labeling. Source J. Am. Stat. Assoc. 82126255, 1147–1149.
Karatzias, A., Power, K., McGoldrick, T., Brown, K., Buchanan, R., Sharp, D., et al., 2007. Predicting treatment outcome on three measures for post-traumatic stress disorder. Eur. Arch. Psychiatry Clin. Neurosci. 257, 40–46.
Kennis, M., van Rooij, S.J.H., Tromp, D.P.M., Fox, A.S., Rademaker, A.R., Kahn, R.S., et al., 2015. Treatment outcome-related white matter differences in veterans with posttraumatic stress disorder. Neuropsychopharmacology 40, 2434–2442.
Klaassens, E.R., van Noorden, M.S., Giltay, E.J., van Pelt, J., van Veen, T., Zitman, F.G., 2009. Effects of childhood trauma on HPA-axis reactivity in women free of lifetime psychopathology. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 33, 889–894.
Klaassens, E.R., Giltay, E.J., Cuijpers, P., van Veen, T., Zitman, F.G., 2012. Adulthood trauma and HPA-axis functioning in healthy subjects and PTSD patients: a meta-analysis. Psychoneuroendocrinology 37, 317–331.
de, C.S., Vermetten, E., Heijnen, C.J., Geuze, E., Lentjes, E.G.W.M., Westenberg, H.G.M., 2007. Enhanced cortisol suppression in response to dexamethasone administration in traumatized veterans with and without posttraumatic stress disorder. Psychoneuroendocrinology 32, 215–226.
Meewisse, M., Reitsma, J.B., de Vries, G.-J., Gersons, B.P.R., Olff, M., 2007. Cortisol and post-traumatic stress disorder in adults: systematic review and meta-analysis. Br. J. Psychiatry 191, 387–392.
Meinlschmidt, G., Heim, C., 2005. Decreased cortisol awakening response after early loss experience. Psychoneuroendocrinology 30, 568–576.
Miller, G., Chen, E., Zhou, E., 2007. If it goes up, must it come down? Chronic stress and the hypothalamic-pituitary-adrenocortical axis in humans. Psychol. Bull. 133, 25–45.
Morris, C., Rao, U., 2013. Psychobiology of PTSD in the acute aftermath of trauma: integrating research on coping, HPA function and sympathetic nervous system activity. Asian J. Psychiatry 6, 3–21.
Morris, M.C., Compas, B.E., Garber, J., 2012. Relations among posttraumatic stress disorder, comorbid major depression, and HPA function: a systematic review and meta-analysis. Clin. Psychol. Rev. 32, 301–315.
National Institute for Health and Care Excellence, 2005. Posttraumatic Stress Disorder: Management. Guidance and Guidelines, London: NICE.
Nijdam, M.J., Van Amsterdam, J.G.C., Gersons, B.P.R., Olff, M., 2015. Dexamethasone-suppressed cortisol awakening response predicts treatment outcome in posttraumatic stress disorder. J. Affect. Disord. 184, 205–208.
Norrholm, S.D., Jovanovic, T., Gerardi, M., Breazeale, K.G., Price, M., Davis, M., et al., 2016. Baseline psychophysiological and cortisol reactivity as a predictor of PTSD treatment outcome in virtual reality exposure therapy. Behav. Res. Ther. 82, 28–37.
Olff, M., de Vries, G., Güzelcan, Y., Assies, J., Gersons, B., 2007. Changes in cortisol and DHEA plasma levels after psychotherapy for PTSD. Psychoneuroendocrinology 32, 619–626.
Pacella, M.L., Feeny, N., Zoellner, L., Delahanty, D.L., 2014. The impact of PTSD treatment on the cortisol awakening response. Depress. Anxiety 31, 862–869.
Patel, R.S., Shaw, S.R., MacIntyre, H., McGarry, G.W., Wallace, A.M., 2004. Production of gender-specific morning salivary cortisol reference intervals using internationally accepted procedures. Clin. Chem. Lab. Med. 42, 1424–1429.
Pruessner, J.C., Wolf, O.T., Hellhammer, D.H., Buske-Kirschbaum, A., Von Auer, K., Jobst, S., et al., 1997. Free cortisol levels after awakening: a reliable biological marker for the assessment of adrenocortical activity. Life Sci. 61, 2539–2549.
R Core Team, 2016. A Language and Environment for Statistical Computing. https://www.r-project.org/.
Rauch, S.A.M., King, A.P., Abelson, J., Tuerk, P.W., Smith, E., Rothbaum, B.O., et al., 2015. Biological and symptom changes in posttraumatic stress disorder treatment: a randomized clinical trial. Depress. Anxiety 32, 204–212.
Reijnen, A., Geuze, E., Vermetten, E., 2015. The effect of deployment to a combat zone on testosterone levels and the association with the development of posttraumatic stress symptoms: a longitudinal prospective Dutch military cohort study. Psychoneuroendocrinology 51, 525–533.
van Rooij, S.J.H., Kennis, M., Sjouwerman, R., van den Heuvel, M.P., Kahn, R.S., Geuze, E., 2015a. Smaller hippocampal volume as a vulnerability factor for the persistence of post-traumatic stress disorder. Psychol. Med. 1–10. http://dx.doi.org/10.1017/S0033291715000707.
van Rooij, S.J.H., Kennis, M., Vink, M., Geuze, E., 2015b. Predicting treatment outcome in PTSD: a longitudinal functional MRI study on trauma-unrelated emotional processing. Neuropsychopharmacology 41, 1156–1165.
Rothbaum, B.O., Price, M., Jovanovic, T., Norrholm, S.D., Gerardi, M., Dunlop, B., et al., 2014. A randomized, double-blind evaluation of D-cycloserine or alprazolam combined with virtual reality exposure therapy for posttraumatic stress disorder in Iraq and Afghanistan War veterans. Am. J. Psychiatry 171, 640–648.
Schelling, G., Kilger, E., Roozendaal, B., de Quervain, D.J., Briegel, J., Dagge, A., et al., 2004. Stress doses of hydrocortisone, traumatic memories, and symptoms of posttraumatic stress disorder in patients after cardiac surgery: a randomized study. Biol. Psychiatry 55, 627–633.
Stalder, T., Kirschbaum, C., Kudielka, B.M., Adam, E.K., Pruessner, J.C., Wüst, S., et al., 2015. Assessment of the cortisol awakening response: expert consensus guidelines. Psychoneuroendocrinology 63, 414–432.
Steudte-Schmiedgen, S., Kirschbaum, C., Alexander, N., Stalder, T., 2016. An integrative model linking traumatization, cortisol dysregulation and posttraumatic stress disorder: insight from recent hair cortisol findings. Neurosci. Biobehav. Rev. 69, 124–135.
Vermetten, E., Vythilingam, M., Schmahl, C., De, Kloet C., Southwick, S.M., Charney, D.S., et al., 2006. Alterations in stress reactivity after long-term treatment with paroxetine in women with posttraumatic stress disorder. Ann. N. Y. Acad. Sci. 1071, 184–202.
Wahbeh, H., Oken, B.S., 2013. Salivary cortisol lower in posttraumatic stress disorder. J. Traum. Stress 26, 241–248.
Walker, J.J., Spiga, F., Waite, E., Zhao, Z., Kershaw, Y., Terry, J.R., et al., 2012. The origin of glucocorticoid hormone oscillations. PLoS Biol. 10.
Walsh, K., Nugent, N.R., Kotte, A., Amstadter, A.B., Wang, S., Guille, C., et al., 2013. Cortisol at the emergency room rape visit as a predictor of PTSD and depression symptoms over time. Psychoneuroendocrinology 38, 2520–2528.
Watson, D., Weber, K., Assenheimer, J.S., Clark, L., Strauss, M.E., McCormick, R., 1995. Testing a tripartite model: I. Evaluating the convergent and discriminant validity of anxiety and depression symptom scales. J. Abnorm. Psychol. 104, 3–14.
Weathers, F.W., Keane, T.M., Davidson, J.R.T., 2001. Clinician-administered PTSD scale: a review of the first ten years of research. Depress. Anxiety 13, 132–156.
Wilcox, R., 2016. Introduction to Robust Estimation and Hypothesis Testing. Academic press.
Wilhelm, I., Born, J., Kudielka, B.M., Schlotz, W., Wüst, S., 2007. Is the cortisol awakening rise a response to awakening? Psychoneuroendocrinology 32, 358–366.
Wingenfeld, K., Wolf, O.T., 2015. Effects of cortisol on cognition in major depressive disorder, posttraumatic stress disorder and borderline personality disorder – 2014 Curt Richter Award Winner. Psychoneuroendocrinology 51, 282–295.
Wust, S., Wolf, J., Hellhammer, D., 2000. The cortisol awakening response-normal values and confounds. Noise Health 2, 79–88.
Yehuda, R., Golier, J.A., Kaufman, S., 2005. Circadian rhythm of salivary cortisol in Holocaust survivors with and without PTSD. Am. J. Psychiatry 162, 998–1000.
Yehuda, R., Pratchett, L., Elmes, M., Lehrner, A., Daskalakis, N., Koch, E., et al., 2014. Glucocorticoid-related predictors and correlates of post-traumatic stress disorder treatment response in combat veterans. Interface Focus 4, 20140048.