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Salivary Cortisol and Alpha-Amylase in Posttraumatic Stress Disorder and Their Potential Role in the Evaluation of Cognitive Behavioral Treatment Outcomes

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Alterations in HPA-axis and autonomic nervous system activity have been associated with posttraumatic stress disorder (PTSD) development and maintenance and are potentially associated with trauma-focused cognitive behavioral therapy (TF-CBT) outcomes. We examined the role of salivary cortisol (sCort) and alpha-amylase (sAA) in PTSD and TF-CBT outcomes in German Armed Forces service members ($N = 100$). Participants categorized as PTSD patients ($n = 39$), previously deployed healthy controls ($n = 33$), and nondeployed healthy controls ($n = 28$) provided diurnal profiles of sCort and sAA; PTSD patients provided samples before, immediately after, and 3 months after an internet-based TF-CBT intervention. No group differences emerged regarding total daily sCort and sAA output or daily slopes, ps = .224–.897, fs = 0.05–0.24. Participants with PTSD demonstrated a significantly attenuated sCort awakening response compared to deployed, $p = .021$, $d = 0.59$, but not nondeployed controls, $p = .918$, $d = 0.08$. Moreover, a significantly steeper sAA awakening response emerged in PTSD patients, $p = .034$, $d = 0.67$, and deployed controls, $p = .014$, $d = 0.80$, compared to nondeployed controls. From pretreatment to posttreatment ($n = 21$) and posttreatment to follow-up ($n = 14$), stable sCort, ps = .282–.628, fs = 0.34–0.49, and sAA concentrations, ps = .068–.758, fs = 0.24–1.13 paralleled a nonsignificant treatment effect. Both PTSD and trauma exposure were associated with alterations in awakening responses, but further investigation is needed to determine whether the observed correspondence remains when PTSD symptoms significantly decline.

Posttraumatic stress disorder (PTSD) is characterized by intrusions, avoidance, negative alterations in cognition and mood, and alterations in arousal and reactivity that cause persistent stress in affected individuals (American Psychiatric Association, 2013). Investigating biological systems that are involved in the pathogenesis and perpetuation of these symptoms can help researchers and clinicians gain a more comprehensive understanding of the disorder (Fischer & Ehlert, 2019). Researchers have repeatedly postulated that PTSD-related alterations in the

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stress-responsive hypothalamic–pituitary–adrenal (HPA) axis activity exist, especially regarding its end-product cortisol. In particular, evidence points toward higher glucocorticoid receptor sensitivity in PTSD (Daskalakis et al., 2013), which leads to enhanced negative feedback sensitivity (de Kloet et al., 2006) and eventually attenuated basal cortisol levels, although reports of the latter have been less consistent. Given the role of cortisol in memory processes, lower basal cortisol concentrations are thought to impede inhibition of the fear memory, leading to characteristic PTSD symptoms, such as reexperiencing of the traumatic event (e.g., flashbacks, intrusive thoughts; de Quervain et al., 2017). The results of meta-analytic evidence, however, has indicated that the hypocortisolaemic pattern is only found in certain subgroups (Meewisse et al., 2007; Morris et al., 2012) or is undetectable (Klaassens et al., 2012), giving rise to a search for moderators to explain the heterogeneity of the study findings (Schumacher et al., 2019). One such moderator could be the time elapsed since exposure to a traumatic event. In their model, Steudte-Schmiedgen et al. (2016) proposed that trauma-exposed individuals' initial upregulation of basal cortisol secretion might turn into a dose-dependent decrease, resulting in diminished circulating basal cortisol levels. Further, this hypocortisolaemic pattern has been suggested as a risk factor for PTSD development. The empirical support for this presumption is currently mixed, with studies linking higher basal (e.g., Cieslak et al., 2011; Pacella et al., 2017) and reactive (e.g., Schultebraucks et al., 2019) cortisol levels or lower basal cortisol levels (e.g., Ehring et al., 2008; Mouthaan et al., 2014), measured posttrauma, to the subsequent development of PTSD symptoms. Consequently, depending on the timing of the measurement, aberrant cortisol levels could also be prevalent in trauma-exposed but healthy individuals. Studies that have allowed for a more precise differentiation of the impact of trauma exposure and PTSD symptoms on cortisol concentrations are still lacking.

Chronic autonomic nervous system (ANS) hyperactivity, particularly of its sympathetic branch, has been considered to be a key pathological mechanism in PTSD (Wolf, 2008). Reduced heart rate variability (Alvares et al., 2016; Chalmers et al., 2014) and elevated noradrenaline concentrations (Kalk et al., 2011) have been identified as correlates of PTSD. Moreover, in an initial study on salivary alpha-amylase (sAA), a surrogate marker of ANS activity, Thoma et al. (2012) observed an altered circadian secretion pattern in PTSD patients compared to non–trauma-exposed individuals, supporting the hypothesis of a dysregulated noradrenergic system in PTSD. At the same time, trauma exposure also appears to be linked with changes in sympathetic activity, with varying findings for different peripheral markers: Meta-analytic evidence suggests that a higher heart rate, but not higher blood pressure, in trauma-exposed individuals might be associated with the severity of subsequent PTSD symptoms (Morris et al., 2016). In addition, Rombold-Bruehl et al. (2019) found that lower heart rate variability was also found to be a predictor of the number of intrusions in an analog trauma study, underscoring its potential as a biological vulnerability factor for subsequent posttrauma PTSD symptoms. Overall, the findings from these studies suggest that the utility of HPA-axis and ANS markers to distinguish between PTSD patients and trauma-exposed and non–trauma-exposed healthy individuals has not yet been conclusively resolved due to heterogeneous study results, insufficient consideration of past trauma exposure among healthy individuals, or the preliminary nature of the evidence. Furthermore, few studies to date have investigated both stress systems in parallel, using single measures of ANS activity in the blood (Inslicht et al., 2006) or a summary measure retrieved from urine sampled over 12 h (Glover & Poland, 2002) or 24 h (Wingenfeld et al., 2015). With the quick and noninvasive measurement of sAA, it is possible to study both systems simultaneously and more in-depth, thereby allowing researchers to gain a better understanding of the coordinated function and dysfunction between the HPA axis and ANS (Ali & Nater, 2020).

Besides their potential for indicating PTSD-related dysregulation, HPA-axis and ANS markers could also play an important role in psychotherapeutic treatment. Trauma-focused cognitive behavioral therapy (TF-CBT) is the gold-standard treatment for PTSD (National Institute for Clinical Excellence, 2018). New ways of disseminating TF-CBT have been introduced in the last decade, with evidence showing that TF-CBT can effectively be administered via the internet (Kuester et al., 2016). The clinical evaluation of such treatments can be supported by incorporating biological markers, which may indicate whether treatment effects on PTSD symptoms can be reflected on a biological and, thus, more objective level. Thereby, biological markers could contribute to a more differentiated evaluation of treatment outcomes by complementing self-report symptom measures and clinical rating scales (Fischer & Ehlert, 2019; Yehuda et al., 2006).

The findings from a systematic review of the literature revealed that thus far, few studies have examined HPA-axis or ANS activity over a full course of PTSD treatment (Schumacher et al., 2018). Only three studies have investigated basal cortisol concentrations before and after treatment. The results of two of these studies demonstrated significant changes in treatment responders, although their direction remained ambiguous (Gerardi et al., 2010; Olff et al., 2007). Furthermore, in two studies, the cortisol awakening response (CAR) was found to predict symptom reduction, with a lower CAR (Rapcencu et al., 2017) or a less dexamethasone suppressed CAR (Nijdam et al., 2015) predicting poorer treatment outcomes.

To elucidate the role of the HPA axis and ANS relative to trauma exposure as well as in PTSD and PTSD treatment, we integrated markers of both systems, specifically basal salivary cortisol (sCort) and sAA, in a randomized controlled trial (RCT) that aimed to evaluate the efficacy of a 5-week internet-based TF-CBT intervention for German Armed Forces service members who were seeking treatment for PTSD. In brief, participants were randomly assigned to an immediate treatment group (IT) or a waitlist control group (WL); participants assigned to the WL condition received the TF-CBT

intervention after the 6-week waiting period. The therapist-guided internet-based TF-CBT intervention was based on 10 writing assignments, followed by written, individualized feedback from a study therapist. An analysis of the efficacy of the treatment revealed no significant change in mean PTSD severity from pre- to posttreatment and to a 3-month follow-up (for details regarding the treatment and its evaluation, see Niemeyer et al., 2020). For cross-sectional analyses of the biological markers, two healthy control groups were additionally recruited, including (a) active service members who reported deployment-related trauma exposure (deployed CG) that qualified for Criterion A according to the PTSD criteria outlined in the *Diagnostic and Statistical Manual of Mental Disorders* (*DSM-5*) and (b) service members who had never been deployed abroad and reported no exposure to *DSM-5* Criterion A traumatic events (nondeployed CG).

The present study was the first PTSD psychotherapy study of which we are aware to investigate markers of both stress systems in parallel. This secondary analysis aimed at differentiating the impact of trauma exposure and PTSD on HPA-axis and ANS markers as well as examining the potential contribution of these markers to the clinical evaluation of TF-CBT. We expected that cross-sectionally, PTSD patients would show lower sCort (i.e., flattened awakening response and slope, lower daily output) and higher sAA concentrations (i.e., steeper awakening response and slope, higher daily output) compared to individuals in the HC groups, with the highest sCort and lowest sAA in nondeployed controls. Longitudinally, we intended to describe sCort and sAA courses during the TF-CBT intervention and explore whether PTSD symptoms, sCort, and sAA were related over the course of treatment.

Method

Participants

Participants were recruited via printed advertisements in military journals, military health service centers, and postdeployment seminars and military mental health conferences. Moreover, advertisements were distributed on websites. Overall, 100 male German Armed Forces service members were included. Of these individuals, 39 were active and former service members with clinical or subclinical PTSD who received the TF-CBT. In addition, 33 active service members were included in the deployed CG, and 28 were included in the nondeployed CG. Participants in the healthy CGs did not fulfill the criteria for PTSD or any other mental disorder. All participants provided written informed consent.

For the longitudinal part of the study (i.e., the RCT), we conducted an a priori power analysis based on the results of previous meta-analyses. The results of the power analysis indicated that at least 100 participants with PTSD were needed to estimate a moderate between-group difference with a Cohen's d effect size of 0.7 with two groups, the power set at .95, and alpha set at .05. For the cross-sectional portion of the study, moderate between-group differences (i.e., $f = 0.25$) were estimated with three groups, the power set at .80, and alpha set at 0.05. These results revealed a required sample size of 158 participants; hence, we aimed to include an additional 58 healthy control participants (i.e., 29 participants per group).

The study was approved by the Institutional Ethics Committee of the Department of Education and Psychology at Freie Universität Berlin, Germany (reference numbers: 85/2014, 116/2016) after internal approval by the German Armed Forces. The study was performed in accordance with the ethical standards as laid down in the latest version of the Declaration of Helsinki.

Procedure

Data were collected between July 2016 and July 2018. All participants attended face-to-face diagnostic assessments at the German Armed Forces Hospital in Berlin, Germany. The IT and WL groups participated in pre- and posttreatment assessments as well as a 3-month follow-up assessment. Participants in the WL group received an additional pre–wait-time assessment 6 weeks before the pretreatment assessment. The control groups attended one diagnostic assessment. Saliva samples were collected for the analysis of sCort and sAA immediately after each diagnostic assessment.

For a detailed description of the sampling protocol, see the Supplementary Materials. All participants were provided with the same detailed instructions in oral and written forms. The SaliCap® system (IBL; Hamburg, Germany) was used for saliva collection. Considering both minimal participant burden due to repeated saliva sampling (Hoyt et al., 2016) and an adequate level of temporal resolution of the diurnal profile (Granger et al., 2007; Stalder et al., 2016), the participants collected 12 saliva samples (i.e., six per day) over 2 consecutive (Hellhammer et al., 2007) workdays (Skoluda et al., 2016). Each day, samples were taken immediately upon awakening, 30 min later, and at 11 a.m., 2 p.m., 6 p.m., and 9 p.m. Participants were asked to follow their normal daily routines but avoid any behavior that could impact sCort or sAA concentrations for 1 hour before sampling, including eating, drinking anything other than water, smoking, sleeping (except before the awakening samples), consuming caffeine or alcohol, and engaging in physical exercise. In addition, they were instructed not to drink alcohol and to refrain from physical exercise for 24 hr before the first sampling day. To assess compliance, participants filled out a diary. After collection, saliva samples were mailed to the study team.

Before and after sampling, SaliCaps® tubes were weighed to assess the saliva flow rate (SFR). The samples were stored and gradually frozen at -80°C at the German Armed Forces hospital and sent to the laboratory for analysis.

Measures

PTSD Symptoms and Diagnosis

The Clinician-Administered PTSD Scale for *DSM-5* (CAPS-5; Weathers et al., 2018) was used to diagnose PTSD. This interview is used to assess all PTSD domains and includes all the *DSM-5* criteria for PTSD. The items represent past-month severity of PTSD symptoms, with clinicians rating each item on a 5-point scale ranging from 0 (*not present*) to 4 (*extremely pronounced*). A sum score can be calculated (range: 0 – 80) to indicate PTSD severity, and subscale scores reflect symptom severity in each PTSD symptom cluster (i.e., Criterion B: Rexperiencing, 0–20 points, Criterion C: Avoidance, 0–8 points, Criterion D: Negative Alterations in Cognition and Mood, 0–28 points, Criterion E: Alterations in Arousal and Activity, 0–24 points). The CAPS-5 has demonstrated good psychometric properties (Weathers et al., 2018).

Psychological Disorders

The Mini-International Neuropsychiatric Interview (M.I.N.I.; Sheehan et al., 1998) is a structured clinician-administered diagnostic interview that was used to diagnose comorbid disorders and exclude the presence of mental disorders in the control groups. The interview consists of 16 chapters. Clinicians rate the presence of symptoms of psychological disorders, with items based on the *DSM-IV* and *International Classification of Diseases and Disorders* (10th rev.) criteria. Each item is rated with "yes" if the symptom is present and "no" if the symptom is not present. At the end of each chapter, cutoff scores indicate the number of items that must be answered "yes" to diagnose a psychological disorder.

Biochemical Analyses

All biochemical analyses were conducted at the Biochemical Laboratory of the Department of Clinical Psychology of Adulthood at the University of Vienna in Vienna, Austria. Participants' sC concentrations were measured using a commercially available enzyme-linked immunoassay (IBL; Hamburg, Germany), and sAA concentrations were assessed using an enzyme kinetic colorimetric test and reagents from DiaSys Diagnostic Systems (Holzheim, Germany). Inter- and intraassay variations were below 10%, respectively.

Data Analysis

For six participants in the PTSD group, no saliva data were available at any diagnostic assessment due to early dropout ($n = 4$), difficulties with sample collection ($n = 1$), or sample loss during postal delivery ($n = 1$). In the control groups, no saliva data were available for four participants in the deployed CG and two participants in the nondeployed CG due to refusal to provide the samples ($n = 4$) or sample loss during postal delivery ($n = 2$). All available sCort and sAA data were checked for incompliance with the protocol based on the diary records. As recommended by Stalder et al. (2016), the following deviations were considered as incompliant: (a) more than 5 min elapsed between awakening and the first sample, (b) more than 45 min elapsed between the first and second samples, and (c) for all other samples, deviations of more than 60 min from the intended time. Consequently, in the cross-sectional part of the study, 70 values out of 1,056 (i.e., 88 participants with available saliva data x 12 samples) were eliminated, as they had been exposed to noncompliance. In the longitudinal part of the study, 26 out of 228 values at posttreatment (i.e., 19 PTSD patients with available saliva data x 12 samples) and 14 out of 168 values at 3-month follow-up (i.e., 14 PTSD patients with available saliva data x 12 samples) were removed. Missing or eliminated samples were replaced by available parallel samples of the respective other day.

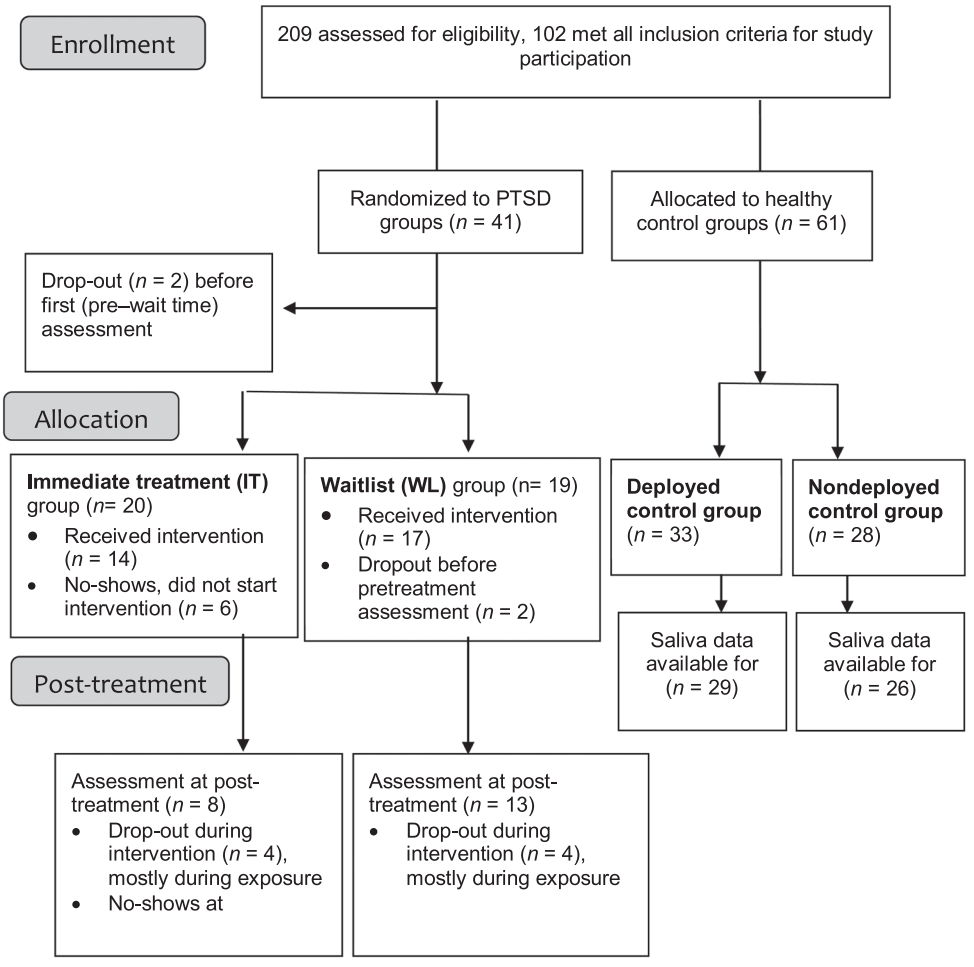
Due to high interindividual differences in saliva secretion, we calculated the SFR for each saliva sample from each individual. The SFR is obtained by subtracting the sample's presampling weight from the postsampling weight. Given that sAA is synthesized and secreted by the salivary glands, it has been suggested that sAA is likely to be affected by the amount of saliva secretion (i.e., SFR). Thus, sAA data (U/ml) were corrected for SFR by multiplying each sAA value by the SFR of the respective sample, which is interpreted as sAA output (U/min; Beltzer et al., 2010).

Participants' sC and sAA data were averaged across the two sampling days to reduce situational influences and increase measurement accuracy due to multiple days of measurement (Hellhammer et al., 2007; Stalder et al., 2016). The data were then ln-transformed to achieve normal distribution. We calculated three indices. First, the total daily sCort and sAA output were estimated by the areas under the curve (AUCs) with respect to the ground (Pruessner et al., 2003). Second, we calculated the CAR and the sAA awakening response (AAR) by subtracting the first from the second measurement time point. The CAR reflects a distinct rise in cortisol 30 to 45 min after awakening (Pruessner et al., 1997), whereas the AAR reflects a steep decline in sAA concentrations within the same time (Nater et al., 2007). Third, the slope, indicating the daily secretion pattern was calculated. It was operationalized as a change score by subtracting the last sample of the day (i.e., 9 p.m.) from the awakening sample and then dividing the value by the number of hours separating these two samples. We thereby adjusted for variations in the total wake time and, thus, the time over which sCort had the chance to decline and sAA to rise (Adam & Kumari, 2009; Ross et al., 2014).

Data analyses were performed with SPSS (Version 25.0). With regard to sAA, the data corrected for SFR are reported (i.e., sAA output). In addition, the sensitivity analyses for uncorrected sAA data (i.e., sAA activity) are presented in the Supplementary Materials so the data can be compared to and integrated into previous study results, as sAA activity that has not been corrected for SFR has been commonly used in a large body of previous studies.

For the cross-sectional comparisons, we used the saliva data of each group's respective first assessment. The IT and WL groups were collapsed into a PTSD group. Group

Figure 1
Participant Flow Through the Study Protocol



Note. PTSD = posttraumatic stress disorder.

differences in demographic characteristics were analyzed using chi-square tests; independent samples *t* tests, when data were available in only two groups, and univariate analyses of variance (ANOVAs). For chi-square tests, Cramer's *V* was used as an effect size estimate, with *V* values of 0.1, 0.3, and 0.5 indicating small, medium, and large effects, respectively. Cohen's *d* was used to calculate effect sizes for *t* tests, with values of 0.2, 0.5, and 0.8 indicating small, medium, and large effects, respectively. Finally, Cohen's *f* effect sizes were calculated for ANOVAs, with values of 0.10, 0.25, and 0.40 indicating small, medium, and large effect thresholds (Cohen, 1992). Univariate analyses of covariance (ANCOVAs) were used to test for group differences in sCort and sAA parameters (i.e., awakening response, AUC, slope), with age and body mass index (BMI) entered as covariates due to their known impact on sC and sAA concentrations (Strahler et al., 2017). Post hoc pairwise comparisons were calculated based on estimated marginal means.

For the longitudinal analyses, the IT and WL groups were again collapsed due to the small sample sizes. Only treatment completers who had available data for pretreatment, posttreatment, and follow-up assessments were included. Repeated-measures ANCOVAs were calculated, with time (pre- vs. post-treatment vs. follow-up) as within-factor variables, age and BMI as covariates, and sCort and sAA parameters as dependent variables, respectively. If the results of Mauchly's test indicated violations of the assumption of sphericity or if the test was considerably underpowered, a Greenhouse–Geisser correction was applied. We also explored the associations between sCort or sAA with PTSD symptom severity; however, due to the small number of treatment completers and the resulting lack of power, these associations were analyzed descriptively, and the results of these analyses are reported in the Supplementary Materials.

Results

Sample Characteristics

The flow of participants is presented in Figure 1. The demographic, biological, and psychological characteristics of the sample at the respective first assessment are shown in Table 1 and Table 2. The three groups did not differ significantly with

Table 1
Demographic Characteristics and Psychological Variables, by Group

	PTSD group ($n = 39$)			Deployed control group ($n = 33$)			Nondeployed control group ($n = 28$)				
Variable	*M*	*SD*	%	*M*	*SD*	%	*M*	*SD*	%	Statistical test	Effect size
Age[a], years	37.69	9.49		37.67	8.40		27.18	5.43		$F(2, 97) = 16.65^{***}$	$f = 0.59$
BMI[b], kg/m^2	27.01	3.17		26.30	9.23		2.39	2.39		$F(2, 92) = 1.37$	$f = 0.17$
Marital status										$\chi^2(6, N = 96) = 34.63^{***}$	$V = 0.43$
Single			10.3			0.0			17.9		
In a relationship			12.8			33.3			67.9		
Married			48.7			57.6			7.1		
Separated/divorced			20.5			9.1			3.6		
Educational attainment										$\chi^2(12, N = 99) = 10.72$	$V = 0.23$
Lower secondary			15.4			12.2			14.3		
Secondary			48.7			45.4			42.9		
Polytechnic degree			17.9			18.2			14.3		
Higher education certificate			15.4			24.2			28.6		
Number of deployments	2.84	3.05		3.21	2.76					$t(69) = -0.53$	$d = 0.11$
Time since most severe traumatic event, years	9.07	6.18		10.5	7.59					$t(63) = -0.84$	$d = 0.21$
Psychotropic medication use	36.8										
Habitual smoker			48.7			30.3			21.4	$\chi^2(2, N = 91) = 5.21$	$V = 0.24$
Psychological variables											
CAPS score	35.05	14.71		1.88	4.08					$t(44.8) = 13.48^{***}$	$d = 3.07$
CAPS PTSD diagnosis			64.1			0.0					

Note. PTSD = posttraumatic stress disorder; BMI = body mass index; CAPS = Clinician-Administered PTSD Scale.
[a]The age ranges were 19–70 years for the PTSD group, 23–54 years for the deployed control group, and 19–44 years for the nondeployed control group. [b]The body mass index (BMI) ranges were 21.74 – 36.72 for the PTSD group, 22.09–34.82 for the deployed control group, and 21.53–31.90 for the nondeployed control group.
$^{***}p < .001$.

Table 2
Psychological and Biological Variables, by Group

	PTSD group ($n = 39$)		Deployed control group ($n = 33$)		Nondeployed control group ($n = 28$)			
Variable	*M*	*SD*	*M*	*SD*	*M*	*SD*	Statistical test	*f*
CAR	0.14	0.19	0.30	0.22	0.14	0.22	$F(2, 69) = 3.38^*$	0.32[d]
sCort AUC[a]	279.97	77.90	294.25	61.32	293.51	113.16	$F(2, 73) = 0.11$	0.05
sCort slope[b]	0.04	0.02	0.03	0.02	0.04	0.02	$F(2, 64) = 0.20$	0.08
AAR[c]	−0.32	0.33	−0.41	0.48	−0.08	0.43	$F(2, 61) = 3.49^*$	0.34
sAA AUC[a]	905.67	201.10	923.32	207.04	904.89	179.96	$F(2, 67) = 0.24$	0.08
sAA slope[b]	−0.007	0.02	−0.009	0.02	−0.018	0.02	$F(2, 55) = 1.54$	0.24
SFR[e]	0.44	0.18	0.41	0.16	0.42	0.18	$F(2, 72) = 0.16$	0.06

Note. CAR = cortisol awakening response; sCort = salivary cortisol; AUC = area under the curve; AAR = amylase awakening response; sAA = salivary alpha-amylase; SFR = aliva flow rate.
[a]Total daily output. [b]Daily slope. [c]All sAA data were corrected for the saliva flow rate. For uncorrected sAA data, see the Supplementary Material. [d]The analyses of covariance (ANCOVA) analyses testing sCort and sAA were controlled for age and BMI. CAR: $F(1, 69) = 1.61$, $p = .209$, $f = 0.15$ for age, $F(1, 69) = 2.57$, $p = .113$, $f = 0.19$ for BMI; sCort AUC: $F(1, 73) = 0.27$, $p = .608$, $f = 0.06$ for age $F(1, 73) = 0.16$, $p = .694$, $f = 0.04$ for BMI; sCort slope: $F(1, 59) = 0.93$, $p = .340$, $f = 0.12$ for age, $F(1, 59) = 2.07$, $p = .156$, $f = 0.18$ for BMI; AAR: $F(1, 61) = 0.92$, $p = .342$, $f = 0.12$ for age, $F(1, 61) = 0.24$, $p = .625$, $f = 0.06$ for BMI; sAA AUC: $F(1, 67) = 0.78$, $p = .380$, $f = 0.11$ for age, $F(1, 67) = 0.03$, $p = .868$, $f < 0.01$ for BMI; sAA slope: $F(1, 55) = 0.18$, $p = .672$, $f = 0.05$ for age, $F(1, 55) = 0.38$, $p = .536$, $f = 0.08$ for BMI. [e]Value represents the saliva flow rate, calculated as mean of the 12 saliva samples per person and averaged over all participants in one group.
$^*p < .05$.

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regard to demographic variables, with the exception of age and family status. Participants in the nondeployed CG were significantly younger than those in the two other groups. This was somewhat inevitable as deployment eventually occurs in most military careers and, thus, nondeployed service members are usually at early career stages and younger in age. In line with this, about half of the participants in the PTSD and deployed CGs were married, whereas approximately 7% of participants in the younger, nondeployed CG were married.

Compliance with the behavioral restrictions on the saliva sampling days was acceptable. Affected sample rates ranged from 1.2% of all instances in which alcohol had been consumed to 20.2% in which drinking any liquids except water had occurred (for further details, see the Supplementary Material).

Cross-Sectional Analyses

Salivary Cortisol

No significant differences were detected between participants in the PTSD, deployed CG, and nondeployed CG groups with regard to the AUC or slope (see also Table 2). However, the rise in cortisol after awakening differed significantly between the groups. The results of post hoc comparisons revealed that individuals in the deployed CG showed a significantly higher CAR compared to those in the PTSD group, $\Delta M = 0.14$, $p = .021$, $d = 0.59$, whereas the difference with those in the nondeployed CG did not reach statistical significance, $\Delta M = 0.14$, $p = .051$, $d = 0.76$. No significant differences emerged between participants in the PTSD group and nondeployed CG, $\Delta M = -0.01$, $p = .918$, $d = 0.08$ (for a descriptive presentation of the averaged daily profile see Figure 2, Panel A).

Salivary Alpha-Amylase

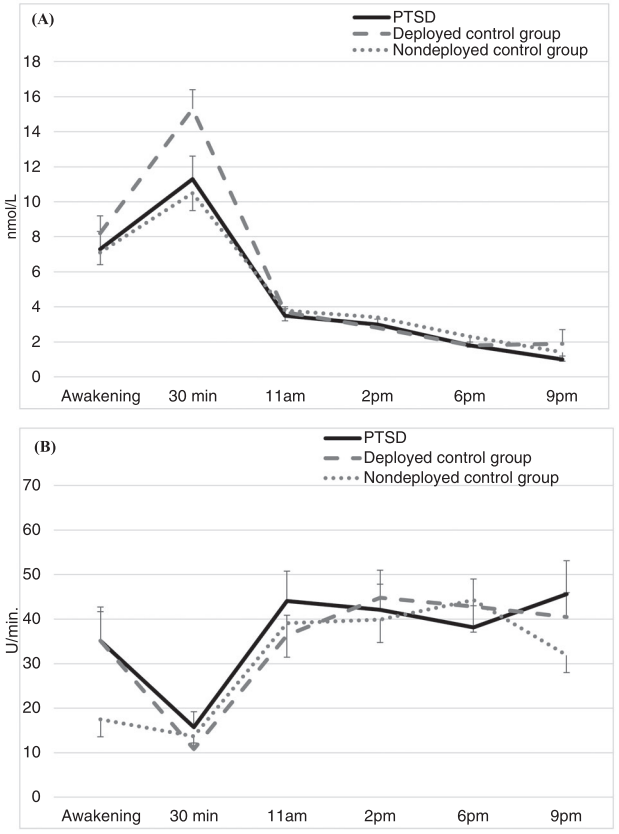
We did not find any significant differences in the AUC or the slope concerning sAA output (see Table 2). Again, the groups differed significantly in their awakening responses. The results of post hoc comparison tests indicated a more pronounced decline among participants in the deployed CG compared to those in the nondeployed CG, $\Delta M = -0.36$, $p = .014$, $d = 0.80$, but not to the PTSD group, $\Delta M = -0.06$, $p = .622$, $d = 0.22$. Participants in the PTSD group showed a significantly steeper decrease than those in the nondeployed CG, $\Delta M = -0.30$, $p = .034$, $d = 0.67$ (for a descriptive presentation of the averaged daily profile see Figure 2, Panel B).

The sensitivity analyses investigating sAA activity yielded comparable results regarding the AUC and the AAR. Concerning the slope, a significant effect emerged, showing a flattened increase in sAA concentrations in PTSD patients compared to participants in the nondeployed CG. Differences between PTSD patients and those in the deployed CG as well as differences between individuals in the deployed and nondeployed CGs were nonsignificant (see Supplementary Materials).

To critically test whether these results were robust against the biases related to multiple testing, we applied an additional correction (i.e., the Bonferroni–Holm correction) to the ANCOVA models. The results of the cross-sectional analyses did not survive such a correction for multiple testing. Consequently, the ANCOVA results were nonsignificant concerning group differences in the CAR, $p = .200$, or the AAR, $p = .222$.

Figure 2

Diurnal Profiles of (A) Salivary Cortisol Concentrations and (B) Salivary Alpha-Amylase Concentrations, by Group



Note. The figure presents the mean observed raw values, corrected for saliva flow rate. Bars indicate standard errors. PTSD = posttraumatic stress disorder.

Longitudinal Analyses

Paralleling the stability of PTSD symptoms (Niemeyer et al., 2020), no significant changes emerged in the sCort or the sAA parameters from pretreatment to posttreatment and from posttreatment to follow-up among participants who completed the treatment (Table 3). The results of sensitivity analyses revealed no significant changes in any of the sAA activity parameters (see Supplementary Materials). The findings from descriptive exploratory investigations suggested that on a descriptive level, PTSD symptom severity was negatively associated with the CAR and positively associated with the sAA AUC both cross-sectionally and longitudinally (see Supplementary Materials).

Discussion

The present findings suggest an attenuated CAR among participants with PTSD compared to trauma-exposed but not

Table 3
Longitudinal Biological Data of Treatment Completers

	Pretreatment		Posttreatment		3-month follow-up					
	M	*SD*	*M*	*SD*	*M*	*SD*	*F*	*df*	*p*	*f*
CAR	0.17	0.21	0.16	0.23	0.20	0.18	1.42	1.7,10.3	.282	0.49
sCort AUC[a]	301.83	78.51	305.99	105.09	285.53	120.40	1.29	2, 20	.296	0.36
sCort slope[b]	0.04	0.02	0.04	0.03	0.02	0.03	0.46	1.8, 7.1	.628	0.34
AAR[c]	−0.31	0.19	−0.23	0.25	−0.13	0.30	5.13	1.3, 5.1	.068	1.13
sAA AUC[a]	900.14	191.68	887.85	239.27	859.72	272.64	1.09	2, 18	.359	0.35
sAA slope[b]	−0.002	0.019	−0.008	0.018	−0.016	0.020	0.17	1.3, 3.9	.758	0.24

Notes. $n = 14$. AAR = amylase awakening response; AUC = area under the curve; CAPS = Clinician-Administered Posttraumatic Stress Disorder Scale; CAR = cortisol awakening response; sAA = salivary alpha-amylase; sCort = salivary cortisol;
[a]Total daily output. [b]Daily slope. [c]All sAA data were corrected for the saliva flow rate. For uncorrected sAA data, see the Supplementary Material.

non–trauma-exposed healthy control participants and a steeper AAR in PTSD patients and trauma-exposed controls compared to non–trauma-exposed controls. However, these results did not survive the correction applied for multiple testing, implying that they were not sufficiently stable. Neither sCort and sAA total daily output nor their diurnal slope differed significantly between the groups. Adding biological markers to the clinical evaluation of the internet-based TF-CBT (Niemeyer et al., 2020) showed that, in tandem with stable PTSD symptoms, sCort and sAA parameters did not change over the course of TF-CBT and to follow-up in treatment completers.

Taken together, our examinations of these three indices of HPA-axis and ANS activity contribute to the current literature regarding the differential impact of trauma exposure and PTSD on HPA-axis and ANS activity. In particular, few studies have focused on the CAR; this index was included only in one of the available meta-analyses (Schumacher et al., 2019), which aggregated effects of six primary studies, with the results pointing to a trauma exposure– and PTSD-related hypocortisolaemic pattern. In contrast, our data suggested an attenuated CAR in PTSD patients but not in trauma-exposed healthy individuals. Moderators could explain these conflicting findings. In their model, Steudte-Schmiedgen et al. (2016) suggested that the time elapsed since trauma exposure may influence cortisol concentrations. Importantly, this moderator could not be considered in the previous meta-analysis because many of the primary studies did not report this information. In the present study, the time that had elapsed since the traumatic event was comparable between participants in the deployed CG and PTSD group, suggesting that the observed differences in the CAR were independent of this variable. The results related to the attenuated CAR among participants in the nondeployed CG were inconclusive. One possible explanation is that these findings might be explained by acute distress in daily life (Powell & Schlotz, 2012) or further moderators that we did not investigate.

Our finding of a more pronounced AAR in PTSD patients compared to non–trauma-exposed healthy individuals corresponds well with models proposing hyperactivity of the noradrenergic system in PTSD (Kalk et al., 2011). At the same time, the stronger decline was also prevalent in trauma-exposed healthy individuals, which would indicate an impact of trauma exposure on the AAR. Thus far, only two other studies of which we are aware have examined basal alpha-amylase concentrations in PTSD patients compared to non–trauma-exposed controls (Keeshin et al., 2015; Thoma et al., 2012). This impedes the investigation of a differential impact of trauma exposure on sAA and thereby limits the comparability with our data.

In contrast to the evidence regarding the efficacy of internet-based TF-CBT (Kuester et al., 2016), this treatment was not shown to be effective (Niemeyer et al., 2020). Our longitudinal analyses showed that among patients who completed the treatment, this null finding on the psychological level corresponded with absent effects on the biological level as indicated by

stable sCort and sAA parameters over the course of the TF-CBT. Potential reasons for the current null result concerning the efficacy of the TF-CBT include sample characteristics, such as low tolerance for negative feelings during exposure; trauma avoidance; concerns regarding confidentiality; chronic symptoms, ongoing pension procedures; and low expectations about the efficacy of psychotherapy in general, as well as specific intervention characteristics, such as demanding and time-consuming assessment days, a writing-based intervention that required participants to have a general interest in writing, and the probably insufficient possibility to address individual fears to confront with traumatic memories (for an in-depth discussion, see Niemeyer et al., 2020). Yet, the small sample size limited potential thorough interpretations of this result. Due to the lack of a treatment effect, we cannot establish whether significant changes in psychological symptoms would also be mirrored in alterations in HPA-axis and ANS activity. Furthermore, it remains unclear when exactly these changes would occur. Previous researchers have posited that therapeutic changes in biological systems emerge with a time delay (Laufer et al., 2018), suggesting that potential alterations in sCort and sAA might have been detectable in the follow-up period. Thus, the clarification of the temporal dynamics between changes on the psychological and biological levels is still pending.

There were several strengths to the present study. First, we investigated both major stress systems (i.e., the HPA axis and ANS) in parallel (Nater et al., 2013). Because the HPA axis and the ANS interact in response to stress, the simultaneous examination of both systems allowed for a deeper insight into the underlying neurobiological underpinnings of PTSD symptoms. Second, we used a comprehensive methodology to assess saliva-based biological markers; that is, we collected daily profiles over 2 consecutive days (Hellhammer et al., 2007), investigated all three recommended indices (Adam & Kumari, 2009; Rohleder & Nater, 2009), and carefully considered confounding influences that might bias sCort and sAA concentrations (Strahler et al., 2017), thereby ensuring the reliability and validity of biological measurements. Third, our study included a follow-up period, which allowed us to capture the long-term outcomes of the intervention.

At the same time, several limitations should be acknowledged. Due to a number of obstacles and barriers during the recruitment process, we did not succeed in reaching the originally targeted sample size according to the a priori power analysis, leading to a small sample of PTSD patients included in the RCT. Given the small sample size, the initial RCT design was dropped, and all PTSD patients were merged into one group, which impeded testing against the waitlist condition. Furthermore, due to considerably low adherence and high dropout rates during the treatment, the number of completers was low, resulting in underpowered longitudinal analyses. Moreover, because we performed completer analyses, the reported results are only valid for the patients who completed the treatment. Consequently, our results need to be considered preliminary and require replication in a larger, sufficiently powered sample. That said, sufficiently powered studies remain a major challenge in this field of research, as has been previously emphasized (Schumacher et al., 2018). In addition, the significant cross-sectional group differences regarding the awakening responses did not survive the correction applied for multiple testing. Currently, biological markers are often implemented as secondary outcomes in psychotherapy studies, which was also true for the present study. As such, these studies are usually powered for the primary outcome (i.e., the clinical measures), and the problem of multiple testing arises in secondary analyses. Future research will need to find solutions to this problem.

It must be noted that the groups differed significantly with regard to demographic variables, such as age and family status. This is because we aimed to examine a group that had no previous deployment experience. As the probability of deployment increases with progression in one's military career, it was inherent that this group would be younger in age. At the same time, age is closely related to family status, which is why differences between the groups were consequently also evident regarding this variable. Finally, as the current study aimed to investigate the diurnal rhythm of salivary markers, we chose the total number of samples and relative time sampling according to the required level of temporal resolution and minimal participant burden due to repeated saliva sampling. However, future studies with the main focus on the awakening responses could potentially include an additional saliva sample (i.e., 45 min after awakening) for the estimation of the CAR and AAR according to the guidelines outlined by Stalder et al. (2016).

The results of the present study suggest that alterations in HPA-axis and ANS activity associated with trauma exposure and PTSD might be reflected in the sCort and sAA awakening responses but not in other parameters, such as the total daily output or slope. Longitudinally, stability in psychological symptoms corresponded with unchanged HPA-axis and ANS activity among treatment completers in the present sample. However, it remains unsolved whether a significant treatment effect would also manifest in biological changes and which sCort and sAA parameters are especially suitable for depicting these changes, thereby complementing the clinical evaluation of TF-CBT. In light of the obvious need to replicate the present findings in optimally powered studies, such results may provide important implications for future studies and guide researchers who intend to integrate these measures in psychotherapy studies.

Open Practices Statement

The study was preregistered at the Australian Clinical Trials Registry (ACTRN12616000956404). The data that support the findings of this study are available upon reasonable request from the German Federal Ministry of Defence (BMVg FÜSK III 5).

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