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Repeated stress leads to enhanced cortisol stress response in child social anxiety disorder but this effect can be prevented with CBT

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ABSTRACT

Social anxiety disorder (SAD) is associated with continual social stress in everyday life. Two physiological components of stress are the hypothalamus–pituitary–adrenal axis, as captured by cortisol reactivity, and the autonomous nervous system, as captured by salivary alpha amylase (sAA) reactivity. In children with SAD, initial evidence points to dysregulated physiological stress reactivity for both systems. Furthermore, hardly any studies have assessed stress reactivity twice, including exploring possible changes after cognitive behavioral therapy (CBT). Children with SAD ($n = 65$; aged 9–13 years) and healthy controls (HCs, $n = 55$) participated in a social stress task (Trier Social Stress Test for Children, TSST-C), which was repeated with children with SAD after either 12 sessions of CBT or a waiting period to explore possible habituation or sensitization effects. Before treatment, children in the SAD and HC groups did not differ in their cortisol stress reactivity toward the TSST-C but did differ in their sAA response with a more pronounced response in the SAD group. After treatment, children with SAD in the waitlist group differed from children with SAD in the CBT group by showing stronger cortisol reactivity and a higher responder rate, indicative of a possible sensitization to stress. No difference was found for sAA. Future research should compare children with SAD and HC children concerning the effect of repeated stress on sensitization.

1. Introduction

Social interactions can be highly stressful, even more so in social anxiety disorder (SAD), one of the most common mental disorders in children and youth (Kashdan and Herbert, 2001). SAD and, subsequently, the experience of stress in social situations are detrimental to child development, as physiological and socioemotional milestones such as building peer relationships cannot be met (Beidel and Turner, 2007).

1.1. Physiological stress response in anxiety

The exact nature of the physiological reactivity of both the hypothalamus–pituitary–adrenal (HPA) axis and the autonomic nervous system (ANS) in SAD has yet to be clarified (e.g., Klumbies et al., 2014). For example, studies of both girls only (Martel et al., 1999) and mixed samples (Krämer et al., 2012) did not find differences between participants with and without SAD in salivary cortisol in response to social

stress. However, other studies reported an elevated cortisol response to a social stressor in 25 children diagnosed with SAD compared to control children (e.g., van West et al., 2008).

As the HPA axis is finely attuned to specific environmental factors, a number of moderators should be considered (Kudielka et al., 2009). First, age can influence HPA effects. Van West et al. (2008) found higher cortisol responsiveness to stress in children with SAD aged 6–12 years compared to healthy controls. The nonsignificant findings in Martel et al.'s (1999) sample refer to adolescent girls (mean age 15.6 years), while Krämer et al. (2012) examined boys and girls aged 8–12 years. Further, SAD is more common in girls and women compared to boys and men (Asher et al., 2017). Thus, age and gender should be considered, especially in the context of pubertal development (Allen et al., 2017). It has been hypothesized that pubertal changes in HPA activity increase vulnerability to psychiatric disorders such as depression by increasing stress reactions of the HPA axis (for an overview see Gunnar et al., 2009). Additionally, basal cortisol levels have been shown to increase from childhood to adulthood (Gunnar et al., 2009). While this

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maturation hypothesis is not gender specific, greater stress reactivity has been observed in adult men than women (for an overview, see Kudielka and Kirschbaum, 2005). However, while there is no detailed research on children, preliminary evidence of a lack of difference between boys and girls during social stress has been found (Kudielka and Kirschbaum, 2005). Further, the *type of stressor* used to study the response to stress has differed, from public speaking to a social competence interview to the highly standardized Trier Social Stress Test (TSST; Allen et al., 2017). For example, Dieleman et al. (2016) did not find a significant increase in mean cortisol compared to baseline or significant alterations in mean heart rate in a mental arithmetic task and a social competence interview. In addition to development, *disorder severity* has to be taken into account, as Dieleman et al. (2015) reported a significant relation between severity of anxiety and a lower diurnal cortisol profile at noon. Further, the *type of assessment* should be considered, as some studies relied on diurnal cortisol (e.g., Dieleman et al., 2015) while others examined a stress response (e.g., Dieleman et al., 2016; Krämer et al., 2012; van West et al., 2008).

For the ANS, salivary alpha amylase (sAA), an enzyme released by the salivary glands, has been shown to be a valid marker for a stress response in healthy adults (Nater and Rohleder, 2009) that can be measured noninvasively and indirectly. Results are inconclusive concerning possible sAA alteration in SAD (Schumacher et al., 2013): For example, we did not find a difference in sAA reactivity toward social stress in children with and without SAD but rather a chronic hyperarousal in children with SAD (Krämer et al., 2012). Overall, there has been a tendency to cautiously assume generally elevated sAA levels in anxiety disorders (Schumacher et al., 2013).

Thus, to draw conclusions about physiological activity and its relation to social anxiety, a standardized stressor in a clearly defined sample (i.e., only clinical SAD, close age range with monitored pubertal status) may be necessary to allow for testing assumptions about physiological stress reactivity and its relation to social anxiety. Additionally, repeated assessment before and after treatment is advised to consider possible treatment effects and to understand the pathophysiology of SAD (Krämer et al., 2012).

1.2. Possible changes in physiological stress responses

There are few studies on repeated stress and physiological stress responses (cf. Kudielka et al., 2007) and even fewer that include clinical samples: In a comparison of cognitive behavioral therapy (CBT) and mindfulness-based stress reduction, adult patients with SAD were asked to perform a social stress task (speech task) before and after treatment. A clinical superiority of CBT was shown, while cortisol levels did not change from baseline to posttreatment (Faucher et al., 2016). Faucher et al. (2016) suggested that in future studies, sAA (a sympathetic measure) might be a better indicator after CBT in patients with SAD. However, research on repeated social stress (e.g., pre- and post-intervention) and physiological effects in children, especially with clinical anxiety, has not yet been conducted. Direct implications from adult research should not be drawn given the known limitations of age and development in psychophysiological reactivity (Allen et al., 2017; Kudielka et al., 2007).

The current study therefore aimed primarily to shed light on sAA and cortisol responses in children with and without SAD and secondarily to explore effects of CBT on both physiological markers to elaborate on their potential for etiology and maintenance. Using a highly controlled set-up (Kudielka et al., 2009), we expected a different pattern in the HPA axis response in children with and without SAD. Further, we expected to find elevated sAA levels in children with SAD compared to those without SAD (cf. Schumacher et al., 2013). As no clear indication can be given concerning the direction of differences in the HPA axis before treatment, in a secondary analysis, we did not expect a habituation or sensitization effect but hypothesized that the group receiving treatment would show altered cortisol levels compared to a group

waiting for treatment. Similarly, a change in sAA levels as indicator of ANS activation was expected.

2. Method

2.1. Trial design

The study was designed as a randomized controlled trial (block randomization, in which half of the participants were allocated by drawing from a hat to an experimental condition receiving immediate treatment and the other half to a waitlist control condition receiving treatment about 16 weeks later. Randomization was conducted at two research centers, each performing the task in a concealed fashion for the other center, based on subject codes, as soon as there were enough participants for one experimental and one waitlist-control allocation. Eligibility criteria were specified and registered with the German Research Foundation (TU 78/5-2, HE 3342/4-2) prior to recruitment and not changed during the study. The variables included in this study were included as primary outcome variables for a comparison between children with and without SAD. Concerning CBT effects, variables were not previously defined as primary outcome measures but were based on an exploratory approach. In consideration of length restrictions, primary outcome variables will be reported elsewhere.¹ As no previous study with a laboratory stressor has included sAA and cortisol as stress markers, the sample size was determined based on theoretical assumptions, and a power analysis was conducted for two groups and six measurements ($1-\beta = 0.95$, $f = 0.15$, $\alpha = .05$, $r_{\text{between measures}} = 0.0$) and set at $n = 98$. As the current study was part of a larger research project requiring a larger sample size of at least $N = 110$, all children were included to increase power.

2.2. Participants

Families with children aged 9–13 years were approached in two larger German cities (Freiburg, Bielefeld) through advertisements in schools and newspaper articles. In compensation for participation, parents received €35 cash, children €25 in vouchers. The study was approved by an independent ethics committee (of the German Society for Psychology). Written informed consent was obtained from both children and parents. The current study was part of a larger project examining different maintaining factors of childhood SAD, which will be presented elsewhere (Asbrand et al., 2019a).

After a screening for social anxiety symptoms and general psychopathology by phone, 177 of 311 initially interested families were invited to one of the two universities (see the flowchart in the online supplementary material). Trained graduate students assessed the diagnostic status of eligible children by using a modified version of the Anxiety Disorders Interview Schedule for children (Schneider et al., 2009). Afterward, a licensed clinical psychologist supervised all diagnostic sessions. Inclusion criteria consisted of SAD as primary diagnosis for the SAD group ($n = 65$) and no lifetime diagnosis of mental disorder for the healthy control (HC) group ($n = 55$). Exclusion criteria included health problems (e.g., asthma, cardiac arrhythmia) or medication (e.g., methylphenidate, psychotropic medication, beta blockers) that could have interfered with psychophysiological assessment. Children in the SAD and HC groups did not differ concerning age, gender, or pubertal

¹ The overall project aimed to measure treatment success by including several outcome variables (state anxiety, negative cognitions, physiological arousal, perception of and worry about physiological symptoms, perception of academic performance, negative postevent processing, parental cognitions, parental fear of negative child evaluation and related predictions). Results are reported elsewhere (postevent processing: Asbrand et al., 2019) or are being prepared for submission. All articles will include cross-references to the other outcome measures.

Table 1
Characteristics of Participants With Social Anxiety Disorder and Healthy Controls.

Characteristic	Social anxiety disorder group	Healthy controls	Statistics
n^a	65	55	
Age (in years) ^b	11.7 (1.34)	11.3 (1.36)	$t_{(118)} = 0.11$, n.s.
% female	63.6	60.0	$\chi^2_{(1)} = 0.17$, n.s.
SPAI-C ^b	23.26 (9.03)	4.2 (5.35)	$t_{(118)} = -13.71^{***}$
PDS ^c			$\chi^2_{(1)} = 1.32$, n.s.
Pre to early puberty	84.5%	92.3%	
Mid to postpuberty	15.5%	7.7%	

Note. For comparisons between groups, continuous data were analyzed using t tests for independent samples, and categorical data were analyzed using χ^2 tests; n.s. = not significant; PDS = Puberty Developmental Scale; SPAI-C = Social Phobia and Anxiety Inventory for Children.

^aSample sizes differ as not all questionnaires were completed correctly.

^bMean (SD).

^cGroups did not differ concerning girls post menarche ($n_{SAD} = 8$, $n_{HC} = 3$, $\chi^2_{(1)} = 1.29$, n.s.).

*** $p \leq .001$.

status, but children in the SAD group reported higher social anxiety (see Table 1).

Children with SAD were randomized after the first laboratory session (see procedure) into a treatment (CBT) and a waitlist control (WLC) group that received treatment later on (see Table 2). Efficacy of the treatment was shown by reduced SAD severity based on a structured interview in children receiving treatment in comparison to children in the WLC group; details concerning treatment and study design are provided elsewhere (Asbrand et al., 2019b).

2.3. Procedure

Following the diagnostic interviews, children participated in the first laboratory session, in which the Trier Social Stress Test for Children (TSST-C; Buske-Kirschbaum et al., 1997), consisting of a speech and a math task (see Fig. 1), was administered. To control for diurnal changes in cortisol secretion (Wust et al., 2000) and meal-related salivary cortisol increases (Kudielka et al., 2009), all sessions were conducted during the same time period in the afternoon, roughly 1.5 h after lunch. In the speech task, children were asked to continue narrating a story in front of two observers after listening to the beginning of the story. In the following mental arithmetic task, children were asked to serially subtract the number 7 from 758 (9- to 11-year-olds) or the number 13 from 1023 (12- to 13-year-olds) as fast and as accurately as possible. Both observers were instructed and trained to give neutral verbal and nonverbal feedback. Anxiety and salivary probes were assessed repeatedly throughout the TSST-C (see Fig. 1). Children rated their anxiety using developmentally appropriate 11-point Likert scales taken from the Scales for Iconic Self-Assessment of Anxiety in Children (Schneider et al., 2005). All anxiety ratings referred to the maximum anxiety in the last period. Due to the slow adaptation of the cortisol response, which peaks 15–20 min after the start of an effective stressor (Kirschbaum and Hellhammer, 1994), a recovery phase (30 min) was included (see Krämer et al., 2012). After participating in a 12-week CBT program (CBT group) or waiting without treatment (WLC group), all children performed a parallel version of the first testing session. Following the protocol of the original TSST-C (Buske-Kirschbaum et al., 1997), the speech task was changed to a different story that was evaluated as being similarly interesting and difficult in a preevaluation. The math task was changed to a different start number (+10).

Table 2
Characteristics of Participants in the CBT and WLC Groups at Baseline.

Characteristic	Treatment group (CBT)	WLC group	Statistics
n^a	32	33	
Age (in years) ^b	11.5 (1.33)	11.2 (1.36)	$t_{(63)} = 0.84$, n.s.
% female	51.6	67.6	$\chi^2_{(2)} = 1.88$, n.s.
SPAI-C ^b	21.9 (10.20)	24.5 (7.74)	$t_{(63)} = 1.17$, n.s.
PDS ^c			$\chi^2_{(1)} = 0.35$, n.s.
Pre to early puberty	87.1%	81.5%	
Mid to postpuberty	12.9%	18.5%	

Note. For comparisons between groups, continuous data were analyzed using t tests for independent samples, and categorical data were analyzed using χ^2 tests; n.s. = not significant; PDS = Puberty Developmental Scale; SPAI-C = Social Phobia and Anxiety Inventory for Children.

^aSample sizes differ as not all questionnaires were returned.

^bMean (SD).

^cGroups did not differ concerning girls post menarche ($n_{CBT} = 4$, $n_{WLC} = 4$, $\chi^2_{(1)} = 1.55$, n.s.).

*** $p \leq .001$.

2.4. Psychometric measures

2.4.1. Social phobia and anxiety inventory for children (SPAI-C)

To further describe social anxiety symptoms on a continuous measure, we used the SPAI-C (Beidel et al., 2001), which measures behavioral characteristics, cognitions, and physiological symptoms of SAD. Validity and reliability have been confirmed in both the original sample (Beidel et al., 2001) and a German sample (Melfsen et al., 2011). The internal consistency of the SPAI-C in the current sample was excellent ($\alpha = 0.98$).

2.4.2. Pubertal developmental scale (PDS)

The PDS (Petersen et al., 1988) measures self- and parent-reported pubertal status for boys and girls using gender-appropriate sketches. Both children and parents were asked to select which of the sketches “looked most like them.” Classifications were based on the average of self- and parent ratings as Tanner stage I–V. Subjects were then grouped according to their status of puberty development: Tanner stages I and II for pre- to early puberty and Tanner stages III–V for mid- to postpuberty.² The PDS has previously been used to describe puberty influences during public speaking (Sumter et al., 2010) and has shown good psychometric properties in the current German version (Watzlawik, 2009) as well as moderate to substantial correlations with physical examination (Chan et al., 2010).

2.5. Salivary biomarkers: sAA and cortisol

Children provided six saliva samples during the TSST-C for analysis of salivary cortisol and sAA concentration. Collection took place parallel to anxiety ratings (see Fig. 1). Children were asked to chew on a Salivette (SarstedtNumbrecht, Germany) for 1 min. Then Salivette collection devices were stored at -20°C until assayed. Both study sites sent the collected saliva samples to the laboratory of Professor Kirschbaum at the Technical University of Dresden, Germany, where they were analyzed collectively. For sAA analyses a quantitative enzyme kinetic method was used as described in detail elsewhere (Van Stegeren et al., 2006). Salivary cortisol levels were measured by use of commercial immunoassay with chemiluminescence detection (IBL Hamburg, Hamburg, Germany). Intra- and interassay precision expressed as percent coefficient of variation was below 10% for both cortisol and sAA assays. Cortisol responders to the TSST-C in both test sessions were determined to be those who showed a 1.5-nmol cortisol increase between baseline and 10 min poststress (recovery 1; Miller et al., 2013).

² Menstrual cycle and intake of oral contraceptives were not further assessed.

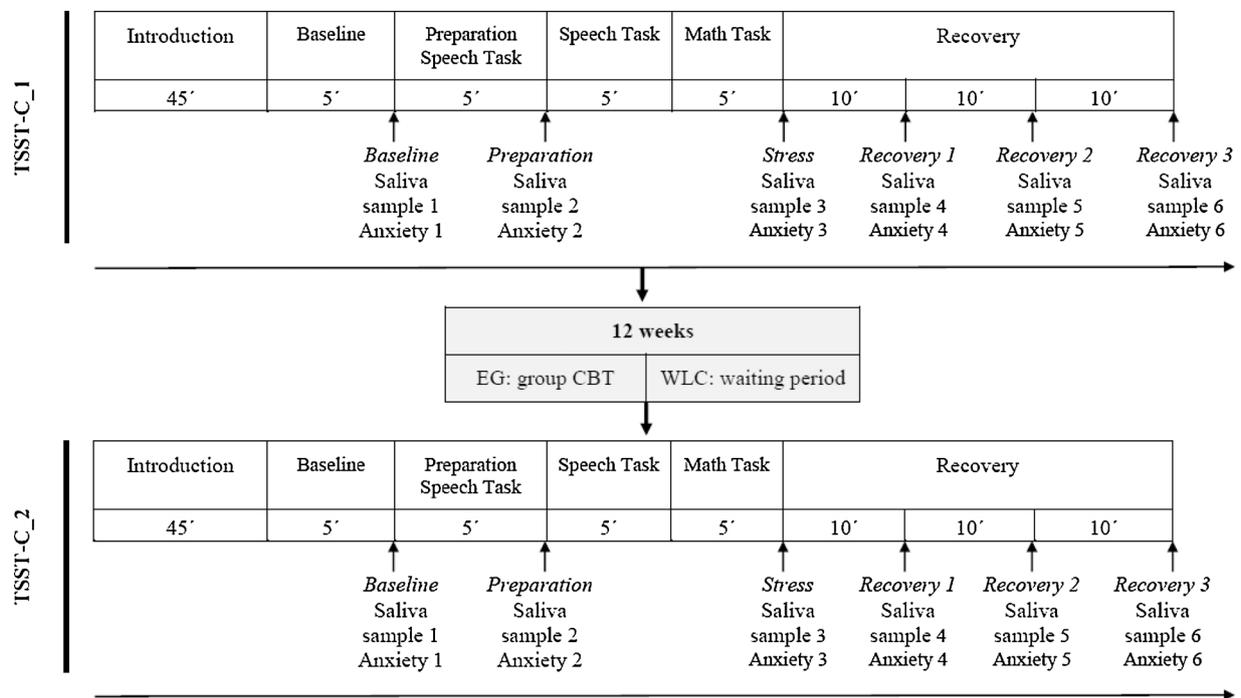


Fig. 1. Procedure of the first and second (designated _1 and _2) Trier Social Stress Test for Children (TSST-C; lab task) and subjective and saliva assessment before and after treatment. CBT = cognitive behavior therapy; EG = experimental group; WLC = waitlist control.

2.6. Statistical analysis

For the analysis of subjective stress (i.e., state anxiety), a 2 (group: SAD, HC) × 6 (time: 1–6) analysis of variance (ANOVA) with repeated measures on time was calculated. For the separate analyses of cortisol and sAA, the open-source statistics software R was applied (R Core Team, 2013) using the mixed-model packages lme4 (Bates et al., 2014) and lmerTest (Kuznetsova et al., 2015)³. Outliers were calculated separately for group and time and excluded.⁴ Both models were fitted with one between-subjects factor group (SAD, HC), one within-subject factor time (1–6), one z-standardized continuous variable puberty status (PDS), and all possible interaction terms as fixed effects. Furthermore, intercepts for every participant were modeled as random effects. Responders and nonresponders (see Section 2.5) in each group (SAD, HC) were analyzed with a χ^2 test.

For the analysis of treatment effects on subjective stress, a 2 (group: CBT, WLC) × 2 (session: TSST-C_1, TSST-C_2) × 6 (time: 1 to 6) ANOVA with repeated measures on session and time was calculated. For analyses of treatment effects on cortisol and sAA, mixed models were used once again. Outliers were calculated and excluded separately for groups, time, and session. Both models were fitted with one between-subjects factor group (CBT, WLC), the two within-subject factors time (1 to 6) and session (TSST-C_1, TSST-C_2), one z-standardized continuous covariate puberty status (PDS), and all possible interaction terms as fixed effects. Furthermore, intercepts for every participant were modeled as random effects. Responders and nonresponders (CBT, WLC) were again analyzed with a χ^2 test. Additionally, we compared the number of participants who changed from responder to nonresponder or from nonresponder to responder or had no change between groups with a χ^2 test.

³ Importantly, mixed models consider nonlinearity: Group, time, and session were modeled in a factorial and, hence, nonlinear fashion. Only the continuous variable PDS and its interactions are affected by this question. We did not account for nonlinearity of PDS as it was not considered in the a priori power analysis.

⁴ Statistical outliers were identified as lying 2.5 SDs above or below the mean. The proportions of outliers for all analyses were below 5%.

The degrees of freedom for all mixed-model analyses were calculated with Satterthwaite approximation. As debates about effect sizes in mixed models are still ongoing, no effect size could be reported. Significant main effects and interactions were further analyzed (if relevant for the hypotheses) with post hoc t tests for independent groups for the group comparisons and with t tests for dependent groups for time and session comparisons. Cohen’s d effect sizes are reported for the post hoc tests. The post hoc analyses of significant interactions involving the continuous measurement PDS were performed with correlation analyses.

3. Results

3.1. Descriptive analysis

Children with and without SAD did not differ on any of the socio-demographic variables or puberty status (see Table 1). Children with SAD reported more SAD symptoms than children from the HC group. Children with SAD in the treatment group did not differ on any variable from children with SAD in the WLC group (see Table 2).

3.2. Children with SAD versus children in the HC group

3.2.1. Subjective stress

The analysis of state anxiety showed a significant main effect of time, $F_{(5, 113)} = 63.28, p < .001, \eta_p^2 = .737$, and group, $F_{(1, 117)} = 20.50, p < .001, \eta_p^2 = .149$, and a significant interaction of Group × Time, $F_{(5, 113)} = 3.03, p = .013, \eta_p^2 = .118$. The post hoc analyses of Group × Time showed higher state anxiety in the SAD group at baseline, $t_{(105)} = 4.06, p < .001, d = 0.76$, and during preparation, $t_{(117)} = 4.66, p < .001, d = 0.86$, stress, $t_{(118)} = 2.90, p = .004, d = 0.53$, recovery 1, $t_{(118)} = 2.05, p = .042, d = 0.38$, and recovery 3, $t_{(110.1)} = 2.50, p = .014, d = 0.45$ (see Fig. 2A). Thus, children in the SAD group reported higher state anxiety almost throughout the stress test.

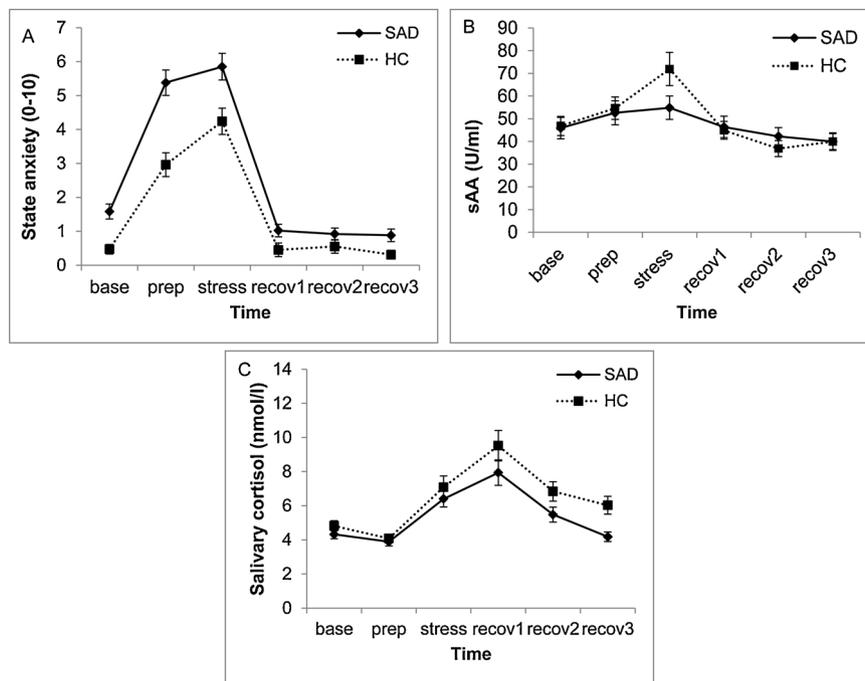


Fig. 2. Means and standard errors of state anxiety (A), salivary alpha amylase (sAA) biomarkers (B), and cortisol (C) during TSST-C.1. SAD = Social anxiety disorder group; HC = healthy control group; recov = recovery time.

3.2.2. Sympathetic reaction to social stress (sAA)

The analysis of sAA levels yielded a significant main effect of *time*, $F_{(5, 446)} = 19.84, p < .001$, two 2-way interactions of *Group* \times *Time*, $F_{(5,446)} = 3.22, p = .007$, and *Time* \times *PDS*, $F_{(5,445)} = 2.52, p = .023$, and the three-way interaction of *Group* \times *Time* \times *PDS*, $F_{(5, 445)} = 2.34, p = .041$ (see Fig. 2B). The post hoc analyses of *Group* \times *Time* showed no significant group differences in any of the times (all *ts* < 1.65, all *ps* > .104, all *ds* < 0.38). Thus, the two groups did not differ in their sAA response to the TSST-C.

The post hoc analyses of the *Group* \times *Time* \times *PDS* interaction revealed no significant correlation between PDS and sAA at any time point for the SAD group (all *rs* \leq .179, all *ps* \geq .183). In the HC group, there was a significant correlation only between PDS and sAA for recovery 1, $r_{(35)} = .347, p = .041$; none of the other correlations reached significance (all *rs* \leq .292, all *ps* \geq .084). Higher PDS scores corresponded to higher sAA levels only in the HC group.

3.2.3. HPA reaction to social stress (cortisol)

The analyses of cortisol levels revealed a significant main effect of *time*, $F_{(5,442)} = 34.38, p < .001$, and a significant two-way interaction of *Group* \times *PDS*, $F_{(1, 84)} = 4.46, p = .038$. None of the remaining main effects or interactions were statistically significant, all *Fs* \leq 1.35 (all *ps* \geq .242; see Fig. 2C). Thus, reactivity to the TSST-C did not differ between groups. A TSST-C response analysis showed that the two groups consisted of equal numbers of responders and nonresponders (see Table 3).

The post hoc analysis of the *Group* \times *PDS* interaction revealed a significant positive correlation between PDS and the cortisol levels for the SAD group, $r_{(325)} = .272, p < .001$, but not for the HC group, $r_{(223)} = -.047, p = .482$. That is, in the SAD group, higher scores on the

PDS corresponded to higher cortisol levels.

3.3. Children with SAD: CBT versus WLC

3.3.1. Subjective stress

The repeated assessment for state anxiety showed a significant main effect of *time*, $F_{(5, 51)} = 64.05, p < .001, \eta_p^2 = .863$, and *session*, $F_{(1,55)} = 7.09, p = .010, \eta_p^2 = .114$. Further, the two-way interactions *Time* \times *Group*, $F_{(5, 51)} = 2.64, p = .034, \eta_p^2 = .205$, and *Time* \times *Session*, $F_{(5, 51)} = 2.55, p = .039, \eta_p^2 = .200$, reached significance (see Fig. 3A, B). None of the remaining main effects and interactions were significant (all *Fs* < 2.68, all *ps* > .107).

The post hoc analyses for time *Time* \times *Group* did not show significant group differences during any phase of the TSST-C.1 (all *ts* < 1.26, all *ps* > .214). State anxiety was, however, significantly higher in the CBT group during recovery 1 of the TSST-C.2, $t_{(41.8)} = 2.15, p = .038, d = 0.56$ (all other *ts* < 1.91, all *ps* > .060). Therefore, self-reported state anxiety was not lower in the CBT group after treatment than in the WLC group after the waiting period. In fact, at certain times during the TSST-C, state anxiety increased in the CBT group.

3.3.2. Sympathetic reaction to social stress (sAA)

The repeated assessment for sAA yielded a significant main effect of *time*, $F_{(5, 588)} = 13.47, p < .001$, a significant two-way interaction of *Session* \times *PDS*, $F_{(1, 593)} = 11.64, p < .001$, and a significant three-way interaction of *Group* \times *Session* \times *PDS*, $F_{(1, 593)} = 13.98, p < .001$. None of the remaining main effects and interactions were significant (all *Fs* < 3.91, all *ps* > .05, see Fig. 3C, D).

The post hoc analyses for *Group* \times *Session* \times *PDS* showed a significant correlation between sAA and PDS only for the CBT group in

Table 3

Responder Analysis of Cortisol Comparing the Social Anxiety Disorder Group and Healthy Controls.

Cortisol response status	Social anxiety disorder group (n = 65)	Healthy controls (n = 55)	Statistics
Responder	31	34	$\chi^2_{(1)} = 2.39, p = .143$
Nonresponder	34	21	

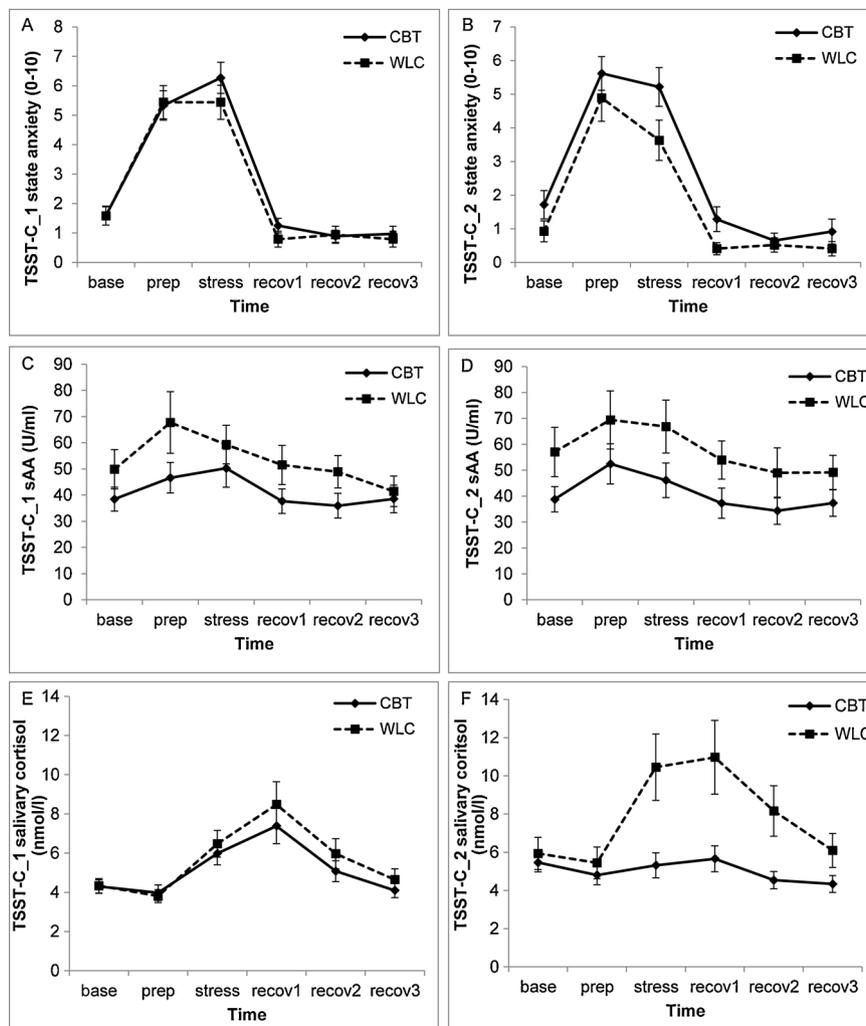


Fig. 3. Means and standard errors comparing treatment (CBT) and waiting (WLC) on state anxiety (A, B), salivary alpha amylase (sAA) biomarker (C, D), and cortisol (E, F) during TSST-C_1 and TSST-C_2. CBT = Cognitive behavior therapy; WLC = waitlist control; recov = recovery time.

session 2, $r_{(171)} = .294, p < .001$, and not in session 1, $r_{(179)} = .084, p = .265$. No significant correlations were found for the WLC group in session 1, $r_{(152)} = .062, p = .445$, or session 2, $r_{(145)} = .007, p = .929$.

The post hoc analyses for the *Group × Session* interaction were not significant for the group comparison in session 1, $t_{(42)} = 1.62, p = .112, d = 0.34$, or session 2, $t_{(45)} = 1.55, p = .127, d = 0.31$. Summing up, the CBT and WLC groups did not differ before and after treatment on sympathetic reactivity.

3.3.3. HPA reaction to social stress (cortisol)

The analysis of treatment effects on cortisol revealed significant main effects of *time*, $F_{(5, 583)} = 26.41, p < .001$, and *session*, $F_{(1, 589)} = 10.34, p = .001$, as well as a statistical trend for *group*, $F_{(1, 57)} = 3.64, p = .061$. Moreover, the two-way interactions *Group × Time*, $F_{(5, 583)} = 5.61, p < .001$, *Group × Session*, $F_{(1, 589)} = 8.94, p = .003$, and *Session × PDS*, $F_{(1, 593)} = 8.00, p = .005$, were significant, as well as the three-way interactions *Group × Time × Session*, $F_{(5, 583)} = 2.56, p = .026$, and *Group × Session × PDS*, $F_{(1, 593)} = 13.29, p < .001$ (see Fig. 3E, F). None of the remaining effects and interactions were significant (all $F_s < 2.24$, all $p_s > .140$). A responder analysis of the TSST-C_2 showed that the WLC group consisted mainly of responders, while children in the CBT group were mostly nonresponders, $\chi^2 = 6.43, p = .015$ (see Table 4). Compared to the TSST-C_1, in the TSST-C_2 there was a trend of a significant change from responder to nonresponder (n_{CBT} : 24.14%,

Table 4

Responder Analysis of Cortisol Comparing the CBT and WLC Groups at Both Testing Sessions.

Test session	Cortisol response status	CBT group (n = 29)	WLC group (n = 26)	Statistics
TSST-C_1	Responder	14	14	$\chi^2_{(1)} = 0.17, p > .999$
	Nonresponder	15	12	
TSST-C_2	Responder	7	15	$\chi^2_{(1)} = 6.43, p = .015$
	Nonresponder	22	11	

Note. CBT = cognitive behavior therapy; WLC = waitlist control; TSST-C = Trier Social Stress Test for Children.

n_{WLC} : 11.54%) and from nonresponder to responder (n_{CBT} : 0%, n_{WLC} : 15.38%), $\chi^2 = 5.67, p = .059$.

The post hoc analyses of *Group × Time × Session* revealed in session 2 significant group differences during stress, $t_{(31)} = 2.59, p = .014, d = 0.74$, recovery 1, $t_{(30)} = 2.48, p = .019, d = 0.71$, and recovery 2, $t_{(30)} = 2.47, p = .020, d = 0.71$. None of the remaining group comparisons in session 1 or 2 yielded significant differences (all $t_s < 1.63$, all $p_s > .113$, all $d_s < 0.46$). Thus confirming the response analysis, the WLC group showed a stronger reactivity to the second TSST-C than the CBT group.

The post hoc analysis for *Group × Session × PDS* revealed a

significant positive correlation between cortisol and PDS in session 1 for both groups, CBT, $r_{(174)} = .174$, $p = .022$; WLC, $r_{(151)} = .344$, $p < .001$. That is, higher cortisol levels corresponded to higher PDS scores. In session 2 no effects were observed (CBT group, $r_{(167)} = .133$, $p = .087$, WLC group, $r_{(149)} = -.041$, $p = .619$).

4. Discussion

Similar to in Krämer et al. (2012), children in the SAD group reported more state anxiety than children in the HC group, but groups did not differ in general level of sAA or cortisol activity. At second assessment, the WLC group showed stronger reactivity than the CBT group on cortisol levels during stress and at the beginning of recovery from the TSST-C, thus indicating a possible sensitization to stress. Further, the responder analyses showed on the basis of cortisol levels that—in contrast to the WLC group—several children in the CBT group no longer reacted to the TSST-C (Miller et al., 2013). The sAA levels indicate the groups did not differ in the sympathetic reaction to the TSST-C. PDS scores corresponded positively to cortisol levels in the SAD group at TSST-C_1 but not at TSST-C_2.

State anxiety did not decrease in the CBT group, which corresponds to findings on self-reported trait anxiety (Gallagher et al., 2004). This effect might have come from a strong avoidance in children that does not allow the perception of anxiety. Applied to our results, this explanation could indicate that children in the WLC group were quicker to avoid the unpleasant feeling and, therefore, reported less anxiety, whereas children in the CBT group were able to experience and express their anxiety.

4.1. Physiological arousal in response to social stress in children with SAD

While the omnibus test for TSST-C_1 pointed to higher sAA reactivity in children with SAD, post hoc analyses did not confirm this finding. This corresponds to results from a similar task for which no elevated sAA levels in children with SAD were found (Krämer et al., 2012). Other studies reported increased sympathetic activity in SAD using different measures such as skin conductance level (e.g., Schmitz et al., 2013) but not as a reactivity parameter in a social stress task. Interestingly, higher sAA levels corresponded to higher PDS levels in the HC group. As PDS levels were controlled (i.e., groups did not differ), possible interaction effects between the physiological development and psychopathology have to be taken into account. Puberty is a critical phase for both the physiological stress response system and emotional development (Gunnar et al., 2009). Therefore, SAD symptoms might interfere with the physiological development and vice versa. Since sAA is a fast-changing parameter, this finding could point to more flexible adaptation and thus more autonomic flexibility in older children in the HC group (Schmitz et al., 2013).

Even though we carefully controlled for possible covariates (Kudielka et al., 2009), no differences appeared between children with SAD and HC children on cortisol levels. These results are in line with previous findings with a similar set-up using the TSST-C but a younger sample (Krämer et al., 2012) but contradict others (van West et al., 2008). Interestingly though, a correlation between cortisol levels and puberty status appeared only in the SAD group. As puberty status did not differ between groups, this finding could point to more stress in more developed children with SAD. Recently, Rapee et al. (2018) argued that puberty—on both social and hormonal levels—has a central role in the etiology of SAD. As previously pointed out, puberty-specific changes in affective neural systems increase (social) stress reactivity and, thus, add to the risk of developing psychopathological symptoms in youth (Dahl and Gunnar, 2009).

4.2. Effects of repeated assessment of physiological reactivity in children with SAD

Faucher et al. (2016) assumed that social-evaluative situations might be associated with greater ANS arousal in sensitive individuals, which is captured by sAA and not cortisol. Our study investigated sAA but did not find significant group differences. Still, as no stable differences appeared between children with SAD and HC children before treatment, changes in the SAD group might not be expected. Previous studies have mostly explored the predictive quality of physiological assessment. For example, Dieleman et al. (2016) showed that high sympathetic reactivity in children with anxiety disorders before treatment is related to less treatment success. Further, lower cortisol reactivity before treatment was related only to a less positive outcome concerning depressive symptoms. This approach does not allow conclusions about changes in physiological measures and a possible alignment to HC levels.

While the two patient groups did not differ in their cortisol response to the first stressor, significant differences emerged after the interventions. Patients from the WLC group showed an enhanced cortisol response, possibly reflecting sensitization. Further, in the responder analysis based on cortisol, the two groups did not differ in the distribution of responders versus nonresponders during the TSST-C_1 but did differ during the TSST-C_2. Interestingly, our results point to a stabilization or even lower response (cf. Section 3.3.3 responder analysis) of cortisol reactivity in treated children while untreated children experienced a major sensitization. This finding is in line with results from an adult sample with SAD (Faucher et al., 2016), for whom changes after treatment were found in psychopathology but not in physiological measures. However, Faucher et al. (2016) did not compare treatment effects in a WLC group. Therefore, our study extends these findings to repeated measurements of social stress in a clinical sample that showed a stronger reactivity to the TSST-C in the WLC group in the second session. In contrast to this finding, studies in healthy adults with a repeated stress task showed cortisol habituation (Kudielka et al., 2007). Wüst et al. (2005) offered the explanation that a potential vulnerability to stress-related diseases could lead to an increased salivary cortisol response to psychosocial stress. Similarly, Pruessner et al. (1997) found a negative relation between personality traits such as positive self-concept or perceived control and cortisol levels in repeated social stress. The authors claimed that an inability to cope with a stressful situation might lead to a lack of habituation of cortisol levels in subsequent stress. Thus, results from the WLC group could reflect a lack of coping mechanisms.

4.3. Limitations, strengths, and future implications

Overall, it should be cautioned that while we found effects on multivariate levels, they did not always reach significance in the post hoc analyses. Even though the sample was large enough to detect medium effects, an even larger sample might be necessary to detect small effects. While it is impossible to control for all potential confounding variables, we acknowledge the most pressing matters previously mentioned, such as time of day, inclusion of both boys and girls, and control of puberty status (Kudielka et al., 2009). Further, the included groups were as distinct as possible (SAD status based on a structured interview, HC status based on a structured interview and low social anxiety scores). As puberty status is an important covariate, some previous studies have included only boys (cf. Chen et al., 2014) for a more coherent sample. However, to exclude girls would make it a nonrepresentative sample, especially as SAD is more common in females (Asher et al., 2017). We did not assess details on menstrual cycle and hormonal contraceptives as the prescription of oral contraceptives is uncommon in this age group in Germany. However, interactions based on these covariates cannot be ruled out (Kirschbaum et al., 1999), which is why future studies should include these factors even in

younger children. Because of the complex set-up of the overall study, children in the HC group participated in only one TSST-C, similar to in previous studies (Faucher et al., 2016). Future studies could include a second TSST-C for children without SAD, as no repeated measure took place to control for habituation and/or sensitization effects in the general sample. Additionally, it should be considered that the TSST-C is a very strong social stress task (Allen et al., 2017) that reliably induces stress even in samples without social anxiety. As such, the HC group reacted quite strongly, possibly leading to a lack of group differences in comparison to the SAD group, as both perceived the task to be very difficult (Richter et al., 2008). Possibly, in addition to conducting the TSST-C, it could be useful to add a parallel paradigm including weaker stressors such as a conversation task that could be more similar to everyday stress and resemble stress that had been targeted in treatment (Allen et al., 2017). Most importantly, several outcomes point to the importance of the assessment of not only age but puberty status. Therefore, puberty status should be considered as key covariate in all physiological research including children and youths. Further, as we opted to assess the beginnings of SAD (earliest onset described at 8–9 years; Beidel et al., 1999), future studies could shed more light on this question by including slightly older age groups to balance pubertal status. Finally, a combined analysis of clinical treatment response and physiological treatment response went beyond the scope of this paper. Future studies could include daily cortisol levels, which can be easily traced for an even longer time, for example, 1 year (cf. Dierckx et al., 2012). Additionally, the interplay between sAA and cortisol could be the subject of future studies in relation to child psychopathology (El-Sheikh et al., 2008). While sAA is widely used as a marker for stress reactivity (e.g., Kuras et al., 2017), it has been argued that the secretion of sAA might not only be related to sympathetic, but also parasympathetic activity (Bosch et al., 2011). Thus, the discussion of possible influences on sAA activity is ongoing (e.g. Strahler et al., 2017).

Summing up, we found differences in autonomic but not endocrine physiological reactivity to social stress between children with and without SAD. Interestingly, a second social stress task led to a sensitization in children who had not received treatment while children in the CBT group remained stable. In general, these findings should be considered relating to research showing that subjective stress perception does not typically align with the physiological stress response in both adults (e.g., Klumbies et al., 2014) and children (e.g., Schmitz et al., 2012). Repeated stress leads to enhanced cortisol stress response in child social anxiety disorder but this effect can be prevented with CBT.

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Ethics approval and consent to participate

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. An independent ethics committee (ethics committee of the German Society for Psychology) granted ethical approval for this study.

Informed consent was obtained from all individual participants (both parents and children) included in the study in written form

Declaration of Competing Interest

The authors declare no conflict of interest

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the

online version, at doi:<https://doi.org/10.1016/j.psyneuen.2019.06.003>.

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