Received: 22 March 2018 | Revised: 13 June 2018 | Accepted: 4 July 2018

DOI: 10.1111/psyp.13273

ORIGINAL ARTICLE

WILEY PSYCHOPHYSIOLOGY SPR

Cortisol response to awakening in prepubertal children and adults: Magnitude and variability

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Abstract

Within the first 30–45 min after awakening, there is a characteristic rise in cortisol that is referred to as cortisol awakening response (CAR). Over the past decades, the CAR has become an important biomarker, mainly because of its reported association with health and disease. Previous research showed that the CAR can already be reliably assessed in infants and children. Yet, earlier findings on the influence of age have been inconsistent, and limited attention has been devoted to prepubertal children. Here, we aimed to contrast the magnitude and stability of the CAR in prepubertal children and adults. To this end, 24 healthy adults between 35 and 50 years of age and 24 healthy children between 6 and 9 years of age collected four salivary cortisol samples within 45 min after awakening on 4 separate days, 2 weekdays, and 2 weekend days. Our results showed that there was a marked CAR on weekdays and weekend days in both adults and children. In children, however, the CAR was overall significantly attenuated relative to adults. Moreover, while the cortisol increases after awakening were, both on weekdays and weekend days, highly correlated in adults, there were no such associations in children. Together, these data suggest that the CAR is less pronounced and less stable in prepubertal children compared to adults. Such age differences need to be taken into account when using the CAR as a biomarker in clinical settings.

KEYWORDS

age differences, children, cortisol awakening response, glucocorticoids, hypothalamus-pituitary-adrenal axis

1 | INTRODUCTION

The release of the steroid hormone cortisol, known to play an important role in health and disease (de Kloet, Joels, & Holsboer, 2005; de Quervain, Schwabe, & Roozendaal, 2017; McEwen, 2008), follows a diurnal rhythm with a morning peak and an evening nadir (Tsigos & Chrousos, 2002). In addition to this characteristic circadian cycle, there is a discrete and distinct increase in cortisol within the first 30–45 min after awakening that is referred to as the cortisol awakening response (CAR; for an overview, see Clow, Hucklebridge, Stalder, Evans, & Thorn, 2010; Fries, Dettenborn, & Kirschbaum, 2009; Stalder et al., 2016). First described more than 20 years ago (Pruessner et al., 1997), the CAR has been demonstrated in numerous studies since then (Hucklebridge, Hussain, Evans, & Clow, 2005; Kudielka & Kirschbaum, 2003; Kunz-Ebrecht, Kirschbaum, & Steptoe, 2004; Schmidt-Reinwald et al., 1999; Stalder, Hucklebridge, Evans, & Clow, 2009; Steinheuser, Ackermann, Schönfeld, & Schwabe, 2014; Wilhelm, Born, Kudielka, Schlotz, & Wüst, 2007). The CAR has received considerable attention over the past decades as it is thought to be a noninvasive and easily accessible potential indicator of hypothalamus–pituitary–adrenal (HPA) axis function and dysfunction. Previous research

suggested that the CAR may be negatively associated with fatigue, burnout, or exhaustion (Pruessner, Hellhammer, & Kirschbaum, 1999; Roberts, Wessely, Chalder, Papdopoulos, & Cleare, 2004; for a review, see Chida & Steptoe, 2009). Furthermore, the CAR has been shown to be altered in post-traumatic stress disorder (PTSD) patients (Wessa, Rohleder, Kirschbaum, & Flor, 2006). Even more interestingly, the CAR has been suggested to be a predictor of major depression and schizophrenia, as well as treatment success (Berger et al., 2016; Meuret et al., 2015; Vrshek-Schallhorn et al., 2013).

Accumulating evidence indicates that the CAR develops early in life and that a reliable CAR can be already assessed in infants at only a few months of age (Stalder et al., 2013; Tegethoff, Knierzinger, Meyer, & Meinlschmidt, 2013). Nevertheless, there appear to be age-related differences in the CAR. In particular, it was reported that the CAR increases linearly over the first 7 years of life (Bäumler, Kirschbaum, Kliegel, Alexander, & Stalder, 2013). For instance, in an elegant longitudinal study, a positive CAR was found in about two thirds of 60-month-old children, and this increased to more than 93% at a second assessment 8 months later (DeCaro & Worthman, 2008). Another study suggested that only about half of the children between 5 and 11 years of age show a CAR (Michels et al., 2012). A subsequent study that employed specific tools to verify the time of sampling suggested that the CAR is already present in many children before the age of 12 months (Stalder et al., 2013). Age-related differences in the CAR may be due to the ongoing maturation of the hippocampus in this period of life (Keresztes et al., 2017), given that an intact hippocampus is assumed to be essential for the CAR (Buchanan, Kern, Allen, Tranel, & Kirschbaum, 2004; Pruessner, Pruessner, Hellhammer, Pike, & Lupien, 2007). Understanding the age-related dynamics of the CAR is important because the CAR may be a valuable biomarker of children's adverse experience (Kumsta et al., 2017) as well as a relevant predictor of cognitive functioning (Bäumler et al., 2014) and future mental health (Adam et al., 2010). While several earlier studies focused on the CAR in younger children (Bäumler et al., 2013; Stalder et al., 2013; Tegethoff et al., 2013) and adolescents (Bouma, Riese, Ormel, Verhulst, & Oldehinkel, 2009; Greaves-Lord et al., 2007; Platje et al., 2013), the CAR is less well studied in prepubertal children. This age group, however, is of particular interest because at the age of 6 to 9 years the hippocampus is typically largely developed, children's days become more and more structured as they enter school, but puberty and the associated hormonal changes have not yet begun.

The present study aimed to directly compare the magnitude and stability of the CAR in prepubertal children and adults. Furthermore, as previous research suggested that the CAR is influenced by sleep- and stress-related factors as well as by the day of the week in adults (Fries et al., 2009; Schlotz, Hellhammer, Schulz, & Stone, 2004; Stalder et al., 2016) but not in younger children (Bäumler et al., 2013), we examined whether these factors affect the CAR in prepubertal children. To this end, healthy children between 6 and 9 years of age as well as healthy adults between the ages of 35 and 50 collected four saliva samples across the first 45 min after awakening on 4 different sampling days, 2 weekdays and 2 weekend days. In addition, we collected sleep- and stress-related data on all 4 sampling days.

2 | METHOD

2.1 | Participants

We tested a total of 24 healthy children between the ages of 6 and 9 (12 boys, 12 girls; mean age = 7.58 years, *SEM* = 0.26 years; mean body mass index [BMI] = 15.28 kg/m^2, *SEM* = 0.33 kg/m^2) and 24 healthy adults between the ages of 35 and 50 (12 men, 12 women; mean age = 41.33 years, *SEM* = 0.79 years; mean BMI = 25.19 kg/m^2, *SEM* = 0.82 kg/m^2), which corresponds roughly to "middle adulthood" as a stage of relative stability (Santrock, 2009), including twelve parent-child dyads. All participants had a Caucasian ancestral background (for ethnicity differences in cortisol rhythmicity, see Martin, Bruce, & Fisher, 2012) and were in good mental and physical health (e.g., no mental disorder, no endocrine, cardiovascular, or any other physical illness), free of medication, and had no history of any mental or neurological disorders or any severe stressor during the previous months. Furthermore, smokers and women who were pregnant or used hormonal contraceptives were excluded from participation. We did not control for menstrual cycle phase in women as previous research indicated that the menstrual cycle phase does not affect the CAR (Kudielka & Kirschbaum, 2003, but see Ozgocer, Ucar, & Yildiz, 2017). This sample size was based on an a priori sample size calculation with the software G*Power (Faul, Erdfelder, Lang, & Buchner, 2007) showing that a sample of 48 participants is needed to detect a medium-sized effect with a power of 0.95. All participants provided written informed consent before participation in the study, which was approved by the local ethics committee and performed in accordance with the Declaration of Helsinki. For participating children, at least one parent provided written informed consent.

2.2 | Design and procedure

Participants and parents of participating children were first extensively informed by the experimenter about the study procedure and study material, including an on-site demonstration of how to collect saliva samples. They were explicitly instructed that it is critical to adhere strictly to the timings

of the sampling protocol (e.g., that it is critical to take the first saliva sample immediately after awakening) and that the compliance with the sampling protocol can be assessed from the saliva samples. In line with the recommendations for CAR research (Stalder et al., 2016), participants and participating parents were further instructed that they or their children should not brush their teeth, not have breakfast, not have any sugared or caffeinated drinks, or do any physical exercise before the last saliva sample of a sampling day. Moreover, participants and parents of participating children received several questionnaires and a sampling protocol that should be completed for each sampling day (see below). All testing took place between July and September to keep possible seasonal variations of the CAR (Thorn, Hucklebridge, Esgate, Evans, & Clow, 2004) to a minimum.

2.3 | Saliva sampling and analyses

All children and adults collected saliva samples on 4 days: on 2 weekdays and on 2 weekend days. Participants were not explicitly instructed on which weekdays they should collect the samples (i.e., weekday sampling days could vary from Monday to Friday). On each day, participants took four saliva samples: the first immediately after awakening, while still lying in bed, as well as 15, 30, and 45 min after awakening. Saliva samples were taken by means of Salivette collection devices (Sarstedt, Germany), which were kept for about 90 s in the mouth, and stored at −18°C until analysis (for 2–6 weeks). After data collection was completed, cortisol concentrations were analyzed from saliva using a luminescence assay (IBL, Germany). Intra- and interassay coefficients of variance were below 12% (for other references using this assay in children, see Bäumler et al., 2013, and Stalder et al., 2013).

2.4 | Control variables

As the CAR is assumed to be influenced, for instance, by sleep parameters and stress levels (Clow et al., 2010; Fries et al., 2009; Stalder et al., 2016), we asked participants or parents of participating children to complete for each sampling day a protocol reporting the time the participant or child went to bed, the sleep duration, the subjective sleep quality: on a scale from 0 (*very bad*) to 10 (*very good*), the subjective stressfulness of the previous day, as well as the expected stressfulness of the current day (both on a scale from 0 (*not at all stressful*) to 10 (*very stressful*). In addition, participants and parents of participating children were required to indicate the exact time at which they or their child woke up and at which times the different saliva samples were taken. For children, parents were requested to ask the children how well they slept and how stressed they felt and to indicate these responses of the children in the questionnaire; if children did not understand the concepts (e.g., stress), they were rephrased in a child-appropriate manner. In addition, adult participants completed the Beck Depression Inventory (BDI; Beck, Hautzinger, Baller, Worall, & Keller, 1995), the State-Trait Anxiety Inventory (STAI; Spielberger, Gorsuch, Lushene, Vagg, & Jacobs, 1983), and the Trier Inventory of Chronic Stress (TICS; Schulz & Schlotz, 1999) to assess participant's depressive mood and chronic stress levels and to determine whether these variables were related to the individual CAR.

2.5 | Statistical analyses

In line with a recent consensus paper on the CAR (Stalder et al., 2016), we used the area under the curve with respect to the increase (AUCi) after awakening, calculated according to the formula provided by Pruessner and colleagues (Pruessner, Kirschbaum, Meinlschmid, & Hellhammer, 2003):

$$\mathrm{AUCi} = \left(\sum_{j=1}^{n-1} \frac{(m\,(j+1) + mj}{2} \right) - (n-1)\,m1$$

with m*j* denoting the single measurements and *n* denoting the total amount of measurements.

In order to analyze age group differences as well as influences of weekday versus weekend and participants' gender, we subjected the AUCi data to a mixed analysis of variance (ANOVA) with the between-subjects factors group (children vs. adults) and gender (male vs. female) as well as the within-subject factors day (weekday vs. weekend) and sampling day (first vs. second sampling day). Significant interaction effects were pursued by appropriate post hoc tests that were Bonferroni corrected, if required. To assess the stability of the CAR, we correlated the AUCi scores for the 2 weekdays and weekend days, respectively. Further correlations were performed to assess associations with stress, sleep, or questionnaire data. In addition to the AUCi, we also analyzed the basal cortisol sample immediately after awakening as well as the increase within the first 30 min after awakening (i.e., the difference between the cortisol concentrations 30 min after awakening and immediately after awakening, to determine whether there was a notable CAR or not). All statistical analyses were performed with SPSS for Windows, version 22 (IBM, USA). In case of violation of sphericity, Greenhouse-Geisser correction was used. AUCi for weekdays in adults did not meet the normality assumption. However, as all other data met this assumption and the ANOVA appears to be relatively robust against violations of normality (Glass, Peckham, & Sanders, 1972), we still subjected our data to the ANOVA model. All reported *p* values are two-tailed.

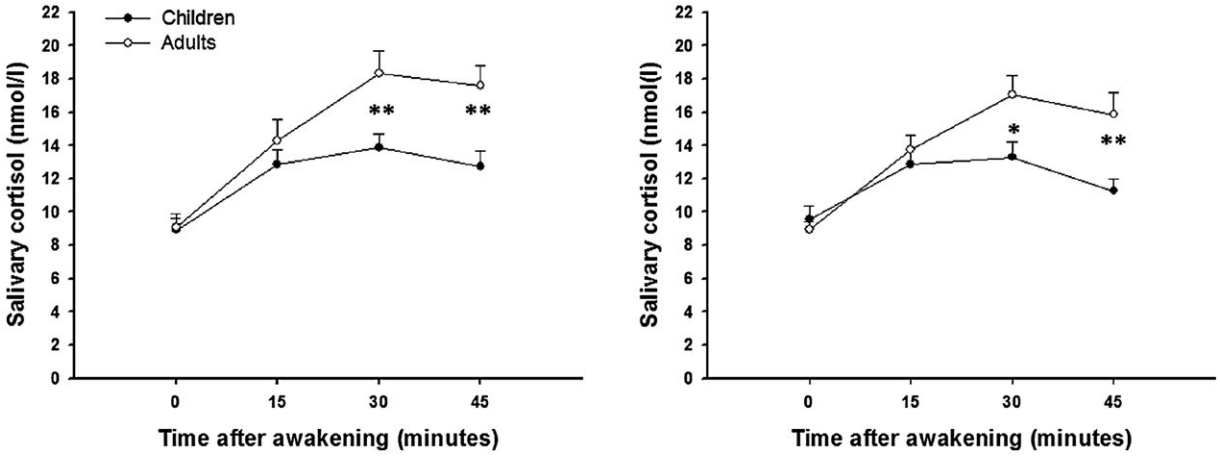

FIGURE 1 Cortisol awakening response in adults and children. Shown are averaged data for the four time points of measurement across the 2 weekdays and weekend days, respectively. There was a marked increase in salivary cortisol after awakening in both adults and children, yet this increase was significantly smaller in children compared to adults on both weekdays and weekend days. Note that analyses focused mainly on the area under the curve with respect to the increase (AUCi; see Method). Data represent means ± standard error of the mean. $^{**}p < 0.01$; $^{*}p < 0.05$

3 | RESULTS

3.1 | More variable and less pronounced magnitude of the CAR in prepubertal children compared to adults

As shown in Figure 1, there was a marked increase in cortisol in response to awakening, defining the CAR, in children and adults on weekdays and weekend days. In both age groups, the minimum cortisol increase within the first 30 min after awakening was, on all four test days, at least 38% relative to the baseline at awakening. Previous studies categorized participants into those that show a CAR and those that do not, depending on whether the increase from the awakening baseline to 30 min postawakening was above or below 2.5 nmol/l (Rosmalen et al., 2005; Wüst et al., 2000). Based on this criterion, about 42% of the participants, across both groups, showed a CAR on all four test days.

In order to assess the stability of the CAR across testing days and whether this stability is comparable in children and adults, we correlated the AUCi after awakening, our main measure of the CAR, on the 2 weekdays and weekend days, separately, both for adults and children. As displayed in Figure 2, the AUCi values were significantly correlated for both weekdays ($r = 0.448$, $p = 0.028$) and weekends ($r = 0.540$, $p = 0.006$) in adults. In children, however, the correlations between AUCi values after awakening were rather low, both on weekdays ($r = 0.162$, $p = 0.461$) and weekends ($r = 0.251$, $p = 0.237$), suggesting a less stable CAR in children. In line with this interpretation, 75% of the adults showed a marked CAR response (i.e., above 2.5 nmol/l) on both weekdays and still 70% of them on both weekend days. In children, in turn, 60% showed a marked CAR on both weekdays and only 52% on both weekend days. One possible explanation for the differences in the stability of the CAR in the two age groups might be that factors that have been shown to affect the CAR, such as sleep duration, sleep quality, stress level at the previous day, or stress level at the present day (for an overview, see Fries et al., 2009), varied more strongly between testing days in children than in adults. However, when we compared these factors between the 2 weekdays and weekend days, respectively, we did not obtain significant differences between sampling days in terms of those factors, neither in children nor in adults (all $t < 1.76$, all $p > 0.092$, all $d < 0.287$; see Table 1). Only for adults, the factor stress level of the previous day differed between the two weekend sampling days, $t(23) = 2.75$, $p = 0.011$, $d = 0.576$. However, this difference would not survive correction for multiple testing, and it would further implicate a higher variability in the CAR in adults, whereas our findings show a high correlation between weekend AUCi values in adults than in children.

In a next step, we aimed to determine whether the magnitude of the CAR, expressed as AUCi, differed in children compared to adults and whether potential group differences were modulated by participants' gender or by the test day (weekday vs. weekend). To this end, we subjected the AUCi data to an Age Group × Gender × Test Day × Sampling Day (first vs. second) mixed-design ANOVA. This analysis revealed that the CAR was significantly less pronounced in children than in adults (main effect age group: $F(1, 43) = 9.40$, $p = 0.004$, $\eta^2 = 0.179$), irrespective of participants' gender or the test day (interaction effects: both $F(1, 43) < 0.24$, both $p > 0.627$, both $\eta^2 < 0.01$; Figure 1). On average, the increase in cortisol from awakening to 30 min postawakening was 42% lower in children compared to adults on weekdays, $t(45) = 2.46$, $p = 0.018$, $d = 0.68$%, and 53% lower than in adults at the weekend, $t(46) = 2.87$, $p = 0.006$, $d = 0.80$. The differential magnitude of the

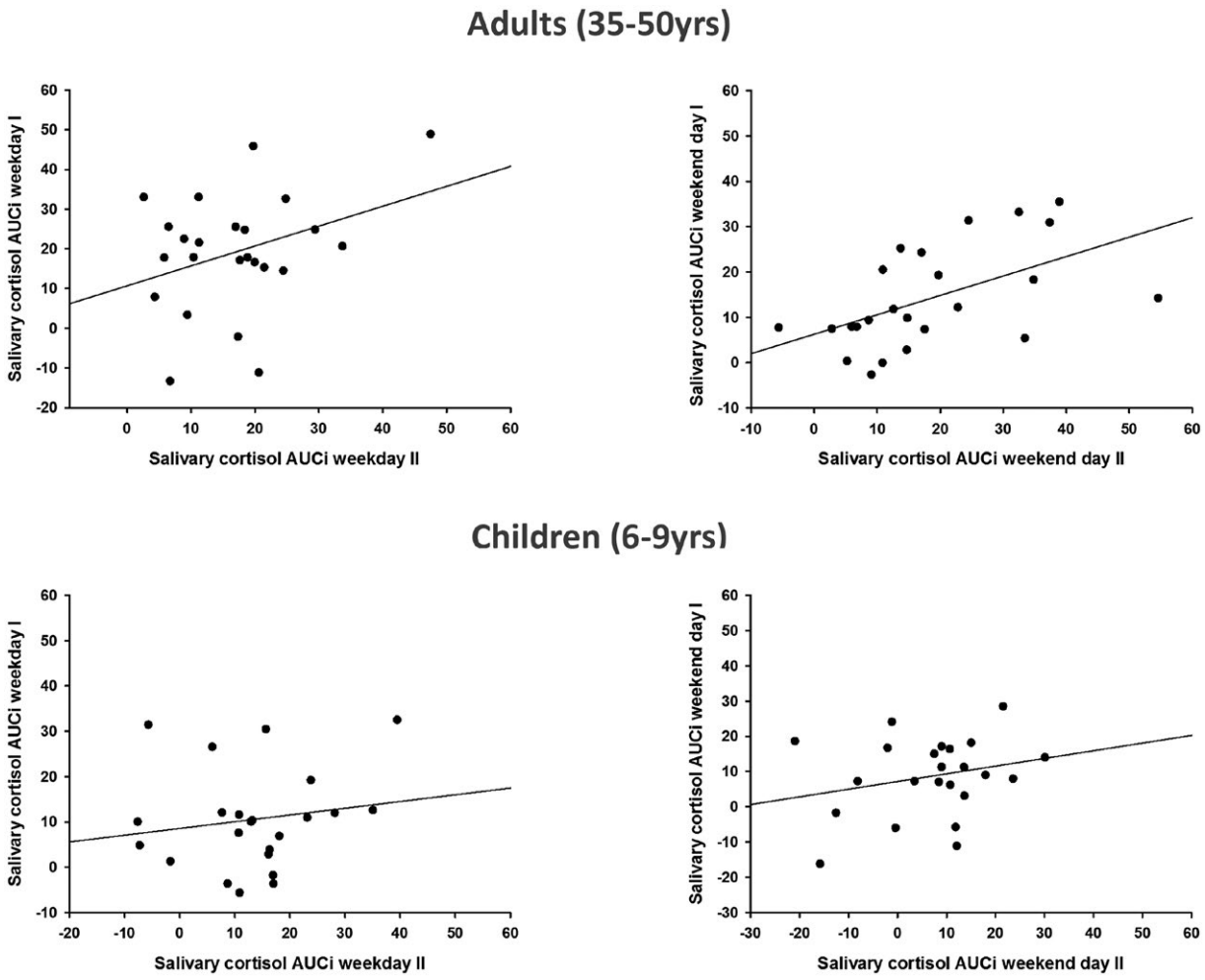

FIGURE 2 Correlation of cortisol awakening responses (CAR), expressed as area under the curve with respect to the increase (AUCi), across sampling days in adults and children. Correlations were performed separately for the 2 weekday measurements of the CAR and the 2 weekend measurements of the CAR. CAR measurements were highly correlated between both the 2 weekday sampling days and the 2 weekend sampling days in adults. In children, there were no such correlations

CAR in prepubertal children and adults might be simply due to the fact that children show a marked CAR less often than adults, as reported above. To address this possible argument, we limited in the next step our ANOVA to participants who did show a CAR above 2.5 nmol/l on all four sampling days ($n = 7$ children, $n = 12$ adults). This analysis with a reduced sample replicated the age group difference in the CAR, showing a large effect size, although this effect was only marginally significant due to the loss of statistical power (main effect age group: $F(1, 15) = 4.39$, $p = 0.053$, $\eta^2 = 0.227$). Notably, when we used the cortisol concentration after awakening, instead of the AUCi, we did not find any difference between children and adults (main effect age group: $F(1, 43) = 0.004$, $p = 0.947$, $\eta^2 < 0.001$, Figure 1; nor was there any influence of gender or test day, all $F(1, 43) < 0.404$, all $p > 0.527$, all $\eta^2 < 0.01$). This result suggests that it was not the basal cortisol concentration that differed between age groups, but specifically the increase after awakening.

Although previous research suggested that the CAR may be different between weekdays and weekend (Kunz-Ebrecht, Kirschbaum, & Steptoe, 2004; Schlotz et al., 2004), our ANOVA model yielded only a nonreliable trend for a lower AUCi after awakening at the weekend relative to weekdays (main effect test day: $F(1, 43) = 2.69$, $p = 0.108$, $\eta^2 = 0.06$; any interaction effects including this factor: all $F(1, 43) < 0.239$, all $p > 0.627$, all $\eta^2 < 0.01$; Figure 2). Furthermore, the CAR was not affected by participants' gender (all main and interaction effects: all $F(1, 43) < 0.66$, all $p > 0.423$, all $\eta^2 < 0.02$).

3.2 | Impact of control variables

Not surprisingly, adults woke up earlier than children, reported higher stress levels at the day before the sampling day, and expected higher stress levels associated with the coming day (see Table 1). To test whether these differences could explain the observed differences in the CAR between children and adults, we first matched our samples with respect to the time

TABLE 1 Sleep and stress level data for adults and children on all sampling days

	Adults				Children				Adults versus children	
	Weekday I	Weekday II	Weekend Day I	Weekend Day II	Weekday I	Weekday II	Weekend Day I	Weekend Day II	F	p η^2
Wake-up time (hr:min)	06:03 (0:09)	06:01 (0:09)	06:54 (0:13)	7:09 (0:16)	06:58 (0:10)	07:02 (0:15)	07:20 (0:10)	07:30 (0:11)	11.58	0.001 0.21
Sleep duration (hr)	7.09 (0.23)	6.86 (0.26)	7.41 (0.26)	7.53 (0.26)	10.15 (0.18)	10.25 (0.28)	10.19 (0.12)	10.40 (0.18)	177.70	< 0.001 0.80
Sleep quality	6.50 (0.43)	6.75 (0.33)	6.71 (0.45)	6.88 (0.33)	7.67 (0.44)	7.37 (0.43)	7.17 (0.46)	7.46 (0.49)	1.29	0.262 0.03
Stress level previous day	3.83 (0.42)	4.25 (0.44)	3.79 (0.50)	2.83 (0.41)	2.63 (0.50)	2.71 (0.46)	2.58 (0.43)	2.71 (0.41)	4.32	0.043 0.09
Expected stress level upcoming day	4.54 (0.43)	4.66 (0.44)	3.04 (0.44)	2.83 (0.47)	2.29 (0.43)	3.33 (0.57)	1.88 (0.37)	1.63 (0.26)	11.83	0.001 0.21

Note. Data represent means (*SEM*). Sleep quality and stress level ratings were given on a scale from 0 to 10.

TABLE 2 Standard questionnaire data of the adult sample

Variable	Score
Beck-Depression Inventory	2.92 (0.74)
State Anxiety (t score)	48.00 (1.82)
Trait Anxiety (t score)	47.00 (1.59)
Chronic Stress Screening Scale (t score)	48.71 (1.89)

Note. Data represent means (*SEM*).

of awakening by excluding the five children with the latest average time of awakening and the five adults who woke up earliest across the 4 sampling days (main effect of age group for wake-up time in this sample: $F(1, 34) = 1.49$, $p = 0.231$, $\eta^2 = 0.04$). This matching procedure also led to more similar stress levels on the day before sampling in children and adults (main effect age group: $F(1, 34) = 1.18$, $p = 0.285$, $\eta^2 = 0.03$). Interestingly, the AUCi values after awakening were still higher in adults than in children in this matched sample (main effect age group: $F(1, 33) = 4.22$, $p = 0.048$, $\eta^2 = 0.11$), despite the reduction in statistical power, suggesting that the time of awakening and the stress level of the previous day are at least not the main factors driving the age group differences in the CAR. In support of this view, neither the time of awakening nor the subjective stress level on the previous day correlated with the AUCi at weekdays or weekends across groups (all $|r| < 0.21$, all $p > 0.167$), nor in children or adults alone (all $|r| < 0.15$, all $p > 0.549$). The expected stress level of the upcoming day, however, was still higher in adults than in children in the reduced sample matched for wake-up time (main effect age group: $F(1, 34) = 5.15$, $p = 0.030$, $\eta^2 = 0.13$). Furthermore, the expected stress level of the coming day correlated, across groups, with the AUCi at weekdays ($r = 0.34$, $p = 0.019$; but not at the weekend: $r = 0.11$, $p = 0.466$). Follow-up tests revealed that this association for weekdays was present in children ($r = 0.50$, $p = 0.012$) but not in adults ($r = 0.17$, $p = 0.44$), albeit this apparent difference was only a statistical trend ($z = 1.21$, $p = 0.113$).

Standard questionnaires that were completed by the adult participants showed that the participating adults were in the healthy control range with respect to their depressive mood, chronic stress level, state, and trait anxiety (see Table 2). Correlational analyses showed that the AUCi at weekdays was significantly correlated with depressive mood ($r = 0.45$, $p = 0.028$) and state anxiety ($r = 0.48$, $p = 0.017$; all other correlations: all $r < 0.31$, all $p > 0.138$). However, these correlations would not survive the correction for the number of correlations performed and are thus to be interpreted only with great caution.

4 | DISCUSSION

The CAR is an important biomarker that has been related to health and well-being (Chida & Steptoe, 2009; Clow et al.,

2010; Fries et al., 2009). In the present study, we aimed to contrast the CAR in healthy prepubertal children and adults. Our data show that the CAR, although clearly present in prepubertal children, was less stable and less pronounced in children compared to adults.

How can the attenuated CAR in 6- to 9-year-old children be explained? In the face of obvious differences in the hormonal status of prepubertal children and adults, one might assume that lower sex hormone concentrations may explain the smaller CAR in children. Sex hormones may modulate the cortisol response to stress (Kirschbaum, Kudielka, Gaab, Schommer, & Hellhammer, 1999) and the CAR appears to be indeed higher during female ovulation (Wolfram, Bellingrath, & Kudielka, 2011), yet the data on the role of the menstrual cycle phase in the CAR are inconsistent (Bouma et al., 2009; Kudielka & Kirschbaum, 2003; Ozgocer et al., 2017). Thus, different sex hormone levels might contribute to age differences in the CAR. Alternatively, it might be argued that the smaller CAR in prepubertal children is owing to methodological issues. Noncompliance with the sampling protocol, in particular a delay in the first sample after awakening, may lead to a seemingly smaller CAR (Broderick, Arnold, Kudielka, & Kirschbaum, 2004; Kudielka, Broderick, & Kirschbaum, 2003). Thus, the attenuated CAR in children might have been due to a systematic delay in sampling in children relative to adults. We consider this explanation to be rather unlikely because all participants and participating parents confirmed in the aftermath of the data collection that they strictly adhered to the instructions (see also below). Moreover, if the first samples after awakening were collected considerably later in children than in adults, one would expect to see different baseline cortisol concentrations in children and adults, yet such differences were not observed.

We did observe, however, significant age group differences in sleep parameters and stress levels. As expected, adults slept less and woke up earlier than children, they felt more stressed on the previous day and predicted higher stress levels for the upcoming day. Matching the groups for wake-up time and stress level of the previous day indicated, in line with correlational analyses, that these factors did not drive the age differences in the CAR. An influence of the predicted stress level of the current day, however, could not be ruled out. Beyond age-dependent differences in sleep duration or sleep quality, there are also characteristic differences in the sleep architecture of children and adults. For instance, children typically show more slow-wave sleep activity (Ohayon, Carskadon, Guilleminault, & Vitiello, 2004). As HPA axis activity during the night is under control of sleep factors (Born & Fehm, 1998) and may influence the cortisol rise after awakening, it might well be that changes in the sleep architecture between children and adults translate into differences in the CAR. Indeed, the CAR in children was recently linked to sleep duration and slow-wave sleep (Lemola et al., 2015). Lastly, the brain of prepubertal children is obviously not yet fully mature. Although the hippocampus appears to be largely developed at this age, the prefrontal cortex is fully mature only in early adulthood (Gogtay et al., 2004). Whereas previous research linked in particular the hippocampus to the CAR (Buchanan et al., 2004; Pruessner et al., 2007), the prefrontal cortex has also a well-documented impact on the regulation of the HPA axis (Diorio, Viau, & Meaney, 1993; Ulrich-Lai & Herman, 2009). Thus, there may also be age-related neural changes in the control of the HPA axis that contribute to the attenuated CAR in prepubertal children compared to adults.

Although previous research suggested that the subjective stress level may influence the CAR and that the CAR is smaller at weekends relative to weekdays (Fries et al., 2009; Kunz-Ebrecht, Kirschbaum, Marmot, & Steptoe, 2004; Schlotz et al., 2004), our data did not confirm these findings. The lack of such effects, however, may be at least partly due to insufficient statistical power to detect these effects, as previous studies showing, for instance, differences between weekdays and weekend days tested typically significantly larger sample sizes. In line with previous research (Bouma et al., 2009), we did not find a modulation of the CAR by participants' gender, neither in adults nor in children. In light of inconsistent findings on the influence of the menstrual cycle on the CAR (Bouma et al., 2009; Kudielka & Kirschbaum, 2003; Ozgocer et al., 2017), future studies in larger samples are required to assess the menstrual cycle phase as a potential modulator of the CAR. Another interesting factor that might affect the CAR in children and would be worth investigating in future studies is the mental state of the parents (Pine et al., 2005).

Finally, a limitation of this study may be seen in the lack of objective verification of awakening and sampling times (see also Stalder et al., 2016). We did instruct all participants and participating parents explicitly and in a separate session that it is critical to adhere strictly to the sampling protocol and that we would assess compliance from the saliva samples. The sampling protocols indicated that participants did comply with the instructions. According to those protocols, only very few samples were taken shortly (i.e., less than 3 min) after the planned time point of saliva collection. Moreover, the magnitude of the CAR (in adults) was very similar to the magnitude of the CAR that was reported in earlier studies that employed objective monitoring devices to verify compliance (Dockray, Bhattacharyya, Molloy, & Steptoe, 2008; Stalder, Evans, Hucklebridge, & Clow, 2011), lending further support to the assumption of high compliance rates in the present study. Nevertheless, we cannot completely rule out a lack of compliance in some participants on some of the sampling days.

In sum, we show here that there is a characteristic rise in cortisol in response to awakening (i.e., a CAR) in prepubertal children. Compared to the CAR in adults, however, the CAR

is less stable and less pronounced in prepubertal children between 6 and 9 years of age. These age differences should be taken into account when using the CAR as a biomarker of well-being in children or even as a predictor of mental health.

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How to cite this article: Bernsdorf M, Schwabe L. Cortisol response to awakening in prepubertal children and adults: Magnitude and variability. *Psychophysiology*. 2018;55:e13273. https://doi.org/10.1111/psyp.13273