The cortisol awakening response in infants: Ontogeny and associations with development-related variables

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Received 4 April 2012; received in revised form 24 July 2012; accepted 25 July 2012

KEYWORDS
Cortisol awakening response; Ontogeny; Development; Infant; Sleep

Summary The cortisol awakening response (CAR) is a frequently used measure in psychoneuroendocrinological research, however, some of its more fundamental aspects still require attention. An important question in this respect concerns the ontogeny of the CAR. Data from two recent reports suggest that the CAR may only emerge relatively late during child development (≥16 months of age). However, as both enquiries did not use objective means of verifying participant adherence or infants’ awakening times, it is unclear whether methodological factors may have contributed to these results. Here, we report data from a study on 33 infants aged 2–12 months with close care being taken to ensure the accuracy of sampling times by using wrist actigraphy and electronic monitoring containers. Salivary cortisol levels were assessed at 0 and 30 min post-awakening over three study days. Results revealed evidence for a significant CAR (≥2.5 nmol/L) in 32 (out of 33) infants and on a total 86.9% of study days, with a marked magnitude of the CAR across infants (mean estimated increase = 12.54 nmol/L). In addition, the cortisol level on awakening and the CAR were found to be associated with different aspects of infant’s physical and sleep-related development as well as with their weight and body mass index (BMI) at birth. Contrary to previous reports, the current results thus indicate that the ontogeny of the CAR occurs at an early stage of development and that it is present from as early as two months of life. The data also suggest that post-awakening cortisol secretion may undergo considerable changes during the first year of life associated with different aspects of infant development.

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1. Introduction

The cortisol awakening response (CAR), the marked increase of cortisol secretion following morning awakening, is frequently assessed in psychoneuroendocrinological research (see Fries et al., 2009; Clow et al., 2010). Despite a growing body of research examining the CAR in relation to different psychosocial and health-related variables, fundamental

Please cite this article in press as: Stalder, T., et al., The cortisol awakening response in infants: Ontogeny and associations with development-related variables. Psychoneuroendocrinology (2012), http://dx.doi.org/10.1016/j.psyneuen.2012.07.015
knowledge regarding the CAR is still fragmented (Clow et al., 2010). An area of investigation which has received little attention so far is the ontogeny of the CAR, i.e., its first occurrence during child development as well as the main correlates of this process. Enhancing current understanding of this issue may not only increase basic knowledge about the CAR but could also help to provide further insights into the functional role of this still poorly understood aspect of endocrine activity.

Information regarding the ontogeny of the CAR may be indirectly derived from research into the development of overall circadian cortisol rhythmicity. Here, most evidence suggests that the development of a stable circadian pattern of cortisol secretion tends to fall into the first half year of life (Price et al., 1983; Spangler, 1991; Antonini et al., 2000), even though with considerable individual differences (De Weerth et al., 2003). A factor which appears to be closely related to the appearance of a circadian cortisol rhythm is the development of a stable 24-h sleep—wake cycle (Price et al., 1983; Spangler, 1991; Antonini et al., 2000; De Weerth et al., 2003; but see Santiago et al., 1996). The relationship with sleep-related development may be particularly strong for cortisol secretion during the morning hours; e.g., the shifting of peak basal cortisol concentrations into the early morning period has been found to concur with the entrainment of a nighttime sleep rhythm between 12 and 16 weeks of age (Spangler, 1991; Larson et al., 1998). Infants who sleep through the night are also more likely to show an early morning peak (Larson et al., 1998). Similarly, more efficient and less fragmented sleep in toddlers aged 12–36 months has been found to be associated with lower awakening cortisol levels, perhaps due to a suppressive effect of unfragmented sleep on cortisol secretion (Scher et al., 2010).

The above evidence may suggest that, similar to the development of circadian cortisol rhythmicity, the ontogeny of the CAR could also take place during the first half year of life in concurrence with the establishment of a 24-h sleep—wake cycle. However, as the CAR is known to be a distinct aspect of basal cortisol secretion (Wüst et al., 2000a; Edwards et al., 2001; Wilhelm et al., 2007), it is unclear how well evidence on the development of general circadian rhythmicity translates to the CAR. To date only a small number of studies have directly examined the CAR in infants or preschool age children. A study on 366 infants aged 12–20 months revealed that on average infants below 16 months of age did not show a positive CAR (Saridjan et al., 2010). In line with this, a recently published study on 32 infants aged 7–17 months also failed to provide evidence for a positive CAR in this age group and, indeed, reported a declining pattern of cortisol levels from awakening to 30 min post-awakening (Bright et al., 2011). In a longitudinal study on somewhat older children, a positive CAR was only found in 68% of 60-month-old children and this increased to 93% at a second assessment eight months later (DeCaro and Worthman, 2008). While these results may suggest that in most children the CAR emerges only relatively late in development, it is important to note that the above studies did not employ means to objectively verify children’ awakening times and/or times of saliva sampling. As participant non-adherence constitutes a major problem in CAR research (Kudielka et al., 2003; Broderick et al., 2004; Kupper et al., 2005), it is conceivable that the low responder rates in these studies may have also been influenced by methodological factors. This is also supported by recent evidence from a small but well-controlled study, using polysomnographic sleep recordings and wrist actigraphy, in which all of the seven examined children aged 30–48 months exhibited a robust CAR (Gribbin et al., 2011). To the best of our knowledge, no published research has examined the CAR in children below 12 months of age carefully controlling for the accuracy of saliva sampling in relation to awakening time.

Taken together, while evidence regarding the development of overall circadian cortisol rhythmicity suggests that the CAR might also develop early during the first year of life, this possibility is yet to be carefully examined. Here, we thus set out to provide first data on the ontogeny of the CAR in 2–12-month-old infants using repeated CAR assessments over three study days and rigorous control for participant adherence to the saliva collection protocol. In addition, as previous research had indicated potential influences of both infant-related and environmental factors on the ontogeny of the CAR (e.g., Saridjan et al., 2010), cortisol associations with different sleep-related, behavioural and anthropometric parameters of the infant as well as with relevant parent data were also examined.

2. Methods

2.1. Participants

The study sample comprised 33 healthy infants aged 2–12 months from the greater Dresden area (see Table 1 for sample characteristics). Only term-born infants (after 37 weeks gestational age) who were currently in good mental and physical health and not taking medication (based on parents’ self-report) were included in the study. Families were recruited at local child care centres, paediatric practices, breastfeeding groups or parent—child courses. Of the participating infants, 30 were exclusively cared for by their primary caregiver (their mother in all instances) while 3 infants also received additional care by an extra-familial childminder. For all participating infants, at least one parent provided written informed consent. The study protocol was approved by the local ethics committee and carried out in accordance with the Declaration of Helsinki. Each parent—child pair received 20 Euro for participation in the study.

2.2. Design and procedure

Interested parents were first informed about the study via email or telephone. Those willing to participate were then invited to the Technical University of Dresden or visited at their homes by a researcher. Here, extensive oral and written information about the study was provided and informed consent was obtained. Parents were familiarised with the study package comprising saliva sampling materials, the study questionnaires (see below) and an instructional DVD on saliva sampling in infants. Careful on-site training on how to obtain saliva samples in young infants was then provided and the importance of strict adherence to the study protocol was emphasised to parents.

Following the initial visit, parents were asked to collect saliva samples from their infants on three non-consecutive
Table 1 Means, standard deviations (SD) and ranges of (a) infants’ anthropometric data, sleep-related information and cortisol concentrations as well as (b) parents’ self-report data.

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>SD</th>
<th>Min</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Infants</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (months)</td>
<td>6.52</td>
<td>2.84</td>
<td>2</td>
<td>12</td>
</tr>
<tr>
<td>Birth height (cm)</td>
<td>51</td>
<td>2</td>
<td>48</td>
<td>56</td>
</tr>
<tr>
<td>Birth weight (kg)</td>
<td>3.51</td>
<td>.42</td>
<td>2.80</td>
<td>4.60</td>
</tr>
<tr>
<td>BMI at birth</td>
<td>13.45</td>
<td>1.05</td>
<td>11.28</td>
<td>15.65</td>
</tr>
<tr>
<td>Duration of pregnancy (weeks)</td>
<td>39.88</td>
<td>1.57</td>
<td>36</td>
<td>44</td>
</tr>
<tr>
<td>Current height (cm)</td>
<td>68</td>
<td>5</td>
<td>56</td>
<td>76</td>
</tr>
<tr>
<td>Current weight (kg)</td>
<td>8.06</td>
<td>1.62</td>
<td>4.80</td>
<td>12.00</td>
</tr>
<tr>
<td>Current BMI</td>
<td>17.19</td>
<td>1.90</td>
<td>12.49</td>
<td>20.78</td>
</tr>
<tr>
<td>Number of daytime naps (median)</td>
<td>2.92</td>
<td>.92</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Sleep onset (hh:mm)</td>
<td>20:13</td>
<td>0:57</td>
<td>18:13</td>
<td>22:59</td>
</tr>
<tr>
<td>Awakening time (hh:mm)</td>
<td>6:44</td>
<td>0:39</td>
<td>5:24</td>
<td>8:17</td>
</tr>
<tr>
<td>Sleep through night (in %)</td>
<td>39</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cortisol 0 min (nmol/L) a</td>
<td>12.33</td>
<td>5.31</td>
<td>3.67</td>
<td>25.36</td>
</tr>
<tr>
<td>Cortisol 30 min (nmol/L) a</td>
<td>24.42</td>
<td>9.22</td>
<td>3.71</td>
<td>47.88</td>
</tr>
<tr>
<td>(b) Parents</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PSS</td>
<td>23.21</td>
<td>7.72</td>
<td>9</td>
<td>43</td>
</tr>
<tr>
<td>Socioeconomic ladder (cm)</td>
<td>5.19</td>
<td>1.46</td>
<td>.50</td>
<td>7.20</td>
</tr>
</tbody>
</table>

Abbreviations: BMI: body mass index; PSS: Perceived Stress Scale.

a Based on n = 28.

b Descriptive information, not based on model estimations.

weekdays (two weekdays, one weekend day) across a two-week period. They were also asked to fill out a short sampling protocol recording their infant’s bed and awakening times, times of sample taking and any difficulties with the sample collection. Parents were also actively encouraged to contact the research team in case of any difficulties with the saliva sampling. Following collection, saliva samples were stored in the parents’ home freezers and transferred to the laboratory along with the other study materials after the final sampling day.

2.3. Saliva sampling in infants and adherence control

On each of the three non-consecutive study days, saliva samples were taken immediately upon morning awakening of the infant and at 30 min post-awakening. To reduce the risk of infants waking up spontaneously prior to the first saliva sampling, parents were asked to gently wake-up their child themselves and to then directly take the first saliva sample. In case there was any indication that the child had already woken up spontaneously, parents were told to postpone saliva collection until the following day. Mothers were also asked not to breastfeed their infants or to provide them with food or drink within 2 h prior to sampling as feeding has been suggested to inhibit infants’ adrenocortical activity (Spangler, 1991). During the 30 min post-awakening period, mothers were asked to continue with their normal routines (except for feeding) but to make sure that their infant did not fall asleep again. Verbal and written feedback of parents consistently indicated that the sampling procedure, including saliva sampling (see below), was well tolerated by the infants and did not result in any apparent negative emotion responses, e.g. strong crying, during the post-awakening periods.

Saliva sampling was carried out using eye sponges (bvi Beaver Visitec, Waltham, USA). These are collection devices which are well-suited for the use in infants and younger children and have been validated for collecting saliva for cortisol determination (De Weerth et al., 2007). Each eye sponge consists of a plastic shaft with an arrowhead made of absorbing sponge material. To obtain a sample, parents placed the sponge part under the infant’s tongue until it was saturated with saliva and visibly swelled many times over. The top part of the saturated eye sponge was then cut off and placed into a pre-labelled, sealable tube (Swab Storage Tubes, Salimetrics Europe Ltd., UK). At each time point, saliva was sampled using four eye sponges to obtain a sufficient amount of saliva. The whole sampling procedure took approximately 1–2 min per sampling point. Saliva samples were stored at −20 °C until assaying. After thawing, saliva samples were centrifuged for 10 min at 4000 rpm. Salivary cortisol concentrations were analysed via a commercially available chemiluminescence immunoassay with an analytical sensitivity of 2.46 ng/mL and inter and intra-assay coefficients of variance below 8% (CLIA, IBL, Hamburg, Germany).

Objective measures were employed to verify both (i) infants’ awakening times and (ii) times of saliva sampling. Motility readings from wrist-worn Actiwatch 2 devices (AW2; Phillips Respironics, Murryville, PA), which infants wore on each of the three nights prior to morning sampling, were utilised to determine the moment of awakening (as previously reported in Stalder et al., 2010b; Gribbin et al., 2011). These devices use piezoelectric sensors to record movement intensity and have previously been validated against polysomnography (Lichstein et al., 2006). AW2 data were analysed using Respironics Actiware software (Phillips Respironics, Murryville, PA). To objectively verify times of sample taking, MEMS 6 TrackCap containers (Aardex Ltd.,
Switzerland) were used. These devices electronically detect and store times of container openings and thus allow verification of participants’ adherence to the sampling regime. The use of MEMS containers has previously been shown to increase adherence to the assessment protocol (Kudielka et al., 2003). Parents were fully informed about the nature of both monitoring devices.

2.4. Self-report measures

Infant sociodemographic and anthropometric information (age, sex, current weight and height, birth weight and length), sleep-related characteristics (number of daytime naps, nighttime sleep onset, lightening and noise conditions in the infants’ bed room) and pregnancy duration were obtained using a self-developed questionnaire. Furthermore, parents’ subjective socioeconomic status (SES) was assessed using the socioeconomic ladder of Adler et al. (1994), scored as a visual analogue scale ranging from 0 cm (very low SES) to 10 cm (very high SES). The 14-item Perceived Stress Scale (PSS; Cohen et al., 1983) was used to assess perceived chronic stress of the infants’ primary caregiver over the period of the previous month.

2.5. Data exclusion and statistical analysis

Non-adherence criteria were specified combining information from parents’ self-reports and the objective means of verifying awakening and sampling times. Following Okun et al. (2010), cortisol data of a study day were excluded if a difference > 15 min was found between (i) self-reported awakening time and AW2-verified awakening time and/or between (ii) pre-specified sampling times (based on AW2-data) and MEMS-verified sampling times. Based on these criteria, at least one compliant study day was found for each of the 33 participants. Of the 99 study days (N = 33 × 3 study days), 77 were classified as compliant and 17 as non-compliant. For the remaining five study days, AW2 and/or MEMS data were missing and they were hence excluded from analyses. An insufficient amount of saliva was further provided on 12 of the 77 compliant study days, leading to a total of 65 study days remaining for analyses. These comprised 11 infants with one compliant study day, 12 infants with two compliant study days and 10 infants with three compliant study days. Interestingly, the number of missing study days was found to be positively associated with infants’ current age, height and weight (r between .38 and .52, all p < .05), indicating that it was more difficult to obtain valid cortisol data from older and more physically developed infants. For all parent–infant pairs, except for one which had to repeat a measurement, compliant study days were carried out within the designated two-week time period with a mean (range) difference of 3.1 (1–6) days between the first and second assessment and of 3.3 (1–8) days between the second and third assessment.

Statistical analyses were performed using JAGS 3.2.0 (Plummer, 2003) with R 2.15.0 statistical software (R Development Core Team, 2010). Cortisol data were found to be normally distributed. To account for the relatively high number of missing values due to strict exclusion of non-compliant study days, analyses were conducted using a Bayesian mixed effects model framework with uninformative priors (Christensen et al., 2011). The model fitted to the data² entailed a fixed-effect coefficient (βi) reflecting the magnitude of changes in cortisol concentrations over the post-awakening period (i.e., the CAR) as well as two fixed-effect coefficients (β2 and β3) jointly reflecting a main effect of study day. As part of the Bayesian mixed effects model, Gibbs sampling was performed from two Markov chains comprising 5,000,000 iterations each, thinned by intervals of 50. Gelman–Rubin diagnostics (Gelman andRubin, 1992) indicated convergence of both chains. While inferential statistics were based on model estimations, for descriptive purposes in tables and figures, original cortisol data accumulated across the available compliant study days of each participant are shown.

Study days on which cortisol concentrations increased by at least 2.5 nmol/L were classified as responder days (Wlist et al., 2000b). Two composite measures were used to quantify post-awakening cortisol levels: the level of cortisol on awakening (S1) and the difference in cortisol concentrations between S1 and the second saliva sample (0–30 delta; see Clow et al., 2010). Associations between these measures and sociodemographic, sleep-related and psychosocial variables were examined using Bayesian correlation analyses. If more than one variable was found to be significantly related to cortisol measures, the intercorrelation patterns of the respective variables were examined using principal component analysis with orthogonal rotation (varimax) and parallel analysis being used to decide on the number of common factors (Bujas and Ebyoboglu, 1992).

3. Results

3.1. Cortisol awakening response in young infants

Descriptive information on child and parent characteristics is provided in Table 1. Fig. 1a and b depicts mean post-awakening cortisol concentrations over the three study days across the whole sample and for specific age groups, respectively. A significant CAR (increase ≥ 2.5 nmol/L) was seen on 86.9% of study days and 32 (out of 33) infants were able to mount a CAR on at least one of the three study days. The Bayesian mixed effects model of changes in cortisol levels over the post-awakening period revealed a mean cortisol increase (β1) of 12.54 (CI: 9.72, 15.36) nmol/L. No significant effect of study day was found (β2 = .26; CI: −1.48, 1.99 and β3 = .43; CI: −2.55, 1.70).

Test–retest associations for cortisol assessments at the three study days did not reach the level of statistical significance for both cortisol on awakening (S1; r’s between .23 and .37, all n.s.) and the CAR (0–30 delta; r’s between −.22 and −.13, all n.s.). An inverse relationship between estimated mean values of S1 and the CAR also closely missed statistical significance levels (r = −.24; CI −.47, .01).

² cortisol_{ijk} = (β_0 + γ_j) + β_1 t_i + (β_2 + β_3) d_{ijk} + ε_{ijk} Denotation: i = infants, j = sampling time point, k = study day, t_i = manifestation of ‘post-awakening sample’ at time point ‘i’, d_{ijk} = manifestation of study day variables (study day is entered as two separate effect-coded variables; d_{i} and d_{k}).
Ontogeny of the cortisol awakening response

3.2. Associations with development-related parameters

Table 2 presents the results of Bayesian correlation analyses between cortisol measures and sociodemographic, sleep-related and psychosocial variables. The level of cortisol on awakening (S1) was found to show significant negative associations with infants’ current height and weight as well as a positive relationship with infants number of daytime naps. The magnitude of the cortisol awakening response (0–30 delta) was found to be inversely associated with infants’ age as well as with their current weight and body mass index (BMI). In addition, positive associations were found between the 0 and 30 delta and infants’ birth weight and BMI, the number of daytime naps and time of sleep onset.

The examination of intercorrelations between different predictors using exploratory principal component analysis (Kaiser–Meyer–Olkin = .57, Bartlett sphericity test: p < .001) suggested that variables loaded onto two main factors which together explained 67.1% of the total variance. Factor 1 comprised six variables relating to different aspects of infant’s current life, i.e. current age, weight, height, BMI, number of daytime naps and time of sleep onset (Eigenvalue: 3.41; factor loadings between .48 and .94). Factor 2 included infants’ birth weight and birth BMI (Eigenvalue: 2.00; factor loadings .92 and .91, respectively).

Table 2  Relationships between cortisol measures and sociodemographic, sleep-related and psychosocial variables.

<table>
<thead>
<tr>
<th></th>
<th>S1</th>
<th>0–30 delta</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>−.17 [−.43, .10]</td>
<td>−.36 [−.57, −.13]</td>
</tr>
<tr>
<td>Birth height</td>
<td>−.10 [−.37, .18]</td>
<td>.12 [−.14, .38]</td>
</tr>
<tr>
<td>Birth weight</td>
<td>−.09 [−.35, .19]</td>
<td>.28 [.02, .52]</td>
</tr>
<tr>
<td>BMI at birth</td>
<td>−.02 [−.29, .25]</td>
<td>.29 [.04, .51]</td>
</tr>
<tr>
<td>Duration of pregnancy</td>
<td>.24 [.02, .48]</td>
<td>−.07 [−.16, .30]</td>
</tr>
<tr>
<td>Current height</td>
<td>−.48 [−.66, −.26]</td>
<td>−.14 [−.38, .10]</td>
</tr>
<tr>
<td>Current weight</td>
<td>−.41 [−.61, −.18]</td>
<td>−.31 [−.48, −.13]</td>
</tr>
<tr>
<td>Current BMI</td>
<td>−.13 [−.39, .14]</td>
<td>−.26 [−.45, −.07]</td>
</tr>
<tr>
<td>Number of daytime naps</td>
<td>.34 [−.11, .55]</td>
<td>.23 [.05, .42]</td>
</tr>
<tr>
<td>Sleep onset</td>
<td>.14 [−.14, .39]</td>
<td>.24 [.02, .43]</td>
</tr>
<tr>
<td>Sleep through night</td>
<td>.21 [−.06, .46]</td>
<td>.02 [−.22, .26]</td>
</tr>
<tr>
<td>Awakening time</td>
<td>.14 [−.13, .39]</td>
<td>−.07 [−.31, .17]</td>
</tr>
<tr>
<td>PSS</td>
<td>−.06 [−.34, .21]</td>
<td>.00 [−.24, .25]</td>
</tr>
<tr>
<td>SES ladder</td>
<td>.02 [−.27, .31]</td>
<td>.21 [.04, .45]</td>
</tr>
</tbody>
</table>

Values in brackets denote 95% confidence intervals (highest posterior density regions) of correlation coefficients. Bold writing signals statistically significant associations. Abbreviations: BMI: body mass index; SES ladder: socioeconomic ladder; PSS: Perceived Stress Scale.

4. Discussion

In this study, we set out to provide first data on the ontogeny of the CAR in 2–12-month-old infants using a rigorous methodological approach. Carefully monitoring infants’ awakening times and the accuracy of saliva sampling, our results suggest that virtually all examined infants were able to mount a significant CAR (≥2.5 nmol/L). In addition, the present data provide first tentative evidence of cortisol associations with development-related parameters during infancy, suggesting a decline of both the cortisol levels on awakening and the CAR with infants’ physical and sleep-related development as well as an increased CAR in infants with a higher birth weight and BMI.

The current findings for the first time suggest that infants from as early as two months of age are able to mount a significant CAR. The observed evidence for this was remarkably consistent, with 32 (out of 33) infants aged 2–12 months exhibiting a CAR on at least one of the three study days. Moreover, the magnitude (mean increase: 12.5 nmol/L) and response rate (86.9%) of the CAR which was seen in the present infant data, closely parallels or even exceeds previous data from adult participants (mean increase: 9.3 nmol/L, see Clow et al., 2004; response rate: 85.3%, Dockray et al., 2008). Overall, the present findings thus stand at variance with previous reports showing no evidence of a positive CAR in children below the ages of 16 (Saridjan et al., 2010) or...
17 months (Bright et al., 2011). To compare these data with the current results, it is crucial to note that both previous studies had not objectively verified infants’ times of awakening or parents’ adherence to the sampling protocol. Both are known to pose a considerable threat to the validity of CAR data (Kudielka et al., 2003; Kupper et al., 2005) which can be further amplified in young infants who may wake up spontaneously prior to saliva sampling without their parents being aware of this (Gribbin et al., 2011). In addition, the current observation of a positive association between the number of missing study days and aspects of infant development suggests that it may be even more difficult to obtain valid post-awakening cortisol data in older and more developed infants. It is conceivable that this could have posed an additional challenge to the above-mentioned studies which had examined somewhat older infants and that this may have further contributed to their negative CAR findings. This situation together with the clarity of the present findings favours the interpretation that previous evidence of no positive CAR in infancy may have been associated with methodological issues and that, indeed, the ontogeny of the CAR occurs at a very early developmental stage, i.e., during the first two months of life.

The timing of CAR development indicated by the present data concurs with the results of previous research placing the emergence of overall cortisol circadian rhythmicity within the first six months of life (Price et al., 1983; Spangler, 1991; Santiago et al., 1996; Antonini et al., 2000; but see Kiess et al., 1995, Lewis and Ramsay, 1995). In addition, the data are also partly in line with previous research suggesting a link between the development of cortisol circadian rhythmicity and sleep-related development (Price et al., 1983; Spangler, 1991; Antonini et al., 2000; De Weerth et al., 2003). The current findings tentatively suggest positive associations between infants’ number of daytime naps and both the level of cortisol on awakening and the CAR as well as positive association between the CAR and the timing of sleep onset. On the other hand, no cortisol associations with awakening time or sleep through the night were seen. Importantly, as the results of principal component analysis indicate that the examined sleep-related variables also shared substantial variance with infants’ current age and physical development (current height, weight and BMI), some caution is required when interpreting bivariate associations with cortisol data. Given that these variables all loaded onto a common factor, the current data may be best interpreted as suggestive of a general relationship between the CAR and interrelated aspects of infants’ sleep-related and physical development. Future longitudinal research more carefully assessing individual time courses of different developmental components will be required to more firmly distinguish these individual associations.

Irrespective of this, it is interesting to note that the finding of inverse associations between the level of cortisol on awakening and infants’ current height and weight can be seen as concurring with previous evidence showing a general decrease in basal cortisol levels over the first six months of life (Price et al., 1983; Lewis and Ramsay, 1995). Specifically, the first awakening sample marks the endpoint of the pre-awakening cortisol increase which, in turn, is assumed to be influenced by inhibitory influences involving the hippocampus as well as extra-pituitary modulation of adrenal sensitivity to ACTH (see Clow et al., 2010). It is tempting to speculate that the current finding is related to an ongoing maturation of these mechanisms during early infancy in parallel to infants’ general physical development. This would result in gradually increasing pre-awakening inhibition of cortisol secretion and decreasing cortisol levels on awakening with infant development. Although being speculative, this interpretation is also in line with evidence showing that particularly the hippocampus is a structure undergoing major changes during the first nine months of life (see Arnold and Trojanski, 1996; Lavenex et al., 2007).

While hippocampal maturation may help to explain the current findings regarding the first sample on awakening, this does not provide an explanation for the age-related decline of the CAR. On the contrary, as pre-awakening inhibition of cortisol secretion is assumed to pave the way for the subsequent CAR (see Clow et al., 2010), lower awakening cortisol levels should indeed be related to an increased magnitude of the CAR. Consistent with this notion, a trend for an inverse association between the cortisol level on awakening and the CAR was also observed in the present infant data. On a phenomenological level, the finding of an attenuation of the CAR during early infancy may be seen as concurring with previous findings showing a decrease in cortisol responsivity to physical stressors with infant age, particularly during the first six months of life (see Jansen et al., 2010). While the CAR does not provide a marker of acute stress responsivity in adult participants (e.g., Schmidt-Reinwald et al., 1999; Prüssner et al., 2007), our findings are in line with the notion that the development of a general hypo-responsivity of the HPA axis during later infancy (see Gunnar and Donzella, 2002) may also result in an attenuation of the CAR during this period. Combining the current findings of an age-related decline in CAR magnitude over the first year of life with previous data failing to show consistent evidence of a CAR in slightly older infants (Saridjan et al., 2010; Bright et al., 2011), may even suggest the development of a relative ‘quiescence period’ of the CAR in infants older than 12 months of age. However, first data from an ongoing study by our research group strongly suggest that when using the same rigorous methodological approach described here, a significant CAR is also consistently observable in two to six year-old children (n = 48, unpublished observation).

The notion that the CAR develops at an early stage of human development also holds indirect implications regarding current hypotheses about the regulation of the CAR in adults. Specifically, it has been suggested that the CAR is linked to cognitive processes involving “an activation of prospective memory representations at awakening enabling individual’s orientation about the self in time and space as well as anticipation of demands of the upcoming day” (p. 71, Fries et al., 2009). In linking this hypothesis to the present findings, it is important to note that implicated cognitive abilities, such as prospective memory and episodic future thinking, are only assumed to develop around the age of three years (see Atance and O’Neill, 2001; Kliegel and Jäger, 2007). As this is considerably later than the emergence of the CAR indicated by the current data, the ability to anticipate the day ahead is unlikely to constitute a sine qua non for the CAR. Hence, our data appears inconsistent with parts of the above hypotheses assuming that the CAR results from
 activation of memory representations which through their content lead to a stimulation of the HPA axis, such as anticipations of negative events or upcoming demands (e.g., Schlotz et al., 2004; Adam et al., 2006; Wilhelm et al., 2007; Stalder et al., 2010a). Importantly, however, this does not preclude the possibility that such processes are involved in the adaptive modulation of the day-to-day variability in CAR magnitude in later life. In addition, while arguing against a fundamental role of post-awakening anticipations, the present data suggesting a very early developmental occurrence of the CAR further supports the notion that it fulfils a fundamental physiological function for the organism (e.g., in energy provision) and may be triggered by basic brain systems involved in awakening and the establishment of wakefulness (see Clow et al., 2010).

The present study has a number of limitations that should be acknowledged. The results are based on cross-sectional data and thus cannot provide information on intraindividual aspects of CAR development. To account for this, future research should aim to incorporate longitudinal assessments, focusing specifically on the ‘development’ of the CAR during the very early period of infancy. In addition, the current research did not include diurnal cortisol assessments and thus could not answer the question whether there is concurrence in the ontogeny of the CAR and of general circadian cortisol rhythmicity. Furthermore, it cannot be excluded that aspects of the morning study protocol may themselves have influenced the study results. Specifically, the act of being gently woken up by a parent (instead of waking up spontaneously) or the postponement of feeding until 30 min post-awakening could have caused some form of stress to the infant due to a change in the normal morning routine. However, as parents unanimously reported that the overall study procedure was well-tolerated by their infants, without obvious signs of negative emotional reactions, it appears unlikely that this has constituted a strong influence on the present results. This notion is further strengthened by adult CAR research which has shown that the magnitude of the CAR is unaffected by participants’ mode of awakening (spontaneous vs. externally induced; e.g., Wüst et al., 2000b; Stalder et al., 2009, 2010a,b) or by changes to their morning routine (Wilhelm et al., 2007). Nevertheless, future research should aim to also incorporate more systematic assessments of infants’ post-awakening behaviour and emotional expression in order to fully rule out a potential influence on the CAR in this age group. Finally, the finding of only relatively weak and statistically non-significant test—retest associations for the two post-awakening cortisol measures tentatively suggests that these measures may be intraindividually less stable in young infants than in adult participants (e.g. Hellhammer et al., 2007). Hence, future research may also choose to use an increased number of sampling days per infant in order to obtain more reliable information on trait-like aspects of cortisol secretion for between-subject analyses.

Despite these limitations, the salience of the present data obtained under conditions of strict methodological control speaks for the current main conclusion of a very early developmental occurrence of the CAR. Future expansions of this evidence may help to further enhance fundamental knowledge of this unique aspect of endocrine activity and enable a more meaningful interpretation of CAR data obtained in specialised and applied research contexts.

Conflicts of interest

The authors have no conflicts of interest to declare.

Role of the funding source

The research described here was supported by a grant from the German Research Foundation (DFG Ki 537/28-1).

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Please cite this article in press as: Stalder, T., et al., The cortisol awakening response in infants: Ontogeny and associations with development-related variables. Psychoneuroendocrinology (2012), http://dx.doi.org/10.1016/j.psyneuen.2012.07.015


