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The cortisol awakening response in toddlers and young children

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Summary The cortisol awakening response (CAR) is frequently assessed in psychoneuroendocrinological research on adult participants. However, knowledge on the development of the CAR during early life is scarce and characterized by inconsistent findings. We have recently shown that a positive CAR is readily observable in young infants under conditions of strict methodological control. However, it still remains unknown whether a significant CAR is maintained consistently throughout toddler- and childhood. Here, we report data from 150 toddlers and young children aged 12—87 months in whom salivary cortisol levels were assessed 0 and 30 min post-awakening over three non-consecutive study days. High quality of data was ensured by the use of objective measures to verify children's awakening times (wrist actigraphy) and sampling times (electronic monitoring containers). Results revealed the presence of a significant CAR (>1.5 nmol/L) in 142 (out of 150) children and on a total of 82% of study days. A marked CAR was consistently observed throughout all examined age groups (mean increase: 8.73 nmol/L). In addition, the level of cortisol on awakening was found to increase linearly with children's age (r = .17, p = .04). Overall, the current findings strongly suggest that, contrary to previous propositions, the CAR is maintained consistently throughout toddler- and childhood.
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1. Introduction

The cortisol awakening response (CAR) describes the period of increased cortisol secretion 30—45 min post-awakening (Pruessner et al., 1997) which is frequently used as a measure in psychoneuroendocrinological research, mostly focusing on adult participants (see Fries et al., 2009; Clow et al., 2010). Only recently, research has begun to explore the CAR and associated factors in infants and young children (DeCaro and Worthman, 2008; Freitag et al., 2009; Saridjan et al., 2010; Bright et al., 2012; Michels et al., 2011; Stalder et al., 2013; Tegethoff et al., 2013). Despite a growing evidence base, knowledge regarding the CAR and its development in infancy and early childhood is still fragmented and characterized by inconsistent findings.

A particular focus of previous research relates to the question at what time the CAR first emerges and how it

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develops during infancy, toddlerhood and early childhood. Recent evidence suggests that a positive CAR is reliably detectable in infants aged 2—12 months (Stalder et al., 2013; Tegethoff et al., 2013). By contrast, other research has failed to show a post-awakening cortisol increase in toddlers aged 12—17 months (Saridjan et al., 2010; Bright et al., 2012) while a more robust CAR was again observed in toddlers aged 16—20 months (Saridjan et al., 2010) and young children aged 2—12 years (DeCaro and Worthman, 2008; Freitag et al., 2009; Michels et al., 2011; Gribben et al., 2012). Overall, the rates of infants/toddlers/children exhibiting a positive CAR vary substantially between studies. Importantly, low CAR responder-rates have emerged particularly from studies in which no objective control of participants' awakening and/or sampling times was employed (e.g., Tegethoff et al., 2013; Bright et al., 2012; Saridjan et al., 2010; DeCaro and Worthman, 2008; Freitag et al., 2009; Michels et al., 2011). Conversely, two published studies in which objective verification of awakening *and* sampling times were used have reported high CAR response-rates of 100% (Gribben et al., 2012) and of 86.15% (Stalder et al., 2013).

Together, the above data can be seen as indicating that a significant CAR emerges during early infancy and is maintained throughout childhood. Thus, failure to show a consistent CAR in previous research may, indeed, have been due to participant non-adherence, which is a major problem in CAR research (e.g., Kudielka et al., 2003; Broderick et al., 2004). By contrast, there is also data pointing to the possibility that the CAR may *not* be consistently seen throughout child development: In our recent study, a significant CAR was consistently observed in infants aged 2—12 months; however, the magnitude of the CAR was found to linearly decrease with age in this sample (Stalder et al., 2013). This concurs with evidence suggesting a period of cortisol hyporesponsiveness in toddlers older than 12 months (Gunnar et al., 2009; Jansen et al., 2010) which, in turn, could explain previous findings of a negative CAR in this age group (Saridjan et al., 2010; Bright et al., 2012). To date, a larger, systematic and well-controlled examination of the CAR in toddlers and young children has not been conducted yet.

The current study aims to address this issue by extending our recent data on infants (Stalder et al., 2013) with a detailed enquiry of post-awakening cortisol secretion across toddlerhood and early childhood (12—87 months). To ensure high quality of data, we used a rigorous methodological approach with objective verification of awakening- and sample collection times and repeated CAR assessments over three study days. In additional analyses, exploiting the advantage of the same methodology and to allow for the first comprehensive evaluation of developmental CAR trajectories across the first 7 years of life, we further extend the examined age-range by combining the current data with previous cortisol data from our infant sample (Stalder et al., 2013).

2. Methods

2.1. Participants

A total of 193 healthy children between 12 and 87 months were recruited using an identical procedure as described in our earlier work (Stalder et al., 2013). In brief, this focused on children from the greater Dresden area who were in good mental and physical health and who were not taking any medication. From this sample, data of 43 children were lost due to discontinuation of participation ($n = 11$) or due to provision of an insufficient amount of saliva on each of the study days ($n = 32$). Finally, 150 healthy children (74 female) aged 12—87 months were included in the study (see Table 1 for sample characteristics). For all participating children, 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 as an incentive for participation in the study.

2.2. Design and procedure

Study design and procedure resembled the previously reported approach in Stalder et al. (2013). Parents were carefully familiarized with the study procedure, including the showing of an instructional DVD and on-site training on the collection of saliva samples in toddlers or children. The importance of strict adherence to the study protocol was highlighted. Parents were asked to collect saliva samples from their children on three non-consecutive weekdays (two weekdays, one weekend day) within a two-week period. On each study day, they were instructed to fill out a short sampling protocol, recording children's bed- and awakening times, sampling times and any difficulties with the sample collection. Parents also filled out the study questionnaires (see below). Saliva samples were stored in the parents' home freezer and delivered to the laboratory after the last sampling day.

2.3. Saliva sampling in young children and adherence control

Parents were asked to gently wake up their child (to reduce the risk of missing the first saliva sample due to prior awakening) and to take two saliva samples, immediately upon awakening and 30 min post-awakening (as in Stalder et al., 2013). If the child had woken up spontaneously, parents were told to postpone saliva collection until the following day. Food and drink were withheld 2 h prior to sampling and parents were instructed not to brush their child's teeth prior to sampling, to avoid contamination of samples (Egliston et al., 2007). Saliva sampling was carried out using *eye sponge* devices (bvi Beaver Visitec, Waltham, USA) which are well suited for the use in young children (De Weerth et al., 2007). Each eye sponge consists of a plastic shaft with an arrowhead made of absorbing sponge material. Parents were asked to place the sponge part under the child's tongue until it was saturated and visibly swelled many times over. Saliva samples were stored at −20 °C until assaying using a commercially available chemiluminiscence assay (CLIA, IBL, Hamburg, Germany). Awakening times were verified using motility readings from *Actiwatch 2* devices (AW2; Phillips Respironics, Murryville, PA, USA) and accuracy of sample taking was controlled by placing eye sponges in electronic monitoring containers (*MEMS 6 TrackCap*; Aardex Ltd., Switzerland). MEMS devices electronically detect and store times of container openings and thus allow verification of participant adherence to the sampling instructions. The use of MEMS containers has been shown to increase adherence to the assessment protocol (Kudielka et al., 2003).

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