ABSTRACT: Development of the hypothalamic–pituitary–adrenocortical (HPA) axis was examined using salivary cortisol levels assessed at wake-up, midmorning, midafternoon, and bedtime in 77 children aged 12, 18, 24, 30, and 36 months, in a cross-sectional design. Hierarchical linear modeling (HLM) analyses were used to characterize cortisol production across the day and to examine age-related differences. Using area(s) under the curve (AUC), cortisol levels were higher among the 12-, 18-, and 24-month children than among the 30- and 36-month children. For all five age groups, cortisol levels were highest at wake-up and lowest at bedtime. Significant decreases were noted between wake-up and midmorning, and between midafternoon and bedtime. Unlike adults, midafternoon cortisol levels were not significantly lower than midmorning levels. Over this age period, children napped less and scored increasingly higher on parent reports of effortful control. Among the 30- and 36-month children, shorter naps were associated with more adultlike decreases in cortisol levels from midmorning to midafternoon. Considering all of the age groups together, effortful control correlated negatively with cortisol levels after controlling for age. These results suggest that circadian regulation of the HPA axis continues to mature into the third year in humans, and that its maturation corresponds to aspects of behavioral development. © 2004 Wiley Periodicals, Inc. Dev Psychobiol 45: 125–133, 2004.

Keywords: cortisol; infants; toddlers; children; development; temperament; effortful control; napping
6 postnatal weeks, an early morning peak and an evening nadir in cortisol production can be observed when measures are averaged across infants (Larson, White, Cochran, Donzella, & Gunnar, 1998). By 3 postnatal months, this pattern is more obvious (Price, Close, & Fielding, 1983), with individual differences becoming increasingly more stable between the fourth and sixth postnatal months (Gunnar, Brodersen, Krueger, & Rigatuso, 1996; Lewis & Ramsey, 1995). Despite the clear emergence of a diurnal rhythm over the first 6 postnatal months, the infant’s daytime pattern of cortisol production is still immature. Specifically, the average salivary cortisol level midafternoon to late afternoon is as high as that obtained midmorning (Larson et al., 1998). Infants retain this pattern throughout most of the first year of life (Spangler, 1991). The current study assessed cortisol patterning among older infants and toddlers to examine when the transition from the infant pattern to a more adultlike pattern might emerge.

We expected that changes in cortisol production over the daytime hours might be related to changes in sleep/wake (i.e., napping) patterns with development. In humans and other mammals, mutual influences between sleep and the HPA axis have been observed (Daly & Evans, 1974; Follenius, Brendenberger, Bandesapt, Libert, & Ehrhart, 1992). In infants, daytime patterns of cortisol production fluctuate with daytime naps. Specifically, cortisol levels decrease while the infant naps and then rebound after the infant wakes up, returning to levels 45 min after the nap that are equivalent to prenap levels (Larson, Gunnar, & Hertsgaard, 1991). Longitudinal studies of sleep have shown that the most prominent decline in napping during childhood occurs between 18 months and 4 years (Iglowstein, Jenni, Molinari, & Largo, 2003). If the lack of an average decrease in cortisol levels between midmorning and midafternoon is related to napping during the day, we might expect to observe the emergence of lower midafternoon than midmorning cortisol concentrations as some children begin to give up daytime naps.

Along with increasing periods of daytime alertness and reduced periods of napping, the transition from infancy to early childhood is marked by the development of self-regulatory competencies that are hypothesized to reflect the continuing maturation and myelization of the prefrontal cortex (Dawson, Panagiotides, Klinger, & Hill, 1992). These competencies include the ability to sustain attention despite distractions and to inhibit prepotent responses, responses that have been frequently executed and are therefore easily recruited. These competencies are supported by the capacity for effortful control, as described by Posner and Rothbart (2000). Regions in the prefrontal and cingulate cortex involved in the effortful regulation of behavior also may contribute to regulation of the HPA axis (Sullivan & Gratton, 2002). Thus, we might expect that young children with a more developed capacity for effortful control may exhibit more mature patterns of cortisol production.

**METHOD**

**Participants**

Families were recruited by telephone from a list of potential participants who indicated interest in research participation at their child’s birth and who had children within 2 weeks of their 12-, 18-, 24-, 30-, or 36-month birthday. Of these, 77 families returned some saliva samples, and full cortisol data were available for 69 children: 18, 14, 14, 10, and 13 children per age group, respectively. An additional 24 families returned questionnaire data. Saliva and/or questionnaire data were available for 32 twelve-month-olds (16 female), 24 eighteen-month-olds (18 female), 18 twenty-four-month-olds (8 female), 12 thirty-month-olds (7 female), and 15 thirty-six-month-olds (8 female). A total of 86 families were recruited by telephone but did not return any data within the 6-week study time frame.

**Measures**

Parents were asked to collect saliva samples at wake-up, approximately 10 a.m., approximately 4 p.m., and bedtime on 2 days within 2 weeks of their child’s birthday, without disturbing their child’s normal schedule. Saliva sampling times reported by parents were wake-up (mean sample time = 7:43 a.m., range = 5:01 a.m.–11:00 a.m.), 10 a.m. (mean sample time = 10:07, range = 9:24 a.m.–12:27 p.m.), 4 p.m. (mean sample time = 4:11 p.m., range = 3:18 p.m.–6:40 p.m.), and bedtime (mean sample time = 8:11 p.m., range = 6:24 p.m.–10:35 p.m.). We recognized that these guidelines would result in a range of sample times, but felt it was important to capture realistic ambulatory levels. All analyses excepting those using hierarchical linear modeling (HLM) were performed twice, once with the full range of samples and once restricted to samples collected within 1 hr of the mean sampling time. Results for the analyses used in this study did not differ between those conducted on the full and time-restricted datasets, therefore only results from the full dataset are presented. HLM analyses could not be performed on the restricted dataset due to sample size.

Parents were asked to record sleeping and waking on the 2 saliva-sampling days in 15-min intervals using a 24-hr diary. Parents also completed the 155-item Toddler Behavior Assessment Questionnaire (TBAQ; Goldsmith, 1996; with additional scales by Rothbart, personal communication, June 8, 1997). Measures of effortful control were obtained from this instrument using the inhibitory control, attention shifting, attention focusing, and interest scales (four scales, α = .70). Parents reported on any health conditions, current medications, and illness symptoms, and were asked to sample only on days when their child did not have a fever. Data from 2 children using an inhaler or nebulizer for asthma were not included. Finally, for descriptive purposes, parents were asked to report the number of hours of nonparental care per week, what type of child care was used, and in cases where more than 10 hr per week were used, when the child began the care arrangement.
Parents were contacted by telephone, at which time the purposes and procedures were described. Those agreeing to participate were mailed a packet with the parent-report questionnaires and saliva-collection materials. Materials were sent to arrive 2 weeks before the child’s birthday. Parents were contacted by telephone weekly for a maximum of 6 weeks until saliva samples were returned or participation was discontinued, and the first author was available by telephone for questions during workday, evening, and weekend hours. Of the 77 children who returned at least partial cortisol data, 62 took saliva samples within 2 weeks of their child’s birthday, 13 within 3 weeks, and 2 within 4 weeks.

Due to difficulties encountered with saliva collection in this age range, two types of collection procedures were used. At first, families were asked to sample saliva from their child using the methods typically used in our laboratory with older children. Thus, parents gave the child a very small amount of premeasured, cherry-flavored Kool-Aid (.025 g), asked them to swallow the crystals, and then mouth a dry cotton dental roll; however, after attempting to sample 51 children using these methods, we noted that nearly 60% of families were unable to provide any of the requested samples. Of those children returning some samples, an additional 30% were missing some of the samples requested. Thus, it was apparent that these procedures were not working well with this age group. A number of parents reported that their children simply refused to mouth the dry cotton roll, and eight samples (4.9%) were of insufficient volume to assay or to assay in duplicate. At this point, we revised our sampling procedure to one we hoped would increase our success rate. We conducted tests of the effects of pretreating the cotton rolls with saliva from three adults using the CIBA magic assay, and with pretreated cotton rolls which were sent to Dr. Douglas A. Granger (College Park, PA) for testing with the electroimmunoassay (EIA) and the radioimmunassay (RIA) by his Salimetrics laboratory. Mean cortisol levels in μg/dl using the CIBA magic assay from the three adults using both a dry cotton roll and a pretreated cotton roll expressed with a syringe fitted with a filter were .22 (dry cotton roll) and .23 (pretreated and filtered). There was no significant difference between the methods, Wilcoxon signed rank test, z = 27, n.s. Using the EIA and RIA assays and cherry-flavored pretreated cotton rolls, no significant differences were found; however, other flavors did contaminate the samples (D. A. Granger, personal communication, November 3, 1998).

Regardless of collection method, parents were instructed to trim the wetted part of the cotton roll, insert it into the syringe, and express the saliva into the vial. The samples were refrigerated until mailed to us in preaddressed, prestamped, bubble-padded mailers, and samples were frozen until assayed. Samples were assayed using a modified version of the Corning CIBA magic cortisol RIA kit (Kirschbaum & Hellhammer, 1989). All samples from an individual child were assayed in the same batch. Inter- and intraassay coefficients of variation (CV) using samples of known cortisol determination were 12.33 and 5.53%, respectively. The mean intraassay CV for samples in this study was 9.8%, and the correlation between duplicate assays was r(540) = .99, p < .0001. To stabilize values, the samples were averaged within time point across the 2 days of sampling. In 84% of the cases, both samples were available and averaged. In the remaining cases, the single value was used. When data were analyzed using a Collection-Procedure Group (2) × Cortisol Sample Time (4) repeated measures analysis of variance, no main effect of collection-procedure group was found, F(1, 69) = .51, n.s. Thus, all further analyses were performed collapsed across collection-procedure type.

RESULTS

Preliminary analyses assessed potential child care or sex effects. Analyses were then conducted on the cortisol data to examine age and time-of-day effects. Next, the relations between napping and daily cortisol production were analyzed, and finally, we explored effortful control–cortisol relations. All available data were used for each analysis, resulting in small fluctuations in sample size per analysis.

Preliminary Analyses

Preliminary repeated measures analyses of the Cortisol Sample (4) × Child Care Group (exclusively by parent or less than 10 hr/week nonparental care, n = 41 vs. 10 hr/week of child care or more, n = 28) revealed no significant
effect of child care, $F(1, 66) = .64$, n.s. Similarly, seven $t$ tests performed to examine potential sex differences in cortisol values at any time point, cortisol AUC, effortful control, or nap duration, revealed no significant differences, $t_s < 2, ps > .05$. Thus, child’s sex or child care attendance was not included as a factor in any subsequent analyses.

**Cortisol Patterning**

The descriptive statistics for cortisol data are shown in Table 1 and Figure 1.

**Age Effects.** Hierarchical linear modeling (HLM) was used to characterize cortisol production over the day and to examine differences in trajectories based on age. HLM was considered appropriate because there was considerable variation in individuals’ cortisol production, and HLM utilizes this individual variation in estimating parameters. Moreover, this method is particularly useful for these data because dependency of observations that occurs when samples are collected only a few hours apart is accommodated with HLM by specifying an autocorrelation covariance structure. Based on known changes in the daytime pattern of cortisol production in infants and adults, the nature of change in cortisol across the day was expected to have linear and nonlinear components. An unconditional model was tested including parameters for intercept as well as linear, quadratic, and cubic change. Missing data met criteria for being missing at random. Parameters were estimated using maximum likelihood estimation, which are valid under missing data. Because the model was saturated, an autoregressive covariance structure was utilized to minimize the number of parameters estimated.

The results indicated that the omnibus test for the unconditional model was significant, $F(3, 73) = 144.45$, $p < .0001$. Significant effects for intercept and change parameters are indicated in Table 2. The significant linear change indicated that on average, cortisol declined over the day. The significant cubic change parameter suggested that while cortisol levels decreased during the morning hours, they rose or leveled off midday and then continued to decline towards the end of the day (see Figures 1 & 2).

A covariate model also was tested with age as a predictor of intercept, linear, quadratic, and cubic change parameters. The omnibus test indicated a significant effect for age, $F(4, 72) = 7.10$, $p < .0001$. Tests for specific effects indicated an Age x Intercept interaction, suggesting that individual children’s average cortisol levels were negatively related to age (see Table 2). An Age x Linear Change interaction also was observed, suggesting that the younger the child, the steeper the drop in cortisol from wake-up to bedtime.

### Table 1. Summary Statistics on Cortisol Values at Each Time Point and Nap Duration by Age Group

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Wake-Up Cortisol (µg/dl)</th>
<th>10 a.m. Cortisol (µg/dl)</th>
<th>4 p.m. Cortisol (µg/dl)</th>
<th>Bedtime Cortisol (µg/dl)</th>
<th>AUC Cortisol (µg/dl)</th>
<th>Average Nap Duration (hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>12 Months</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>0.72 (0.30)</td>
<td>0.39 (0.19)</td>
<td>0.38 (0.29)</td>
<td>0.19 (0.21)</td>
<td>4.79 (2.48)</td>
<td>2.11 (0.65)</td>
</tr>
<tr>
<td>Median</td>
<td>0.68</td>
<td>0.29</td>
<td>0.29</td>
<td>0.11</td>
<td>3.94</td>
<td>2.13</td>
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<td></td>
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<td>$n = 21$</td>
<td>$n = 21$</td>
<td>$n = 18$</td>
<td>$n = 26$</td>
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<tr>
<td>18 Months</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>0.68 (0.28)</td>
<td>0.29 (0.13)</td>
<td>0.30 (0.22)</td>
<td>0.16 (0.19)</td>
<td>3.13 (0.77)</td>
<td>2.38 (0.53)</td>
</tr>
<tr>
<td>Median</td>
<td>0.62</td>
<td>0.26</td>
<td>0.26</td>
<td>0.06</td>
<td>3.23</td>
<td>2.44</td>
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<td>$n = 14$</td>
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<td>$n = 16$</td>
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<tr>
<td>24 Months</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>0.72 (0.37)</td>
<td>0.29 (0.16)</td>
<td>0.34 (0.33)</td>
<td>0.13 (0.19)</td>
<td>4.23 (3.99)</td>
<td>1.81 (0.72)</td>
</tr>
<tr>
<td>Median</td>
<td>0.64</td>
<td>0.25</td>
<td>0.24</td>
<td>0.10</td>
<td>3.41</td>
<td>2.00</td>
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<td>$n = 15$</td>
<td>$n = 14$</td>
<td>$n = 16$</td>
</tr>
<tr>
<td>30 Months</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Mean</td>
<td>0.49 (0.10)</td>
<td>0.18 (0.08)</td>
<td>0.18 (0.08)</td>
<td>0.05 (0.03)</td>
<td>2.29 (0.52)</td>
<td>1.79 (0.93)</td>
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<tr>
<td>Median</td>
<td>0.51</td>
<td>0.19</td>
<td>0.21</td>
<td>0.05</td>
<td>2.37</td>
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<td>$n = 11$</td>
<td>$n = 11$</td>
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<tr>
<td>36 Months</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>0.53 (0.16)</td>
<td>0.23 (0.08)</td>
<td>0.20 (0.12)</td>
<td>0.11 (0.20)</td>
<td>2.92 (1.00)</td>
<td>0.86 (0.85)</td>
</tr>
<tr>
<td>Median</td>
<td>0.47</td>
<td>0.22</td>
<td>0.16</td>
<td>0.05</td>
<td>2.54</td>
<td>1.00</td>
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<tr>
<td></td>
<td>$n = 13$</td>
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<td>$n = 13$</td>
<td>$n = 13$</td>
<td>$n = 13$</td>
<td>$n = 13$</td>
</tr>
</tbody>
</table>

AUC, area under the curve.
SDs of the mean are given in parentheses.
With age, the variance of cortisol values also decreased (see Figure 2). Using Levene’s test for equality of variance, this difference was significant at the wake-up, $F(4, 66) = 2.62, p < .05$, and midmorning sampling times, $F(4, 66) = 3.25, p < .05$. This may have been due, in part, to the fact that the mean levels were decreasing with age; however, coefficients of variation decreased as well, particularly when comparing the three younger age groups with the two older age groups. The mean CV for the three younger age groups was 44.7 at wake-up and 49.6 at midmorning while for the two older age groups the mean CV was 25.3 at wake-up and 39.6 at midmorning.

**Napping Effects.** For each child, we calculated the amount of sleep between 10 a.m. and 4 p.m., and averaged this across 2 days (average nap duration, see Table 1). While all of the 12- and 18-month-old children napped both days, 4 of the 24-month-olds napped only 1 day (25%). Among the 30- and 36-month children, 6 children napped neither day (25%), 5 children napped 1 of 2 days (21%), and 13 children napped both days (54%). In addition, average nap length and age in months were negatively correlated among the 30- and 36-month-olds, $r(24) = -.54, p < .01$, but this was not true among the younger children, $r(37) = .26$, n.s. Using partial correlations to control for age, among the older children, nap length was negatively correlated with the cortisol drop from 10 a.m. to 4 p.m., $pr(21) = -.46, p < .05$. That is, older children who napped less exhibited more of a decrease in cortisol between midmorning and midafternoon. In contrast, among the younger children, nap length was not correlated with the drop in cortisol from 10 a.m. to 4 p.m., using either bivariate correlations, $r(51) = -.12$, n.s., or using partial correlations controlling age, $pr(46) = -.14$, n.s. For the 30- and 36-month-olds, we calculated the mean cortisol at 10 a.m. and 4 p.m. for the groups of children who napped neither day, napped 1 day, and napped both days. For the six 30- and 36-month-olds who napped neither day, mean cortisol at 10 a.m. was .22(.03), and at 4 p.m. was .15(.01). For the four 30- and 36-month-olds who napped 1 day, mean cortisol at 10 a.m. was .26(.07), and at 4 p.m. was .22(.09). For the remaining thirteen 30- and 36-month-olds who napped both days, mean cortisol at 10 a.m. was .19(.02), and at 4 p.m. was .22(.03). Thus, those 30- and 36-month-olds who napped both days showed increasing cortisol across the day while those napping only 1 or neither day showed the more mature decreasing cortisol pattern.

**Effortful Control.** The 155-item TBAQ yields four scales that were expected to reflect effortful control: inhibitory control, attention shifting, attention focusing, and interest. These scales were standardized and averaged to compute effortful control. As expected, reported...
effortful control increased with age, \( F(4, 96) = 14.28, p < .001 \). Mean effortful control scores and SEs for all 12-, 18-, 24-, 30-, and 36-month-olds were \(-.56(10), -.02(12), .18(16), .44(12), \) and \(.63(15)\), respectively. Next, we examined whether effortful control would be related to cortisol production. Controlling statistically for age, effortful control was negatively correlated with cortisol AUC, \( pr(63) = -.31, p < .05 \) (see Figure 3). Examining the data within age group, effortful control and cortisol AUC controlling age were significantly correlated for 24-month-olds, \( pr(11) = -.62, p < .05 \). At no other age was the relation significant, although the sample sizes are notably very small and partial correlations for the 12- and 18-month-olds were \( pr(15) = -.27, \) n.s., and \( pr(8) = -.37, \) n.s., compared to those of the 30- and 36-month-olds, \( pr(7) = .02, \) n.s., and \( pr(10) = -.10, \) n.s., respectively. Comparing the three younger age groups with the two older age groups, as above for cortisol patterning and napping, the relation between effortful control and cortisol AUC controlling age was significant for the three younger age groups, \( pr(40) = -.39, p < .05 \), but not for the two older age groups, \( pr(20) = -.09, \) n.s.

**DISCUSSION**

The results of the present study suggest that the HPA system is continuing to mature during the infant and toddler periods in human children. Home baseline production of salivary cortisol decreased between 12 and 36 months, with children 30 to 36 months exhibiting lower levels of cortisol, particularly near wake-up, than children 12, 18, or 24 months of age. With age, we also observed a decrease in the variability of cortisol values at

**FIGURE 3** Partial correlation between Effortful Control and Cortisol AUC, controlling age.

**FIGURE 2** Cortisol patterning for individual children across the day by age group.
wake-up and midmorning, even after correcting for the age-related decreases in mean cortisol levels. Again, among the 12-, 18-, and 24-month-olds, there was significantly greater variability in cortisol levels at wake-up and midmorning than among the 30- and 36-month-olds. This suggests that baseline cortisol production, at least during the morning hours, may become more stable during the toddler period.

Across all of these ages, however, there was a clear daytime rhythm in cortisol production. On average, the highest baseline values were obtained near wake-up and the lowest values at bedtime. Across all age groups studied, cortisol levels decreased significantly between wake-up and midmorning, and again between midafternoon and bedtime. Nonetheless, even among the oldest age group (36-month-olds), we did not obtain a significant group difference in cortisol levels between midmorning and midafternoon. On average, by the time children are 36 months of age, the daytime production of cortisol is still not yet fully consistent with the mature pattern.

There was some suggestion that the mature pattern of a decrease in cortisol levels between midmorning and midafternoon might emerge as children give up their daytime naps. All 12- and 18-month-old children in this study were napping each day, and among the 24-month-olds, a few napped only 1 of 2 days (25%); however, by 30 months, nearly half the children napped neither or only 1 day, and the duration of naps had begun to decrease. Among the 30- and 36-month-olds, those who napped less were more likely to exhibit a decrease in cortisol between midmorning and midafternoon. We have previously shown that among infants, napping is associated with a significant decline in cortisol levels from pre- to immediately postnap, with a marked rebound in levels by 45 min after the nap (Larson et al., 1991). Recently, we found that at child care, cortisol levels for nearly all children were lower immediately following their afternoon naps than they were late in the morning and still later in the afternoon (Watamura, Sebanc, & Gunnar, 2002).

Relatively little is known about the neurobiology of daytime napping as it relates to regulation of the HPA system; however, the Larson et al. (1991) and Watamura et al. (2002) studies suggested that the process of waking up from a nap stimulates the HPA system. This nap-waking stimulation may obscure the diurnal decrease in cortisol over the midportion of the day. It is thus possible that as children sleep for shorter and shorter durations with development, the underlying diurnal decline in activity of the HPA system can be more readily detected. This view is consistent with the argument that processes regulating the HPA system in response to sleep and activity are somewhat independent of the processes regulating the diurnal rhythm (Czeisler & Klerman, 1999). Our evidence that age was negatively correlated with napping time among the older, but not younger, children was consistent with previous data indicating that reduction in nap duration due to giving up the afternoon nap occurs postinfancy, during the later toddler and preschool years (Iglowstein et al., 2003). This also may explain why we observed a negative correlation between napping and cortisol levels among only the older children, as they were in the age period when children transition into the more adultlike day–night sleep pattern.

The results of the present study also suggested an inverse association between cortisol levels and children’s self-regulatory abilities. Specifically, between 12 and 36 months, parents described children as exhibiting greater effortful control competencies. That is, with age, they were better able to sustain interest in tasks, regulate their attention, and inhibit prepotent responses. These abilities presumably involve the development of the anterior cingulate and prefrontal cortices (Posner & Rothbart, 2000). Children who exhibited more mature effortful control competencies also produced lower concentrations of cortisol. This was the case even after controlling statistically for age, suggesting that some of this effect was due to individual variation within age groups. This finding suggests several hypotheses, none of which can be determined based solely on the present results. First, increased effortful regulatory competence might reduce the number of conflicts that children are involved in. To the extent that such encounters activate the HPA axis, the negative association between effortful control and cortisol might reflect reduced stressful encounters for children with greater effortful control abilities. Although the sample size for the within-age analyses was small—and thus the results tentative—the fact that the association between cortisol AUC and effortful control was observed among the younger, but not older, children cannot be easily reconciled with the reduction of stressful interactions hypothesis. Effortful control competencies are hypothesized to rely on the development of anterior cingulate and prefrontal circuitry (Posner & Rothbart, 2000). Presumably, these circuits begin to exhibit marked development in the second year of life (Dawson et al., 1992). Thus, a second hypothesis might be that the development of these circuits during the second year of life is sufficient to begin to lower the set point of the HPA axis while continued development beyond the second year does not provide additional input to basal HPA functioning. This hypothesis, while admittedly highly speculative, would place cortical maturation as the cause of altered basal HPA axis regulation. A third, equally speculative hypothesis is that decreasing levels of basal cortisol production play a role, perhaps a permissive one, in the initial development of the neural system underlying effortful self-regulation. Of course, a fourth hypothesis is that the changes in basal cortisol production observed
in this study and changes in effortful control reflect the operation of variables that were not measured. Nonetheless, the literature on effortful control suggests that more effective self-regulation is associated with the development of conscience (Kochanska & Knaack, 2003), and with lower externalizing problems (Eisenberg et al., 2004; Kochanska & Knaack, 2003), and the current results suggest that lower basal cortisol concentrations may be associated with this profile of adaptive self-regulation during the toddler and preschool years.

The results of this study also shed light on methodological issues in the collection of salivary cortisol data on children of this age. In previous work with infants and with older children, the procedures used in the initial phase of this study have been effective. That is, we used dry cotton rolls and asked parents to swab their infant’s mouth, and we asked children as young as 3 to first chew and swallow a small amount of Kool-Aid and then chew on a dry cotton roll. Both methods worked well; however, we found that parents were having difficulty getting saliva samples from these toddler-age children using dry cotton rolls. We therefore pretreated the cotton rolls to add color and flavor, and provided parent-practice samples. The return rate for samples and the amount of saliva per sample increased, and anecdotally, parents reported more compliance and less distress. The pretreated cotton rolls did not produce a difference in the results obtained; therefore, this method might be useful in more easily collecting saliva samples for cortisol determination with children of this age.

Limitations

There are several limitations for the present study. First, a cross-sectional rather than a longitudinal design was used. It is possible that a different developmental picture would emerge if children were followed across the toddler period. Second, the sample was composed of middle-class, Caucasian children with parents willing and able to complete the home-sampling and diary procedures. Thus, caution is warranted in generalizing these findings. Third, the correlational design does not allow a consideration of the direction of effect for the napping and effortful control findings. Finally, sleep and effortful control were assessed using parent report. Using actigraphy for sleep measures and laboratory assessments of effortful control would allow more accurate assessments of these variables.

NOTES

The authors thank the parents and children for their participation and feedback, Darcy List for 30-month data collection, Erika Riggs for assistance with data collection, and Dr. Douglas Granger for assistance in establishing the reliability of the pretreated cotton rolls. This research was supported by a University of Minnesota Undergraduate Research Opportunities Program award to Sarah E. Watamura, and a National Institute of Mental Health Research Scientist Award (MH 00946) to Megan R. Gunnar.

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