Published in final edited form as:

Dev Psychobiol. 2010 January ; 52(1): 44–53. doi:10.1002/dev.20410.

Sleep Quality, Cortisol Levels, and Behavioral Regulation in Toddlers

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Abstract

This study examines the association between nighttime sleep characteristics and cortisol levels and how these variables relate to aspects of children's temperament and behavior. Twenty-seven healthy children, aged 12–36 months, attending group childcare settings, participated in the study. Each child's sleep was measured at home with actigraphy over three nights. Saliva samples were collected by the mothers at bedtime and within 30 min of awakening. In addition, both the mother and the daycare teacher rated the child's behavioral difficulties and negative emotionality. It was found that children with more fragmented sleep displayed higher awakening cortisol levels compared to children with more efficient sleep. Moreover, elevated awakening cortisol levels were correlated with teachers' ratings of internalizing behavior and negative emotionality. These preliminary findings suggest that awakening cortisol may serve as a useful index of adrenocortical reactivity in young children, signaling a disturbance in physiological regulation, and underscore the need for more research pertaining to the dynamic associations between sleep and HPA-axis across the 24-hr period.

Keywords

fragmented sleep; awakening cortisol; temperament; daycare

INTRODUCTION

In children, as in adults, the sleep–wake rhythm is governed primarily by two bio-regulatory processes: homeostatic and circadian (Borbély, 1982). The homeostatic process is dependent on previous sleep–wake periods, whereas the circadian process is a clock-like regulator bound to the light–dark cycle. The circadian pacemaker also regulates hypothalamic–pituitary–adrenal (HPA) activity, which mediates metabolic and stress-sensitive processes.

The circadian rhythm of secretion of cortisol, the primary hormonal end-product released from the adrenal cortex, is characterized by high secretory activity during the morning hours, with a peak just after waking, and a nadir occurring during the first few hours of nocturnal sleep (Weitzman et al., 1971). Newborns typically show two cortisol peaks 12 hr apart, that do not depend upon the time of day (Klug et al., 2000), but the temporal pattern of high early morning and low evening hormone levels is established by 3 months of age (Larson, White, Cochran, Donzella, & Gunnar, 1998), and by the middle of the first year, cortisol production follows a rhythm that parallels that of the sleep–wake cycle (Spangler, 1991).

The relationships between sleep and the HPA axis have been extensively investigated in adults, but surprisingly, the association between cortisol production and sleep states has received little attention in developmental psychobiology research. Only a few studies have explored links between sleep and cortisol in infants and young children. For example, White, Gunnar, Larson, Donzella, and Barr (2000), studying infants with colic, found a blunted cortisol rhythm and less sleep in this group compared to control infants. In contrast, Larson et al. (1998) found that young infants who slept through the night showed an early-morning peak in cortisol levels, suggesting earlier onset of a normal circadian rhythm. Similarly, Larson, Gunnar, and Hertzgaard (1991) reported that morning naps in 9-month-olds were associated with significant decreases in salivary cortisol levels. In the preschool years, problem nappers were shown to demonstrate high afternoon cortisol levels but their morning levels did not differ from a nonproblem group (Ward, Gay, Alkon, Anders, & Lee, 2008).

A number of studies have compared cortisol levels at home and in daycare. In daycare, some children showed an unexpected rise in cortisol production across the day, with increased levels from mid-morning to pre-rest, decreased cortisol production over the rest period, and increased production post-rest (Watanabe, Seabanc, & Gunnar, 2002). Moreover, afternoon cortisol levels were higher at daycare than at home, suggesting that the children may be experiencing stress while at daycare. Thus, during days spent at the daycare, children experienced a rise in cortisol levels from mid-morning to afternoon (Watanabe, Donzella, Alwin, & Gunnar, 2003); this pattern was age-related and appeared among toddlers as compared to younger infants. Together these studies suggest that basal cortisol levels and the pattern of decrease over the day are altered in young children attending daycare.

Furthermore, daytime naps appear to moderate basal cortisol production, but the effect may be age-related. These findings must be interpreted with some caution, however, as in most of the above studies sleep behavior was based on parental reports.

A few recent investigations obtained objective sleep recordings and measured cortisol secretion in older children (Hatzinger et al., 2008; El-Sheikh, Buckhalt, Keller, & Granger, 2008). Hatzinger et al. (2008) found higher levels of morning cortisol in 5-year-olds with poor sleep, as measured with polysomnography, compared to those found in good sleepers. Similarly, actigraph recordings conducted with 7–11 year olds, showed that poor sleep quality was associated with higher afternoon cortisol levels (El-Sheikh, Erath, Buckhalt, Granger, & Mize, 2008). While there are some inconsistencies that could be attributed to age differences as well as to different definitions and measures of sleep quality, overall, poor sleep quality appears to be associated with higher levels of basal cortisol secretion, and

conversely, good sleep quality has been related to lower cortisol levels or better HPA regulation.

The association of cortisol secretion with temperament and emotional behavior is a topic of ongoing biobehavioral research. For example, pre-school children with more negative emotionality exhibited less decrease in cortisol levels during rest periods (Watamura et al., 2002) and, in toddlers, effortful control was negatively correlated with cortisol levels (Watamura, Donzella, Kertes, & Gunnar, 2004). Similarly, links between cortisol levels and behavioral and emotional problems (e.g., Forbes et al., 2006) and adverse early experiences (e.g., Dozier et al., 2006; Fisher, Stoolmiller, Gunnar, & Burraston, 2007) have been documented. Young children with depressive symptoms showed greater cortisol responses to stressful situations than controls (Luby et al., 2003), and increased social isolation and internalizing problems were associated with high cortisol levels during the transition to school (Turner-Cobb, Rixon, & Jessop, 2008). Studies in clinical populations of older children similarly suggest a link between cortisol secretion and internalizing problems (e.g., Feder et al., 1996; Forbes et al., 2006) and inhibition and shyness (Schmidt, Fox, Rubin, Sternberg, & Gold, 1997), which are risk factors for later anxiety disorder. However, little is known about the links between cortisol levels and internalizing behavior in very young children.

Two main objectives guided the present study: (1) to explore relationships between nighttime sleep and cortisol levels in children aged 1–3 years, a group that has generally attracted less sleep-related research; and (2) to examine the links among sleep, cortisol levels, and children's temperamental and behavioral characteristics. Based on the literature, we hypothesized that consolidated and restful sleep patterns would contribute to better HPA regulation and that poor sleep and high levels of cortisol would be associated with more behavioral difficulties, particularly difficulties in regulating emotional states. The target age was toddlers and the context of the current study was attendance at daycare. To realize our objectives, we obtained sleep recordings (actigraphy), collected saliva samples at bedtime and at awakening, and administered questionnaires to both mothers and daycare teachers.

METHODS

A subgroup of parents of toddlers who participated in a community-based sleep survey of children attending daycare facilities (Hall et al., in preparation), volunteered to participate in the present study. Ethical approval was obtained from the University of British Columbia Behavioral Ethics Review Board and the Community Ethics Review Board. Inclusion criteria were: children from 12 to 36 months of age, without developmental delays and/or severe medical conditions, who were in a group daycare a minimum of 3 days a week, and whose parents and principal teacher gave informed consent to participate in the research. Twenty-seven parents, and the respective 27 teachers from 15 daycare facilities, agreed to take part in the study. The 15 facilities represented a range from family daycares with six to eight children to university-based centers. All of the centers were licensed. The data collection involved three night objective sleep recordings (actigraphy) in the children's homes, saliva samples on those days, and questionnaires completed by the mothers and daycare teachers. Sleep recordings took place Mondays to Thursdays, the corresponding

cortisol samples were collected between Monday evening to Friday mornings, and the child's behavior at daycare referred to any school day except Monday.

To ensure that children who participated in the present study were not a self-selected subgroup of children with sleep and/or behavioral difficulties, we compared them to the larger group of children who participated in the survey study (Hall et al., in preparation). The subgroup was comparable with respect to sleeping difficulties as measured by the Infant Sleep Questionnaire (Morrell, 1999), which provides a measure of parental perception of the child's sleep difficulty ($t = .94$, n.s.) and teachers' evaluations of adjustment and coping in the childcare context ($t = .39$, n.s.), as measured by the Preschool Adjustment Questionnaire (Behar, 1977). For each child, behavioral information was obtained from both the mother and the teacher.

Sample Characteristics

The 27 children (12 boys and 15 girls) in this study ranged in age from 12 to 36 months (mean = 24.5, $SD = 6.9$). Mothers' ages were between 30 and 42 years (mean = 35.4, $SD = 3.0$). Their education levels ranged from some college to postgraduate university degrees; 89% were currently working. The majority of the parents self-identified as Canadian or European (70%). The teachers ranged in age from 22 to 64 years (mean = 36, $SD = 17.7$). All but two of the teachers were female; 71% identified their ethnicity as Canadian or European; 82% had completed college education or a university degree; 64% had worked in the daycare facility for more than 4 years, and the majority (80%) worked full time.

Measures and Procedure

Sleep—Actigraphy: A wristwatch-like activity monitor (actigraph), which is a noninvasive and user-friendly tool that provides a reliable and valid measure of sleep in infants (Sadeh, Acebo, Seifer, Aythur, & Carskadon, 1995), was the tool used for measuring sleep in the home context. The model used in the present study was the mini-motion logger[®] in a zero crossing mode. The actigraphs were attached prior to bedtime and removed in the morning of each day of data collection. Recordings were made for three consecutive week nights. Mothers recorded in a diary the time of attaching the actigraph, children's bed time, and the time of actigraph removal in the morning.

The Action-W[®] software (Sadeh algorithm for infants) was applied to score the data. Due to a technical failure, the sleep data of one child were lost. The following parameters were used in the present study ($n = 26$): **DUR**—the entire sleep period duration (in minutes) from sleep onset to sleep end time; **ACT**—percentage of activity per minute of sleep; **WAKE**—The number of awakenings episodes longer than 5 min, and **SEF**—Sleep efficiency defined as time spent asleep out of the total sleep period, which is an index of overall sleep quality. The correlations between **SEF** and the other measures were, $r = -.12$, n.s., $r = -.89$, $p < .001$ and $r = -.86$, $p < .001$, with **DUR**, **ACT**, and **WAKE**, respectively. For data reduction purposes, we focused on two variables **DUR**, which reflects circadian and homeostatic aspects (mean = 600.0 ± 48.3 min), and **SEF**—which measures consolidation and provides, as stated above, an overall measure of sleep quality ($m = 92.8 \pm 3.97\%$). In addition, in light of the current emphasis on developmental research on intraindividual variability, we used the

coefficient of variation (*SD*) in sleep efficiency scores across the three nights as an index of night-to-night variability in sleep quality (mean = 4.32, *SD* = 3.15).

Cortisol—Mothers collected samples for salivary cortisol in their homes for 3 days in the evening and morning. They were instructed by the research assistant about how to collect saliva samples from their children with a cotton dental roll. Mothers were asked to place the wet swab in an empty plastic syringe tube, to clearly mark the designated tubes with date and the time of collection, and to store the tubes in the refrigerator, until returning them to the daycare for collection.

Bedtime samples were collected at least 30 min following any food or drink. The time of the last meal, the time of saliva collection, and the child's bedtime were recorded. Similarly, morning samples were collected within 30 min of awakening, prior to breakfast or any food or fluid intake. Mothers recorded the time of sample collection and the timing of breakfast. The research assistant phoned each evening to remind the parents to collect the saliva (and apply the actigraph), and to answer any questions concerning the procedure. The group mean hour of bedtime collection was 8.24 (*SD* = .93, range = 6.92–10.75), and the mean morning collection time was 7.30 (*SD* = .78, range = 5.92–9.17).

On the morning of the fourth day, following completion of three nights of recording, the actigraph and package with six saliva tubes were returned to the daycare and collected by the principal investigator. Typically, data collection took place over three consecutive week days. If the child was sick on one of the days or if family activities prevented measurement, in consultation with the research assistant, the measurement period was extended in order to obtain complete data for that child. Salivary cortisol levels were analyzed in Dr. Weinberg's laboratory at UBC, using the Salimetrics High Sensitivity Salivary Cortisol Enzyme Immunoassay Kit (Salimetrics LLC, Philadelphia, PA). All samples were analyzed in duplicate; intraassay and interassay coefficients of variation were 3.64% and 5.66%, respectively.

Saliva specimens were obtained from all 27 subjects. Out of the 162 saliva samples (27 subjects $\times$ 6 samples (3 morning, 3 evening samples), 36 samples (20%) did not have sufficient amounts of saliva for quantification. For five of the children, only one or two of the six saliva samples could be quantified. When checking for outliers, defined as >2 standard deviations above or below the mean, two children had one outlying value; these were replaced by the individual's mean PM or AM value, as required.

Across all participants, bedtime (PM) cortisol levels (mean $\pm$ *SD*) were $.03 \pm .02$ $\mu\text{g/dl}$, and awakening (AM) cortisol levels were $.35 \pm .14$ $\mu\text{g/dl}$. Coefficients of variation in cortisol levels, denoting intraindividual variability across days, were: AM ($.16 \pm .09$) and PM ($.01 \pm .02$). In addition, we calculated the nocturnal change in cortisol levels from bedtime to morning awakening (PM – AM = $-.30 \pm .14$ $\mu\text{g/dl}$). As the exact time of collecting the AM sample was not correlated with cortisol level, as previously reported in the literature (Spath-Schwalbe et al., 1992), it was not necessary to include the time of sampling as a covariate.

Behavior and Emotional Regulation—*The Toddlers' Behavior Assessment*

Questionnaire (TBAQ) is a measure of temperament-related behavior designed to be completed by parents of toddlers (Goldsmith, 1996). Mothers completed the short version of the instrument, which includes 83 items, rated on a Likert-scale, from 1 to 7. The items are divided into 9 scales: *activity*, *anger*, *social fear*, *interest*, *pleasure*, *inhibitory control*, *soothability*, *sadness*, and *social desirability*. These scales show high levels of convergence with other parental reports of temperament such as the IBQ (Rothbart, 1981), as well as acceptable reliabilities (Goldsmith, 1996). In line with the focus of the present study on the regulation of negative emotionality, we included in the analysis the following sub-scales: *anger*, *inhibitory control*, and *soothability*. The *anger* scale consists of 10 items [e.g., When you tried to remove something your child should not have been playing with, how often did s/he try to grab the object back?; the mean score was $3.64 \pm .73$], the *inhibitory control* scale includes 9 items (e.g., When you said no to your child, how often did your child ignore your warning? [reverse scored]; mean = $3.64 \pm .73$), and the *soothability* scale consists of 10 items (e.g., Following an exciting event, how often did your child remain excited for a long time? Mean = $4.89 \pm .67$).

The Child Behavior Checklist (CBCL) for Ages 1.5–5 (Achenbach & Rescola, 2000) is the most widely used assessment of behavioral problems. This instrument includes 100 descriptions of children's behavior. The respondent (parent/teacher) marks "0" if the item is not true of the child, "1" if somewhat or sometimes true and "2" if it is very true or often true. The items are grouped into the following sub-scales: emotionally reactive, anxious/depressed, somatic complaints, withdrawn, attention problem, aggressive behavior, and sleep problem, where higher scores indicate more behavioral difficulties. The subscales are grouped into two scores: internalizing and externalizing behavior problems (neither the internalizing scale nor the externalizing scale included sleep related items). The mothers rated their children's behavior using the parent form, and the teachers completed the teacher form. The internalizing mean score was 7.04 ± 6.23 and 6.08 ± 6.12 , respectively, according to the mother and the teachers, and the mean externalizing score was 11.32 ± 6.04 and 8.20 ± 7.81 .

The Teacher Daily Report (TDR) (Bates, Viken, Alexander, Beyers, & Stockton, 2002) is a daily report of the child's behavior at pre-school focused on positive behaviors, such as compliance or enthusiasm to learn, and negative behaviors such as arguing or fighting. The teacher marks on a scale, from 1 to 5, how often the behavior occurred on a specific day (1: not at all today to 5: very often today) and how typical or unusual this behavior is for the child (e.g., much less than usual). The teachers completed the report with respect to a designated day for which sleep data was to be collected in the night preceding and following diary completion (thus Mondays and Fridays were not included in the measurement window). Given our focus on behavioral and emotion regulation, we grouped the age appropriate items into two subscales. The *negative emotionality* subscale included three items (mean = $2.10 \pm .85$) pertaining to the child's responses to minor stressors and challenges (e.g., "When minor stressors occurred, this child became upset or tearful), where high scores denote reactivity to daily stressors. Although Cronbach's Alpha was low ($\alpha = .56$), we decided to include the scale in further analyses rather than to examine individual

items. The second subscale, *attentive behavior* included four items (mean = $3.56 \pm .73$; $\alpha = .68$), pertaining to regulation of attention and task-related behavior (e.g., “During structured group activities [e.g., circle time or meals], this child appeared to be paying attention and participating in an active, focused manner”).

Data Analysis

Statistical analysis was performed using SPSS16. *t*-Tests were applied for analysis of gender and age effects. Research hypotheses were examined using correlation analyses as well as by multivariate analyses of variance, both within and between comparisons were applied. The level of alpha was set at .05. We did not include a Bonferroni correction adjustment for multiple comparisons or for correlations, as this would be too conservative a test of hypotheses. The outcome of these conservative tests is a reduced statistical power (Zumbo & Hubley, 1998). Hence, we examined the effect size for our findings using the benchmarks of Cohen (1992): *r* of .10–.29 is a small effect, *r* = .30–.49 is a medium effect, and *r* = .50 a large effect size.

RESULTS

An initial comparison of boys and girls revealed that there were no significant differences in their sleep patterns or cortisol levels. Hence, we did not include gender in further statistical analyses. To examine age effects, the participants were divided into two age groups: 12–24 months (*n* = 13) and 25–36 months (*n* = 14). The two groups were comparable in terms of cortisol levels and sleep duration, with the exception that the sleep of the older group was less fragmented and more efficient (SEF: $t_{(24)} = 2.45$, $p < .05$); hence, age was included as a covariate when correlating sleep with the cortisol measures. Because partial correlations did not change the pattern of results obtained with simple correlations (e.g., the simple correlation between awakening cortisol and sleep was $r = .33$ and the partial correlation was $r = .35$), we report only the Pearson correlation coefficients.

The main finding of the correlational analysis was that variability in sleep efficiency was significantly associated with higher AM cortisol ($r = .54$, $p < .01$), and with steeper nocturnal change in cortisol from bedtime to awakening ($r = -.51$, $p < .05$). Night-to-night SEF variability also significantly correlated with variability in PM cortisol levels ($r = .45$, $p < .05$).

To explore further the relationships between sleep quality and cortisol, we divided the sample by a median-split of SEF scores. Figure 1 depicts the comparison between cortisol levels in the High (*n* = 13) versus Low (*n* = 13) SEF groups. A MANOVA indicated that the two groups differed significantly from each other ($F_{(1, 19)} = 5.13$, $p < .05$, partial $\eta^2 = .35$). AM cortisol levels of the High SEF group (mean = .28, *SD* = .10) were significantly lower than those of the Low SEF group (mean = .44, *SD* = .14, $F_{(1, 20)} = 10.73$, $p < .01$, partial $\eta^2 = .35$); the two groups were comparable with respect to PM levels (mean = .03, *SD* = .02, and mean = .04, *SD* = .03, respectively, for the High and Low groups. An additional *t*-test analysis revealed that the nocturnal cortisol rise from bedtime to awakening (PM – AM) was significantly higher in the group with the less efficient sleep ($t_{(20)} = 3.41$, $p < .01$). A further comparison of the temperamental characteristics of these two groups showed that

children with better sleep quality scored lower in *anger* ($F_{(1,24)} = 4.52, p < .05$, partial $\eta^2 = .16$) and higher in *inhibitory control* ($F_{(1,24)} = 6.71, p < .05$, partial $\eta^2 = .22$).

Turning to the associations between the behavioral measures and the cortisol levels (see Table 1), it was found that internalizing behavior, specifically, the teachers' ratings on the CBCL, was significantly correlated with higher levels of AM cortisol ($r = .47, p < .05$). The change in cortisol levels from bedtime to awakening (PM – AM) was negatively associated with the internalizing score, so that a sharper rise was significantly associated with more internalizing problems ($r = -.46, p < .05$ [note: a steeper slope is expressed negatively]). Analysis of the individual subscales that constitute the internalizing construct indicated that higher *emotional reactivity* was positively correlated with elevated AM ($r = .49, p < .05$) and PM ($r = .42, p < .05$) cortisol levels, and that higher scores on the anxious-depressed scale were associated with a steeper PM-AM slope ($r = -.44, p = .05$). Mothers' ratings of children's internalizing behavior on the CBCL were not associated with cortisol levels, with the exception of one significant correlation (PM – AM with the withdrawn subscale: $r = .46, p < .05$). As to externalizing behavior, cortisol levels were not associated with the overall score, but teachers' ratings of children's attention problems were associated with higher levels of AM cortisol ($r = .43, p = .05$). Finally, it was found that teachers' ratings of negative emotionality were significantly correlated with AM cortisol levels ($r = .51, p < .05$) and with PM – AM ($r = -.58, p < .05$). The higher the morning level, and the steeper the slope from bedtime to awakening, the greater the reactivity to daily challenges.

DISCUSSION

Sleep and the HPA Axis

The main goal of the present study was to examine relationships between nocturnal sleep quality and cortisol levels in toddlers. We found that toddlers who showed consolidated sleep, as measured objectively with actigraphy, had lower awakening cortisol levels compared to children whose sleep was more fragmented. The present finding corroborates Hatzinger et al.'s (2008) study with electroencephalographic sleep profiles, in which 5-year-olds with "poor" sleep showed significantly increased morning cortisol compared to "good" sleepers. Together, these two studies are consistent with the adult literature on the inhibitory effect of unfragmented or "good" sleep on cortisol secretion (e.g., Weitzman, Zimmerman, Czeisler, & Ronda, 1983).

Morning cortisol levels in the present study were sampled within 30 min of awakening, thus reflecting the awakening cortisol response (ACR), defined as the period of cortisol secretory activity in the first 45–60 min immediately post-awakening (Clow, Thorn, Evans, & Hucklebridge, 2004; Edwards, Clow, Evans, & Hucklebridge, 2001). The data showed that awakening cortisol secretion was significantly lower in children whose sleep was more efficient and less fragmented. Pruessner et al. (1997), who measured awakening cortisol by sampling saliva at 10 or 15 min intervals for 30–60 min after awakening, maintained that early morning (i.e., awakening) cortisol levels are reliable biological markers of adrenocortical reactivity. Indeed, the ACR appears to represent a discrete and distinctive part of the cortisol circadian cycle, which may be under regulatory influences different from the rest of the diurnal cortisol secretory cycle (Clow et al., 2004; Edwards et al., 2001).

The exact physiological role of the ACR has still not been clearly defined, and there are many discrepancies within the literature. Nevertheless, a number of studies in adults have found an association between the ACR and stress. Thus the ACR may represent a potentially useful biological marker of psychosocial and health status (Ellenbogen & Hodgins, 2009; Grant, Hamer, & Steptoe, 2009). The present study points to the possible involvement of sleep quality in regulating this early morning neuroendocrine activity. While there are some data in the literature pertaining to the links between parental reports of sleep duration and morning cortisol production in children under 3 years of age (e.g., Silva, Mallozi, & Ferrari, 2007), to the best of our knowledge, this is the first study to show that sleep fragmentation, as measured objectively, is associated with elevated levels of awakening cortisol in 12- to 36-month-olds.

While our study was not directly designed to examine circadian rhythms, the variables and sampling procedure allow us to consider further aspects of the interplay between sleep and HPA-axis in young children. For example, sleep duration was associated neither with other measured sleep variables nor with the cortisol and behavioral measures. Although this null finding could be due to type 2 error, it may suggest that sleep duration is not a marker of HPA activity.

In both “good” and “poor” sleepers, the expected low evening cortisol levels were observed. However, the slope, representing change in cortisol levels from evening to morning, was steeper when sleep was less consolidated and efficiency was lower. This finding demonstrates the entrainment of cortisol to sleep processes, and could be related to pulses in cortisol secretion when periods of wakefulness interrupt nocturnal sleep (Buckley & Schatzberg, 2005). While the mechanism underlying the documented link between sleep quality and change in cortisol level needs to be addressed in future research, we cautiously suggest that more restful sleep contributes to lower levels of cortisol upon awakening. The role of good rest in decreasing cortisol levels in young children was noted by Watamura et al. (2002) who found a decrease in cortisol secretion over a daytime rest period and by Larson et al. (1991) who reported that morning naps in 9-montholds were associated with significant decreases in salivary cortisol levels.

Because we only measured cortisol before bedtime and at awakening, the difference score between PM and AM cortisol levels provides an estimate of the overall nocturnal slope, but not of the actual trajectory of temporal changes in cortisol secretion across the entire night. Further studies are needed to describe more specifically the patterns of nocturnal cortisol secretion in children with good and poor sleep quality.

Behavioral and Emotional Regulation

In the present sample of toddlers, behavioral variables were marginally correlated with sleep patterns, as measured objectively. The only significant link between sleep and mothers’ behavioral ratings was with respect to negative emotionality: children with poorer sleep exhibited more anger and limited inhibitory control compared with those with efficient sleep patterns. This finding is in line with previous reports that point to a link between parental temperament ratings and objective sleep measures (e.g., Scher, Epstein, Sadeh, Tirosh, & Lavie, 1992). A question arises as to why negative emotionality as assessed by the teachers

did not show a similar association with poor sleep. The inconsistency between mothers' and teachers' ratings could stem from differences in the tools (temperament vs. behavior on specific days) or the context of measurement (e.g., mothers observe their child across multiple situations during the day and at night). As in many other reports, the views of mothers and teachers do not necessarily coincide (e.g., Kolko & Kazdin, 2006), but together they provide a more valid assessment of children's behavior.

With respect to teachers' ratings, consistent with the literature on the association between sleep and ADHD (e.g., Owens, 2005), our findings indicated a link between attention behavior and sleep quality. This link was not consistent across measures and reporters, possibly reflecting the young age of the sample and the limited statistical power for revealing a small effect size (Sadeh, Pergamin, & Bar-Haim, 2006).

As to cortisol and behavior, consistent with the findings of Watamura et al. (2003) concerning the association between elevated cortisol levels and fearfulness in the context of childcare, teachers' daily reports in our study supported the hypothesized link between higher cortisol levels and higher reactivity to stressful situations and challenges. Our data set makes a unique contribution by showing that elevated cortisol levels in children with emotional regulation difficulties (Watamura et al., 2002, 2003) can be detected at awakening, prior to children's arrival at daycare. In addition to supporting Watamura et al.'s (2004) finding that difficulty in effortful control in toddlers was associated with higher daytime cortisol levels, our data showed that higher AM cortisol levels and a steeper slope from bedtime to morning were associated with internalizing behavior. This result is consistent with studies of older children where internalizing problems were linked to poor HPA regulation (Forbes et al., 2006; Li, Chiou and Shen, 2007; Li & Shen, 2008). The novel contribution of the present data is in documenting a link between elevated awakening cortisol levels and both internalizing behavior and attention problem behavior as early as the second year of life.

Variability Across Days

The inclusion of an estimate of night-to-night variability in sleep quality revealed important associations with cortisol levels and rhythms. Night-to-night variability in sleep quality was associated with both higher awakening cortisol levels and higher day-to-day variability in PM cortisol levels. Intraindividual variability in cortisol levels was noted by de Weerth, Zijl, and Buitelaar (2003), who studied the early maturation of the cortisol circadian rhythm and highlighted its links with sleep.

Night-to-night variability in children's sleep has been a concern among sleep researchers, leading to guidelines for the required number of days of sampling for obtaining reliable scores (e.g., Acebo et al., 1999; Sadeh et al., 1995). Notwithstanding the metric requirement, in a current developmental approach, intraindividual variability is viewed as an important characteristic of developing systems and a variable to be analyzed methodically (e.g., Adolph et al., 2008; van Dijk & van Greet, 2007). Our variability-related findings, and in particular the associations with internalizing behavior and negative emotionality, suggest that the night-to-night variability in sleep quality and the day-to-day variability in cortisol levels might not be random, but possibly a proxy of physiological and/or contextual

vulnerability. The contextual correlates and causes of the daily variability in cortisol levels and sleep quality should be examined in future investigations.

Fisher et al. (2007), who studied the effects of a family-based therapeutic intervention for preschoolers in foster care, reported day-to-day variability in the PM cortisol level, and showed that in an intervention group in which parents were instructed to set regular bedtimes and develop routines to help settle children in the evenings, the children exhibited reduced day-to-day variability in PM cortisol levels. It would have been interesting to test whether the sleep of the children in their intervention group was of better quality than that prior to intervention as well as that of their control group. Bates et al. (2002), who studied sleep patterns of pre-school children in a low-income community sample, found that inconsistent bedtime and night-to-night variability in sleep duration, as reported by the parents, predicted less optimal day-time adjustment in pre-school. As the present sample was rather heterogeneous and included toddlers from both low-income and more affluent neighborhoods, but was not large enough to address contextual factors, future studies should address the association between cortisol levels and sleep quality in different contexts.

Strengths and Limitations

The present study is the first to show a link between sleep fragmentation and higher levels of awakening cortisol in toddlers. Because this finding was demonstrated with a small sample, more studies with larger sample sizes are necessary to validate the effect. The measurement of both sleep and cortisol was carried out over a period of 3 days. Therefore, the data allowed examination of day-to-day variability, and pointed to possible links between the degree of stability, or lack thereof, in HPA secretion and sleep–wake states. In light of the limited amount of research that has addressed these issues, and the small size of our sample, more research that focuses, in concert, on day-to-day variability in both HPA activity and sleep is essential, particularly in toddlers or very young children, where regulatory systems are just developing. This recommendation is further supported by the growing interest, among developmental scientists, in the significance of intraindividual variability in behavior (e.g., van Geert & van Dijk, 2002).

A number of additional limitations should be noted. The most important of these relates to the correlational design of the study. While the findings pointing to associations between sleep and cortisol are important, the present methodology precludes addressing directional links. In adults, bi-directional associations between sleep and regulation of the HPA axis have been demonstrated, showing that sleep deprivation results in increased levels of cortisol and conversely, alterations in the HPA axis have influences on sleep (Steiger, 2002). Thus, it has been suggested that cortisol-releasing mechanisms may be involved in the regulation of sleep (Follenius, Brandenberger, Badesapt, Libert, & Ehrhart, 1992), and that the endogenous early morning activation of the HPA system (the ACR) is likely to be terminated by mechanisms associated with awakening, resulting in lower cortisol levels by 60 min compared to those immediately after awakening (Spath-Schwalbe et al., 1998). Moreover, while we included PM and AM measures of cortisol, more data points, across the night and day, are necessary in order to trace the patterns of change in secretion. This is clearly a

methodological challenge, which is beyond the scope of the present study and awaits future research.

In conclusion, the results of this study showed a relationship between consolidated and restful sleep and HPA regulation. The finding that higher levels of awakening cortisol were associated with fragmented sleep, as well as with negative emotionality and internalizing behavior, suggests that awakening cortisol may be a useful index or biomarker of altered physiological regulation and/or reactivity in toddlers.

Acknowledgments

The researchers gratefully acknowledge funding from the British Columbia Ministry of Children and Family Development through the Human Early Learning Partnership (HELP). We extend our thanks to the parents, children, and daycare providers who participated in the study.

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FIGURE 1.

Mean values of cortisol (µg/dl) at awakening (AM), bedtime (PM) and the difference score between bedtime and awakening levels (PM – AM) in toddlers with high sleep efficiency (gray) and lower sleep efficiency (black). Stars denote significant difference ($p < .01$).

Table 1
Pearson Correlation Coefficients of Cortisol With Sleep and Behavioral Measures

	Cortisol				
	AM	AM Variability	PM	PM Variability	PM – AM
Sleep					
DUR	.01	.05	.17	.37	.00
SEF	-.33	-.12	-.03	-.14	.38
SEF variability	.54**	.36	.35	.45*	-.51*
Behavior					
Anger (M)	.26	.02	.35	.42*	-.12
Negative Emotionality (T)	.51*	-.10	.18	.12	-.58*
Internalizing (T)	.47*	.09	.37	.45*	-.46*

Variability is defined by coefficient of variation, M, mothers' reports; T, teachers' reports.

* $p < .05$.

** $p < .01$.