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Children's diurnal cortisol activity during the first year of school

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ABSTRACT

The present study examined 4- to 5-year-old British children's diurnal cortisol activity during their first year of school. The children's cortisol was measured before enrollment (baseline), upon enrollment, and both 3 and 6 months after enrollment. On each day, cortisol was sampled four times, providing information about the diurnal amount of cortisol secreted (AUC_G). Mixed-effect models were constructed to examine the way children's cortisol fluctuated over the course of the school year. Physiological activity was greater 3 months after enrollment, suggesting that some children reacted more to the challenge of school later than they did initially. Implications and suggestions for transitional practices and future research are discussed.

Introduction

Cortisol is the primary glucocorticoid produced by the Hypothalamic-Pituitary-Adrenal (HPA) system, activation of which is associated with psychological or emotional responses to everyday demands and challenges. Levels of activation tend to rise in response to novel, challenging, or threatening situations (Gunnar & Quevedo, 2007; Gunnar & White, 2001; Kirschbaum & Hellhammer, 1994). Starting school is exciting, though likely to be demanding too. While children are eager to experience school, they may also find it challenging to accommodate school routines, pay sustained attention to tasks, and play cooperatively with peers. All these novel demands may elicit changes in cortisol secretion.

The first school experience constitutes a "window of opportunity" during which children can start developing participatory learning behaviors that lead to favorable academic trajectories (Alexander, Entwisle, & Dauber, 1993, p. 813). School readiness is thus an important focus in early education and many transition practices are designed to support children's adjustment and readiness to learn in school (LoCasale-Crouch, Mashburn, Downer, & Pianta, 2008). These transition practices might make the new classroom routines easier for young children to cope with, thereby creating a benign experience upon initial days in school. When individuals perceive and interpret situations as benign, their HPA systems typically do not respond with increased cortisol secretion (McEwen, 1998). This might explain why previous studies have yielded inconsistent findings regarding children's cortisol responses when they enter school (e.g., Russ et al., 2012; Turner-Cobb, Rixon, & Jessop, 2008). However, there are indeed many cognitive, social, and behavioral challenges faced by children starting school, such as adjusting to the class schedule and activities, getting to know teachers and peers, and starting literacy and mathematic learning. We thus wondered whether young children's cortisol production might be more apparent months after enrollment, as the demands accumulated, rather than immediately. As the glucocorticoid hormone of the human HPA system, cortisol affects the function of central nervous system activity mediating processes such as learning, memory, and emotion (Sapolsky, Romero, & Munck, 2000). Examining children's diurnal cortisol activity during the course of their first year at school could help identify when children start responding to the associated demands physiologically. Teachers who recognized this could then provide timely and appropriate support that would help make the early school experience positive, thereby fostering an important foundation for future school success.

The current study thus assessed young children before enrollment, upon enrollment, and 3 months and 6 months after enrollment. We observed children's cortisol activity throughout the initial 6 months of school and hoped to clarify the way these young children experienced school physiologically.

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The British context

Unlike children of the same age in many other countries, British children start long days (0900–1500 h) of formal schooling at 4 years of age. The British context thus provides a unique opportunity for a developmental investigation of 4-year-old children's cortisol production in new school environments. In British first-grade classrooms (reception class), there are activity corners set up for children to practice their cognitive, motoric, and social skills, with sections for reading, crafts, and dressing up. There are also carpeted areas for small-group learning and whole-class activities. British schools have three terms each year: Autumn term (September–December), spring term (January–March), and summer term (April–July). The current study followed children for two terms from the autumn term to the spring term, measuring their cortisol in September (enrollment), December (the end of the autumn term), and March (the end of the spring term) as well as before enrollment (baseline). To ease children into new classroom environments, it is common for British teachers to invite the incoming parents and children to visit the classrooms before enrollment to ensure that children are appropriately prepared for school.

Cortisol production at child care or school during the preschool years

In the United Kingdom and the Netherlands, the legal age to start school is four, but in other countries, such as the United States, most children of this age are still attending child care or preschool. There have been numerous studies on preschool-aged (3 to 5 years) children's cortisol production on days in child care or during the transition to child care or school. Many have found that, compared to infant- or school-aged children, children aged 3 to 5 years had elevated cortisol production on days in child care (Bernard, Peloso, Laurenceau, Zhang, & Dozier, 2015; Dettling, Gunnar, & Donzella, 1999; Geoffroy, Côté, Parent, & Séguin, 2006; Vermeer & van IJzendoorn, 2006). As opposed to cortisol levels at home, child care environments, including both home- and center-based child care, elicited more cortisol (Bernard et al., 2015; Geoffroy et al., 2006; Gunnar, Kryzer, van Ryzin, & Phillips, 2010) and the increases were particularly evident in the afternoon levels of preschool-aged children (Bernard et al., 2005; Dettling et al., 1999; Watamura, Sebanc, & Gunnar, 2002). The majority of the children (aged 3 to 4 1/2 years) in Gunnar et al.'s (2010) study, who had attended child care for 2 months or more, still showed a rising pattern of cortisol on days in child care, and the afternoon levels of 40% of the children, as opposed to 10% of the children's morning levels, could be classified as stress responses. Gunnar et al. (2010) suspected that clear cortisol elevations in the afternoon might not simply be momentary responses, but might reflect children's physiological responses to an accumulation of experiences during the day in full-day care.

Fewer studies have examined the transition from home to new child care settings. The toddlers (aged 15 months) examined by Ahnert, Gunnar, Lamb, and Brathel (2004) responded to new care settings with higher cortisol than home levels and this was still evident 5 months after child care began. Bernard et al. (2015) followed a diverse group of children (infants, toddlers, preschoolers, and school-aged children) for 10 weeks through their transition. They found the afternoon levels of cortisol were elevated throughout the 10 weeks for all children and preschoolers had the largest increases.

Informed by two decades of research on cortisol production in child care, the data has consistently shown that levels and patterns of cortisol observed at child care differ from those measured at home. More cortisol was released at child care than at home, and, with evident increases in the afternoon, the diurnal pattern on child care days involved elevated levels from morning to afternoon (e.g., Bernard et al., 2015; Gunnar et al., 2010). Studies of four-year-old children's cortisol in school settings have not reported such consistent findings, however. As mentioned earlier, children in the United Kingdom and the Netherlands start school at 4 years of age. Although first-grade classrooms are usually set up in a friendly way, "school" is a much demanding environment than child care, with time and activities organized so as to facilitate student learning. Students are expected to follow a schedule and show appropriate learning behavior. As a result, it seems plausible that school should evoke more cortisol excretion by 4-year-old school children than would days at home. However, when Turner-Cobb et al. (2008) measured the cortisol levels of British 4-year-old children 4 months before enrollment (home measurement) as well as 2 weeks and 6 months after enrollment (school measurement), they found that the children's awakening levels of cortisol did not significantly increase from before enrollment to 2 weeks after enrollment, but the awakening and evening levels of cortisol 6 months after enrollment were lower than the pre-enrollment and enrollment levels. In another British study, Russ et al. (2012) noticed an increasing trend in the morning and afternoon level of cortisol from 1 month before starting, through the first week at school, and until 3 months after enrollment. The cortisol was significantly higher 3 months after (school measurement) than before enrollment (home measurement). In a Dutch sample, Gutteling, de Weerth, & Buitelaar (2005) found no significant differences between the cortisol levels of Dutch 4-year-old children on the first day of school and on another school day 2 weeks later. Groeneveld et al. (2013), on the other hand, found that Dutch children's concentration of hair cortisol was higher during the first 2 months of school as opposed to the level measured 2 months before enrollment.

It thus appears that 4-year-old children's cortisol levels are not always elevated upon initial entry but sometimes fluctuate later during the school year. Could it be that over time young children's cortisol reflected their accumulated experiences since enrollment, ensuring clear cortisol elevations after 2 or 3 months of school (e.g., Bernard et al., 2015; Groeneveld et al., 2013; Russ et al., 2012) while after 6 months or a year in care or school they became better adapted physiologically (e.g., Ouellet-Morin et al., 2010; Turner-Cobb et al., 2008)? When 2-year-old children were followed for a year in the same child care, their cortisol activity after a year in care showed a diurnal decline similar to that observed at home whereas their initial diurnal pattern had been flattened initially (Ouellet-Morin et al., 2010). Prior studies at most included two measures of cortisol after school commenced: one around enrollment and the other weeks or months after enrollment.

To clarify the way young children experienced school physiologically, the current study examined children's cortisol 3 times over the school year (and at baseline before school began), sampling the children's cortisol around enrollment as well as 3 months and 6 months after school began. We hoped by doing so to clarify the way young children's cortisol fluctuated in response to the accumulating demands of the school environment.

The current study

Hypothalamic-pituitary-adrenal (HPA) axis reactivity is an important focus in early childhood research because early experiences provide the context for the developing "phenotype" of adaptive physiological responses (Ellis, Jackson, & Boyce, 2006; Giudice, Ellis, & Shirtcliff, 2011). Starting school is a common early experience shared by most children in the world. School entry thus provides a context for the assessment of young children's physiological adaptation. Although young children may go through a hypo-responsive period of adrenocortical development during which their cortisol activity is dissociated from their behavioral or emotional responses (Gunnar & Donzella, 2002; Gunnar, Talge, & Herrera, 2009), it is believed that the physiological and behavioral response patterns developed in the early years may result in differential sensitivity to stimuli which may in turn alter children's developmental pathways (Boyce & Ellis, 2005; Obradović, Bush, Stamperdahl, Adler, & Boyce, 2010). Furthermore, the extent of children's school participation in the early years predicts their future academic trajectories (Ladd & Dinella, 2009); therefore, it is important to determine when first-grade children might be physiologically most sensitive to challenges in order to ensure that support to facilitate learning and classroom participation can be provided. School transitions may be normative circumscribed stressors for young children, and may even be benign experiences for the majority who have already experienced similar routines while attending day care or preschool. With time, however, formal schooling requires more cognitive concentration and behavioral regulation so that young children can meet the academic demands. Activities such as literacy, mathematics learning, and peer interactions require cognitive, behavioral, and emotional self-regulation, and these abilities are still developing for 4- to 5-year-old British children. We suspected that children's HPA systems might respond more after the effects of school experiences had accumulated. We thus assessed children's diurnal cortisol activity before enrollment, upon enrollment, and both 3 months and 6 months after enrollment. We hypothesized that the children's cortisol levels would be elevated after rather than upon enrollment.

Age, gender, and birth order were not the main focus of this study. We included these factors in the analyses for exploratory purposes, however. Although our study focused on 4- to 5-year-old children, we examined the effect of age because the adrenocortical system is still developing at this age (Gunnar & Donzella, 2002) and previous studies have revealed age effects on levels of cortisol in young children (e.g., Dettling et al., 1999; Watamura, Donzella, Kertes, & Gunnar, 2004). With respect to the effects of gender, previous results are mixed (Kudielka & Kirschbaum, 2005), although gender differences have not been found in research on children's cortisol responses to school (e.g., Quas, Murowchick, Bensadoun, & Boyce, 2002; Turner-Cobb et al., 2008). Birth order was also examined in light of Russ et al. (2012)'s finding that first-born children had higher levels of cortisol in the morning and evening. Children with older siblings might be more familiar with the new school or school routines and this sense of familiarity might buffer their cortisol reactivity to school.

Method

Participants

In the spring, the researchers contacted all primary schools in a south-eastern English city district about a larger study examining factors that might affect the transition to school (Yang & Lamb, 2014). Five schools (8 classrooms) agreed to participate. Letters introducing the study were sent to parents of incoming students via the schools. The researchers also recruited parents at events organized for incoming students and their parents. A total of 231 parents were contacted in person and/or via letter before the children started school and 67 subsequently consented to their children's participation in the study, with 59 of them agreeing to the collection of salivary cortisol.

The children (33 boys) averaged 54.70 months (*SD* = 3.43) when they started school. All children had attended nursery (equivalent to preschool in the United States) prior to school. Of the 56 children whose demographic backgrounds were known, 32 children (54%) were the eldest or the only children. The majority of the children were white (67.8%), while others were from mixed (8.5%), Asian (i.e., Indian, Pakistani, and Bangladeshi; 11.9%), Chinese (3.4%), or Black (1.7%) backgrounds. The majority of the children were from middle to upper-middle class families; most of the parents had at least completed their GCSEs[1] (90%), and 23.7% of the fathers were employed as managers/senior officials, 33.9% as professionals, 23.7% were in skilled trades or technical occupations, and 11.9% were in other occupations. Of the mothers, 6.8% were employed as managers/senior officials, 27.1% as professionals, 5.1% had technical occupations, 8.5% were in administrative or secretarial positions, 11.9% were in other occupations, and 33.9% were stay-at-home mothers.

Although our sample was small, it was relatively representative of the local population. The census statistics showed that more than 80% of the residents had received educational qualifications at the GCSE level or above and that 63% worked in managerial or professional positions, 9.6% in skilled trades, 14.1% in semi-skilled technical or unskilled manual positions, and the other 13.3% received social benefits or worked in jobs at the lowest grades (Cambridgeshire County Council the Research Group, 2003).

Procedure

The children's saliva was collected before school in July or August (baseline), and in September (enrollment), December (3 months after enrollment and around the end of the autumn term), and March (6 months after enrollment and around the end of the spring term). On each sampling day, saliva was collected 4 times: Early morning[2] (EM: before breakfast), mid-morning (MM: between 10 hours and 11 hours), mid-afternoon (MA: between 14 hours and 16 hours), and early evening (EE: before dinner). Thus, with four single days of sampling, and four samples per day, a maximum of 16 cortisol samples were provided by each child.

All saliva was sampled before food intake. Parents were instructed to sample saliva before breakfast and before dinner, and at school, saliva was sampled before snack time. Parents were also instructed not to collect saliva right after the children had been physically active. At school, the afternoon samples were collected at least one hour after lunch period because during lunch period (1200–1255 hours), the children could decide whether to take a nap, read quietly in class, or play in the playground after they finished their meals. Children were also asked to rinse their mouths with water before the procedure (Salimetrics, 2009). If the children were ill (one child in September and five children in December), their saliva was sampled after they had recovered, mostly on the following Thursdays or Fridays.

Because the September (enrollment) samples were taken on the fifth school day, which was at the end of the week, the December and March samples were also scheduled for sampling on Thursdays or Fridays. Almost all September (enrollment) samples (99.5%) were collected on Thursday and Friday, as were 90.2% and 92.6% respectively of the December and March samples. The July/August (baseline) samples were taken at home on various days of the week, however, because they were measured at the parents' convenience (6.2% on Monday, 21.3% on Tuesday, 12.5% on Wednesday, 16.7% on Thursday, 14.6% on Friday, 15.1% on Saturday, and 13.5% on Sunday). Preliminary one-way ANOVAs showed that day of the week had no significant effect on cortisol levels.

The study received ethical approval from the University Psychology Research Ethics Committee.

Measures

Salivary cortisol

Parents were provided with the saliva sampling kit during a home visit in the summer. The kit contained detailed instructions about when and how to collect

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[1] The General Certificate of Secondary Education (GCSE) is the qualification students receive upon completing 11 years of secondary education.

[2] The first sample of the day was taken pre breakfast but not necessarily close to the time of awakening, so levels are not comparable with those reported in studies in which awakening samples were obtained.

their children's saliva and four sets of saliva sampling devices (one set each for July/August, September, December, and March). During the visit, the researcher coached the parent(s) to use Salicaps (IBL, Hamburg, Germany) or Salivettes (Starstedt, United Kingdom), depending on the children's preferences. Of the 59 children, 41 used Salicaps and 18 used Salivettes. T-statistics were conducted to examine sampling-tool differences in cortisol levels, and revealed that there were significant differences between children using Salicaps ($M = 3.31$ nmol/L, $SD = 1.91$) and Salivettes ($M = 5.77$ nmol/L, $SD = 3.96$) when cortisol was measured in September in the mid-afternoon ($t(43) = -3.71$, $p < .01$). There were no significant differences for the other 15 sampling frames, suggesting that the one difference was likely to be spurious.

The parents collected four saliva samples in July/August (baseline) and two samples each in December and March (early morning and early evening samples). Four primary schools in our sample had children initially attended only part of each day (morning or afternoon sessions) for 2 weeks, while one school started with a full-day schedule (0900–1500 hours) immediately. Children in schools with part-day entry schedule were randomly assigned by their school into morning or afternoon sessions. In our sample, there were 30 morning-session children, 17 afternoon-session children, and 12 full-day children. Because some children started school with part day and some with full day programs, during the first 2 weeks of school, some parents collected two saliva samples (full-day children) and some three saliva samples (part-day children) in September. For example, if children were in the morning session, the parents collected the early morning (EM), mid-afternoon (MA), and early evening (EE) samples; if children were in the afternoon session, the parents collected the early morning (EM), mid-morning (MM), and early evening (EE) samples; if children were in the full-day session, the parents collected only the early morning (EM) and early evening (EE) samples. Therefore, some of the September MM and MA samples were collected by the parents and some by the researchers. T-statistics showed that there were no effects of administering person (parents or researchers) on the September MM levels and MA levels.

After parents collected their children's saliva, they were instructed to record the time at which the sample was taken, store it immediately in a freezer, and then bring the samples to school for collection on a designated day. When saliva was sampled at school, the samples were temporarily stored in the school refrigerator before being transferred to the research storage freezer. The transfer from school to the office usually took less than one hour. Samples were then kept in the storage freezer at − 20°C until assayed.

All saliva samples were assayed at Professor C. Kirschbaum's laboratory in Dresden, Germany. The inter- and intra-assay coefficients of variation (CVs) were both less than 10%.

Missing data

Rates of missing samples were 16% in July/August, 13% in September, 8% in December, and 6% in March. The majority of the missing samples were those that should have been collected at home. To determine whether the missing data were missing at random (Schafer & Graham, 2002), we examined the missingness (missing vs. not missing) of cortisol samples at each time point against the demographic variables, including child age, gender, ethnicity, and classroom. Chi-square tests were used when assessing categorical variables (i.e., gender, ethnicity, and classroom) and *t*-tests were used for continuous variables (i.e., child age). None of the test results were significant, suggesting that there were no patterns of missingness for the cortisol samples at each time point and that the missing data were thus missing at random.

Data preparation

Of the 843 saliva samples that were sent for assay, 39 were excluded for various reasons: eight samples did not have sufficient saliva, five samples had extreme values (>139 nmol/L), four samples were not obtained at the correct time, 14 samples were excluded because the child was thought to be ill, and eight home samples appeared to have been diluted by water. After further screening of the remaining samples, an additional 14 samples were excluded because the values were three standard deviations or more from the mean cortisol value. Thus 790 samples remained available for analysis.

Raw cortisol values are reported in nmol/L. The area(s) under the curve (AUC_G) scores were computed for children who provided all four saliva samples on the designated sampling day. The areas under the curve (AUC) were computed using the formula introduced by Pruessner et al. (1997; Pruessner, Kirschbaum, Meinlschmid, & Hellhammer, 2003). Here we use the AUC with respect to ground (AUC_G), which is a measure of the total amount of cortisol secretion during the day. The raw cortisol level and AUC_G values were positively skewed and were normalized by log transformation. Log-transformed cortisol values were used in analyses, whereas raw cortisol values are provided when descriptive statistics (i.e., means and standard deviations) are reported, unless otherwise specified.

Results

Table 1 and Figure 1 provide descriptive information about the salivary cortisol obtained on the 16 sampling occasions as well as the children's AUC_G values. Table 2 shows the correlations among the cortisol indices.

Preliminary analysis

Preliminary analyses were first conducted to examine the effects of demographic variables such as age, gender, and birth order on cortisol. Preliminary analyses, conducted using Pearson's correlations and independent-sample t tests, revealed no significant effects of age and birth order on cortisol levels and AUC_G. However, *t*-statistics revealed significant differences between boys ($M = 4.01$ nmol/L, $SD = 1.46$) and girls ($M = 4.97$ nmol/L, $SD = 1.85$) when cortisol was measured in March in the mid-afternoon (t (56) $= -2.10$, $p < .05$). There were no significant differences for the other 15 sampling frames, suggesting that the one difference might be a chance association.

Table 1. Means and standard deviations for raw cortisol levels and AUCG sampled at each time of day and time of year.

		Time(hour)^a		Cortisol level (nmol/L)	
	n	*M*	SD	*M*	SD
July/August (baseline)					
Early morning	46	0753 h	0040 h	12.62	6.50
Mid-morning	49	1035 h	0018 h	6.07	3.81
Mid-afternoon	48	1539 h	0028 h	3.72	2.12
Early evening	44	1743 h	0103 h	2.22	1.54
$AUC_G{}^b$	40			53.26	18.40
September (enrollment)					
Early morning	43	0739 h	0027 h	11.67	6.17
Mid-morning	55	1008 h	0014 h	5.83	3.27
Mid-afternoon	50	1441 h	0048 h	4.06	2.90
Early evening	44	1743 h	0106 h	2.56	2.27
$AUC_G{}^b$	37			55.36	23.96
December (3 months after enrollment)					
Early morning	45	0743 h	0030 h	15.99	7.29
Mid-morning	55	1014 h	0030 h	6.11	4.38
Mid-afternoon	55	1353 h	0008 h	5.59	4.07
Early evening	45	1734 h	0107 h	3.16	2.13
$AUC_G{}^b$	43			61.22	23.25
March (6 months after enrollment)					
Early morning	49	0738 h	0026 h	15.72	9.31
Mid-morning	56	0957 h	0006 h	4.75	2.35
Mid-afternoon	58	1350 h	0007 h	4.42	1.69
Early evening	48	1734 h	0059 h	2.86	1.96
$AUC_G{}^b$	45			53.44	19.25

[a] Means of Time are shown on a 24-hour clock; standard deviations for Time are shown in hour(s) and minute(s).

[b] Data available only for those with all four samples per day at each sampling months.

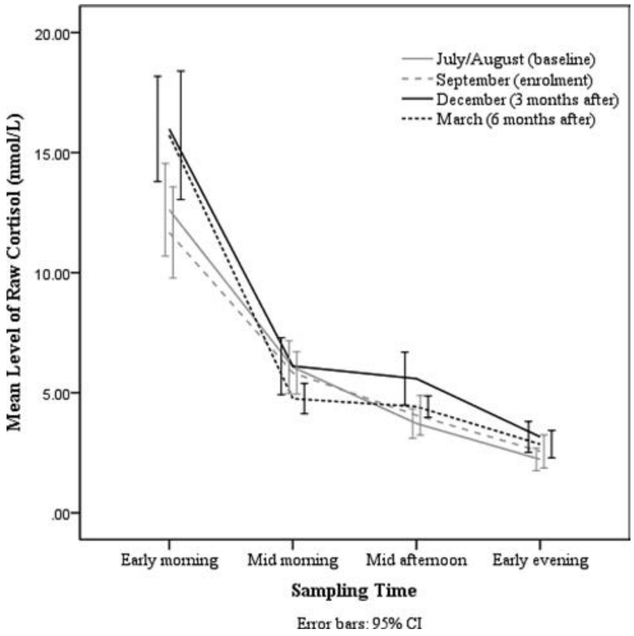

Figure 1. Diurnal cortisol patterns in July/August, September, December, and March.

Mixed-effect modelling

Mixed-effect modeling is increasingly used in research on cortisol levels because it allows simultaneous modelling of cortisol parameters such as elevation and diurnal slope and it has high tolerance for between- and within-individual variations in sampling time (Hruschka, Kohrt, & Worthman, 2005). Also, mixed-effect modeling is preferable to repeated measures analysis because it does not assume the independence of cortisol samples within an individual (Hruschka et al., 2005). Considering our small sample size and the missing data, we conducted mixed-effect model analyses, so factors that might introduce variability could be accounted for and the missing data could also be handled with maximum likelihood parameter estimation.

Below we explain the steps applied to fit the mixed-effect model using the log transformed cortisol levels as the dependent variable. The likelihood function was used to assess the deviance (−2 Log Likelihood) generated by each model; the −2 Log Likelihood ratios (−2LL) were reported to indicate the model's goodness of fit. The *p* value was calculated using the chi-square distribution table based on differences in the divergence between two models (the model chi-square difference) given differences in the number of model parameters (*df*) (Hox, 2002).

In our sample, children who initially attended school part day had one of their September MM or MA samples measured at home. To avoid confusing samples that were sampled at the same time during the day but in

Table 2. Correlations between and among cortisol indices.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Summer (baseline)																				
1. EM	–																			
2. MM	.26	–																		
3. MA	.03	.32*	–																	
4. EE	.11	.22	.18	–																
5. AUCG	.38*	.75**	.67**	.27	–															
September (enrollment)																				
6. EM	.40*	.04	.16	−.29	.16	–														
7. MM	.17	.24	−.01	.08	.22	−.01	–													
8. MA	−.05	.35*	.29*	−.12	.30	−.06	.25	–												
9. EE	−.16	.20	.17	.43**	.11	−.10	.40**	.28	–											
10. AUCG	.05	.18	.12	−.21	.24	.43**	.67**	.62**	.48**	–										
December (3 months after enrollment)																				
11. EM	−.06	−.09	.15	−.09	.09	.17	−.12	−.20	−.35*	−.13	–									
12. MM	.22	.03	−.11	.22	.14	.06	.10	−.09	.09	.01	−.13	–								
13. MA	−.16	.03	.20	.04	.12	.15	−.01	.05	.21	.18	.05	.44**	–							
14. EE	.15	.25	.08	.27	.19	.13	.04	−.12	.001	−.28	.09	.32*	.19	–						
15. AUCG	.04	−.11	.20	−.14	.16	.48**	.00	−.11	.02	.20	.39**	.58**	.73**	.22	–					
March (6 months after enrollment)																				
16. EM	.33*	.24	.18	.14	.34*	.31	−.15	−.22	−.24	−.12	.26	.41**	.36*	.23	.35*	–				
17. MM	.25	.37**	.04	.35*	.42**	−.14	.34*	.31*	.26	.22	−.01	.09	−.02	.10	.06	.13	–			
18. MA	−.20	.11	.05	.05	.04	−.22	−.07	.14	.16	−.12	−.04	.24	.36**	.21	.32*	.28*	.22	–		
19. EE	−.15	−.07	.09	−.04	−.14	.08	−.05	−.06	−.13	−.14	.21	−.07	.23	.11	.26	18	.07	.31*	–	
20. AUCG	.14	.23	.00	.12	.23	.16	.04	.04	.00	.05	.22	.48**	.49**	.21	.52	.73**	.36*	.65**	.44**	–

Note. EM = early morning, MM = mid-morning, MA = mid-afternoon, EE = early evening.
$^{*}p < .05$. $^{**}p < .01$.

different contexts, we decided to examine the cortisol data in two ways: One analysis included only the baseline and September (enrollment) measurements, and the other included the baseline, December (3 months after enrollment), and March (6 months after enrollment) measurements.

Intercept model

The first model tested was the intercept model, including the population mean (β_0), the deviation of each individual's mean from the population mean (b_i), and within-individual variation and measurement error (ε_i):

$$\ln(\text{cort})_i = \beta_0 + b_i + \varepsilon_i \tag{1}$$

Significant fixed effects were reported for the intercept model assessing cortisol levels at baseline and enrollment, $F(1,\ 50.54) = 1066.33$, $p < .001$, $-2\text{LL} = 953.42$., and at baseline, 3 months after enrollment, and 6 months after enrollment, $F(1,\ 53.81) = 2020.82$, $p < .001$, $-2\text{LL} = 1470.71$.

Sampling time

The second model addressed the sampling time, including the exact time the cortisol was obtained and the diurnal slope. Individual differences in diurnal slopes were examined in light of previous research suggesting that individuals may differ with respect to their mean levels and diurnal slopes (Smyth et al., 1997; Stone et al., 2001). The diurnal slope was not included in the model assessing cortisol levels at baseline and enrollment however, because the preliminary results showed that the estimated random slope variance was close to zero.

In the model assessing baseline and enrollment, the natural log of salivary cortisol was predicted by the exact time the cortisol was obtained ($-2\text{LL} = 693.75$, $p(df = 1) < .001$). b_i was the deviation of each individual's mean from the population mean and there was a significant fixed effect of time, $F(1,\ 335.70) = 389.19$, $p < .001$:

$$\ln(\text{cort})_{ij} = \beta_0 + \beta_1(\text{time}) + b_i + \varepsilon_{ij} \tag{2}$$

In the model assessing baseline, 3 months after enrollment, and 6 months after enrollment, in addition to the exact time the cortisol was obtained, the natural log of salivary cortisol was also predicted by the diurnal slope ($-2\text{LL} = 1080.18$, $p\ (df = 2) < .001$). b_{0i} was the deviation of each individual's mean from the population mean and b_{1i} was the individual deviation from the population average slope (β_1). There was a significant fixed effect of time, $F(1,\ 276.22) = 557.13$, $p < .001$:

$$\ln(\text{cort})_{ij} = \beta_0 + \beta_1(\text{time}) + b_{0i} + b_{1i}(\text{time}) + \varepsilon_{ij} \tag{3}$$

Assessment points over time

The third model investigated cortisol levels over time. In the model assessing baseline and enrollment ($-2\text{LL} = 692.81$, $p\ (df = 1) > .05$), the result showed no significant effect of assessment point, $F(1, 335.96) = .94$, $p = .33$:

$$\ln(\text{cort})_{ijk} = \beta_0 + \beta_1(\text{time}) + \beta_2(\text{assessment point}) + b_i + \varepsilon_{ijk} \quad (4)$$

In the model assessing baseline, 3 months after enrollment, and 6 months after enrollment ($-2\text{LL} = 1072.25$, $p\ (df = 2) < .05$), the results showed a significant fixed effect of assessment point, $F(2, 548.19) = 4.00$, $p < .05$. Significant differences were evident 3 months after enrollment (December), $t\ (554.47) = 2.68$, $p < .01$:

$$\ln(\text{cort})_{ijk} = \beta_0 + \beta_1(\text{time}) + \beta_2(\text{assessment point}) + b_{0i} + b_{1i}(\text{time}) + \varepsilon_{ijk} \quad (5)$$

Further post-hoc analyses with Bonferroni correction showed that the mid-afternoon level of cortisol measured after 3 months of school (December) was significantly higher than the baseline level (mean difference = 1.87, SE = .64, $p < .05$) and marginally higher than the level measured in March 6 months after enrollment (mean difference = 1.31, SE = .56, $p = .07$). The early evening level of cortisol measured after 3 months of school (December) was significantly higher than the baseline level (mean difference = 1.08, $SE = .35$, $p < .05$).

Confounding factors

Potential confounds such as sampling tools (Salicap vs. Salivette), day of the week the samples were obtained, and classroom were investigated. The results showed that none of the potential confounds was significant for either model. We further investigated in the model assessing baseline and enrollment whether initial program type (part day vs. full day) and sampling location (home vs. school) affected cortisol levels. There were no significant results of program type, $F(1, 54.26) = 1.00$, $p = .32$, and sampling location, $F(1, 348.78) = 2.72$, $p = 1.00$.

Table 3. Models of diurnal variation in cortisol levels: July/August (baseline) to September (enrollment).

Final model	Estimates[a]	S.E.
Fixed effects		
Intercept[b] (β_0)	3.39***	.11
Overall slope (β_1)	−.15***	.008
Assessmentpoint[c] (β_2)		
Enrollment	−.06	.06
Variance components		
Child-specific means(b_i)	.05*	.02

[a] Estimates are reported in log-transformed cortisol values.
[b] Expected log cortisol value at time before breakfast ($M = 0746$ h, $SD = 34.7$ min).
[c] Baseline (July/August) was assigned as the base category.
*$p < .05$. **$p < .01$. ***$p < .001$.

Table 4. Models of diurnal variation in cortisol levels: July/August (baseline) through December (3 months after enrollment) to March (6 months after enrollment).

Final model	Estimates[a]	S.E.
Fixed effects		
Intercept[b] (β_0)	3.37***	.0900
Overall slope (β_1)	−.15***	.0060
Assessment point[c] (β_2)		
3 months after enrollment	.16**	.0600
6 months after enrollment	.03	.0600
Variance components		
Child-specific means(b_{0i})	.03	.0200
Slopes (b_{1i})	.00004	.0001

[a] Estimates are reported in log-transformed cortisol values.
[b] Expected log cortisol value at time before breakfast ($M = 0744$ h, $SD = 32.4$ min).
[c] Baseline (July/August) was assigned as the base category.
*$p < .05$. **$p < .01$. ***$p < .001$.

The final model assessing cortisol levels at baseline and enrollment was shown in Table 3. The results suggested that there were no significance differences in the children's cortisol levels between baseline and enrollment. The final model assessing cortisol levels at baseline, 3 months after enrollment, and 6 months after enrollment was shown in Table 4. The results suggested that the children had significantly greater cortisol secretion 3 months after enrollment (see Table 1 for the descriptive information on cortisol levels and AUC_G).

Discussion

The present study focused on the transition associated with school enrollment. Previous research on the transition to child care or school has shown cortisol rise across the first 2 or 3 months (Bernard et al., 2015; Groeneveld et al., 2013; Russ et al., 2012), although the cortisol measured upon entry to school was not always elevated significantly relative to levels at home (Gutteling et al., 2005; Russ et al., 2012; Turner-Cobb et al., 2008). It also seemed possible that children's cortisol had become adapted to school after 6 months; the children in Turner-Cobb et al.'s (2008) study had the lowest level 6 months after school began. We suspected that the 4-year-old children in our study might show more cortisol elevation after the effects of school experiences had accumulated because the demands of cognitive concentration and behavioral regulation increased over the school year. In addition to baseline assessments before enrollment, the children's cortisol was measured three times during the school year (during the first week of the autumn term, and around the end of the autumn term and spring term) and, on each measurement day, cortisol was measured four times a day (early morning, mid-morning, mid-afternoon, and early evening). This design thus allowed assessment of the cortisol pattern as the school-related demands accumulated, and the four measurements a day allowed us to calculate the total amount of cortisol secretion daily using the area (s) under the curve (AUC_G) technique.

We examined 4-year-old children's cortisol activity during the first two terms of school using a mixed-effect model because it allowed simultaneous modelling of cortisol parameters such as elevation and diurnal slope and had a high tolerance for between- and within-individual variations in sampling time (Hruschka et al., 2005). The results suggested that children's cortisol levels indeed fluctuated over the school year. In line with our prediction, the elevation of cortisol did not emerge immediately upon enrollment but after 3 months of school. Children's cortisol sampled on school days (after 3 months at school) was higher than on home days (baseline), with higher afternoon and evening levels at school than home.

British children's cortisol levels were also examined by Russ et al. (2012) after 3 months of school and by Turner-Cobb et al. (2008) after 6 months of school. In the present study, the same children were assessed both 3 and 6 months after enrollment, and the results were consistent with those reported by Russ et al. and Turner-Cobb et al. As in Russ et al.'s study, our children showed greater cortisol elevation 3 months after enrollment than at enrollment, suggesting that the children were physiologically responsive to the cumulative effects of school. And, after 6 months of school, we observed, as did Turner-Cobb et al., that the children's cortisol levels appeared to decrease to levels similar to those at the time before enrollment, suggesting that by the end of the spring term the children had adapted to the classroom demands. Our findings thus suggest a physiological pattern of adaptation during the first year of school that involved the re-establishment of cortisol stability through physiological and behavioral changes (Giudice et al., 2011). It appeared that the children in this study might have been adjusting gradually to school throughout the first 6 months, and that by March their cortisol regulation had returned to levels similar to that at the beginning of school. This suggests that the children might have become fully familiarized with the classroom learning and social structure after 6 months of school. Transition policies and practices might want to focus more on the initial 6 months of school during which young children might be reacting to accumulating demands. Classroom practices supporting children's mastery of the necessary social and cognitive skills during this period might help facilitate young children's physiological adjustments to school.

Prior research examining preschool-aged children's cortisol responses to child care had consistently noted a rising pattern of cortisol during days at child care (e.g., Bernard et al., 2005; Dettling et al., 1999; Watamura et al., 2002). Children in the present study appeared to have significantly higher levels of afternoon and evening cortisol on school days (3 months after enrollment) than on home days (baseline), which suggests that children on school days, like their same-age counterparts in child care, had less obvious diurnal decline in their cortisol activity. Although the children's cortisol was not secreted significantly more on school days around the first week of school, it is possible that, upon enrollment, the children's nursery experience and the school's transition practice (e.g., open day for incoming parents and students to visit the classroom) had eased the children into the new school schedule. And, perhaps by the end of the spring term (6 months after school began), the children had become so familiar with the structure of school that their routines were predictable, which might help mitigate the children's cortisol activity at school since unpredictability is known to trigger elevated levels of cortisol (Dickerson & Kemeny, 2004). Overall, our findings suggest that, like the pre-school aged children in child care research, school children at the ages of 4 and 5 years find out-of-home settings more demanding than home and respond with heightened levels of cortisol later during the day.

Activation of the HPA axis is associated with psychological or emotional responses to everyday demands and challenges (Gunnar & Quevedo, 2007). Learning activities at school might be expected to require extensive attention and cooperation from youngsters and it is known that social pressure, such as peer rejection, also triggers more cortisol secretion in 3- to 5-year-old children (Gunnar, Sebanc, Tout, Donzella, & Van Dulmen, 2003). Young children might therefore find it challenging to pay sustained attention in class and to play cooperatively with peers, with associated increases in the secretion of cortisol. Gunnar et al. (2010) has suggested that the high level of cortisol observed later in the day may reflect children's physiological responses to an accumulation of taxing experiences in full-day care. We thus hypothesized that school children's cortisol might reflect the accumulated effects of their experiences over the time at school. Cortisol responses to anticipatory events reflect our memory of prior similar experiences (Dedovic, Duchesne, Andrews, Engert, & Pruessner, 2009). It is possible that children's cortisol activity at school involved anticipatory responses based on their memory of prior school days as well as their momentary responses to daily activities.

The present study followed children for 9 months through the transition to formal schooling, during which time the children's salivary cortisol was assessed on four occasions so that meaningful patterns could be assessed. We were able to identify a physiological pattern of adaptation, showing that cortisol secretion was much greater at the end of the first term (3 months after enrollment) whereas by the end of the second term (6 months after enrollment) the children seemed to be adapting to school. Our results suggested that cortisol levels provided a meaningful assessment of children's physiological activity over time in school. Future research might consider including cortisol when evaluating effects of school support programs, determining which facilitate readily physiological adaptation, for example. The present study contributes to our knowledge of young children's HPA-axis regulation during the first year of school and provides some insights into transition policies and early education practices. Our findings suggest that educators and policy makers might want to consider physiological adjustment when developing transition practices, and to recognize that some children need support with the transition to school beyond the first month.

The present study was limited in a few methodological respects, however. Although we had a representative sample of 4- to 5- year-old children, the sample size (59) was small and their socio-economic backgrounds were quite homogenous. Although the association between cortisol levels and family socio-economic status (SES) is not consistent, children in lower SES families are likely to experience more stressful events that might affect their cortisol activation (Dowd, Simanek, & Aiello, 2009). We did not have a diverse enough sample to investigate effects of SES or early rearing contexts on cortisol regulation, however. Staffing constraints prevented us from sampling the children's saliva on consecutive days as well. Although statistical controls were employed, other factors such as the use of two types of sampling tools (Salicaps or Salivettes) and the varying sampling days may also have affected our results. Dietary choices, such as dairy consumption or meal skipping, might affect cortisol too (Witbracht, Keim, Forester, Widaman, & Laugero, 2015; Witbracht, van Loan, Adams, Keim, & Laugero, 2013). We did not have information about children's food choices and this might limit the interpretation of our results as well.

Nevertheless, the present study contributes to the literature on early childhood in many ways. Although starting school is an important event involving "full alert readiness and mental anticipation" which are usually associated with cortisol release (Flinn, 2006, p. 147), it is possible that many children are familiar with school routines through prior preschool experiences or benefit from high quality support during the transition. In the future, researchers studying the transition to school might want to assess responses after the entry month and to include more measures of cortisol during the school year, in order to elucidate young children's physiological patterns as they cope with the accumulating demands of school. Future research might also include measures of contextual factors, such as teacher quality or classroom support, and personal factors, such as children's academic or social competence. In that way, we could develop a more systematic and dynamic understanding of the factors that influence young children's physiological adaption to school and thus inform transition policies and practices. Lastly, although we did not identify any differences associated with demographic variables such as birth order, gender, and SES, these factors merit consideration in studies involving larger and more heterogeneous samples.

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