Classroom Emotional Support Predicts Differences in Preschool Children's Cortisol and Alpha-amylase Levels

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Abstract:

Accumulating evidence suggests children enrolled in full-time child care often display afternoon elevations of the hormone cortisol, which is an indicator of stress. Recent advances in immunoassays allow for measurement of activity in the hypothalamic‐pituitary‐adrenal axis and the autonomic sympathetic nervous system from saliva, and measurement of both systems provides a more complete understanding of activity in the stress response system. This study is the first to examine both cortisol and alpha-amylase in children attending child care and focuses on the influences of specific indicators of classroom process quality. A diverse sample of 63 preschool children nested in 14 classrooms of varying quality participated in this study; child salivary cortisol and alpha-amylase were collected at six times over 2 days. Results indicate that children in classrooms with higher Emotional Support displayed a greater decline in cortisol from morning to afternoon. Further, children in classrooms with higher Emotional Support exhibited lower total alpha-amylase output while attending child care. Implications for professional development for early childhood teachers and measurement of classroom quality are discussed.

Keywords: Cortisol | Alpha-amylase | Classroom quality | Process quality | Teacher–child interactions | Classroom Assessment Scoring System

Article:

Introduction

Children's responses to their environments encompass a wide variety of developmental domains including social, emotional, and cognitive development. Researchers of child care environments and teacher‐child interactions have often measured these various responses through observation, survey, and interviews (Early et al., 2005 and NICHDECCRN, 2002). Recently, researchers in early care and education have begun to investigate children's physiological responses to interactions within their early care settings, most often in an effort to provide information that cannot be measured through observation (Vermeer & van Ijzendoorn, 2006). Children's stress responses have been of particular interest in conjunction with the quality of child care environments. Such information on children's physiological responses to the environment provides an additional way of understanding how child care quality impacts children's development.

It is well documented that cortisol levels, a biomarker for activity in the hypothalamic‑pituitary‑adrenal (HPA) axis, vary as a function of context. Specifically, children often display elevated levels of cortisol at child care but not at home (Groeneveld et al., 2010 and Watamura et al., 2009). Given that cortisol is expected to decline across the day (Gunnar & Donzella, 2002), interest in differentiating the experiences of children in child care settings often leads researchers to explore indices of classroom quality. Preliminary evidence suggests that classroom process quality, namely the interactions within the classroom, may be central to identifying characteristics of the classroom that lead to elevations in cortisol as increased teacher sensitivity in the classroom is linked to a decrease in cortisol throughout the day when compared to children in classrooms with lower sensitivity (Watamura et al., 2009). Essentially, individual patterns of cortisol activity for children in classrooms that foster community, cohesion, and autonomy closely resemble activity displayed in the home environment. Further, in family child care homes characterized by teachers who provided high levels of verbal and behavioral attention, children's cortisol patterns showed the expected decline throughout the day. Children in those family child care settings showed no difference between cortisol patterns at home and patterns at child care (Dettling, Parker, Lane, Sebanc, & Gunnar, 2000).

Although the current body of literature has identified relationships between child and classroom characteristics that influence activity in the HPA axis, little emphasis has been placed on the functioning of the remaining components of the stress response system. The stress response system is comprised of three integral but unique components: the HPA axis, the parasympathetic nervous system, and the sympathetic nervous system (Del Giudice, Ellis, & Shirtcliff, 2011). In early childhood, the stress response system is immature and is largely influenced by experiences, relationships, and interactions during this period (Levine, 1994 and Meaney et al., 1996). Given that 36% of preschool children experience center-based care (U.S. Department of Education, 2006), the purpose of the current study is to identify indicators of classroom quality that impact preschool children's activity in the stress response system.

Stress response system

Activity throughout the stress response system is essential for human functioning as it allows an individual to cope with positive (e.g., excitement about an upcoming vacation) and negative (e.g., experiencing a car accident) events. Individuals vary slightly in how their stress response system calibrates to the environment due to his or her ability to adapt and modify to the “local conditions of the social and physical environment,” (p. 1563, Del Giudice et al., 2011). The three components of the stress response system (i.e., parasympathetic, sympathetic, and HPA axis) have unique purposes, but the timing of activation for each component is somewhat dependent. Simply, the parasympathetic branch of the stress response system serves as a ‘first responder’ in the event of a challenge, triggering increased attention and vigilance. If the parasympathetic response is not sufficient, the sympathetic system is activated, secreting norepinephrine and epinephrine. Finally, the HPA axis is signaled and provides a more sustained response and helps the sympathetic system recover (for detailed review see Del Giudice et al., 2011 and Gunnar and Quevedo, 2007). These systems converge at the hypothalamus where a behavioral response within the individual is signaled (Palkovits, 1987). Activity in the stress response system is uncorrelated (e.g., Davis and Granger, 2009, El-Sheikh et al., 2009, Fortunato et al., 2008 and Wolf et al., 2008) suggesting that activity in the three components are separate entities. The current study focuses only on how classroom quality relates to activity in two of the components, the HPA axis and the sympathetic nervous system (SNS). Thus, more detail regarding the HPA axis and SNS is provided below.

The HPA axis produces cortisol, a steroid hormone that impacts brain functioning and behavior (Bohus, de Kloet, & Veldhuis, 1982), as well as regulates important bodily functions such as the immune system (Palacios & Sugawara, 1982) and emotional expression (Oberlander, Weinberg, Papsdorf, Grunau, Misri, & Devlin, 2008). Salivary cortisol is a well-established measure of activity in the HPA axis (Granger, Schwartz, Booth, Curran, & Zakaria, 1999) and is strongly correlated with serum cortisol levels (Charmandari, Tsigos, & Chrousos, 2005). Cortisol levels in individuals vary diurnally with levels highest in the morning and gradually declining throughout the day (Gunnar & Donzella, 2002). Deviation in the diurnal patterns of cortisol is linked to internalizing and externalizing behaviors (Shirtcliff, Granger, Booth, & Johnson, 2005), anxiety (Feder et al., 2004), and immune deficiencies (Greenspan, 2003). Until recently, the specific associations of elevated cortisol on child outcomes for children in child care were unclear. There is new evidence that children who display elevations in cortisol at child care are more likely to display lower levels of sIgA antibodies and experience increased illness (Watamura, Coe, Laundenslager, & Robertson, 2010). Lower levels of sIgA are linked to a variety of poor health outcomes such as an increased risk of respiratory illness (Winzer et al., 1999).

Salivary alpha-amylase (sAA) is predictive of norepinephrine levels in humans (Chatterton, Vogelsong, Lu, Ellman, & Hudgens, 1996), and it is considered to be a surrogate biomarker for the SNS (Granger et al., 2006 and van Stegeren et al., 2006). sAA is an enzyme in the mouth that aids in digestion, and the appearance of sAA is associated with the introduction of solid foods into an infant's diet (Davis & Granger, 2009). The salivary glands that produce and secrete sAA are stimulated by the sympathetic (and parasympathetic) nerves, and thus activity in the SNS is related to increases in sAA (Nater & Rohleder, 2009). Further, sAA is also correlated with other measures of activity in the SNS such as skin conductance (El-Sheikh, Erath, Buckhalt, Granger, & Mize, 2008) as well as two cardiac indices of SNS, vagal suppression (El-Sheikh, Mize, & Granger, 2005) and preejection period (i.e., the elapsed time between a heartbeat and the ejection of blood into the aorta; Granger, Kivlighan, El-Sheikh, Gordis, & Stroud, 2007), identifying sAA as a reliable and non-invasive measurement of activity in the SNS. Generally, sAA also follows a diurnal pattern, decreasing 60 min after wake, increasing over the day peaking around 4:30 pm, and then declining (Nater, Rohleder, Schlotz, Ehlert, & Kirschbaum, 2007). This diurnal pattern for sAA is also apparent in children, although overall levels are smaller in magnitude compared to adults (Wolf et al., 2008). Activity in the SNS is related to overall arousal (i.e., negative or positive arousal) in response to environmental stressors (Beauchaine, 2001), as well as behaviors specific to positive affect and surgency. Specifically, during a laboratory task, higher positive affect (e.g., smiling, laughing) and higher frequency of approach behaviors (e.g., boldness/surgency, exuberance) were related to higher baseline sAA in toddlers (Fortunato et al., 2008). Thus, research suggests that sAA is related more to the expression of emotions, both negative and positive, and this may influence relationships that children experience in the classroom, complying with Nigg's (2006) theory that activity in the SNS relates to overall emotional arousal whereas the HPA axis responds to more intense triggers and/or helps the SNS recover. Thus, the measurement of sAA and cortisol provide unique indicators to aid in understanding activity in the HPA axis and SNS.

The patterns of activity described above for cortisol (declining across the day) and sAA (increasing throughout the day) promote homeostasis within the HPA axis and SNS (McEwen & Seeman, 1999). To date, only the associations between activity in the HPA axis and child care quality have been investigated; this study is the first to understand activity of the SNS in the context of child care.

Classroom quality

Children consistently demonstrate higher cortisol levels at child care than at home (Dettling et al., 1999 and Watamura et al., 2003). However, a meta-analysis (Vermeer & van Ijzendoorn, 2006) indicates that only 18% of the variability in cortisol is attributable to the context (home vs. classroom). Investigations into global quality of the child care context reveal that children in classrooms rated as good or excellent by the Early Childhood Environmental Rating Scale-Revised (ECERS-R; Harms, Clifford, & Cryer, 1998) continue to demonstrate a rise in cortisol throughout the day (Rappolt-Schlichtmann et al., 2009, Watamura et al., 2003,Watamura et al., 2010 and Watamura et al., 2002). Given the complex nature of the classroom, a global measurement of quality may not be distinctive enough to specifically relate to children's activity in the HPA axis or identify relations with activity in the sympathetic nervous system. Instead, particular components of classroom quality may differentially impact children's activity in the HPA axis or SNS.

In the field of early care and education, global quality is often categorized into two separate but related components: structure and process. Structural quality refers to elements of the classroom that are constant, materialistic in nature, exist without interactions, and form the foundation for potential learning experiences (Layzer & Goodson, 2006). Research investigating correlates between cortisol and structural quality have identified group size, square feet/individual, parent involvement, and health and safety as salient influences on cortisol levels in young children (Legendre, 2003, Lisonbee et al., 2008, Martimportugués-Goyenechea and Gómez-Jacinto, 2005, Rappolt-Schlichtmann et al., 2009 and Sims et al., 2006). The HPA axis generally interprets crowding or large groups as stressors and responds with elevated cortisol levels (Lisonbee et al., 2008, Martimportugués-Goyenechea and Gómez-Jacinto, 2005 and Rappolt-Schlichtmann et al., 2009). In child care, cortisol levels are lower in classrooms with more than 16.5 ft^2 of useable play space per child and those with less than 15 people (Legendre, 2003). Cortisol levels are also lower for children in classrooms with a parent orientation and teachers who are invested in protecting the health and safety of each child (Sims et al., 2006). Yet, in the other studies focused on understanding links between classroom quality and children's social and academic outcomes, structural quality often demonstrates an indirect effect, whereas process quality has a direct effect (Mashburn et al., 2008 and NICHDECCRN, 2002). Thus, in line with this research, the current study focuses on how specific components of process quality, after accounting for structural quality, may influence child activity in the HPA axis and SNS.

Process quality encompasses interactions and experiences that occur in the classroom that require active human interaction and intent (Cassidy et al., 2005a, Cryer, 1999 and Phillipsen et al., 1997). The mechanisms by which *specific* components of process quality impact cortisol levels have not been explored in depth; relations between classroom quality and children's sAA levels have not been pursued. Given these preliminary findings and improved measurement techniques for classroom process quality and activity in the SNS, it is important to investigate further the relations between specific dimensions of process quality and the activity the HPA axis and SNS. Classrooms high in process quality are likely to have a teacher who (a) has sensitive, warm, and secure interactions, (b) organizes the classroom in such a way that is not rigid or regimented, and (c) engages their students in intentional, instructional discussions, activities, and conversations (Burchinal et al., 2000 and Hamre and Pianta, 2007).

There are associations among these constructs of process quality (i.e., Emotional Support, organization and behavior management, intentional instruction) and activity in the SNS and HPA axis reflected in the parenting and early education literature. Children of parents who are emotionally supportive and responsive are more likely to demonstrate cortisol activity that declines throughout the day (Francis et al., 1999 and Gunnar et al., 1992). Results are similar for child care as children exposed to teachers who are warm and nurturing are more likely to display cortisol levels that resemble levels demonstrated at home (Groeneveld et al., 2010). Further, research examining mother–child interactions and activity in the SNS indicate that children whose mothers are more “in tune’ with their child's emotional needs have better regulation within the SNS” (Moore & Calkins, 2004). This association is also evident in the child care setting; lower sAA was positively associated with a close teacher–child relationship for preschool children (Mize, Lisonbee, & Granger, 2005), implying that children with lower baseline sAA may have fewer moments in the classroom that demand high emotional arousal, possibly due to the close relationship with the teacher. Thus, emotional support, whether provided by the parent or a teacher, seems to influence activity in the HPA axis and SNS. Teachers within classrooms high in process quality also organize the classroom in such a way that is not rigid or regimented, and this structured organization is likely to help support activity in the HPA axis and SNS. Cortisol levels increase in unorganized, chaotic, or noisy environments (Martimportugués-Goyenechea & Gómez-Jacinto, 2005), and maternal negative management of child behavior is associated with poor regulation of SNS (Calkins, Smith, Gill, & Johnson, 1998). Further, children's activity in the SNS increased after hearing an argument between two adults (El-Sheikh et al., 2009), suggesting that conflict is associated with abnormalities in the SNS. Thus, classrooms in which students have engaging activities, are efficiently managed, and where little misbehavior occurs may not elicit increased activity in the HPA axis or the SNS.

Finally, child cortisol is more likely to decline across the day when children are exposed to child care that affords more opportunities for stimulation (Dettling et al., 2000). However, Dettling et al. aggregated instructional stimulation and conversational exchanges with caregiver sensitivity, thus limiting the ability to illustrate specific links between intentional, instructional discussions, activities, and conversations to activity in the HPA axis. This study aims to understand the specific relation between instructional discussion and activity in the HPA axis. Regarding activity in the SNS, higher levels of baseline sAA are tentatively related to poorer scores on standardized tests of achievement in middle childhood (Granger et al., 2007), but this is not a direct association between teacher's instructional skills and facilitation. A major strength of this study is the ability to examine the unique contribution of instructional discussions and facilitation in early childhood classrooms and relations to activity in the HPA axis and SNS via salivary cortisol and sAA, respectively.

Child characteristics

Although characteristics of the classroom environment influence children's cortisol levels, research suggests individual child characteristics, such as gender and experience in the child care environment, also influence associations with cortisol, but not sAA. Regarding gender, new evidence suggests that adolescent males exhibit higher awakening cortisol levels than females (Roisman et al., 2009). Further, in the child care environment, boys demonstrated significantly higher total cortisol activity (Groeneveld et al., 2010 and Ouellet-Morin et al., 2010). Ouellet-Morin et al. contend that children with less child care experience (enrolled in child care after 16 months of age) demonstrated higher cortisol levels at 3 years of age than children who started child care earlier in life. This study acknowledges that these child characteristics along with indicators of classroom quality (i.e., structure and process) are needed to understand better the differences in activity of the HPA axis and SNS.

Aims and hypotheses

Classroom indicators of process quality such as warm, sensitive relationships, effective questioning and promotion of thinking skills, as well as predictable routines and well-managed activities and behaviors are influential for child emotional and academic development (Hamre & Pianta, 2007). Further, caregiver sensitivity is related to activity and development of the SNS and HPA axis in early childhood (Groeneveld et al., 2010 and Mize et al., 2005), but specific associations with intentional classroom instruction and classroom management and behavior have not been explored. The primary aim of this study is to understand how three aspects of classroom process quality (i.e., sensitive and warm interactions, classroom management and behavior, intentional and instructional discussions) impact activity in the HPA axis and SNS after accounting for relations with structural quality and child characteristics. Note that the hypotheses for sAA are largely exploratory, but specific hypotheses are provided and were formulated from knowledge garnered from studies that examined sAA outside of the classroom context.

With regard to the emotional climate and support in the classroom, it is expected that children in classrooms with higher levels of respectful and warm interactions, support for autonomy, and sensitive teachers will demonstrate lower activity in the HPA axis and SNS. This would support previous research claiming associations with sensitive caregivers and the HPA axis and SNS. Second, based on research with adults indicating that chaotic environments influence cortisol activity and that conflict influences activity in the SNS, we hypothesize that this association will also hold for children such that children in classrooms that follow a predictable routine and manage child behavior in an effective, calm manner will have lower activity in the HPA axis and SNS. Given Granger et al.'s (2007) finding suggesting that in middle childhood, there is a positive association between lower sAA and test scores, we expect that activity in the SNS may be lower for children in classrooms with a high frequency of intentional, instructional discussions aimed to further children's learning and understanding. Given the absence of theory or research to suggest a distinct association between instructional dialog and cortisol levels, a null relation is expected.

Methods

Participants

The classrooms in this study were drawn from a random sample of child care centers in one metropolitan county that participated in North Carolina's Division of Child Development (DCD) Star Rated License. Under the Star Rated License, child care centers in North Carolina are awarded 1–5 stars based upon program standards and staff education levels. One hundred centers were randomly selected and divided into two groups (high quality, 4 or 5 stars; low quality, 1–3 stars). Then, in a strategic effort to recruit child care centers from an at-risk population, child care centers in both groups were ranked by per-child subsidy (total yearly subsidy for center/center capacity) using 2008 child care subsidy data from the North Carolina DCD, and centers that received the most funding were contacted first. For the high-quality group, the average per-child subsidy was $2270 and was similar to the average per-child subsidy in the low-quality group (*M* = $2341).

Center directors were initially contacted by mail, and up to three follow-up phone calls were made to the director to provide additional information, answer questions, and determine if the center had an eligible preschool/pre-kindergarten classroom. Classrooms were eligible if at least eight full-time children were between 36 and 59 months of age, and the teacher had served as the primary teacher for at least 8 weeks. Of the 100 centers, only 61 had an eligible classroom. Twenty-three of the remaining classrooms agreed to participate. The most common reason directors gave for not participating was hectic, busy schedules.

Following approval from the director and teacher, parent recruitment packets were distributed to all families in the classroom. Interested parents returned the consent form, and child eligibility was determined using the following criteria: enrolled in the classroom where data collection took place for at least 8 weeks, attended child care for at least 25 h a week, typically developing, from English-speaking households, had not taken an oral steroid in the past 30 days, and between 36 and 59 months of age prior to data collection. A minimum of four and a maximum of five children were required for data collection. If more than five children consented in a classroom, five were randomly selected. Only 14 of the 23 classrooms had at least four eligible children with parent consent. The final sample included five lower-quality centers (*M* = 2.8 Star rating; *M* = $920.26 per-child subsidy) and nine higher-quality centers (*M* = 4.33 Star rating; *M* = $745.43 per-child subsidy).

Sixteen teachers within 14 classrooms participated in the study that included 12 lead teachers and four co-teachers. The teachers were all female (*M* = 33 years, *SD* = 9.88), 21% African

American, 72% Caucasian, and 7% mixed race, and had an average of 10 years experience in the field of early care and education. Eight teachers held a bachelor's degree, two had an associate's degree, five either earned a CDA, a North Carolina Early Childhood Credential, or attended some college, and one was a high school graduate. For additional classroom descriptives, see Table 1.

Table 1. Descriptives and correlations for classroom variables.

	M (*SD*)	1.	2.	3.	4.
1. Teacher–child ratio	1.7 (1.75)	–			
2. Emotional Support	5.19 (.79)	.45*	–		
3. Classroom organization	5.06 (.66)	.49*	.77*	–	
4. Instructional support	2.15 (.65)	.44*	.79*	.87*	–
5. Materials and activities	4.62 (.94)	.71*	.62*	.52*	.58*

*$p < .01$.

Sixty-six typically developing preschool children from 14 classrooms were recruited for participation. However, one child was not present on either day of data collection and was removed from the sample. One child was deleted from the sample as the parent failed to return the questionnaires, and one child refused to participate in saliva collection resulting in a final sample of 63 children (M = 53.92 months; SD = 5.39). Children (46% female) were 42% Caucasian, 34% African American, 3% Latino, and 21% mixed race, from a range of economic backgrounds (23% earned less than $24,000/year, 38% earned between $24,000 and $47,999/year, 15% earned between $48,000 and $83,999/year, and 24% earned over $84,000/year), and the majority (70%) of children lived in married households. Fifteen percent of families reported using child care subsidy and the average child began attending some form of child care at 17 months of age (SD = 17.85). Children spent an average of 38.75 h (SD = 7.84) in child care each week and were enrolled in the classroom for at least 2 months (M = 6.56 months, SD = 6.36) prior to data collection.

Procedures

Classroom assessments and child saliva collection occurred over 2 days in the classroom (see below for specific collection procedures). On the first day of data collection, two observers arrived at the beginning of the classroom's day, and one administered the CLASS Pre-K (Pianta, La Paro, & Hamre, 2008) and the other conducted a modified version of the ECERS-R (Harms et al., 1998). Further detail regarding the observational assessments is provided below. Saliva samples were collected at three time points during each day (shortly after arrival, following morning free play, and in the late afternoon). A parent or guardian (92% mothers, 5% fathers, and 3% other) completed a basic demographic questionnaire as well as a questionnaire summarizing child care experience and enrollment history. On each day of data collection, the parent/guardian completed a brief three-item questionnaire assessing degree to which the child's day was typical (quality of sleep, morning routine, and any medications). Teachers completed a basic demographic questionnaire. Additional questionnaires were collected from the teacher and parent but are not used in the current analyses.

Measures

Cortisol and alpha-amylase levels

Children's activity in the HPA axis and SNS were assessed by measuring salivary cortisol and sAA, respectively. Whole saliva was collected as sAA levels are influenced by placement of the collection device in the mouth (Harmon, Towe-Goodman, Fortunato, & Granger, 2008). Two Sorbettes® (hydrocellulose microsponges), a reliable collection material for collecting both cortisol and alpha-amylase (Granger et al., 2006 and Harmon et al., 2007) were used at each collection and remained under the child's tongue for at least 1 min. Children were not allowed to eat or drink anything 20 min prior to collection. Samples were collected after arrival at the center (*M* = 8:29 AM, range 8:11–8:49 AM), after free play (*M* = 10:48, range 9:58 AM–12:10 PM), and in the afternoon (*M* = 3:27 PM, range 1:53–4:41 PM). The extended range of time for the afternoon collection is due to two classrooms ending their day at 2:00 PM. In the remaining 12 classrooms, the afternoon collection occurred later in the afternoon (*M* = 4:13 PM, range 3:05–4:41). Means of cortisol and sAA levels at the each collection did not significantly differ by time of collection.

Child non-transformed cortisol or sAA levels for each day of data collection did not significantly differ by medication (p = .58–.91 for cortisol; .23–.97 for sAA) or an atypical morning or evening (p = .58–.93 for cortisol; .08–.72 for sAA). The samples were transported on ice and stored in a household refrigerator (Kirschbaum and Hellhammer, 1989 and Rohleder and Nater, 2009) for no more than 3 weeks and then shipped to Salimetrics (State College, PA) on dry ice where the samples were stored frozen at −80 °C until assayed. Samples were assayed for cortisol using an enzyme immunoassay performed in singleton on 25 μL of saliva. The test has a range of sensitivity from .007 to 3.0 μL/dL; when possible classrooms were assayed in the same batch. The mean intraassay coefficient of variation (CV) for cortisol was 3.71%. Saliva samples of 10 μL were assayed for sAA with a kinetic reaction assay. The mean CV for sAA was 4.67%; classrooms were assayed together when possible.

Classroom quality

The intentional measurement of process quality is an essential aspect of understanding possible relations to child activity in the HPA axis and SNS. The Classroom Assessment Scoring System Pre-K (CLASS Pre-K; Pianta, La Paro, et al., 2008) allows for the measurement of observed teacher–child interactions that best promote children's learning and development. The measure is divided into 10 dimensions (e.g., Positive Climate, Quality of Feedback) that form three domains of process quality: Emotional Support, Classroom Organization, and Instructional Support. These domains are informed by research and theory on effective teaching (Hamre, Pianta, Mashburn, & Downer, 2007). Classrooms are observed for 20 min and then all 10 dimensions are coded using a seven-point Likert scale, 1–2 (*Low*), 3–5 (*Mid*), 6–7 (*High*); at least 4 full cycles are completed. In previous research, the measure has demonstrated acceptable reliability (α = .85) and is only moderately correlated with the total ECERS-R (r = .52; La Paro, Pianta, & Stuhlman, 2004). All CLASS Pre-K observations were conducted by the first author, who is a

Master Trainer for the CLASS and completes yearly reliability assessments. In the current study, the three domains demonstrated acceptable reliability (α = .87–.89).

Based on a recent factor analysis (Cassidy, Hestenes, Hedge, Hestenes, & Mims, 2005), a modified version of the ECERS-R (Harms et al., 1998) was used to measure structural quality. The Materials and Activities factor (items 3, 5, 15, 19, 20, 22, 24, 25, and 26) evaluates the range of toys and types of activities available to the children. This factor demonstrates acceptable reliability and is highly correlated with the full ECERS-R score (r = .76; Cassidy, Hestenes, Hedge, et al., 2005). ECERS-R data were collected by one of two graduate students. The primary data collector previously served as an assessor for the North Carolina Rated License Assessment Project and trained the second graduate student to 93.75% agreement (based on scoring within one point of the primary data collector). In the current study, the Activities and Materials factors demonstrated acceptable reliability (α = .84) and is used as a proxy for structural quality.

Data analysis

The purpose of the study was to understand activity in the HPA axis (cortisol) and SNS (sAA) during typical child care days. On a few occasions, deviations from the typical classroom schedule were experienced (e.g., weather did not permit outside play so the daily schedule was altered, assistant teacher not present); only cortisol and sAA levels during typical child care days are included in the analyses. Untransformed cortisol levels were highly correlated (r = .61–.997) at each time point over the 2 day collection period; raw sAA levels were moderately correlated at each collection period (range, r = .51–.79). Thus, cortisol and sAA levels for each day were averaged at each collection time point creating an untransformed average sAA and average cortisol level for each of the three collection times (arrival, free play, and afternoon).

Using these averages for each collection time point, two transformations were executed for cortisol and sAA. First, the mean rate of diurnal change (RDC) illustrates change in levels of cortisol and sAA across the child care day. This affords the opportunity to understand if, for example, a child's cortisol increases from morning to afternoon, or if it decreases from morning to afternoon. To compute RDC, the two morning collections were averaged to obtain a more reliable estimate of morning cortisol levels. Then RCD ((average morning − afternoon)/average morning) was computed for sAA and cortisol. Second, area under the curve with respect to ground (AUC_g) was calculated following the equation recommended by Pruessner, Kirschbaum, Meinlschmid, and Hellhammer (2003). AUC_g creates a summary of individual output while accounting for time between collection points without losing numerous degrees of freedom. This variable accounts for total activity within the HPA axis or SNS across the day in child care. AUC_g was calculated for children who had data for at least three time points (N = 63) following recommendations of previous studies (e.g., Saridjan et al., 2010). RDC allows for change; AUC_g examines total output. For example, a child with low levels at all three collection times would have a low AUC_g, but a child with high levels at all three collection times would have a higher AUC_g. Non-transformed values were used to compute all variables; outliers (< or >3 *SD* from the mean) and distributions were examined for the final four variables (see Table 2). Cortisol AUC_g was positively skewed, and the values were transformed using natural log transformation (Tabachnick & Fidell, 2001).

Table 2. Descriptives for salivary cortisol (μ g/dL) and alpha-amylase (U/mL), non-transformed.

	M (*SD*)	1.	2.	3.	4.	5.	6.	7.	8.	9.
Cortisol										
1. Morning	.35 (.90)	–								
2. Free play	.25 (.71)	.92**	–							
3. Afternoon	.45 (2.37)	.87**	.96**	–						
4. AUC_g	110.36 (377.39)	.91**	.98**	.99**	–					
5. RDC	−.05 (.76)	−.24	−.30*	−.40**	−.37**	–				
Alpha-amylase										
6. Morning	30.09 (19.57)	.02	−.02	−.08	−.04	.25	–			
7. Free play	33.34 (23.45)	.00	−.03	−.07	−.05	.09	.73**	–		
8. Afternoon	41.26 (25.70)	−.11	−.10	−.07	−.08	.17	.73**	.57**	–	
9. AUC_g[a]	138.80 (97.83)	−.09	.08	.01	−.10	.13	.84**	.87**	.79**	–
10. RDC	−.35 (.52)	−.24	−.11	−.10	.04	−.09	.22	.34*	−.37*	.09

A Values presented have been divided by 100 for scaling.
*$p < .05$.
**$p < .01$.

Given the nested nature of the data (children nested within classrooms), HLM 6.06 was used in all analyses. Model building procedures, as outlined by Raudenbush and Bryk (2002), were followed in all analyses. Due to the small sample size and the overall aim of the study, the effect of the level-two classroom predictor was estimated separately for each indicator of process quality (Raudenbush & Bryk, 2002). Ordinary least squares regression models were also executed and findings were parallel to the nested models.

Results

First, descriptive results examining the levels and patterns of cortisol and sAA are presented. Then, the models predicting cortisol AUC_g and RDC for cortisol are presented. Models are presented separately for each indicator of classroom process quality (Emotional Support, Classroom Organization, and Instructional Support) to explore relations to cortisol AUC_g and RDC. Finally, parallel models are repeated predicting sAA.

Descriptives

As illustrated in Fig. 1, cortisol, on average declined from arrival to free play and increased from free play to the final afternoon collection; however, there was marked variability between children, especially in the afternoon. Overall, sAA increased (Fig. 2) across the day mirroring the typical diurnal pattern (Nater et al., 2007). Means and standard deviations for sAA and cortisol are listed in Table 2. The means for cortisol (Groeneveld et al., 2010) and sAA (Fortunato et al., 2008) are similar to those in previous studies.

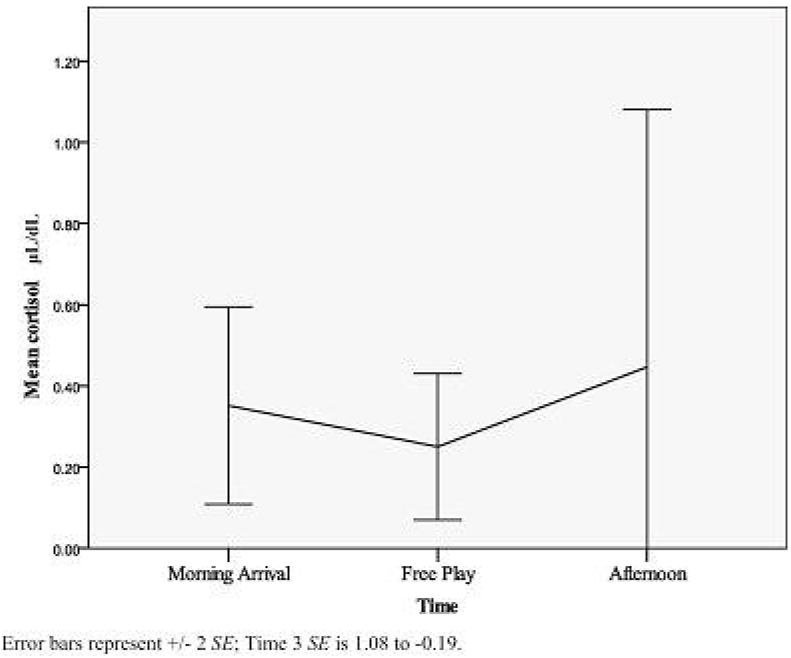

Fig. 1. Patterns of activity for cortisol, non-transformed. Error bars represent ±2 *SE*; time 3 *SE* is 1.08 to −.19.

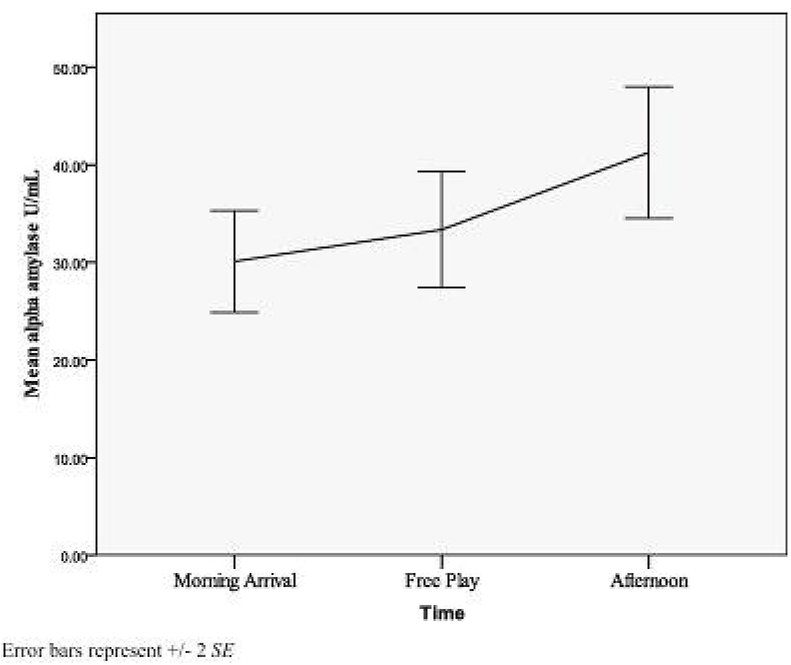

Fig. 2. Patterns of activity for alpha-amylase, non-transformed. Error bars represent ±2 *SE*.

Predictors of cortisol activity

Two unconditional models predicting (1) cortisol RDC and (2) cortisol AUC_g were executed. The unconditional models were built to examine the variability within and between classrooms. The model examining the variability in cortisol RDC was significant ($\mu_0 = .09$, $p < .05$), but the unconditional model for cortisol AUC_g indicated that there was not sufficient variability between classrooms and no further analyses were conducted (μ_0 = .07, p = .09). The intraclass correlation for the model predicting RDC was .14, indicating that 14% of the variability in RDC cortisol was due to differences between classrooms. Coefficients from a random intercept model and slopes model indicated that neither child experience in child care nor gender varied between classrooms; these effects were fixed in subsequent models. Finally, three separate models were built for each indicator of process quality (controlling for structural quality). Children in classrooms with higher levels of Emotional Support demonstrated a decline in cortisol throughout the day (see Table 3). This decline in cortisol is more consistent with the expected diurnal pattern of cortisol activity. Further, the inclusion of Emotional Support in the model reduced the intercept variance by 4%. The other indicators of process quality (i.e., Classroom Organization and Instructional Support) did not demonstrate a significant association to cortisol RDC.

Table 3. Models predicting activity in the stress response system from indicators of process quality.

Outcome	RDC cortisol		AUC_g sAA[a]	
	B	SEB	*B*	SEB
Emotional Support				
Fixed effects				
Intercept (γ_{00})	.23	.54	339.02**	66.12
Gender (γ_{10})	.16	.19	17.88	17.15
Age start (γ_{20})	.00	.01	−.83	.58
Activities and materials (γ_{01})	.22*	.10	−7.55	15.66
Emotional Support (γ_{02})	−.30**	.07	−34.25**	10.07
Random effects				
Level 1 variance (σ^2)	.13*	.35	529.34	23.01
Intercept1 (τ_{00})	.50	.73	8404.95	91.68
Classroom Organization				
Fixed effects				
Intercept (γ_{00})	−.21	.55	233.11*	92.50
Gender (γ_{10})	.19	.18	11.51	16.33
Age start (γ_{20})	.00	.00	−.42	.59
Activities and materials (γ_{01})	.11	.13	−28.15	16.33
Classroom Organization (γ_{02})	−.13	.13	5.31	19.25
Random effects				
Level 1 variance (σ^2)	.13*	.35	1348.77	36.725
Intercept1 (τ_{00})	.50	.70	8249.33	90.83
Instructional Support				
Fixed effects				

Outcome	RDC cortisol		AUC_g sAA[a]	
	B	SEB	*B*	SEB
Intercept (γ_3)	−.59	.57	250.33**	72.82
Gender (γ_{10})	.20	.18	11.35	16.05
Age start (γ_{20})	.00	.01	−.41	.59
Activities and materials (γ_{01})	.17	.15	−28.25	16.05
Instructional Support (γ_{02})	−.27	.16	5.16	21.92
Random effects				
Level 1 variance (σ^2)	.11*	.34	1396.47	37.37
Intercept1 (τ_{00})	.49	.70	8225.84	90.70

a *Note*: Alpha AUC_g term divided by 100 for scaling.
**p* < .05.
***p* < .01.

Predictors of alpha-amylase activity

The analysis plan for understanding the child and classroom influences on child sAA was equivalent to that of cortisol. First, two unconditional models were explored predicting (1) sAA RDC and (2) sAA AUC_g. Only the model predicting sAA AUC_g demonstrated significant variability (μ_0 = 1883.49, $p < .05$) and warranted further model building (sAA RDC, μ_0 = .00, $p > .50$). The model predicting sAA AUC_g indicated that 19% of the variability was between classrooms. A random intercept and slopes model indicated that child experience in child care or gender did not vary between classrooms and these variance coefficients were fixed in the full models. Models predicting sAA AUC_g were fit for each of the three CLASS domains, controlling for structural quality. Only a main effect of classroom Emotional Support emerged as a significant predictor of sAA AUC_g. The models predicting sAA AUC_g from Classroom Organization or Instructional support were not significant. Overall, these results indicate that children in classrooms with higher Emotional Support demonstrated lower sAA AUC_g while attending child care (Table 3), and explained 66% of variance available to explain in the total levels of sAA for children in child care.

Discussion

This study was the first to explore sAA activity within the child care context and the first to measure the relation between classroom interactions, measured by the CLASS, and activity in the SNS and HPA axis. Overall, children in this sample showed an increase in cortisol from morning arrival to early afternoon; this increase in cortisol during the child care day has been replicated in numerous other studies for children in child care (e.g., Sims et al., 2006 and Watamura et al., 2003). This finding is juxtaposed against the rise in sAA across the day, which is in tandem with the expected diurnal pattern found in adults (Wolf et al., 2008). The hierarchical linear models demonstrate that specific aspects of process quality, namely classroom Emotional Support, may be influencing activity in the SNS and HPA axis for preschool children.

Activity in the HPA axis and the SNS was measured via salivary cortisol and sAA, respectively, and the associations with classroom quality were explored for cortisol and sAA using two representations of activity. RDC reflects the change in cortisol or sAA levels from morning to afternoon, while AUC_g is used to understand total levels of cortisol or sAA over the course of the day. Both provide unique information to understand the functioning of the HPA axis and SNS. Our unconditional models indicated that only cortisol RDC and sAA AUC_g demonstrated significantly variability between classrooms. Regarding cortisol, the lack of variability in cortisol AUC_g was also evident in Groeneveld et al. (2010), as quality of care was predictive of cortisol RDC, but not cortisol AUC_g. In adults, activity in the SNS is often understood using AUC_g(Wingenfeld et al., 2010), but there is a dearth of evidence exploring AUC_g or RDC for sAA in early childhood. Results from this study suggest that sAA AUC_g demonstrates the ability to detect differences in children's total levels of sAA in child care. Additionally, it is possible that the rate of increase is the same for children in child care, but that levels of sAA are higher for some children than others.

Children did not differ in their cortisol RDC or sAA AUC_g based on previous experience in child care, which is in contrast to Ouellet-Morin et al.'s (2010) findings for toddlers in child care. This difference may be attributable to age differences between the two studies. This is the first study to examine child care quality with age of entry to further understand the influences on sAA levels, and no significant association was apparent. Child gender also failed to predict differences in child sAA AUC_g and child cortisol RCD, in accordance with Groeneveld et al. (2010). Finally, classroom activities and materials, the proxy for structural quality, was related to an increase in children's cortisol from morning to afternoon, after accounting for classroom Emotional Support. In classrooms where there is a variety of materials and planned activities, but an absence of a warm, supportive teacher with child-centered beliefs about play, children's cortisol increases throughout the day.

Classroom Emotional Support

In the current study, classroom Emotional Support, as measured by the CLASS PreK (Pianta, La Paro, et al., 2008), was the only indicator of process quality that was related to children's cortisol and sAA. Specifically, children in classrooms with higher levels of warm, sensitive interactions with the teacher and peers, more frequent child-initiated activities, and increased sensitivity for children's emotional expression and autonomy displayed a decline in cortisol from morning to the afternoon and lower total sAA over the day. This mirrors previous work highlighting the importance of sensitive caregiving on cortisol patterns for children in child care (Dettling et al., 2000 and Watamura et al., 2009) and that children who have secure, positive relationships demonstrate lower levels of sAA (Granger et al., 2007). The lower overall levels of sAA may be indicative of a child's ability to regulate behavior and stressors, which is supported by a positive classroom environment.

Children in classrooms with higher Emotional Support experience warm relationships with their teacher and peers, have teachers who value children's input and ideas, and due to the teacher's high level of sensitivity, children feel comfortable tackling difficult problems. Thus, children in these environments are better able to interact successfully with their friends and may use the teacher as a secure base. Higher child social competence may be a function of higher process quality, such as supportive teacher verbalizations (Kontos & Wilcox-Herzog, 1997), or individual child characteristics. Further, classroom Emotional Support may be more important for at-risk children (Hamre & Pianta, 2005) as a secure teacher‑child relationship moderates the maladaptive effect of an insecure mother‑child attachment on child outcomes (O'Connor & McCartney, 2006). Including cortisol and sAA in addition to observations and report of children's behaviors in the classroom will assist in the field's understanding of how the teacher‑child relationship and classroom Emotional Support is associated with child behaviors and development, or vice versa.

Classroom Organization

We expected Classroom Organization to be negatively associated with sAA and cortisol, hypothesizing that well-managed classrooms with clear behavioral expectations and a variety of materials would promote children's self-regulatory skills. However, these data did not support our hypotheses. Specifically, neither the change in cortisol levels from morning to afternoon nor total amount of sAA were related to how teachers manage behaviors or routines. This could be due to the fact that, on average, classrooms were fairly high in Classroom Organization and had a low standard deviation, limiting the ability to detect effects. Moreover, it could be that children's cortisol levels are influenced only in extremely chaotic environments. Classroom Organization was not related to child's sAA AUC_g even though previous research indicates that child vagal suppression, a proxy for activity in the SNS, during three emotion-eliciting tasks is related to maternal negative-management of misbehavior (Calkins et al., 1998). However, the current study was interested in typical activity, not reactivity, and measured SNS activity with sAA, not vagal suppression. These methodological differences may explain the incongruence in the findings. It is also possible that the CLASS is unable to detect individual differences in how a teacher manages misbehavior as it is a measure of the average classroom management of behaviors and routines.

Instructional Support

The hypotheses for relations between Instructional Support activity in the SNS or HPA axis were largely exploratory, but based on slim previous research, we hypothesized that higher Instructional Support would be related to lower sAA in and a null relation with higher cortisol. The results failed to support our hypothesis with sAA; Instructional Support did not show significant relation with classroom cortisol RDC or sAA AUC_g. It is possible that given the significant association between classroom Emotional Support and lower cortisol and sAA, children feel protected even in a cognitively challenging exchange with the teacher. Also, while the mean levels of Instructional Support were similar to previous studies (e.g., Mashburn et al., 2008), the mean is below the Instructional Support threshold reported by Burchinal, Vandergrift, Pianta, and Mashburn (2010) related to positive change for children's school readiness skills. Thus, our ability to detect an association between Instructional Support and children's activity in the SNS may also be limited. Further, as with Classroom Organization, these types of teacher‑child interactions and their relation to activity in the SNS and HPA axis may be better understood from an individual child's perspective rather than at a classroom level. Future studies using larger samples may better uncover if specific aspects of teacher questioning techniques and feedback to individual children influence stress responses.

Limitations and conclusions

The results of the present study illustrate the value and importance of examining process quality, namely Emotional Support, and its association with the HPA axis and SNS. However, a few limitations should be noted. First, the small sample size prohibits further exploration of multiple classroom quality variables within the same model, limiting the ability to identify how the indicators of process quality may work in an additive fashion to promote child functioning in the stress response system. The small sample size likely limited the variation between classrooms as well, which is why the unconditional models for sAA RDC and cortisol AUC_g did not have sufficient variability between classrooms to warrant further model building. Second, the data were collected from one southeastern county in the United States, limiting the generalizability of the findings. Thirdly, there is some evidence that suggests the interaction of activity in the HPA axis and SNS illustrates differences in child behavior (Allwood, Handwerger, Kivlighan, Granger, & Stroud, 2011). Our study was underpowered to investigate these relations, but the interaction between the two systems may illustrate additional differences in associations between classroom quality and child behavior.

Finally, the current study is limited as it did not compare cortisol or alpha-amylase levels at child care to those in the home setting. While the relations between cortisol and setting are consistent in the literature, little is known about normative patterns for children concerning sAA. Thus, future research should explore potential differences between home and child care for sAA, and our study suggests that the overall output, and not deviations in the diurnal rhythm, may be most illustrative. Nonetheless, a major strength of this study was the intentional measurement of three indicators of process quality with a high-stakes measurement tool which has been lacking in previous research (Geoffroy, Côté, Parent, & Séguin, 2006). The CLASS is used in many Quality Rating and Improvement Systems in the United States, and classroom scores from the CLASS are tied to Head Start funding (2011). The findings in this study illustrate additional associations between the CLASS and children's activity in the HPA axis and SNS. These results combined with other studies demonstrating associations between CLASS scores and child academic and behavioral outcomes (e.g., Burchinal et al., 2010) have implications for teacher training and professional development as well as quality enhancement and accountability efforts. Professional development opportunities focused on increasing process quality should continue as results of this study suggest that if teachers learn how to be more sensitive and supportive, children's activity in the HPA axis and SNS is supported. Successful professional development programs using the CLASS framework have made significant improvements to classroom quality post-intervention (Pianta, Mashburn, Downer, Hamre, & Justice, 2008), and future research should consider the intervention's impact on children's cortisol and sAA. The use of the randomized control trial framework would allow causal mechanisms to be identified between changes in classroom process quality and child activity in the HPA axis and SNS.
Quality enhancement and accountability efforts may use these findings as an incentive for improving classroom sensitivity and the emotional connection between the teacher and children and among the children. Increased efforts to help teachers create environments that support children's development and learning, while reducing experiences of stress, are highly worthwhile endeavors. These efforts may be directed at lowering the teacher–child ratio as classrooms with a lower teacher–child ratio have more opportunities to expose children to emotion language, assist in self-regulation techniques, and provide more Emotional Support (NICHDECCRN,

1996, NICHDECCRN, 2002 and Shim et al., 2004). Researchers should also focus on teacher's stress and cortisol patterns as there may be implications for teacher‑child relationships (Chang, 2009). Given replication of the results from this study linking process quality to the activity in the HPA axis and SNS and validation of causal mechanisms within a randomized control trial framework to improve classroom quality, quality enhancement efforts and evaluative mechanisms should reflect the importance of this domain of process quality.

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