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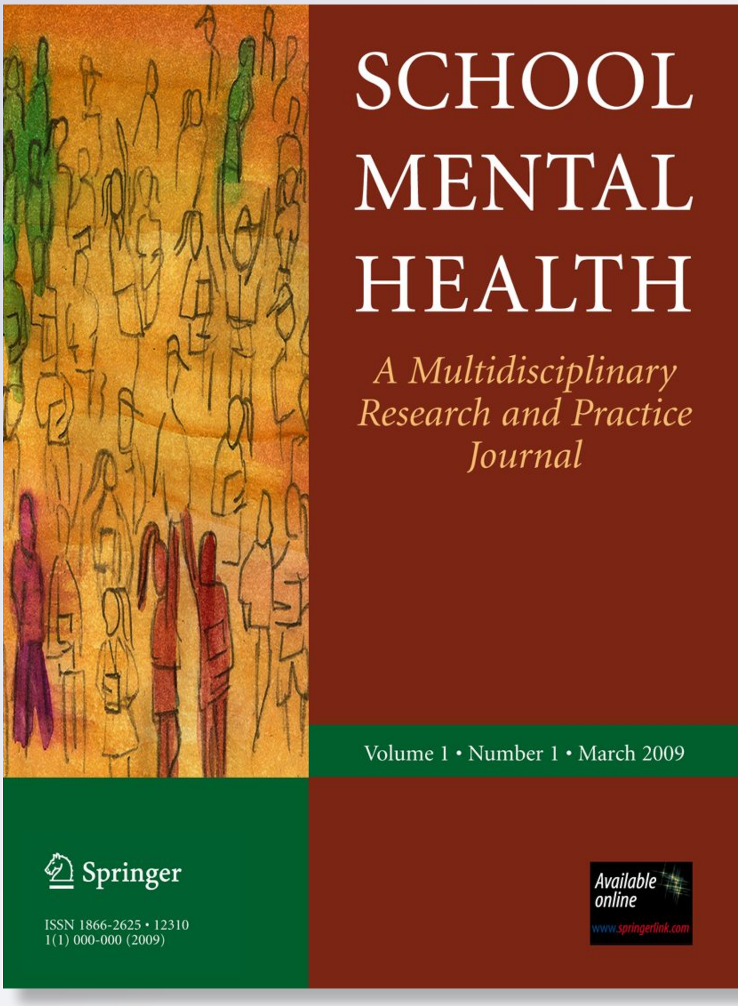

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ORIGINAL PAPER

Afternoon Cortisol in Elementary School Classrooms: Associations with Peer and Teacher Support and Child Behavior

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Abstract In response to a general paucity of research exploring biological markers of behavior in children in elementary school classrooms, this study investigated associations among cortisol, aggression, and prosocial (sharing and helping) behaviors, and peer and teacher supportive relationships in school-aged children. Salivary cortisol was obtained from children ($N = 89$, Mean age = 10.4 years, SD = .62) in classrooms three times a day (9 am, 12 pm, and 3 pm) across four consecutive days. Multiple informants (i.e., peers and teachers) completed questionnaires on children's social behavior, peer acceptance (peers only), and student–teacher closeness (teachers only). The results indicated a unique mediating influence of peer acceptance and teacher closeness on the association of afternoon cortisol with prosocial and specific subtypes of aggressive behavior (proactive, reactive, social). Findings highlight the importance of peer and teacher relationships in studies of biobehavioral development and demonstrate the utility of integrating neurobiological processes in classroom-based research.

Keywords Cortisol · Aggression · Prosocial behavior · Peer acceptance · Teacher closeness · Classroom

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Introduction

Children on average spend at least 15,000 h in classrooms from kindergarten to high school during their education (Hamre & Pianta, 2010). Therefore, research devoted to examining the ways in which children's social behaviors develop within a classroom context is of great importance to school-based mental health researchers (Milkie & Warner, 2011; Roeser, Eccles, & Sameroff, 2000). Research has identified two key factors significantly associated with childhood aggressive behaviors including internal neurobiological processes, such as activity of the hypothalamic–pituitary–adrenal (HPA) axis, and external contextual factors, such as supportive relationships with others (Alink et al., 2008; Ladd & Burgess, 2001).

The HPA axis is part of a larger neurobiological stress response system that functions to maintain a child's ability to respond to acute and prolonged changes in the environment (see Gunnar & Quevedo, 2007). The HPA axis is a primary system of interest in developmental literature due to its established links with behavior and ease of sampling cortisol in children's saliva (e.g., Alink et al., 2008). Cortisol exhibits a typical diurnal pattern of secretion, with levels rising within 20–45 min after waking, then gradually declining across the day (see Gunnar & Quevedo, 2007). Short-term cortisol release in response to stress serves an adaptive function; however, prolonged exposure to chronic stress in early childhood (i.e., maltreatment, peer rejection)

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may result in dysregulation of the HPA axis, possibly due to alteration of the sensitivity of receptors in the HPA axis that affect the production of cortisol (Tarullo & Gunnar, 2006). To date, numerous cross-sectional and longitudinal investigations have shown that dysregulation of basal patterns of daily HPA axis activity (i.e., basal cortisol levels obtained in a non-experimental setting) is associated with, and predictive of, emotional and behavioral problems in preschool and elementary school-age children (e.g., Alink et al., 2008; Gunnar & Vazquez, 2001; Murray-Close, Han, Cicchetti, Crick, & Rogosch, 2008). Of particular note, attenuated cortisol levels following vulnerabilities in early childhood (see Gunnar & Vazquez, 2001) are considered a risk marker for persistent antisocial behavior (Susman, 2006). However, the existing research examining the association between basal HPA axis activity and aggression in middle childhood is limited in three important ways. First, most investigations in this area have used a variety of different indicators of daily patterns in salivary cortisol (e.g., average morning or afternoon values, change across the day, total daily output) obtained in diverse settings (e.g., home, laboratory, day camp) under different conditions (e.g., resting or in response to an experimentally induced stressful task; see Jessop & Turner-Cobb, 2008). Few well-designed studies exist examining daily patterns of cortisol and behavioral functioning using multiple informants (peers and teachers) across multiple methods (salivary cortisol, questionnaires) in elementary school classrooms. Such distinctions not only permit a better understanding of the mechanisms underlying aggression, but may also lead to more effective classroom-based interventions tailored to each form of behavior.

Second, minimal research has examined daily patterns of cortisol in relation to prosocial (sharing, helping, cooperating) behavior or specific subtypes of aggressive behaviors, such as proactive (PA), reactive (RA), or social aggression (SA). Evidence shows that specific subtypes of aggressive behavior are differentially related to children's adaptation (see Vitaro, Brendgen, & Barker, 2006) and may be associated with distinct daily patterns in salivary cortisol. For example, Murray-Close et al. (2008) observed that lower morning cortisol and flatter diurnal slopes were associated with social/relational aggression (e.g., gossip, spread rumors), while higher morning and steeper decline in cortisol across the day was associated with physical aggression (e.g., kicks, hits) in non-maltreated children attending day camp. The authors speculate that the relationship between physical aggression and higher morning cortisol is due to a subset of children displaying a reactive form of physical aggression. However, Murray-Close et al. (2008) failed to measure reactive (e.g., defensive, hot-headed) and proactive (e.g., planned, goal-directed, cold-blooded) subtypes of physical aggression in everyday naturalistic settings, providing clear direction for the current study.

A third limitation is the lack of investigations examining the influential role of supportive relationships with peers and teachers on HPA axis functioning and aggression. Empirical evidence from independent cross-sectional studies suggests that dysregulation of the HPA axis stress response system is associated with victimization by peers (e.g., "said mean things, called you names," Vaillancourt et al., 2008) and social status in school-based hierarchies (West, Sweeting, Young, & Kelly, 2010). Separate but parallel lines of investigation demonstrate that peer acceptance and close, supportive relationships with teachers have an immediate and enduring impact on children's behavioral development (Ladd & Burgess, 2001; Wentzel, Barry, & Caldwell, 2004) and academic achievement (Hamre & Pianta, 2001). In contrast to the relatively few studies in elementary classrooms, there exists a burgeoning literature documenting the impact of child-care on biobehavioral development in the preschool years (see review and meta-analyses by Geoffroy, Côté, Parent, & Séguin, 2006; Vermeer & van IJzendoorn, 2006). Evidence from these studies indicates that some young children demonstrate an atypical secondary increase in average afternoon daily HPA axis activity that is associated with behavioral problems, and is buffered by the quality of relationships with child-care providers (e.g., Badanes, Dmitrieva, & Watamura, 2012; Gunnar, Kryzer, Van Ryzin, & Phillips, 2010). Together, this body of work provides insight into the type of neurobiological investigations needed in older children residing in elementary classrooms.

Healthy HPA axis functioning is thought to require the presence of strong diurnal or daily patterning in cortisol. Deviations from strong daily patterning of the HPA axis may provide valuable information about the role of the HPA axis in the development of children's behavior in middle childhood (see Alink et al., 2008; Gunnar & Quevedo, 2007; Gunnar & Vazquez, 2001; van Goozen, Fairchild, Snoek, & Harold, 2007). Accordingly, the current study examines the associations of daily patterns in HPA axis functioning (i.e., average morning, noon, afternoon, diurnal slope, total cortisol output) with specific behaviors and examines the mediating influence of children's supportive relationships with peers and teachers. Consistent with the methodology employed by investigations of preschool children in child-care settings (i.e., Badanes et al., 2012; Gunnar et al., 2010) and school-age children attending day camps (e.g., Alink, Cicchetti, Kim, & Rogosch, 2012; Murray-Close et al., 2008), the current study sampled cortisol from children (ages 9- to 12-years) residing in their classroom three times across the school day (9 am, 12 pm, and 3 pm) over four consecutive days. The average approximation of daily variations in cortisol is commonly used as an index of long-term patterning of HPA axis functioning (e.g., Shirtcliff & Essex, 2008). Within 2 weeks, multiple informants (i.e., peer, teacher) of behavior were employed to provide unique perspectives of the different forms (i.e., prosocial, SA) and functions (i.e., PA, RA) of behavior. Teachers and peers were asked to rate their experiences within their classroom near the end of the school year when students and teachers were familiar with one another. This methodology is consistent with published investigations in which peer, teacher, or parent judgments of child behavior are linked to average daily variations in HPA axis functioning (e.g., Alink et al., 2012; Murray-Close et al., 2008; Shirtcliff & Essex, 2008). Consistent with theories of arousal and empirical evidence, it was predicted that low cortisol, indicative of biological under-arousal and fearlessness, would be associated with higher ratings of aggression (Alink et al., 2008; Susman, 2006; van Goozen et al., 2007). However, the exact nature of the association between daily patterns of HPA axis activity to specific subtypes of aggression (i.e., RA, PA, and SA), peer acceptance and teacher closeness was exploratory. Associations between daily patterns of cortisol and prosocial behavior were exploratory and not hypothesis-driven. Research indicates that teachers are important to children for different reasons than peers (Darling, Hamilton, & Shaver, 2003); thus, distinct mediating effects of peer acceptance and teacher closeness were predicted.

Method

Participants

Participants were 89 children (49 girls, 40 boys) drawn from four regular education classrooms in the 4th and 5th grades of a public elementary school located in a large Western Canadian city ($M_{age} = 10.44$ years, SD = .63, range = 9.26–12.18). This research was part of a larger study designed to examine biological, social, and emotional correlates of behavioral development in school-age children in elementary school classroom contexts. Classroom teachers were all female (3 Caucasian, 1 Asian). The majority of children reported English (67 %) as their first language followed by Chinese (15 %). The remaining children (18 %) indicated a range of other language backgrounds (e.g., Spanish, Vietnamese, Tagalog) reflective of the cultural and ethnic diversity of the Canadian city in which this study took place. As to participating children's family composition, 62 % ($n = 55$) of children reported living in two parent homes (including both biological and step-parent families), 25 % ($n = 22$) reported living with a mother only, 1 % ($n = 1$) father only, 1 % ($n = 1$) grandmother only and the remainder, 11 % ($n = 10$) reported living in dual custody arrangements (i.e., ½ time mother, ½ father). Children who were identified by teachers as not competent in written and spoken English (1 %; $n = 1$), or learning difficulties (1 %; $n = 1$), or did not receive parental consent (16 %) were excluded from participation, resulting in 89 participants.

Procedure

Following approval by the University Ethics Board and local school board, and upon receiving parental consent and child assent, research assistants administered a series of questionnaires to the children during a regular 40-min class period. The instructions for each questionnaire and the questionnaire items were read aloud to take into consideration any reading difficulties. Student and teacher questionnaire ratings were obtained once. Within 2 weeks, two research assistants visited each individual classroom three times a day over four consecutive days (i.e., Tuesday through Friday) to collect saliva samples. Salivary cortisol samples were obtained from each child at the same time of day: morning, noon, and afternoon (i.e., 9 am, 12 pm, and 3 pm). To ensure that teachers would have adequate time to complete the behavior rating scales for each of their students, each teacher was provided with a half day substitute teacher. Data were gathered in the spring semester of the school year; thus, teachers and students had adequate time to know one another. One student was not present at time of administration of the questionnaires, but was available to complete all questionnaires at a later date, except for peer-nominated behavioral ratings.

Measures

The questionnaire package administered to the children in this study contained previously validated instruments assessing peer-nominated aggression and peer acceptance along with a series of questions on students' demographic information (i.e., gender, date of birth, first language learned, history of illness, and current mediations). Teacher ratings were used to assess student–teacher closeness as well as student prosocial and aggressive behaviors. For all questions, teachers and students were instructed to answer with reference to their experiences in the class they were in at the time of data collection. Data were collected near the end of the school year to obtain student and teacher ratings based on their experiences within their classroom since the beginning of the school year.

Teacher-Reported Social Behaviors

Teachers rated students' prosocial behavior and social aggression (National Longitudinal Survey of Children and

Youth (NLSCY), 2001) and proactive and reactive aggression (Crick & Dodge, 1996; Dodge & Coie, 1987). For each student, teachers rated 27 items on their feelings and beliefs about the student's behaviors on four subscales; prosocial behavior (10 items, e.g., "will try to help someone who has been hurt;" $\alpha = .94$); proactive aggression (9 items, e.g., "plays mean tricks;" $\alpha = .84$); reactive aggression (3 items, "e.g., when teased or threatened, he/she gets angry easily and strikes back;" $\alpha = .86$); and social aggression (5 items, e.g., "when mad at someone, tries to get others to dislike that person;" $\alpha = .85$). Teachers rated how applicable each statement was to the behavior of the particular student on a 3-point scale: *Never or not true* (1), *Sometimes or somewhat true* (2), *Often or very true* (3). Items were averaged to yield a composite score with higher scores representing greater levels of prosocial and aggressive behaviors.

Peer Nominations of Social Behavior

Following the procedures outlined by Parkhurst and Asher (1992), peer nominations were used to obtain independent assessments of four types of social behavior assessed with 2 items each: prosocial behavior (e.g., "shares and cooperates;" $\alpha = .89$; Wentzel & Erdley, 1993); reactive aggression (e.g., "gets mad at kids who hurt them by accident;" $\alpha = .89$); proactive aggression (e.g., "gets other students to gang up on a classmate;" $\alpha = .90$); and social aggression (e.g., "talks behind other people's back;" $\alpha = .70$; Dodge & Coie, 1987; Crick & Dodge, 1996). Below each written question, students were given a list of all classmates who were participating in the study. For each question, students were asked to circle the names of any of their classmates who fit the behavioral description. Students could circle as many or as few names as they wanted. For each question, the percentage of nominations each student received was computed by dividing the number of nominations received by the total number of participating students in the classroom. Behavioral ratings were standardized within classroom. Composite scores were computed by averaging the nominations given by boys and girls. The distribution of students' composite scores was normalized using an arcsine square-root transformation. Higher scores represent greater levels of the behavior.

Peer Nominations of Peer Acceptance

Children's level of acceptance by peers (1 item, i.e., "would like to be in school activities with") was assessed using the same nomination sociometric procedure used for obtaining measures of behaviors (e.g., Oberle, Schonert-Reichl, & Thomson, 2010).

Teacher Ratings of Student–Teacher Closeness

Teachers' perceptions of the quality of relationship with their students were assessed using the closeness subscale of the Student–Teacher Relationship Scale (STRS; Pianta, 2001). The STRS is a self-report measure of teacher-perceived relationships with individual students. The closeness subscale is comprised of 11 items that measure warmth and open communication in the teacher–student relationship (i.e., "I share an affectionate, warm relationship with this child"). Teachers rated how applicable each statement was to their relationship with a particular student. Responses ranged from (1) definitely does not apply to (5) definitely applies. Items were averaged to yield a mean score, where higher scores represent greater levels of student–teacher closeness.

Salivary Cortisol

Salivary cortisol was collected using a plastic Salivette device with a sterile cotton swab held inside a plastic tube (Salimetrics^{TM}). To capture average daily patterns of cortisol in a classroom setting (i.e., average morning, noon or afternoon values, change across the day or diurnal slope, total daily output), salivary cortisol samples were obtained from children while residing in their classrooms three times a day (9 am, 12 pm, and 3 pm) over 4 days (Tuesday–Friday) for a total of 12 samples per subject. Prior to each morning saliva collection (9 am), children recorded what time they woke up that day. Children also recorded recent food intake prior to every sample collection. Salivettes were immediately centrifuged (within 3 h of collection) at 3,000 rpm for 5 min resulting in a clear supernatant of low viscosity. Salivary cortisol levels were measured using a commercial immunoassay with chemiluminescence detection (CLIA; IBL-Hamburg, Hamburg, Germany). The lower concentration limit of this assay was 0.44 nmol/l; intra- and inter-assay coefficients of variance were less than 8 %. Any sample over 50 nmol/l was repeated.

This method of salivary cortisol collection is consistent with numerous published investigations examining associations of average daily patterns of HPA axis functioning with behavior in preschool children in child-care settings (e.g., Gunnar et al., 2010) and school-age children in home or day camps settings (e.g., Alink et al., 2012; Murray-Close et al., 2008; Smider et al., 2002). To illustrate, Murray-Close et al. (2008) examined the association between daily cortisol patterns and aggression using the average of three cortisol samples (morning, pre-lunch, and afternoon) over 5 days obtained from school-age children (6–12 years) while attending a weeklong day camp.

Results

Data Screening and Reduction

The data were analyzed by first screening for accuracy of entry, patterns of missing data, and assumptions of multivariate analysis. One case was identified as a true multivariate outlier using Mahalanobis' distance (Rousseeuw & van Zomeren, 1990). This case scored maximum values on aggressive behaviors and minimum values on prosocial behaviors, but did not significantly differ on any of the demographic variables. All subsequent analyses involving peer-nominated behavioral variables were therefore examined with, and without, the multivariate outlier. A logical decision was made to drop the case from the analyses when found to significantly influence the results (Tabachnick & Fidell, 2001). The cortisol data were screened to ensure each case had complete data for cortisol samples and 'time since wakening' at all time points. Outlier values were "winsorized" to within 3 SD of the mean and transformed to the natural logarithm scale (e.g., El-Sheik, Erath, Buckhalt, Granger, & Mize, 2008). To obtain various indicators of daily patterns of cortisol, cortisol values from across the four testing days were calculated to produce average morning (9 am), noon (12 pm), and afternoon (3 pm) basal values. Diurnal slope was calculated by subtracting the value at the end of the sampling time frame (e.g., 3 pm) from the first sample (e.g., 9 am) and dividing it by the number of hours between the two samples. The average slope across 4 days, controlling for time since awakening was calculated (see Miller, Chen, & Zhou, 2007). The area under the curve (AUC) is a simple formula for the computation of two parameters that reveal different information embodied in repeated measures of cortisol data (see Fekedulegn et al., 2007; Pruessner, Kirschbaum, Meinischmid, & Hellhammer, 2003). The total cortisol concentration secreted between 9 am and 3 pm (i.e., AUC with respect to ground or AUCg) was calculated. The rate of change of cortisol between 9 am and 3 pm taking into consideration baseline values (i.e., AUC with respect to change or AUCc) was also calculated.

Descriptive Statistics

Means, standard deviations, and the possible range of scores are reported in Table 1. To facilitate inter-study comparisons, reported mean cortisol values are non-transformed

Table 1 Descriptive statistics for the study measures

Variable name	*M*	SD	Skew	Kurtosis	α	*N*	Min. to Max.
Age (years)	10.43	.62	.44	.54	N/A	89	9.26 to 12.18
Peer-nominated behaviors							
Prosocial	.59	.18	−.13	−.74	.89	88	.18 to .96
Reactive	.28	.21	.73	.46	.89	88	.0 to .94
Proactive	.19	.18	.63	−.31	.90	88	.0 to .73
Social	.32	.17	−.21	−.45	.70	88	.0 to .64
Teacher-reported behaviors							
Prosocial	2.16	.54	−.29	−.99	.94	89	1 to 3
Reactive	1.42	.58	1.24	.32	.86	89	1 to 3
Proactive	1.15	.24	2.37	5.79	.84	89	1 to 2.1
Social	1.35	.39	1.10	.87	.85	89	1 to 2.6
Supportive relationships							
Teacher closeness	3.48	.81	−.18	−.79	.84	89	1.45 to 4.82
Peer acceptance	.27	.12	−.03	−.14	N/A	88	.0 to .54
Cortisol[a]							
Morning (9 am)	.25	.11	1.27	1.97	.86	89	.07 to .64
Noon (12 pm)	.17	.05	.60	.83	.74	89	.07 to .33
Afternoon (3 pm)	.16	.05	.71	.53	.64	89	.07 to .33
Diurnal slope[b]	−.04	.03	−.33	1.46	–	89	−.13 to .07
AUCg	−4.74	.63	−.28	.29	–	89	−6.42 to −3.21
AUCc	−.80	.89	−.49	.27	–	89	−3.15 to −.80
Average time of awakening	7:30 am	30 min	−.29	−.34	–	89	6 am to 8:30 am

[a] Cortisol values indicate mean of 4 days in μg/dl prior to logarithmic transformation to correct skew

[b] Slope values represent cortisol values following logarithmic transformation

values measured in μg/dl. A series of one-way analyses of variance (ANOVAs) indicated no significant effect of gender, food intake, medical condition, or medications on cortisol levels, although older children had significantly higher afternoon cortisol, $F(1, 86) = 6.62$, $p = .01$. Age and gender significantly influenced the majority of behavioral and relational variables and were included as covariates in all regression analyses. Pearson product-moment correlations were calculated among all variables of interest and are presented in Table 2. These preliminary associations were consistent with theoretically expected relationships and were explored further in a series of regression analyses.

Main Effects: Cortisol and Behavior

The first objective of this study was to examine associations among various indicators of basal salivary cortisol obtained in a classroom context to peer- and teacher-reported social behaviors. A series of independent hierarchical linear regressions were conducted with each of the four behaviors as the dependent variable (i.e., reactive, proactive, social aggression, and prosocial behavior) and each index of cortisol as the independent variable (i.e., morning, noon, afternoon, diurnal slope, AUCg, AUCc). Age and gender (coded as 1 = boy and 2 = girl) were entered in the first step as covariates, followed by cortisol in the second step. To control for the intercorrelation often observed between RA and PA, the alternate subtype of aggressive function was entered in the third step for peer- and teacher-reported PA and RA (Scarpa, Haden, & Tanaka, 2010). Results indicated that afternoon cortisol was significantly and positively associated with peer- ($\beta = .20$, $p = .04$) and teacher-reported ($\beta = .43$, $p = < .001$) prosocial behaviors, and inversely associated with peer-nominated SA ($\beta = -.33$, $p = .003$), and teacher-reported RA ($\beta = -.19$, $p = .04$; see Table 3). No association was observed between cortisol and PA. Steeper cortisol slope (i.e., higher morning and lower afternoon cortisol) was significantly associated with peer-nominated SA ($\beta = -.22$, $p = .047$; see Table 3). Results revealed no significant associations of other indicators of cortisol (i.e., morning, noon, AUCg, and AUCc) with behavior. Only afternoon cortisol was significantly and positively associated with the putative mediators, peer acceptance and teacher closeness. Therefore, all further analyses are discussed with reference to afternoon cortisol as the independent variable. To verify that the mediators were associated with the behavioral outcomes, a series of independent hierarchical linear regressions were run, controlling for age, gender, and afternoon cortisol. Results indicated that peer acceptance and teacher closeness were significantly and positively associated with peer- and teacher-reported prosocial behavior and inversely associated with specific subtypes of aggressive behaviors (see Table 4).

Mediation Analyses: Influence of Peer Acceptance and Teacher Closeness

We predicted a unique mediating influence of peer acceptance and teacher closeness on the association between cortisol and behavior. To investigate this hypothesis, the method of bootstrapping of multiple indirect effects was employed to examine the unique influence of one putative mediator (i.e., peer acceptance), while controlling for the presence of the other mediator (i.e., teacher closeness; Preacher & Hayes, 2004, 2008). Separate mediation models were specified and tested for each of the peer- and teacher-reported behaviors. For all analyses, age and gender were entered as covariates. The results indicated that teacher closeness and peer acceptance were unique mediators of prosocial behavior, that is, they contributed to the indirect effect above and beyond the presence of each other, age, and gender (see Table 5). The results suggest that higher afternoon cortisol is associated with greater ratings of peer acceptance and, separately, teacher closeness, that in turn are associated with higher levels of teacher- and peer-rated prosocial behaviors. For aggressive behaviors, the opposite is true, such that lower ratings of peer acceptance mediate the association between lower afternoon cortisol and higher levels of teacher-rated RA and, separately, teacher-rated SA. Both teacher closeness and peer acceptance were shown to uniquely mediate the association between afternoon cortisol and teacher-rated PA. Follow-up analyses standardizing teacher reports of behavior to each classroom, and testing for possible influence of different classrooms (dummy variable for each of the 4 classrooms as a covariate), ESL, or hours since time of awakening did not alter the significance of any results.

Exploratory Analyses

Given the cross-sectional nature of the study and lack of temporal precedence of variables for mediation analyses, the alternative model with behavior as the independent variable and cortisol as the dependent variable was addressed in a series of exploratory analyses. Findings revealed a small but significant influence of teacher closeness on the association between peer-nominated prosocial behavior and afternoon cortisol ($\alpha\beta = .08$, CI = .02, .18), and teacher-rated PA and afternoon cortisol ($\alpha\beta = .11$, CI = −.32, −.01). Peer acceptance was shown to influence the association between teacher-rated SA and afternoon cortisol ($\alpha\beta = -.02$, CI = −.07, −.002). These preliminary findings highlight the need to include social-

Table 2 Pearson correlations among study variables

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1. Sex	–																
2. Age	.08	–															
Peer-nominated behaviors																	
3. Prosocial	.43*	.31*	–														
4. Proactive	−.29*	−.20	−.47*	–													
5. Reactive	−.22*	−.37*	−.59*	.69*	–												
6. Social	.11	−.13	−.27*	.66*	.59*	–											
Teacher-reported behaviors																	
7. Prosocial	.33*	.30*	.69*	−.46	−.46*	−.29*	–										
8. Proactive	−.08	.11	−.52*	.62*	.49*	.47*	−.54*	–									
9. Reactive	−.21*	−.11	−.54*	.66*	.66*	.40*	−.46*	.60*	–								
10. Social	.07	.15	−.32*	.46*	.42*	.42*	−.22*	.59*	.50*	–							
Supportive relationships																	
11. Peer acceptance	−.06	.32*	.64*	−.26*	−.41*	−.32*	.45*	−.26*	−.43*	−.19	–						
12. Teacher closeness	.36*	.27*	.50*	−.32*	−.21*	−.12	.70*	−.35*	−.16	.04	.26*	–					
Cortisol																	
13. Morning	−.07	.07	−.04	.06	.08	−.02	.07	.12	.03	−.04	.05	.08	–				
14. Noon	.14	.18	.03	−.13	−.12	−.14	.09	.06	−.13	.03	−.02	.05	.23*	–			
15. Afternoon	.16	.28*	.33*	−.38*	−.38*	−.31*	.51*	−.09	−.33*	−.04	.34*	.41*	.25*	.36*	–		
16. Slope	.22*	.09	.26*	−.29*	−.29*	−.19	.27*	−.19	−.24*	−.01	.15	.22*	−.70*	.09	.44*	–	
17. AUCg	.07	.18	.05	−.06	−.06	−.13	.17	.05	−.08	.03	.13	.16	.71*	.78*	.53*	−.26*	–
18. AUCc	.17	.02	.10	−.18	−.12	−.08	.04	−.12	−.13	.02	−.06	.01	−.75*	.23*	.08	.83*	−.30*

$N = 88$ for peer-nominated behaviors; $N = 89$ for teacher-rated behaviors

* $p < .05$

Table 3 Regression of supportive relationships and social behaviors on cortisol

Dependent variables	Afternoon cortisol						Diurnal slope cortisol					
	B	SEB	*β*	*F*(*df*)	ΔR^2	95 % CI	*B*	SEB	*β*	*F*(*df*)	ΔR^2	95 % CI
Peer-nominated behaviors												
Prosocial	.28	.13	.20*	4.40 (1, 84)	.04	.01, .54	.89	.53	.16	2.84 (1, 84)	.02	−.16, 1.93
Reactive	−.13	.13	−.08	1.02 (1, 83)	.01	−.39, .13	−.57	.50	−.09	1.30 (1, 83)	.01	−1.57, .43
Proactive	−.19	.11	−.14	2.73 (1, 83)	.02	−.42, .04	−.41	.45	−.07	.82 (1, 83)	.01	−1.32, .49
Social	−.42	.14	−.33*	9.17 (1, 84)	.10	−.70, −.15	−1.15	.57	−.22*	4.07 (1, 84)	.05	−2.28, −.02
Teacher-reported behaviors												
Prosocial	1.79	.38	.43*	21.64 (1, 85)	.17	1.02, 2.56	3.16	1.67	.19	3.59 (1, 85)	.03	−.16, 6.48
Reactive	−.83	.40	−.19*	4.23 (1, 84)	.03	2.86, 5.5	−1.14	1.65	−.06	.48 (1, 84)	.004	−4.42, 2.1
Proactive	−.02	.06	−.04	.13 (1, 84)	.001	−.13, .09	−.30	.21	−.13	2.0 (1, 84)	.02	−.73, .12
Social	−.29	.34	−.09	.69 (1, 85)	.01	−.96, .41	−.38	1.36	−.03	.08 (1, 85)	.001	−3.08, 2.33
Teacher closeness	1.59	.53	.30*	10.65 (1, 85)	.08	.55, 2.64	3.33	2.52	.13	1.76 (1, 85)	.01	−1.67, 8.33
Peer acceptance	.27	.10	.29*	7.82 (1, 84)	.08	.08, .46	.53	.39	.14	1.85 (1, 84)	.02	−.25, 1.31

$N = 88$ for peer-nominated behaviors; $N = 89$ for teacher-rated behaviors

CI confidence intervals, lower and upper limits

* $p < .05$

Table 4 Regression of social behavior on teacher closeness and peer acceptance

	Teacher closeness						Peer acceptance					
	B	SEB	*β*	*F*(*df*)	ΔR^2	95 % CI	*B*	SEB	*β*	*F*(*df*)	ΔR^2	95 % CI
Peer-nominated behaviors												
Prosocial	.08	.03	.30*	9.28 (1, 83)	.08	.03, .13	.96	.11	.64*	76.68 (1, 83)	.39	.74, 1.18
Reactive[a]	.01	.03	.04	.17 (1, 82)	.00	−.05, .07	−.33	.17	−.20*	3.88 (1, 82)	.04	−.66, −.00
Proactive	−.02	.03	−.07	.38 (1, 83)	.00	−.08, .04	−.25	.16	−.17	2.39 (1, 83)	.02	−.56, .07
Social[a]	.001	.03	.004	.001 (1, 82)	.00	−.06, .06	−.28	.16	−.20	2.99 (1, 82)	.03	−.61, .04
Teacher-reported behaviors												
Prosocial	.45	.06	.58*	51.70 (1, 84)	.29	.33, .58	1.49	.42	.33*	12.96 (1, 83)	.11	.67, 2.32
Reactive	.04	.10	.04	.13 (1, 84)	.00	−.16, .23	−1.97	.51	−.40*	15.06 (1, 83)	.13	−2.99, −.96
Proactive	−.09	.04	−.26*	4.87 (1, 84)	.04	−.17, −.01	−.17	.07	−.27*	5.56 (1, 84)	.06	−.32, −.03
Social	.004	.07	.01	.004 (1, 84)	.00	−.14, .15	−.81	.39	−.25*	4.38 (1, 83)	.04	−1.58, −.04

$N = 88$ for peer-nominated behaviors except [a]reactive and social aggression where $N = 87$; $N = 89$ for teacher-rated behaviors

CI confidence intervals, lower and upper limits

* $p \leq .05$

relational variables in studies of cortisol and behavior, irrespective of the directionality of the model.

Discussion

This study examined the association of basal cortisol with peer- and teacher-reports of specific subtypes of aggression and prosocial behaviors in elementary classroom contexts. Also, it investigated the mediating influence of peer and teacher supportive relationships. This is one of the first studies to demonstrate a consistent association of afternoon cortisol with various markers of social functioning (aggression, prosocial behavior, supportive relationships) rated by multiple informants (self, peers and teachers) across multiple methods (salivary cortisol, questionnaires) in an elementary school classroom context. Three key findings emerged from this study. First, our results are consistent with previous research observing an inverse association between basal cortisol and teacher reports of externalizing behavior problems in children (e.g., Alink et al., 2008; Oosterlaan, Geurts, Knol, & Sergeant, 2005). These findings are also consistent with theoretical (i.e., attenuation hypothesis) and empirical evidence in clinical

Table 5 Multiple mediation of peer acceptance and teacher closeness

	Peer acceptance		Teacher closeness	
	Parameter estimate	95 % BC CI	Parameter estimate	95 % BC CI
Peer-nominated behaviors				
Prosocial	.24*	.05, .45	.09*	.03, .20
Reactive[a]	−.09	−.23, .003	.02	−.09, .15
Proactive	.06	−.2., .011	−.04	−.16, .04
Social[a]	−.08	−.20, .01	.01	−.10, .08
Teacher-reported behaviors				
Prosocial	.31*	.06, .65	.67*	.28, 1.18
Reactive	−.58*	−1.24, −.16	.15	−.19, .63
Proactive	−.04*	−.10, −.01	−.05*	−.13, −.01
Social	−.22*	−.59, −.02	.04	−.29, .30

$N = 88$ for peer-nominated behaviors except [a]reactive and social aggression where $N = 87$; $N = 89$ for teacher-rated behaviors

BC CI = lower, upper limit bias corrected confidence intervals based on 1,000 bootstrap samples

* $p < .05$ (significant indirect effect)

samples of children suggesting that low basal HPA axis activity is a risk factor for persistent antisocial behavior (McBurnett et al., 2000; Popma et al., 2007; Susman 2006). There are two main theoretical interpretations of reduced arousal in antisocial, aggressive children. The stimulation-seeking theory suggests that under-aroused children may seek stimulation to increase their arousal levels to a more optimal state (van Goozen et al., 2007). The fearlessness theory suggests that under-arousal is indicative of fearlessness and a lack of inhibition of being involved in aggressive behaviors (Raine, 2002). In both instances, physiologically under-aroused children appear disinhibited or fearless, and reward/goal achievement takes on more salience (e.g., Scarpa & Raine, 2004). It is recommended that future investigations in clinical populations consider whether comorbid disorders are present because children diagnosed with attention-deficit hyperactivity disorder (ADHD) and comorbid disruptive behavior disorders may exhibit lower cortisol levels upon awakening compared with children diagnosed with ADHD alone or ADHD plus conduct disorder (see Fairchild, 2012; Freitag et al., 2009).

A second key finding was the association of basal cortisol with specific types of aggressive and prosocial behavior. The results from the current study expand on recent work in the field to suggest that afternoon cortisol levels obtained in a classroom context are inversely associated with distinct subtypes of aggression, specifically peer-nominated SA and teacher-reported RA (e.g., Murray-Close et al., 2008). Furthermore, our results emphasize the need to distinguish between reactive and proactive functions of physical aggression in behavioral investigations (see Hubbard, McAuliffe, Morrow, & Romano, 2010). Another key finding was the observed positive association of afternoon cortisol with both peer- and teacher-reported prosocial behaviors. These data may be juxtaposed with results from a recent longitudinal study showing an inverse association between morning (9 am) cortisol levels and prosocial skills 1 year later in school-age children with a history of maltreatment (Alink et al., 2012). Although the determinants of morning and afternoon cortisol are different from each other, both sets of findings suggest that children's basal cortisol levels may play a role in the pathways to positive social and coping skills that facilitate supportive relationships with peers and teachers. Our results contribute to the growing interest in the biological processes underlying social competence in childhood (e.g., Gunnar et al., 2010; Obradovic, Bush, Stamperdahl, Adler, & Boyce, 2010).

Third, our data indicate that peer acceptance and teacher closeness uniquely mediate the association between afternoon cortisol and teacher- and peer-reported prosocial behaviors, and teacher-reported PA. Additionally, lower levels of peer acceptance mediated the association between low afternoon cortisol and higher teacher-rated RA and SA. This research was based on the theoretical model that HPA axis dysregulation in school-age children is predictive of later social functioning (e.g., Alink et al., 2012; Murray-Close et al., 2008). A possible explanation for our findings is found in empirical evidence and theories of arousal suggesting that low cortisol may reflect decreased fear and physiological under-arousal and a propensity for stimulation seeking to increase arousal to more comfortable levels (Raine, 2002; Susman, 2006; van Goozen et al., 2007). Thus, children displaying low afternoon cortisol, or lower arousal, may exhibit a diminished capacity to cope with stress or challenge, placing them at risk for lower quality relationships and an increased propensity for aggression (Locke, Davidson, Kalin, & Goldsmith, 2009). With respect to specific subtypes of aggression, low arousal may promote beliefs that peer's intentions are hostile as well as promote situations in which a child reacts aggressively (RA) to peer provocation (e.g., Crick & Dodge, 1996; Dodge & Coie, 1987). Low arousal combined with low peer acceptance may increase the propensity to engage in SA and PA to regain status and popularity (Murray-Close et al., 2008; Neal, 2010) or social dominance, respectively (Dodge & Coie, 1987; Hubbard et al., 2010). Future longitudinal investigations that assess directionality among biological, behavioral, and social-relational variables are recommended.

Results from the current study have a number of implications for classroom-based research. The mediating influence of peer acceptance suggests that children characterized as RA may benefit from classroom-based behavioral interventions designed to promote peer acceptance (e.g., Witvliet, van Lier, Cuijpers, & Koot, 2009). Whereas the unique mediating influence of both peers and teachers on the association of cortisol with PA and prosocial behavior suggests that peer acceptance and teacher closeness may represent unique pathways for intervention. In the absence of peer acceptance, supportive relationships with teachers may buffer children at risk for engaging in PA (e.g., Ladd & Burgess, 2001). Importantly, these results complement extensive research in child-care settings where it is shown that high-quality child-care environments, characterized with teacher support, buffer some preschool children from a secondary increase in afternoon cortisol (e.g., Badanes et al., 2012). Our data provide clear direction for future investigations examining the interplay among HPA axis, behavior, and social relationships in elementary classroom settings. Whether changes in afternoon basal cortisol levels near the end of a school year reflect a normative adaptive response to classroom-based social interactions in elementary classroom settings is an important question that remains to be addressed.

A number of limitations should be noted including the small sample size and cross-sectional nature of the study. The current study attempted to address some of these issues via exploratory analyses and use of bootstrapping of indirect effects to limit possible Type I error rate due to multiple comparisons. Nevertheless, it should be emphasized that these findings are exploratory in nature given the small sample size, large number of analyses performed and lack of consistency in findings. Longitudinal research is needed given the recent evidence suggesting that variations in basal cortisol may not adequately predict concurrent behavioral problems but could identify those children with a poor prognosis once disruptive behaviors are present (e.g., Haltigan, Roisman, Susman, Barnett-Walker, & Monahan, 2011). A potential limitation was the lack of evidence to suggest that other indicators of basal cortisol, such as morning levels, diurnal slope or total cortisol output, were associated with aggression (e.g., Alink et al., 2008; Gunnar & Vazquez, 2001; Murray-Close et al., 2008). It is possible that more robust indicators of morning cortisol, such as levels upon awakening, are required to accurately index diurnal slope or total cortisol output (see Chida & Steptoe, 2009 for a review).

Future classroom-based investigations would benefit from the addition of at-home measures of cortisol that capture cortisol levels upon awakening (e.g., Shirtcliff & Essex, 2008). Cortisol is only one marker among numerous possible physiological markers of biobehavioral development. Future studies that capture multiple aspects of physiological stress systems (e.g., sympathetic nervous system) and emotion processes such as anger regulation are recommended (see Bauer, Quas, & Boyce, 2002; Hubbard et al., 2002). It is possible that a third unknown variable related to both cortisol levels and behavior is driving the current results. Longitudinal research exploring biological and emotional correlates of behavioral adaptation across the academic year may be of particular interest given the potential stress of peer social hierarchies, academic demands, and familial expectations associated with school (e.g., West et al., 2010). Prospective studies that extend over longer periods of time will permit assessment of whether daily patterns in HPA axis functioning are responsive to change and if so which factors (e.g., improved or worse classroom supportive environment) bring about change (see Bevans, Cerbone, & Overstreet, 2008). Our findings highlight the benefit of using multiple informants of behavior; however, it is possible that our questionnaire items were conceptually distinct for teachers compared with peers. Children may be able to accurately assess subtypes of aggression in their peers, but utilize different criteria for assessing these behaviors that were not captured by our measure. Recently, there has been a call for more specific measures of aggression that tap into children's behavioral motivations rather than subjective expressions of anger (Hubbard et al., 2010). Our study was limited to peer and teacher ratings of behavior at the end of the academic year. Observational measures of behavior may offer greater insight into the dynamic associations of daily patterns of cortisol with prosocial and aggressive behaviors in classroom contexts (see Alink et al., 2012; Gunnar et al., 2010). Another limitation may be that some of the older children were entering puberty, which may have influenced their daily patterns in cortisol (see Gunnar, Wewerka, Frenn, Long, & Griggs, 2009). The current study was limited by a lack of measurement of pubertal timing; however, children's age was included as a covariate for all analyses. A final limitation to the current study was the lack of examination of gender effects. This decision was based on a number of factors, the most salient being the small sample size. Longitudinal evidence suggests that children may follow different developmental trajectories of physical and social aggression irrespective of their gender, suggesting that individual differences are important in predicting children's adaptation (Underwood, Beron, & Rosen, 2009).

In conclusion, these findings contribute to a better understanding of biological and social-relational correlates of social behavior and demonstrate the utility of integrating neurobiological processes into elementary classroom-based research (see Beauchaine, Neuhaus, Brenner, & Gatzke-Kopp, 2008). These findings are exploratory and limited to a cross-sectional sample of children in a Western Canadian elementary school. Longitudinal studies are recommended to identify temporal associations among daily patterns in salivary cortisol, peer and teacher supportive relationships, and behavioral development across middle childhood.

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