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Classroom social experiences in early elementary school relate to diurnal cortisol levels

Pia Behnsen[a,b,*], Marieke Buil[a,b], Susanne Koot[a,b], Anja Huizink[b], Pol van Lier[a,b]

[a] Vrije Universeit Amsterdam, Section of Clinical Developmental Psychology, van Boechorstraat 1, 1081 BT Amsterdam, The Netherlands
[b] Erasmus University Rotterdam, Department of Psychology, Education and Child Studies, Burgemeester Oudlaan 50, 3062 PA Rotterdam, The Netherlands

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ABSTRACT

Social stress has been linked to altered hypothalamic-pituitary-adrenocorticol (HPA) axis activation. During elementary school, children can become exposed to negative peer relations, such as poor appraisal among classroom peers, which is considered a social stressor. However, little is known about the association between classroom peer appraisal and the physiological stress system in children. The goal of this study was to examine the association of peer acceptance and peer non-acceptance with diurnal cortisol concentrations in 222 children from 20 mainstream elementary schools (*M*age = 6.97 years, SD = 0.99, 55% boys) in the Netherlands. Saliva samples were collected at awakening, 30 min post-awakening, at noon and at 8 pm during a weekend day. From these assessments, the Cortisol Awakening Response (CAR), diurnal cortisol concentration (AUC_g) and diurnal cortisol slope were calculated. Peer nominations of peer acceptance (being liked), and peer non-acceptance (being disliked) were collected across a one year interval. Associations were controlled for peer victimization, age, sex and SES and children's levels of emotional problems and behavioural problems. Results showed that low peer acceptance was associated with heightened diurnal cortisol concentration (i.e., heightened AUG_g), lower cortisol reductions across the day (i.e., less decreasing cortisol slope) and heightened cortisol awakening response (i.e., heightened CAR). Peer non-acceptance and the interaction between peer acceptance and peer non-acceptance (known as peer rejection) were not associated with AUCg, cortisol slope or the CAR. The findings emphasize the association between poor appraisal among classroom peers and children's heightened HPA-axis activation. This underscores the importance of the physiological stress system in studying the consequences of negative peer relations in children.

1. Introduction

With the transition to formal schooling, children are exposed to potential social stressors such as negative peer appraisal (Rubin et al., 2006). Exposure to social stress may have long-term consequences on hypothalamus-pituitary-adrenal (HPA) axis functioning (Cohen et al., 2007). Social stress, such as constituted by negative experiences in the peer context (peer victimization) are possibly associated with upregulated HPA-axis functioning in elementary school children (Peters et al., 2011). Approximately 10–15% of all children become poorly accepted or rejected by their peers (Woodward and Fergusson, 2000). Yet, little is known about the association of this type of social stress in early elementary school and the physiological stress system in children. The goal of this study is to assess the unique and combined association between peer acceptance and peer non-acceptance with HPA-axis functioning in early elementary school children in the Netherlands. Dysregulation of the HPA-axis functioning has previously been linked to the onset of problematic behaviour and emotional problems during childhood (Gunnar, 2000; Shirtcliff and Essex, 2008; El-Sheikh et al., 2008; Saridjan et al., 2014). Thus, if research results reveal a biological embeddedness of social stress in children, this would indicate that social environmental factors associated with altered HPA-axis activation in children need to be targeted for early prevention of mental health problems (Adam et al., 2007).

Both higher and lower than average diurnal levels of cortisol have been suggested to be maladaptive. Elevated and lowered diurnal cortisol levels have been linked to memory, attention problems and failures in coping capacities (Flynn and Rudolph, 2007). Cortisol concentrations typically rise directly following awakening and decline throughout the day, with a slight peak around noon (Cohen et al., 2007). The cortisol awakening curve (CAR) is typically assessed by the change in cortisol concentrations from directly following awakening to 30 min after awakening. Two other cortisol measures represent daytime changes in cortisol concentration, namely the daytime cortisol curve

* Corresponding author at: Vrije Universeit Amsterdam, Section of Clinical Developmental Psychology, van Boechorstraat 1, 1081 BT, Amsterdam, The Netherlands.
E-mail addresses: behnsen@fsw.eur.nl (P. Behnsen), buil@fsw.eur.nl (M. Buil), koot@fsw.eur.nl (S. Koot), a.c.huizink@vu.nl (A. Huizink), vanlier@fsw.eur.nl (P. van Lier).

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and the cortisol slope. The daytime cortisol curve (Area Under the Curve with respect to the Ground; the AUC_g) represents an estimation of the total cortisol output during the day. The cortisol slope represents the diurnal change of cortisol concentration (Saxbe, 2008).

The CAR represents the preparation of the body to face daily hassles and is therefore linked to overall diurnal cortisol output (Clow et al., 2010; Adam et al., 2007). A larger than usual CAR may signal anticipation to stressful situations and has been associated with frequent arguments at home in children (Michels et al., 2012). Whether associations between (peer) stressors and CAR exist in childhood is unknown as previous studies in childhood samples (e.g., see Gunnar et al., 2003) did not study CAR but focused on cortisol levels instead. However, a larger CAR than average may be expected in children experiencing frequent peer stressors.

Several studies have linked individual differences in daily cortisol output to (chronic) stress exposure. For instance, negative social experiences, such as frequent arguments between parents at home, were related to a more negative cortisol slope in six-year-old children (DeCaro et al., 2008). Cortisol production might be downregulated after facing extended stress exposure for several months, as the HPA-axis becomes overstrained. Through the negative feedback circuit this results in suppressed output of CRH and ACTH by acting on glucocorticoid receptors in the brain, ultimately leading to cortisol output rebounds below normal (Miller et al., 2007). Indeed, lowered diurnal cortisol levels were observed in 3/4-year-old children after facing adverse early child care for two years (Koss et al., 2014). Additionally, active classroom exclusion by peers was related to downregulation of the cortisol diurnal curve and heightened cortisol levels at school in 8–10 years old children (Peters et al., 2011). Peer rejection was associated with heightened cortisol levels in preschool children (Gunnar et al., 2003). However, in this latter study cortisol levels were only assessed once during the day, thereby impeding studying whether peer rejection was linked to the CAR, cortisol slope and the AUC_g. It needs to be investigated whether the cortisol slope, AUC_g and CAR are similarly influenced by peer appraisal as these indicators of HPA axis functioning have a different connotation (Saxbe, 2008). Thus, although negative peer experiences may represent (prolonged) stressful social experiences (MacDonald and Leary, 2005; , 2001; , 2007), our understanding of the association between peer relations and the cortisol awakening response as well as diurnal cortisol output in early elementary school children is far from complete.

In this study, we associate individual differences in diurnal cortisol concentration in elementary school children with early elementary school classroom peer acceptance and peer non-acceptance. Peer acceptance is typically assessed by asking classroom peers to nominate who is liked in the classroom (Coie et al., 1982). In contrast, peer non-acceptance indicates who is disliked by their classroom peers (Coie et al., 1982). Scoring low on peer acceptance does not necessarily mean that a child is actively disliked. The child may be mostly neglected in the social evaluation (Coie et al., 1990). However, peer-acceptance and peer non-acceptance may also jointly excerpt their influence. That is, low scores on peer acceptance, combined with high scores on peer non-acceptance is referred to as peer rejection (Deater-Deckard, 2001; Coie et al., 1990). Peer rejection has been previously described as especially troublesome for children (Williams, 2001, 2007). Although poor peer appraisal is described as stressful for children, it is important to note that asking children how they like or dislike peers does not necessarily mean that they actively show negative behaviours toward the child. Peer victimization is considered a behavioural manifestation of peer rejection, as it involves actively trying to harm the victim and their social relations with peers (Olweus, 1986, 1993). Previous research suggested that there exists an association between peer victimization and heightened cortisol levels in children (Peters et al., 2011). However, it is still unclear whether mere peer appraisal differences are associated with altered cortisol levels in children. This is important as mere peer appraisal might not always be visible in classroom behavior but might already be associated with altered daily cortisol levels in children as proposed by Gunnar et al. (2003).

In the present study, we aimed to explore the unique and possible joint influence of peer acceptance and non-acceptance with diurnal cortisol output in 222 early elementary school children. We expected that especially peer rejection, that is, the combination of low peer acceptance and high peer non-acceptance, is associated with heightened individual differences in daily cortisol levels (Gunnar et al., 2003). Specifically, we expected peer rejection to be linked to heightened CAR, with lower reductions in cortisol across the day, and with higher cortisol levels across the day. In all models, behavioural and emotional problems, as well as peer relational victimization, will be controlled for as such problems in itself have been repeatedly linked to altered HPA axis activity (Bruce et al., 2002; Peters et al., 2011; Rudolph et al., 2010; Saridjan et al., 2014) and coincide with the peer social stressors rejection and poor acceptance (Blackhart et al., 2007; van Lier and Koot, 2010).

2. Methods

2.1. Participants

Data of this study are part of the Happy Children, Happy Adolescents? (HCHA) project, a longitudinal study among 1624 children on their socio-cognitive and behavioural development in the school context (M age = 6.0 years, SD = 0.46 at the start of the study; 50% boys). Children were first assessed in fall 2011. The participants lived in the east and center of the Netherlands, had mostly a Dutch-Caucasian background (90%), and the family socioeconomic status (SES) was low in 10% of the sample. For reasons of feasibility, only children of whom we had complete family contact information, and whose primary caregiver actively participated in a parent data collection in 2013 (N = 657) were approached for data collection of cortisol. For the current study, 298 children (M age = 6.97 years, SD = 0.99; 55% boys) of the longitudinal study participated in the cortisol assessments between 2013 and 2014. Ethical approval was obtained from the Medical Ethical Review Board of the VU Medical Centre (protocol number: NL37788.029.1).

Children who did and did not participate in the cortisol collection (N = 298) did not differ on peer acceptance $t(499) = -1.28$, $p = 0.20$, peer non-acceptance $t(499) = 1.01$, $p = 0.31$, and age $t(499) = 8.11$, $p = 0.42$ from the rest of the approached children. Children who participated in the cortisol data collection were equal on peer acceptance $t(1197) = -0.0.34$, $p = 0.73$ and non-acceptance $t(1197) = 1.89$, $p = 0.06$, and age $t(1197) = 2.79$, $p = 0.50$ as compared to the original sample. Of these 298 children, data of three children who had outliers of 3 SDs of the mean on cortisol measures were deleted in order to rule out possible influence of outliers by contamination or by medication. Of the remaining 295 children, 282 children provided valid saliva samples. Valid saliva samples for at least two cortisol measurement points were necessary to calculate at least one of the cortisol indicators. Based on this criterion, N = 222 were included for further analyses. Children who had non-usable cortisol data, did not differ from children with complete data on peer acceptance, $t(209) = -0.66$, $p = 0.44$ or peer non-acceptance, $t(210) = 0.73$, $p = 0.47$.

2.2. Design

The current study used data assessed over a one-year period. Children were 5–8 years old (grade 1, 2, 3 and 4). Participants completed nominations procedures in the spring of 2013 and 2014. They were supervised by trained interviewers. Cortisol data was obtained at home by the parents between spring of 2013 and spring of 2014. We collected diurnal cortisol levels at home on a Saturday, as a reflection of HPA axis functioning in everyday life (Saxbe, 2008). To avoid confounding by acute experiences of classroom peer rejection, we tested

our hypotheses on a weekend day to assess whether stable negative peer appraisal is associated with increases of daily HPA-axis functioning. Daily cortisol concentrations have been shown to be reduced on weekend days in comparison to weekdays in adults (Schlotz et al., 2004). Of children attending day-care, 70 percent showed more typical decline of cortisol on weekend days at home than during a week day in day-care (Watamura et al., 2009). However, studies showed heightened cortisol levels at the weekend after a stressful working week in adults (Berset et al., 2009). This suggests that heightened cortisol levels in the weekend may result of less effective recovery from stress experienced during the week. Averaged peer acceptance, peer non-acceptance and control measure scores (emotional and behavioural problems and relational victimization) were computed to achieve robust measures. An additional advantage of this is that measure captures stability of peer appraisal across a one year interval. We hypothesized that especially chronic poor peer experiences link to adjustment in daily stress activity.

2.3. Measures and procedures

2.3.1. Cortisol

Four tubes for assessment of salivary cortisol levels were sent to the home of the children. Detailed written instructions were send to the participants, including visual instructions explaining children and their parents how and when to collect the saliva, and how to preserve the tubes. Participants were instructed to provide the first sample directly at awakening (Cortisol 1), the second sample 30 min afterwards (Cortisol 2), the third at 12 p.m. (Cortisol 3) and the fourth at 8 p.m (Cortisol 4). The average time of the first sample collection was 7.07 a.m. with a margin of 45 min. Compliance was stimulated by calling parents previous to the cortisol collection day to remind them to assist their children with cortisol collection at the specific time points. Cortisol in saliva was collected with the passive drooling method (Schlotz et al., 2004). Parents were instructed to help their children with refraining from brushing their teeth, physical activity, leaving their bed and eating before the first and second cortisol collection time point at 7.00 and 7.30, respectively. They were further asked to not eat directly before collecting saliva at any point and to only drink water directly before the cortisol collection. Children were further instructed to not be physically active two hours before collecting saliva. Parents were instructed to store the cortisol tubes in the freezer before returning them by prepaid mail envelopes. They were asked to send the samples on a weekday and not on a Friday to avoid samples being outside the freezer for more than two days. After receiving the samples, the cortisol tubes were stored at −20 °C. After completion of the data collection, samples were sent on dry ice in one batch to the laboratory of the Department of Biological Psychology, Technical University of Dresden for analysis. Salivary cortisol concentrations were measured using a commercial immunoassay with chemiluminescence detection (CLIA; Hamburg, Germany). Intra- and interassay coefficients of variation were below 7% (Saridjan et al., 2014).

We computed three composite variables of the separate cortisol measures within a day: the cortisol awakening response (CAR), the area under the curve with respect to the ground (AUC_g), and the diurnal cortisol slope. These three independent variables represent different aspects of the HPA axis activity. The CAR was computed by substracting Cortisol 1 from Cortisol 2 (Wust et al., 2000). The AUC_g was calculated as the total area under the curve between the four time points (Saxbe, 2008). The AUC_g represents the total cortisol secretion during the day. The AUC_g was calculated for only those children who provided at least three saliva samples. The diurnal cortisol slope was computed by fitting an individual regression line for each child on samples cortisol 1 and cortisol 4, which predicted the change in cortisol values from time since awakening (Saridjan et al., 2014). The second cortisol sample was not included in the measurement to avoid effects of the CAR. Flatter slopes, as indexed by less negative betas, imply a slower cortisol decline during the day.

2.3.2. Social experiences

Peer acceptance and non-acceptance were assessed using peer nominations. All children completed the peer nomination procedure in the spring of 2013 and again in 2014 in their classroom. Children were presented a list of names of their classmates and were asked to nominate an unlimited number of peers in the classroom who they liked (peer acceptance), and who they disliked (non-acceptance) (Coie et al., 1990). Children were not allowed to nominate themselves. To account for differences in class size, the sum scores of peer acceptance and non-acceptance were divided by the number of participating children in the classroom minus one, because self-nomination was not possible. The correlation of peer acceptance and peer non-acceptance across waves was $r = 0.41$ and $r = 0.44$, p's < 0.01 respectively.

2.3.3. Control variables

Early morning cortisol differences were represented by individual cortisol intercepts ($M = 6.57$ cortisol per n/mol, $SD = 3.09$) at awakening, in order to control for potential cortisol awakening level differences for the diurnal cortisol measures. (Stalder et al., 2011).

Time of first sample was controlled for since differences in time of first cortisol sampling have been shown to influence daily cortisol levels in children (Michels et al., 2012).

Relational victimization was assessed using peer nominations of the relational victimization scale of the Social Experiences Questionnaire (SEQ; Crick and Grotpeter, 1996). Children were asked "About which classmates, mean things are said?" The correlations between relational victimization scores across the two waves was $r = 0.30$, $p < 0.01$.

Emotional problems and behavioural problems were assessed by parents in the spring of 2013 and again in the spring of 2014 using the emotional problems and behavioural problems scales of the Strengths and Difficulties Questionnaire (SDQ; Goodman, 1997). Parents were asked to indicate on a 5-point scale, ranging from not true (0) to definitely true (4) whether their child is for example "scared a lot" ($M = 1.8$, $SD = 0.77$) or whether their child "often has anger outbursts". The correlation between emotional problems and behavioural problems across the waves was $r = 0.6$ and $r = 0.5$, $p < 0.01$ respectively. Cronbach's alphas were 0.62 and 0.64 for emotional problems, and 0.50 and 0.50 for behavioural problems across waves.

Sex of children was dummy coded with 0 = boys and 1 = girls.

Calendar age of the children was used to control for children's chronological age.

Low Socioeconomic Status was assessed through parental occupation using the Dutch Working Population Classifications of Occupations Scheme (Statistics Netherlands, 2001). The highest occupation level (from father or mother) was considered to reflect household SES. Low SES was defined as being unemployed of having an elementary job or less. Household SES was dummy coded as 0 = medium to higher level SES, 1 = unemployed to lower level SES.

2.4. Statistical analyses

We tested for unique and possible interaction effects of acceptance and non-acceptance on CAR, AUC_g and cortisol slope. To this end, a model containing all outcomes (CAR, AUC_g, cortisol slope) was fitted, on which main effects of acceptance and non-acceptance and the interaction acceptance x non-acceptance were tested. Paths were controlled for possible effects of relational victimization, behavioural and emotional problems, early morning cortisol differences, time of first sampling, low-SES, age and sex. To account for possible level differences in cortisol, the cortisol intercept was entered as a control variable in all models. To control for possible covariation between the predictors of interest (acceptance, non-acceptance) and emotional and behavioural problems, the predictors were regressed on emotional problems and behavioural problems. Conclusions on best fitting nested models were based on significant prediction effects (main effects and two-way interaction). Mplus version 7 (Muthén and Muthén, 2005) was used for

Table 1
Means, Standard Deviations and Correlations Between Study Variables.

	M	*SD*	Range	1	2	3	4	5	6	7	8	9	10	11	12	13
1. CAR	0.94	1.37	−2.28 to 9.02	–												
2. AUCg	3.33	0.21	2.72–3.97	0.54**	–											
3. Slope	−5.43	3.77	−14.96 to 17.87	0.34**	0.40**	–										
4. Like	0.31	0.14	0.03–0.78	−0.18*	−0.25**	−0.14	–									
5. Dislike	0.15	0.13	0.00–0.63	0.16*	0.01	0.03	−0.35**	–								
6. Emot. prob.	1.80	0.79	1.00–5.00	−0.01	−0.04	−0.06*	−0.14	−0.13	–							
7. Behav. prob.	2.1	0.99	1.00–5.00	0.03	−0.02	−0.16*	−0.17*	0.21**	0.23**	–						
8. Victimization	0.38	0.95	0.00–1.00	0.06	−0.02	−0.04	0.12	0.22**	0.06	0.05	–					
9. Intercept	6.57	3.09	2.06–18.73	−0.14*	0.25**	0.72*	−0.01	−0.19*	0.03	−0.04	0.06	–				
10. Age	6.97	0.99	5.00–10.00	−0.70	−0.11	−0.03	−0.01	0.06	−0.08	−0.04	0.16*	−0.04	–			
11. Sex	–	–	–	−0.01	0.03	−0.00	0.20**	−0.27**	0.11	−0.10	0.12	−0.05	0.02	–		
12. SES	5.35	1.66	1.00–8.00	0.13	−0.03	0.05	0.00	0.02	0.04	−0.07	−0.16*	0.06	0.32	0.07	–	
13. Time first sampling	7.02	0.74	5.30–9.00	−0.08	−0.12	−0.03	−0.15	0.12	0.04	0.16	0.05	−0.08	−0.01	−0.00	0.13	–

Note. CAR = Cortisol Awakening Response. AUC_g = Area Under the Curve with respect to the ground. Slope = changes of cortisol concentration during the day. Emot. Prob. = Emotional problems, Behav. Prob. = Behavioural problems. Intercept = Early morning cortisol differences.

* $p < 0.05$.

** $p < 0.01$.

fitting the nested regression models. Standard errors were adjusted for clustering of data at the classroom level using a sandwich estimator (Williams, 2000)

3. Results

3.1. Descriptive statistics

Descriptive statistics for the study variables, and the correlations between the study variables are depicted in Table 1. Cortisol concentrations of the four measurement points are presented in Table 2. Results in Table 1 showed significant negative correlations were found between peer acceptance and AUCg ($r = -0.17$, $p < 0.05$), between peer acceptance and the diurnal cortisol slope ($r = -0.17$, $p < 0.05$), and between peer non-acceptance and peer acceptance ($r = -0.35$, $p < 0.01$). Relational victimization was positively correlated with non-acceptance ($r = 0.22$, $p < 0.01$). Emotional problems correlated negatively with the cortisol slope ($r = -0.16$, $p < 0.05$). Behavioural problems correlated negatively with the cortisol slope ($r = -0.16$, $p < 0.05$) and peer acceptance ($r = -0.17$, $p < 0.05$) and correlated positively with non-acceptance ($r = 0.21$, $p < 0.01$). The intercept was negatively correlated with the (CAR $r = -0.14$, $p < 0.01$) and positively correlated with the AUCg ($r = 0.25$, $p < 0.01$) and the cortisol slope ($r = 0.72$, $p < 0.01$). Age was positively correlated with victimization ($r = 0.16$, $p < 0.05$). SES was negatively correlated with relational victimization ($r = -0.16$ $p < 0.05$).

3.2. Associations between peer social stressors and cortisol

Multivariate regression models were fitted to test our hypotheses. The outcome variables CAR, AUC_g and diurnal cortisol slope were simultaneously included in the model. Models containing main effects, and two-way interaction term of peer acceptance and peer non-acceptance were fitted. Results are depicted in Table 3.

The results showed (Table 3, lower portion) that the two-way interaction term between acceptance and non-acceptance was not significant in predicting the CAR, AUC_g or the diurnal cortisol slope. However, results in Table 3, upper portions showed significant main effects of peer acceptance in predicting the CAR(β per nmol/L CAR = −0.13, 95% CI: −0.09; −0.86, $p < 0.05$), AUC_g (β per nmol/L AUC_g = −0.23, 95% CI: −2.21; −0.38, $p < 0.01$) and diurnal cortisol slope (β per nmol/cortisol slope = −0.17, 95% CI: −6.17; −0.31, $p < 0.05$). The model provided a good fit to the data (χ^2 (9, n = 222) = 13.54, SRMR = 0.023, RMSEA = 0.048, CFI = 0.97). With respect to the CAR, results implied that children with lower peer acceptance scores had a higher cortisol awakening response. With respect to the AUC_g, results implied that children with lower peer acceptance scores had higher overall cortisol output across the day. Given that cortisol levels decreased across the day (Table 2; Fig. 1), the negative estimate of peer acceptance implies that higher levels of peer acceptance were associated with more negative beta's, and thus higher declines in cortisol across the day (Fig. 1). In addition to these effects, higher levels of emotional problems were associated with more negative beta's, thus steeper declines in the cortisol slope (β per nmol/L cortisol slope = −0.14).

Table 2
Cortisol measurements.

Variable	Measurement	Mean	Std. Deviation
Cortisol four timepoints	Cort 1 (7.00)	6.57	3.09
	Cort 2 (7.30)	7.51	5.65
	Cort 3 (12.00)	2.63	2.16
	Cort 4 (20.00)	1.48	4.33

4. Discussion

This study examined the association between peer appraisal and diurnal cortisol levels in a sample of children in the early years of mainstream elementary schools in the Netherlands. Our results revealed that low peer acceptance (scoring low on being liked) was associated with heightened CAR, heightened AUC_g and attenuated decreases in cortisol across the day, as indexed by the diurnal cortisol slope. Non-acceptance by peers (being disliked) or peer rejection (scoring low on being liked and high on being disliked) were not related to HPA activity. Collectively these results suggested heightened diurnal cortisol output on a weekend day among children who had lower peer acceptance in the classroom across a one year interval.

Our results showed that low peer acceptance was associated with increased cortisol awakening response in our sample. Waking time was not significantly correlated with peer acceptance (r = 0.04). Therefore, we do not expect differences in waking time cortisol collection between peer accepted and peer non-accepted children. We further compared the magnitude of our CAR measure to previous studies. The range of our CAR is comparable to previous studies assessing the CAR in young children (for example Sarijdjan et al., 2015). Moreover, a previous study among children showed an awakening response (defined as an increase by at least 1.5 n/mol/L from awakening to 30 min later) in 90% of the sample (Bäumler et al., 2013). We had CAR scores of the same magnitude in 90% of our sample. Therefore we are confident that

Table 3
Associations Between Peer Appraisal and Diurnal Cortisol Outcome Parameters.

		CAR				AUCg				Slope			
		B	*SE(B)*	*Beta*	*p*	*B*	*SE(B)*	*Beta*	*p*	*B*	*SE(B)*	*Beta*	*p*
Step 1: Main effects	Age	−0.06	−0.84	0.07	0.40	−0.08	−1.39	0.06	0.16	0.03	0.26	0.13	0.79
	Sex	0.05	0.29	0.16	0.76	0.09	0.59	0.16	0.55	0.13	0.30	0.44	0.76
	SES	0.47	1.05	0.44	0.29	0.31	0.84	0.36	0.39	0.53	0.69	0.76	0.48
	Emotional prob.	−0.04	−0.61	0.07	0.54	−0.11	−1.79	0.06	0.07	−0.38	−2.69	0.14	0.01
	Behavioural prob.	0.13	1.16	0.11	0.25	0.13	1.46	0.09	0.14	0.37	1.57	0.23	0.12
	Victimization	0.39	0.42	0.95	0.67	0.93	1.07	0.87	0.28	2.12	1.16	0.84	0.25
	Intercept	−0.06	−0.96	0.07	0.34	0.15	2.52	0.06	0.01	−0.03	−0.18	0.14	0.86
	Time first sampling	−0.22	−0.95	0.23	0.34	−0.21	−1.14	0.18	0.25	−0.24	−0.71	0.34	0.47
	Like	−1.24*	−2.02	0.61	0.04	−2.09**	−3.28	0.63	0.00	−3.43*	−2.31	1.49	0.02
	Dislike	0.49	0.46	1.06	0.64	−0.37	−0.58	0.65	0.65	−1.50	−1.16	−1.29	0.25
	R^2	0.06				0.23				0.04			
Step 2: 2-way interaction	Age	−0.07	−1.05	0.07	0.28	−0.08	−1.42	0.06	0.15	0.04	0.31	0.13	0.75
	Sex	0.03	0.16	0.16	0.87	0.09	0.57	0.17	0.44	0.15	0.33	0.44	0.74
	SES	0.48	1.07	0.45	0.28	0.31	0.84	0.36	0.39	0.52	0.68	0.76	0.49
	Emotional prob.	−0.05	−0.66	0.07	0.51	−0.12	−1.79	0.06	0.07	−0.37**	−2.70	0.14	0.01
	Behavioural prob.	0.13	1.14	0.12	0.26	0.13	1.45	0.09	0.15	0.34	1.56	0.24	0.12
	Victimization	0.23	0.23	0.99	0.82	0.90	1.02	0.88	0.31	2.23	1.21	1.84	0.23
	Intercept	−0.06	−0.93	0.07	0.35	0.16	2.51	0.06	0.01	−0.03	−0.18	0.14	0.89
	Time first sampling	−0.20	−0.87	0.23	0.38	−0.19	−1.09	0.18	0.27	−0.24	−0.69	0.34	0.49
	Like	−0.32	−0.38	0.84	0.70	−1.79	−2.48	0.72	0.01	−3.87	−2.19	1.76	0.03
	Dislike	2.59	1.27	2.04	0.70	0.30	0.21	−2.22	0.80	−2.51	−1.26	1.99	0.21
	Like* Dislike	−8.73	−1.62	5.38	0.10	−2.84	−0.88	3.19	0.37	4.09	0.83	4.90	0.40
	R^2	0.08				0.25				0.07			

Note. CAR = Cortisol Awakening Response. AUC_g = Area Under the Curve with respect to the ground. Slope = changes of cortisol concentration during the day. Intercept = Early morning cortisol differences.

* $p < 0.05$.

** $p < 0.01$.

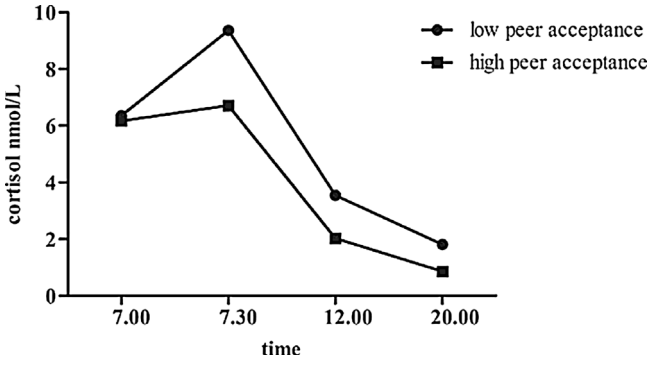

Fig. 1. Cortisol concentation in nmol/L according to low and high peer acceptance.The x-axis depicts the timepoints of cortisol measurement(cortisol 1-cortisol 4).
Note: Cortisol 1 and cortisol 2 are significantly different from each other.

we actually captured a valid assessment of CAR in our sample.

Low peer acceptance was associated with diurnal cortisol in our sample. Low peer acceptance was associated with a flattened diurnal cortisol slope and heightened overall cortisol output. Poorly accepted children in our sample showed high cortisol levels in the morning, with less decline of cortisol concentration during the day as compared to the rest of the sample, resulting in heightened evening cortisol levels. This failure of decrease of cortisol concentration during the day constituted the increased overall cortisol output.

Poorly accepted children in our sample showed lower-than-expected cortisol changes during the day. Similar to Peters et al. (2011) findings, low peer acceptance was associated with a flattened cortisol slope. Low peer acceptance is likely experienced during multiple school years as the stability of negative peer appraisal across school years is quite high (Coie and Dodge, 1990). Our peer appraisal assessment was based on two consecutive school years. The stability of our measure was a similar stability as found in earlier studies. Therefore, negative peer appraisal might represent a chronic stressor as children are exposed to such peer stress throughout multiple school years. Extended periods of stress have earlier been related to changes in the cortisol slope (Wolf et al., 2008).

Our hypothesis regarding peer rejection and the association with increased CAR as well as heightened diurnal cortisol was not confirmed. We used linear regression analysis, measuring associations, to test our hypotheses. Based on comparable research in an adult sample, we do not expect that level differences between week and weekend cortisol levels influence associations between peer relations and daily cortisol levels. Therefore, we suggest that our non-significant findings are not a result of possible cortisol level weekend-weekday differences. Earlier research underscores the notion that chronic stress during the week is significantly associated with altered cortisol levels during the weekend in adults (Berset et al., 2009; Geurts and Sonnentag, 2006).

We found that higher levels of emotional problems were associated with more negative betas, thus higher declines in the cortisol slope. This was not the focus of our study but still constitutes an interesting finding. Hyperactive HPA axis functioning has often associated with emotional problems in similar studies in children (Greaves-Lord et al., 2007; Feder et al., 2004; Hartman et al., 2013). More insights into the cortisol slope and internalizing problems in children are needed to draw knowledgeable conclusions about related associations. Self-report of emotional problems were not available for the current study since the children were too young. We suggest to compare our results with self-reports of internalizing problems and related associations with daily cortisol levels in a sample with older children.

Our results did not support earlier findings of Gunnar et al. (2003) who found that peer rejection was related to heightened cortisol levels in pre-schoolers. Daily cortisol concentration should be tested repeatedly to assess whether up-regulation of the daily HPA axis functioning is a relatively enduring, habitual condition of the HPA-axis.

It is further possible that children become even more susceptible to non-acceptance and rejection by peers in adolescence. Peer appraisal grows more important during adolescence and not fitting in with the peer group becomes a highly salient stressor during puberty (Brown, 1990). Additionally, more pronounced alteration of cortisol rates in

pubertal as compared to pre-pubertal children have been reported (Gandia et al., 1990). Therefore, it needs to be assessed, whether peer non-acceptance and peer rejection might become significantly related to diurnal cortisol rates in puberty.

Our findings extend previous studies by showing that upregulation of daily cortisol is present in the early years of elementary school. Moreover, we firstly assessed the association between peer appraisal and CAR, AUC_g and cortisol slope to investigate the distinct association between negative peer appraisal and overall HPA axis functioning. We found upregulation of overall cortisol output, i.e. no blunted diurnal cortisol output, as a function of negative peer appraisal in this study. A steep cortisol slope, or strong decrease of cortisol concentration during the day, has been linked to general health, social support and wellbeing in adults (Sjogren et al., 2006; Miller et al., 2007). A flattened slope (associated with low peer appraisal in our sample) is maladaptive, as variability in cortisol concentration is needed in order to respond to different experiences during the day (Saxbe, 2008).

We do not have data to test whether manifest stress in the week prior to our assessments of cortisol during the weekend day affected our results. However, based on related findings in adults (Berset et al., 2009), and given that we anticipate poor peer appraisal to constitute a chronic stressor, we did expect to find a significant association between peer social stress during the week and salivary cortisol during the weekend. Moreover, for reasons of feasibility and adherence to assessment protocol by instructing parents to assist their children in the cortisol collection during the weekend day, we choose to use this approach. The data suggests that heightened cortisol output on a weekend day might be linked to extended exposure to negative appraisal during the week. The pattern of low peer appraisal in the classroom during the week and the impossibility to recover from this during the weekend, may be linked to chronic stress exposure (Wolf et al., 2008). It has been suggested that experiences of the preceding day activate the CAR to prepare the body based on prior day experiences (Clow et al., 2010; Adam et al., 2007). Increases of the CAR have been reported to be a valid indication for chronic influences of social stress (Fries et al., 2009; Pruessner et al., 1997). Possibly as a consequence, the CAR shows higher intraindividual stability than the AUC_g and slope (Pruessner et al., 1997). An increase of the CAR after adverse experiences with peers during the previous day might consequently prepare the individuals to meet the demands of stress related to low peer acceptance during the upcoming day, even on a weekend day.

Our results underscore the notion that chronic negative peer appraisal is associated with increases of daily HPA-axis functioning even when children are outside of their classroom peer context. Consistent upregulation associated with negative peer appraisal might indicate programming of the daily axis functioning based on negative social experiences during childhood (Meaney et al., 2007). Our results suggest that influences on HPA axis functioning are mostly associated with peer appraisal in children of this age group. Evidence that negative peer appraisal is associated with higher cortisol levels is consistent with the findings of Gunnar et al. (2003). Thus, it is possible that absence of peer support might influence children's adrenocortical activity in peer settings. However, this would need to be examined directly by assessing the association between perceived peer support and cortisol secretion. Thus, altered HPA-axis functioning may constitute the link between negative social experiences and the onset of psychopathology. We therefore suggest that future research should investigate whether upregulation of the HPA-axis mediates the link between negative peer appraisal and psychopathology in elementary school children in a longitudinal setting. The association between adverse peer experiences and AUCg, cortisol slope and CAR should be studied by logging these data on several consecutive days, including both weekdays and weekend days. Upregulation of the HPA-axis constitutes a risk factor for emotional problems in children and adolescents (Bruce et al., 2002). It is possible that after prolonged hypersecretion of cortisol for several months, the HPA-axis becomes downregulated as a result of reduced expression of the glucocorticoid receptor (Miller et al., 2007). Therefore, changes of heightened HPA- axis activation towards downregulation might only be visible after chronic exposure to peer rejection in late childhood.

4.1. Limitations

We averaged the peer nomination scores of 2013 and 2014 since cortisol was collected between these two time points for the current study. Therefore, we cannot draw a knowledgeable conclusion on the direction of the relation between peer appraisal and cortisol. However, we are convinced that averaging the peer nomination scores for our purposes is favorable since this approach guarantees a more stable measure of peer appraisal.

As suggested previously in adult samples, a flattened cortisol slope can also be an indicative of negative experiences during the day. Overall cortisol output has been shown to be influenced by daily hassles as well as activity during the day (Adam et al., 2007; Miller et al., 2007). High cortisol levels are linked to negative affect (e.g. anger or tension) and may become cumulative during a day, resulting in heightened morning and evening cortisol values during an overall "bad day" (Saxbe, 2008; van Eck and Nicolson, 1994). This may thus suggest that poorly accepted children experience more daily hassles when compared to better accepted peers. We suggest that future studies focus on a design including daily experiences through Ecological Momentary Assessment, which can provide valuable information on interindividual differences in negative social experiences (Steptoe et al., 2007). In this manner, daily hassles and other daily social stress, for example in the family context that might influence daily cortisol output, can be controlled for. To distinguish between within-person state variation and chronic alterations of the AUC_g and cortisol slope, cortisol samples on consecutive days, also during the week, need to be collected. Future studies should focus on exploring the role of altered stress activation as a potential mediator between negative peer experiences and the onset of psychopathology (Luman et al. 2010; Saridjan et al. 2014).

Swapping of first and second sample could have resulted in obtained negative values for CAR (20% of the sample). Furthermore, no information on daily activities, time between waking and first sampling, medication use, sleeping pattern, or food intake, were collected. Self-reports might provide additional insights into individual daily experiences of negative peer appraisal in children. However, self-reports on peer appraisal and victimization often do not correspond with peer nominations on victimization (Scholte et al. 2013). Self-reports of peer appraisal might mediate the relationship between sociometric peer status reports and diurnal cortisol change.

A genetic predisposition could underlie our observed association. Variants in genes involved in endocrine development, (Goodyer et al., 2010), give rise to individual differences in cortisol levels. Higher initial cortisol levels might lead to increased perceived stress and might reinforce avoidance or little contact with peers in the classroom, resulting in low peer acceptance. Nevertheless, molecular genetic variances influencing cortisol levels have not been reliably demonstrated (Saridjan et al. 2014), therefore our results emphasize the notion of negative peer appraisal being associated with heightened daily cortisol levels.

4.2. Conclusion

Our findings suggest that low appraisal by the peer group constitutes a stressful experience that is associated with heightened HPA-axis activation in early elementary school children. The current study evidences a psychobiological mechanism that might explain how peer appraisal at the group level might contribute to alterations of stress system functioning in children.

The findings emphasize that preventive interventions need to be directed at peer processes in elementary school. We firstly assessed whether mere negative peer appraisal is initially associated with HPA-

axis functioning in children in primary school. More specifically, teachers need to monitor peer appraisal processes in the classroom. Teachers need to be further trained in helping children with developing successful coping strategies for stressful social experiences such as low peer appraisal in the peer context. Consequently, social competence training should be embodied in the school curriculum to prevent detrimental influences on the stress system activation.

Our findings suggest that further studies should research the associations between behavioural manifestations of peer rejection and HPA-axis functioning. Importantly, no reference value for maladaptive overall cortisol output and cortisol slope changes are available for children, so more insights into maladaptive daily cortisol patterns are needed (Ice et al. 2004). Our results do not provide insights concerning healthy or unhealthy daily cortisol patterns. However, heightened cortisol levels might represent an endophenotype for psychological disorders (Mannie et al. 2007). In line with this notion, our results suggest an association between the daily cortisol slope and internalizing problems in our sample. Future studies should focus on exploring the role of altered stress activation as a potential mediator between negative peer experiences and the onset of psychopathology (Luman et al. 2010; Saridjan et al. 2014). These studies are needed to gain a better understanding of the developmental cascades.

Disclosure

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Conflict of interest

None.

Authorship

Pia Behnsen: (1) the conception and design of the study, analysis and interpretation of data, (2) drafting the article (3) final approval of the version to be submitted. Marieke Buil: (1) the conception and design of the study and analysis and interpretation of data, (2) revising the article critically for important intellectual content, (3) final approval of the version to be submitted. Susanne Koot: (1) interpretation of data, (2) revising the article critically for important intellectual content, (3) final approval of the version tobe submitted. Anja Huizink: (1) the conception and design of the study and interpretation of data, (2) revising it critically for important intellectual content, (3) final approval of the version to be submitted. Pol van Lier: (1) the conception and design of the study, analysis and interpretation of data, (2) revising it critically for important intellectual content, (3) final approval of the version to be submitted.

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