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The interaction of social risk factors and HPA axis dysregulation in predicting emotional symptoms of five- and six-year-old children

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Objective: We examined the links of social relational (family environment and peer victimization) and neuroendocrinological (HPA axis dysregulation) risk factors to children's emotional symptoms. We placed special emphasis on the joint effects of these risk factors with respect to the emergence and course of the emotional symptoms.
Methods: One hundred and sixty-six children were interviewed (Berkeley Puppet Interview) at age 5 and 6. Teachers and parents completed the Strengths and Difficulties Questionnaire. Parents completed the Family Environment Scales. Peer victimization was assessed by teacher and child reports. Children's saliva cortisol was measured before and after a highly structured story completion task which targeted their cognitive emotional representations of family conflicts.
Results: In the cross-sectional analyses, negative family environment, peer victimization, and cortisol increase during the story completion task independently contributed to the variance of emotional symptoms. There was a significant interaction effect between family environment and cortisol increase: those six-year-olds who had experienced an unfavorable family environment only showed high levels of emotional symptoms if they exhibited a cortisol increase during the story completion task. In the longitudinal analysis, peer victimization at age 5 predicted an increase of emotional symptoms at age 6, but only for those children who exhibited a blunted cortisol response a year earlier.
Conclusions: Negative family environment and peer victimization proved to be independently associated with emotional symptoms. HPA axis reactivity differentially moderated these associations. Therapeutic strategies should take the interaction between negative relational experiences and biological susceptibility to stress into account.

1. Introduction

Depression and anxiety disorders are clear-cut entities with well-researched aetiologies in adolescence and adulthood (Birmaher et al., 1996; Zahn-Waxler et al., 2000; Zalsman et al., 2006). In school-age children, perturbations of the family and peer environments figure prominently in the formation and maintenance of these forms of psychopathology (Cowan and Cowan, 2006; Juvonen et al., 2003; Parker et al., 2006; Rigby, 2003). Also, dysregulation of the stress-response systems has been proposed as an intervening biological mechanism, such that it moderates the impact of stressful life events on the emergence of anxiety and depression (McEwen, 2007). In recent studies, children with abnormal functioning of the neuroendocrine and autonomic stress-response systems have proven more vulnerable for developing anxiety and depressive symptoms following exposure to deleterious peer and family experiences (Badanes et al., 2011; Obradovic et al., 2011; Rudolph et al., 2011).

In preschoolers, these risk factors and mechanisms remain understudied (Luby, 2009; Luby et al., 2003b; Stalets and Luby, 2006). Complicating matters, symptoms of anxiety and depression – while already somewhat distinct in the preschool period – are nevertheless intimately related at this age, with the former often found to predate the latter (Snyder et al., 2009). Due to this

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high overlap researchers and clinicians often use the umbrella term "emotional symptoms", to simultaneously refer to both dimensional symptom types in young children (e.g. Garber et al., 1998; Goodman et al., 2010; Goodman, 1997). In the current study, we set out to address the question of whether some of the same sociobiological risk factors and mechanisms observed in older children are involved in the formation and course of emotional symptoms at preschool age.

1.1. Negative family environment

Disrupted family environments have been consistently linked to increased risk even at younger ages. Several studies report links between family relations/parenting styles and young children's behavioral or emotional problems (Campbell, 1995; Eisenberg and Fabes, 1998; Laible et al., 2004). The general emotional climate and relationship quality of families were also associated with child behavior outcomes. Various studies indicate that offspring of families with higher levels of conflict and lower levels of expressiveness and cohesiveness develop more internalizing and externalizing problems (Halpern, 2004; Slee, 1996; Stadelmann et al., 2007). We therefore sought to confirm the importance of family risk factors in the evolution of emotional symptoms for preschoolers.

1.2. Peer victimization

Peer victimization (i.e. being a target of peer aggression or bullying) has been linked to increased risk for the development of emotional symptoms mainly from school age onwards as children spend increasing amounts of time with same-age children and their victim-status tends to stabilize (Monks and Coyne, 2011). Meta-analyses suggest that children who receive many negative and few positive nominations by their classmates display a high degree of withdrawn behavior (Newcomb et al., 1993) and depression (Hawker and Boulton, 2000). Both lack of peer group acceptance and victimization contribute to loneliness, social dissatisfaction, and social withdrawal (Coie et al., 1990; Kochenderfer and Ladd, 1996). There is a well-documented link between peer relationship problems and the development of psychopathology (Deater-Deckard, 2001; Perren and Alsaker, 2009, Perren et al., 2006) which implies a reciprocal relationship (Hay et al., 2004). Recent findings extend this link, also implicating peer victimization during preschool as a correlate of emotional symptoms (Perren and Alsaker, 2009; Perren et al., 2006). One aim of this study, was therefore to test whether – in parallel to older children (Reijntjes et al., 2010) – peer victimization functions not only as a correlate, but also as an antecedent of internalizing problems in the preschool period.

1.3. Emotional symptoms and HPA axis dysregulation

Neuroendocrinological mechanisms are thought to be critical in mediating the genotype-phenotype transmission of affective disorders (Holmans et al., 2007; Holsboer and Ising, 2010; Levinson, 2006; von Klitzing, 2007). Compelling evidence indicates that the hypothalamic-pituitary-adrenocortical (HPA) axis is compromised in adult major depressive (Goodyer et al., 2000; Harris et al., 2000; Holsboer, 2000; Holsboer et al., 1995) and bipolar disorders (Rybakowski and Twardowska, 1999), as well as in post-traumatic stress disorders (Heim et al., 2001; Kessler et al., 1995).

Cross-sectional studies implicate the HPA system regulation as involved in children's behavioral and psychological abnormalities: task-related increases in salivary cortisol were linked to low levels of belief in the ability to cope with stressful situations (Granger et al., 1996), as well as with internalizing symptoms (Zahn-Waxler et al., 2000). Similarly, high basal levels of cortisol (particularly in the early morning) and heightened stress reactions to unfamiliar events have both been noted in young children described as behaviorally inhibited and shy (Kagan et al., 1988; Schmidt et al., 1997). Luby et al. (2003a) found altered HPA axis reactivity in depressed preschool children indicated by a pattern of increased cortisol levels in response to separation and frustration stressors. Several studies point to stress-related cortisol responses also in younger children (Buske-Kirschbaum et al., 1997; Dahl et al., 1992; Gunnar et al., 2003; Hatzinger et al., 2007; Khilnani et al., 1993; Lundberg, 1983).

1.4. Associations between relational adversities and HPA axis reactivitiy

In contrast to the findings of elevated cortisol reactions in children with emotional problems, recent studies demonstrated blunted cortisol reactions in children living in adverse environmental conditions. In a study on 200 toddlers, Sturge-Apple et al. (2011, online first) found that greater levels of adversity in the family environment were associated with children's lower cortisol reactivity to family stressors. As the children were still very young, the authors could not establish a link to possible psychopathological outcomes in these children.

Furthermore, there are hints in the literature that peer victimization may also be associated with HPA axis dysregulation when the experience is persistent (Vaillancourt et al., 2008). In a recent behavior genetic study (Ouellet-Morin et al., 2011), 30 bullied 12-year-old twins exhibited attenuated cortisol in response to the Trier Social Stress Test relative to their nonbullied MZ co-twins. This difference could not be attributed to children's genetic makeup, their family environments, pre-existing and concomitant individual factors. As a consequence, the authors assumed a causal effect of adverse childhood experiences, especially peer victimization on the neuroendocrine response to stress. Notably, Badanes et al. (2011) suggest a distinction between pre- and postpubertal reactions to stressors in keeping with their finding of blunted cortisol reactivity following family stress as a predictor of emotional symptoms in early childhood. In particular, stressors that are substantial and inescapable for the young child (e.g. adverse family environment) and occur prior to puberty-onset, may lead to attenuated cortisol reactivity in an attempt to protect the child from the health risks arising from a chronically hyperactivated stress-response system. Once children mature, they may have more resources at their disposal to tackle or flee from these stressors, making hyperactivation a more adaptive stress response (Badanes et al., 2011).

With this study, we also sought to inform this burgeoning field of the interplay between biological and social factors and how this interplay gives rise to children's early emotional symptoms. One possible pathway may lead from negative environmental experiences to neurobiological dysregulation which, in turn, culminates in emotional symptoms. To test this developmental cascade, long-term studies beginning in early childhood would be necessary. Alternatively, environmental influences on developmental outcomes may be moderated by neurobiological susceptibility to the environment as proposed by differential susceptibility theory (moderation hypothesis, Belsky et al., 2007; Ellis et al., 2011).

1.5. Study aims

This study examined (a) direct effects of family environment, peer victimization and HPA axis dysregulation on the level of emotional symptoms in a community sample of kindergarten children at ages 5 and 6; and (b) how these biological and social risk factors interact with each other to predict children's emotional

symptoms at ages 5 and 6. Specifically, we hypothesized that each of these factors would predict the level of emotional symptoms as orthogonal risk factors. For the interaction of social-relational and endocrinological risk factors, we hypothesized that the effects of an unfavorable family environment and peer victimization on emotional symptoms would be particularly pronounced in those children who also revealed signs of HPA axis dysregulation.

2. Method

2.1. Sample and procedure

The sample consisted of 166 five-year-old children (95 boys; mean age 5.23, $SD = 0.35$) visiting public kindergarten classes in different city districts of a medium-sized Swiss city (Basel). In this city, more than 90% of children enter the public school system at age 5 and have two years of kindergarten (at ages 5 and 6).

The participating families were mainly middle class, German speaking, and of European origin with nearly 50% of the parents holding university or college degrees.

All participants gave written informed consent after the nature of the procedures had been fully explained. The study was carried out in accordance with the latest version of the Declaration of Helsinki and approved by the ethics committee Basel.

The data were collected during a home or kindergarten visit. The children were longitudinally assessed at two assessment points, one in their first (age 5) and one in their second (age 6; mean age 6.2, $SD = 0.35$) year of kindergarten. A multi-informant assessment of children's emotional symptoms and social risk factors was applied. All children were interviewed individually in a separate room in their kindergarten or at home by a trained psychologist. After a warming-up activity (20 min), a story completion task (35 min) and a child interview were conducted. Immediately before and 30 min after the story completion task, saliva samples were collected to assess children's cortisol level.

Teachers and parents completed questionnaires.

2.2. Instruments and measures

2.2.1. Emotional symptoms

Parents and teachers completed the Emotional Symptoms scale of the Strengths and Difficulties Questionnaire (Goodman, 1997) which specifically comprises internalizing symptoms (anxiety symptoms, mood symptoms). The scale consists of five items that are rated on a 3-point scale (not true, somewhat true, definitely true). Internal consistency of the scale was acceptable ($\alpha = 0.65–0.75$).

To assess the child's self-report, we used the Berkeley Puppet Interview. This interview developed by Measelle et al. (1998), blends structured and clinical interviewing techniques to elicit children's self-perceptions. The interview was carried out using two identical hand puppets that made two opposing statements on a topic: e.g. "I am (not) a happy child". Then the child provided his or her own statement. The interview was videotaped and afterward scored by independent raters, who were blind to all other data. Each item was rated on a 7-point scale (1–3 = strong to mild agreement with the positive statement, 4 = neither positive nor negative, 5–7 = mild to strong agreement with the negative statement) plus 8 = uncodable, <0.3% of all items). Our interviewers were trained by the authors of the instrument. Inter-rater reliability was first established with the authors of the instrument and then for the raters of the research group (average intraclass correlation = 0.97, range 0.79–1.00). To link child reports of behavioral/emotional difficulties with parent and teacher reports, we aggregated the three Berkeley Puppet Interview subscales "depression", "separation anxiety", and "overanxiety" (Cronbach's $\alpha = 0.70–0.76$) to an Emotional Symptoms compound (20 items).

2.2.2. Combining multi-informant data

As recommended by Kraemer et al. (2003) and used before (Perren et al., 2006) we aggregated child, parent and teacher reports. To combine child, parent and teacher reports on symptoms, the average symptom scores of each informant were first z-standardized ($M = 0$, $SD = 1$). Then scores were averaged across informants (mean scores). The multi-informant scores were built when information from at least two different informants was available.

2.2.3. Social-relational risk factors

To assess the *emotional family environment*, parents completed the Family Climate Scales, a German version of the Family Environment Scales (Moos and Moos, 1981; Schneewind et al., 1985). We used the subscales cohesion (6 items, $\alpha = 0.62–0.68$), expressiveness (4 items; $\alpha = 0.52–0.58$) and conflict (8 items, $\alpha = 0.79$). Items were rated on a 4-point scale. The mean score of the subscales was used to assess the overall negative emotional family environment (high conflict, low cohesion, low expressiveness). *Peer victimization* was assessed by teacher and child reports. Teachers completed Peer Victimization scales (Perren and Alsaker, 2006) rating each child on four victimization items (physical, verbal, object-related, exclusion; e.g. "child is victimized verbally") on a 5-point rating scale (never, seldom, once or several times a month, once a week, several times a week; $\alpha = 0.62–0.65$). Children completed the Peer Victimization scale (e.g. "Kids at school tease me") of the Berkeley Puppet Interview. The scale consists of four items ($\alpha = 0.65–0.73$). The mean of the standardized teacher and child scores was used to indicate level of peer victimization.

2.2.4. Cortisol

In order to assess the children's cortisol response to a challenging task, we used the MacArthur Story Stem Battery (MSSB) which is extensively described elsewhere (von Klitzing et al., 2003) and validated for HPA responses Hatzinger et al., 2007. MSSB demands high ego involvement by confronting the child with standardized, developmentally appropriate beginnings of stressful stories to elicit relevant play narratives. The pre-task cortisol sample (baseline) was collected immediately prior to the story stem task, approximately 20 min into the visit. The post-test cortisol sample was collected about 30 min after completion of the task.

2.2.5. Saliva cortisol sampling technique and cortisol analysis

Saliva samples were obtained using the "Salivette" device for quick and hygienic sampling (Sarstedt, Nümbrecht/Germany), stored and analyzed as described in Hatzinger et al., 2007. This device consisted of a small cotton swab on which the subject gently chewed for 0.5–1 min. The swab was then transferred into a small plastic tube, the Salivette container, and stored in the freezer. Saliva samples were returned to the laboratory, where samples were centrifuged at 4 °C (2000 rpm, 10 min) and stored at −20 °C until assay. Free salivary cortisol concentrations were analyzed using a time-resolved immunoassay with fluorometric detection: "Coat-A-Count" Cortisol RIA from DPC (Diagnostics Products Corporation; obtained through H. Biermann GmbH, Bad Nauheim/Germany) as described in detail elsewhere (Dressendorfer et al., 1992; Tunn et al., 1992).

Most MSSB tests were conducted in the early afternoon, but because of the challenges of different kindergarten and home environments, not all assessments could take place at exactly the same time of day. To minimize the influence of different assessment times and settings, we controlled all cortisol-related analyses for time and place of assessment (home vs. kindergarten).

The first sampling was defined as *cortisol baseline*. In order to obtain cortisol reaction scores that were controlled for baseline values, we used the regression method described by Twisk (2003) and Smeekens et al. (2007): regression analysis was performed with post-task cortisol as the dependent variable and pre-test cortisol as the independent variable. Residuals were retained and defined as *cortisol increase*, which by definition was uncorrelated with cortisol baseline. Cortisol baseline and increase were computed for both assessment points (age 5 and age 6).

2.3. Statistical analysis

We calculated ANOVAs to analyze sex differences and Pearson correlation coefficients to analyze bivariate correlations between the different study variables. We used all available data for the bivariate analyses (N between 133 and 151). We executed linear regression analyses to analyze the main and the interaction effects of the hypothesized predictors of emotional symptoms. To include the interaction terms in the regression models, all variables were centered around the mean. In these multivariate analyses, we included only children with complete data-sets (listwise deletion; $N = 133$ at age 5, $N = 139$ at age 6). A level of $p < .05$ was considered as significant. All analyses were controlled for time and place of assessment as well as of children's sex.

3. Results

3.1. Participants

Based on the SDQ norms (Goodman, 1997), reports of parents and teachers were categorized as normal, borderline and abnormal scores. According to parent reports (teacher reports in parentheses), 10.5% (9.4%) of children had emotional symptoms in the abnormal range at age 5, 7.0% (5.3%) at age 6. According to child and teacher reports, 19.3% of the children were sometimes victimized by their peers at age 5, 12.3% at age 6 and 11.1% at both assessment points. According to parents' reports, 28.1% of the children were exposed to a negative family environment (mean >2) at age 5, 24.0% at age 6, and 11.7% at both assessment points. The average (absolute) increase in cortisol between pre- and post-test was slightly negative ($M = -0.15$ at age 5 and -0.07 at age 6), reflecting a slight average cortisol decrease from pre- to post-test, but with considerable variation between children ($SD = 1.06$ at age 5 and $SD = 0.77$ at age 6).

Girls and boys did not differ from each other in terms of emotional symptoms, family climate, peer victimization, cortisol baseline, or cortisol increase. At age six, but not at age five the cortisol baseline levels were significantly lower in children tested in the home environment as compared to children tested in the kindergarten environment. In contrast, the cortisol increase was not significantly different between the home and the kindergarten assessments.

3.2. Correlation analyses

To assess the stability of the emotional symptoms, cortisol levels and social-relational factors from age 5 to 6, and the associations between these variables, we computed bivariate Pearson correlations.

As can be seen in Table 1, negative family environment and peer victimization and cortisol levels were not significantly interrelated at age 5 or at age 6.

3.2.1. Cross-sectional at age 5

Emotional symptoms were significantly positively correlated with both negative family environment and peer victimization at age 5. The more the children experienced negative family environments and peer victimization, the higher were their levels of emotional symptoms. Neither cortisol baseline nor cortisol increase at age 5 were significantly correlated with emotional symptoms at age 5.

3.2.2. Cross-sectional at age 6

At age 6, emotional symptoms were significantly correlated with peer victimization, negative family environment, and cortisol increase, but not with cortisol baseline.

3.2.3. Longitudinal age 5 to age 6

Emotional symptoms, negative family environment and peer victimization showed moderate to high stability from the age of 5–6 years. There were no significant correlations between cortisol values at ages 5 and 6. Emotional symptoms at age 5 were significantly correlated with cortisol increase at age 6 and with negative family environment but not with peer victimization at age 6. Negative family environment and peer victimization at age 5 significantly predicted emotional symptoms at age 6. Children whose parents had described a negative family environment or who were frequently victimized at the age of 5 showed more emotional symptoms at the age of 6.

3.3. Regression analyses

3.3.1. Cross-sectional analyses

To answer the main research questions, we computed linear regression analyses to compare the specific predictive value of each of the social risk factors and cortisol for children's emotional

Table 1
Cross-sectional and longitudinal correlation between emotional symptoms, psychosocial risks and cortisol (baseline and increase).

	Age 5					Age 6				
	Emotional symptoms	Peer victimization	Negative family env.	Cortisol baseline	Cortisol increase	Emotional symptoms	Peer victimization	Negative family env.	Cortisol baseline	Cortisol increase
Age 5										
Emotional symptoms		.363**	.261**	−.044	.128	.583**	.051	.177*	.096	.240**
Peer victimization			−.031	.003	−.021	.263**	.334**	−.016	.080	.132
Negative family env.				−.164	.066	.144	−.062	.617**	−.048	−.010
Cortisol baseline					.000	−.028	.038	−.202*	.112	.055
Cortisol increase						.084	.126	−.027	.069	−.026
Age 6										
Emotional symptoms							.184*	.162*	.116	.311**
Peer victimization								.024	−.086	.155
Negative family environment									−.108	.053
Cortisol baseline										.000
Cortisol increase										

*$p < .05$; **$p < .01$.

symptoms at age 5 and age 6 separately, when controlled for the other variables. Peer victimization, family environment, cortisol baseline and increase, and children's sex served as independent variables. We also analyzed whether there were significant interactions between the social-relational risk factors and cortisol levels.

The results are shown in Tables 2 and 3.

At both assessment points, the analyses showed independent main effects of peer victimization ($\beta = .42$, $p < .001$ at age 5, $\beta = .21$, $p = .012$ at age 6) as well as of negative family environment ($\beta = .29$, $p < .001$ at age 5, $\beta = .20$, $p = .014$ at age 6). Peer victimization and negative family environment both independently predicted the level of emotional symptoms. The more frequently a child was victimized by peers or the more negative the family climate was, the more emotional symptoms were manifest in the child. At age 6 (not at age 5) we found a significant main effect of cortisol increase ($\beta = .23$, $p = .006$), but only when the interaction terms were not entered into the analysis. At no time point was cortisol baseline associated with emotional symptoms.

At age 6, a significant interaction effect of negative family environment × cortisol increase emerged ($\beta = .17$, $p = .045$). The negative family environment × cortisol baseline showed a trend toward significance ($\beta = .15$, $p = .086$). Entering the interaction terms reduced the predictive main effect of cortisol increase ($\beta = .16$, $p = .097$). The analyses did not show other significant interaction effects.

To demonstrate the significant interaction effect, we used the procedures of Aiken and West (1991), see Fig. 1. The lines represent associations (slopes) between the independent and dependent variables for high and low levels (±1 SD) of the moderator.

As can be seen in Fig. 1, in the group of children with high cortisol increases, a strongly negative family environment predicted high levels of emotional symptoms. In the group of children who showed a low or negative cortisol increase, negative family environment did not predict emotional symptoms. The children with high cortisol increase but low negative family environment exhibited the lowest number of emotional symptoms. No interaction effects between peer victimization and cortisol increase appeared (see Fig. 2).

3.3.2. *Longitudinal analyses*

Next, we computed a regression analysis to investigate whether risk factors at age 5 predicted the level of emotional symptoms at age 6 when controlled for the level of emotional symptoms at age 5 (predicting effect of emotional symptoms age 5: $\beta = .48$, $p < .001$). The results are shown in Table 4. The analysis showed a significant main effect of peer victimization ($\beta = .16$, $p = .047$) over and above the effect of emotional symptoms at age 5. Consequently, peer victimization predicted the increase of emotional symptoms from age 5 to 6. The higher the level of peer victimization at age 5, the higher the increase of emotional symptoms from age 5 to 6. Negative family environment showed a trend toward significance ($\beta = .14$, $p = .072$) in predicting the increase of emotional symptoms. There were no predictive associations between cortisol baseline or increase at age 5 and emotional symptoms at age 6.

Furthermore, the longitudinal analysis yielded a significant interaction between peer victimization and cortisol increase ($\beta = .27$, $p = .001$) which is portrayed in Fig. 3. In the group of children with low or negative cortisol increases at age 5, a high level of peer victimization at age 5 predicted an increase of emotional symptoms from age 5 to 6. In the group of children who showed high cortisol increases, peer victimization did not predict an increase of emotional symptoms. The children with low cortisol increase and low peer victimization exhibited a decrease of emotional symptoms.

Table 2
Regression analysis for emotional symptoms at age 5 (cross-sectional).

	B	SE B	β
Constant	0.20	0.30	
Peer victimization	0.38	0.07	.42**
Negative family environment	0.63	0.17	.29**
Cortisol baseline	−0.01	0.07	−.01
Cortisol increase	0.07	0.06	.10
Sex	−0.23	0.12	−.16 (*)
Peer victimization × Cortisol increase	−0.01	0.08	−.12
Negative family environment × Cortisol increase	−0.01	0.17	−.01

$R^2 = .27$**.
Note: (*)$p < .10$, *$p < .05$, **$p < .01$, $N = 133$.
Analyses were controlled for assessment time (morning, afternoon), place (home, school) and duration of the MSSB. None of these variables yielded significant results. All other interactions were not significant.

Table 3
Regression analysis for emotional symptoms at age 6 (cross-sectional).

	B	SE B	β
Constant	0.15	0.34	
Peer victimization	0.17	0.07	.21*
Negative family environment	0.36	0.14	.20*
Cortisol baseline	0.10	0.08	.12
Cortisol increase	0.10	0.06	.16 (*)
Sex	−0.21	0.11	−.17 (*)
Peer victimization × Cortisol increase	0.05	0.07	.07
Negative family environment × Cortisol increase	0.36	0.18	.17*

$R^2 = .25$**.
Note: (*)$p < .10$, *$p < .05$, **$p < .01$, $N = 139$.
Analyses were controlled for assessment time (morning, afternoon), place (home, school) and duration of the MSSB. None of these variables yielded significant results. All other interactions were not significant.

4. Discussion

The aim of this study was to evaluate the role of social-relational and neuroendocrinological risk factors in the development of emotional symptoms at preschool and early school age. In *cross-sectional analyses*, we found clear indications that a negative family environment and peer victimization experienced by the five- and six-year-olds were independently associated with emotional symptoms. The results for cortisol were less clear: the baseline

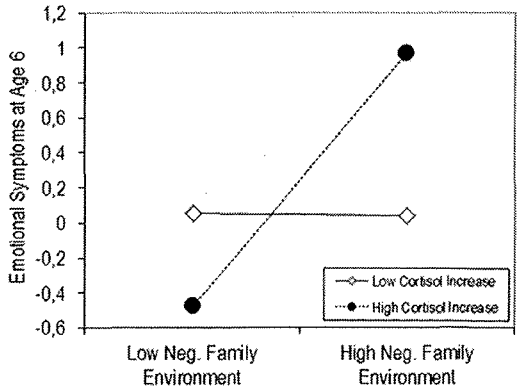

Fig. 1. Interaction effect negative family environment × cortisol increase (cross-sectional: age 6). To demonstrate the significant interaction effect, we used the procedures of Aiken and West (1991). The lines represent associations (slopes) between the independent and dependent variables (negative family environment and emotional symptoms) for high and low levels (±1 SD) of the moderator (cortisol increase).

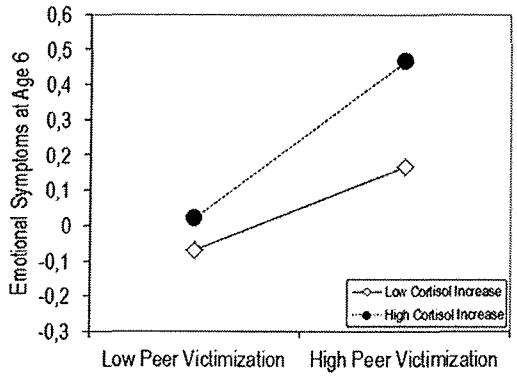

Fig. 2. Peer victimization × cortisol increase (cross-sectional: age 6). To demonstrate the absent interaction effect, we used the procedures of Aiken and West (1991). The lines represent associations (slopes) between the independent and dependent variables (peer victimization and emotional symptoms) for high and low levels (±1 SD) of the moderator (cortisol increase).

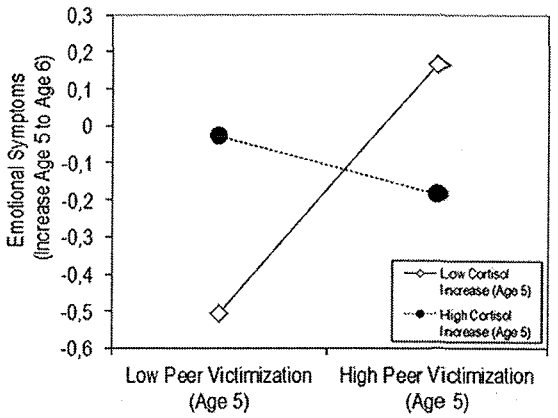

Fig. 3. Peer victimization × cortisol increase (longitudinal: age 5 to age 6). To demonstrate the significant interaction effect, we used the procedures of Aiken and West (1991). The lines represent associations (slopes) between the independent and dependent variables (peer victimization and increase of emotional symptoms) for high and low levels (±1 SD) of the moderator (cortisol increase).

saliva concentrations did not predict emotional symptoms at age 6, but the increase of cortisol during the narrative task did. Furthermore, cortisol increase moderated the association between negative family environment and emotional symptoms at age 6: the negative family environment was associated with emotional symptoms only in those children who showed a cortisol increase during the task. Accordingly, the increase in cortisol levels during and after the MSSB task was a more valid indicator of HPA axis dysregulation than baseline cortisol, which could be influenced by all kinds of factors, including the home vs. kindergarten environment.

Our study replicated the findings of several other studies (Coie and Dodge, 1998; Darling and Steinberg, 1993; Eisenberg and Fabes, 1998; Halpern, 2004; Laible et al., 2004), suggesting that the experience of disharmonic relationships in families puts children at risk of developing emotional symptoms such as depressive mood and/or anxiety. But not all children who experience this type of family environment manifested emotional symptoms. Living in an unfavorable family climate may have led to stress, and the way this stress was handled was crucially associated with these symptoms. Those children who exhibited low biological stress reactions while handling family conflict situations in narratives did not evidence emotional symptoms even when living in an unfavorable family environment. In contrast, those children who suffered from an unfavorable family environment and exhibited a strongly reactive HPA axis system during the narrative task showed higher symptom levels. The apparently meaningful differences of reactivity in the biological stress system seemed to represent differences of the neurobiological susceptibility to environmental influences leading to different pathways from family stress to developmental outcome. Interestingly, those children who exhibited a high HPA axis reactivity and lived in a positive family environment had the lowest levels of symptoms. At a first glance, this result may come as a surprise. Yet, it is in keeping with recent conceptualizations of differential susceptibility which claim that some individuals are "disproportionately vulnerable to adversity as also disproportionally likely to benefit from supporting and enriching environments" (Ellis et al., 2011, p. 14). Our findings support the view of Stadelmann et al. (2007) that the experience of an unfavorable family environment places children at risk of developing behavioral symptoms, especially when it is associated with the development of stressful internal representations and cognitive emotional styles.

Table 4
Regression analysis for emotional symptoms at age 6 (longitudinal).

	B	SE B	β
Constant	−0.14	0.24	
Emotional symptoms age 5	0.42	0.07	.48***
Peer victimization	0.13	0.06	.16*
Negative family environment	0.26	0.15	.14 (*)
Cortisol baseline	0.02	0.06	.03
Cortisol increase	0.03	0.05	.05
Sex	−0.05	0.10	−.03
Peer victimization × Cortisol increase	−0.21	0.06	−.27***
Negative family environment × Cortisol increase	0.16	0.14	.10

$R^2 = .43^{**}$.
Note: (*)$p < .10$, *$p < .05$, **$p < .01$, ***$p < .001$, $N = 129$.
Analyses were controlled for assessment time (morning, afternoon), place (home, school) and duration of the MSSB. None of these variables yielded significant results. All other interactions were not significant.

In the *longitudinal analyses* we found contrasting results: the experience of peer victimization (to a lesser, yet not significant extent also negative family environment) predicted an increase of emotional symptoms from age five to six. This predictive association was only extant in children who revealed a blunted cortisol response during the task at age 5. Thus, only a combination of victimization and blunted cortisol reaction at age 5 gave rise to increasing symptoms of anxiety, shyness, and depressive mood at age 6.

This result stands in contrast to our expectations that children with high HPA axis reactivity would be more susceptible to the stressful experience of peer victimization, similar to what we had found in the cross-sectional analysis with respect to the experience of negative family environment. But this finding is in accordance with the findings of Ouellet-Morin et al. (2011) that the experience of being bullied can lead to a blunted HPA axis activity and that this mechanism "is not restricted to persons with post-traumatic stress disorders" (p. 579). Also, it meshes with Badanes, Watamura, and Hankin's findings (2011), with the primary difference that their finding of hypocortisolism is on family not peer environments. Children whose experience of peer victimization is associated with an (excessive) down-regulation of the biological stress response may thus not be reacting appropriately to stress and therefore show elevated risk of developing further emotional symptoms.

The study has several *strengths*. First, the assessment of young children by interviewing multiple informants — parents, teachers, and the children themselves — (Kraemer et al., 2003) strengthens

the validity of the observed levels of emotional symptoms. Second, by using two longitudinal assessment points during an important developmental phase we could show the relative stability of the symptoms, as well as social risk factors. Third, the assessment of both biological *and* social-relational factors in the same study enabled us to investigate not only their direct effects, but also how they act in concert in a dynamic way.

Equally, some *limitations* deserve attention. First, the study was conducted in a mainly middle class community sample, in which only a relatively small number of children showed clinically relevant emotional symptoms. This certainly limits the power to inform risk for psychopathology. Second, as the study was executed in a sample of very young children, the possibilities of using well-validated stress tests in highly standardized settings was restricted. Instead, we had to accept compromises with respect to amount of triggering stress as well as standardization of time and place of the experimental situation. As expected, the baseline cortisol levels of the children tested in the home environment were lower compared to the children in the kindergarten setting, probably because they felt safer and less distressed. This may have concealed direct cortisol effects. The fact that the cortisol increase in response to the task did not differ significantly between home and kindergarten environment suggests that this measure was the more valid parameter with respect to the HPA axis function in the context of our study.

There are important clinical and public health implications: the finding that early emotional symptoms are significantly associated with adverse environmental contexts (negative family environment and peer victimization) urges clinicians to include the family and peer relationships into their diagnostic evaluations and intervention strategies. Especially the peer problems in preschool and kindergarten classes contributing to the development of emotional symptoms should not be discounted by teachers and preschool/school principals. The conclusion that children's variable susceptibility to adverse relational experiences seems to cause differential developmental outcomes presents clinicians with the challenge to improve their instruments of early diagnostic evaluation, especially with respect to the assessment of stress reactivity.

The complex interplay between social-relational risk factors, biological patterns, and internal cognitive emotional styles demands further study. Additional longitudinal research will help determine the influence of subsequent development (for example, into middle childhood and adolescence) on the socio-emotional as well as biological systems which may engender emotional symptoms and suffering.

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Contributors

Kai von Klitzing, Martin Hatzinger, Edith Holsboer-Trachsler, Serge Brand, Stephanie Stadelmann, and Sonja Perren designed the study and wrote the protocol. Annette Klein and Lars White managed the literature searches and analyses. Sonja Perren, Maureen Groeben and Annette Klein undertook the statistical analysis, and Kai von Klitzing wrote the first draft of the manuscript.

All authors contributed to and have approved the final manuscript.

Conflict of interest

All authors declare that they have no conflicts of interest.

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