J Abnorm Child Psychol (2016) 44:859–870
DOI 10.1007/s10802-015-0087-7

Physiological and Behavioral Vulnerability Markers Increase Risk to Early Life Stress in Preschool-Aged Children

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Published online: 30 September 2015

Abstract The study examined whether child physiological (cortisol reactivity) and behavioral (negative emotionality) risk factors moderate associations between the early rearing environment, as measured by child exposure to maternal depression and stressful life events, and preschool psychopathology and psychosocial functioning. A sample of 156 preschool-aged children (77 boys, 79 girls; age M= 49.80 months, SD=9.57, range: 36–71) participated in an observational assessment of temperament and was exposed to a stress-inducing laboratory task, during which we obtained five salivary cortisol samples. Parents completed clinical interviews to assess child and parent psychopathology and stressful life events. Results indicated that the combination of a blunted pattern of cortisol reactivity and recent stressful life events was associated with higher levels of preschoolers' externalizing symptoms and lower psychosocial functioning. In addition, greater life stress was associated with higher levels of preschoolers' internalizing symptoms. Lastly, children with high levels of negative emotionality and who were exposed to maternal depression had the lowest social competence. Our findings highlight the critical role of the early environment, particularly for children with identified risk factors, and add to our understanding of pathways involved in early emerging psychopathology and impairment.

Keywords Risk · Vulnerability · Preschool psychopathology · Cortisol reactivity · Temperament · Negative emotionality

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Children's emotional and behavioral problems are ranked among the top five causes of impairment among chronic conditions in the United States (Halfon et al. 2012). Developmental psychopathology research has begun to focus attention on early childhood as findings suggest that psychiatric illnesses have their roots early in life. Psychopathology in preschoolers occurs at similar rates to those observed in older youth, is associated with significant impairment, and persists across childhood (Dougherty et al. 2015). Given the greater behavioral and neural plasticity during early childhood, there is a pressing need to identify the mechanisms and processes underlying risk to inform the development of more effective interventions for young children.

Environmental Risk

Life stress has been shown to play a key role in the etiology and course of psychopathology across the lifespan (e.g., Monroe and Reid 2009). Specifically, adverse early life experiences consistently have been linked to increased risk for emotional and behavioral problems and poorer psychosocial functioning in children, adolescents, and adults (for a review see Goodyer 1993). Although research with preschoolers is limited, some emerging evidence has demonstrated that exposure to early stressful life events (e.g., household financial strain, death of a loved one, peer rejection, parent–child conflict) plays a significant role in the development of preschool psychopathology (e.g., Luby et al. 2006). Maternal depression history is another potent predictor of offspring's long-term negative outcomes, including increased rates of psychopathology, social impairment, physical illness, suicide, and mortality (Weissman et al. 2006). Notably, a child's exposure to maternal depression, particularly during early childhood, has been liked to increased risk for emotional and behavioral problems

in early childhood (Brennan et al. 2008; Dougherty et al. 2013), as well as later childhood and adolescence (Bagner et al. 2010; Hay et al. 2008). This is likely due, at least in part, to the associated disruptions in parenting and the early rearing environment, including marital and financial problems and inadequate social support (Goodman and Gotlib 1999). Taken together, although there is support that exposure to stress in the early rearing environment increases a young child's risk, exposure to these stressors does not universally lead to maladjustment, highlighting the complexity of early stress-psychopathology associations.

Vulnerability-stress models of psychopathology (Monroe and Simons 1991; Zuckerman 1999) have proposed that person-level characteristics increase certain individuals' risk for maladaptation when exposed to environmental adversity. A considerable research base has supported vulnerability-stress models in older youth and adults for multiple forms of psychopathology (Belsky and Pluess 2009; Ingram and Price 2009); however, a limited amount of research has extended these models to preschool mental health problems. Thus, the current study examined whether early biological and behavioral child-level risk factors moderate associations between the early rearing environment, as measured by recent life stress and child exposure to maternal depression during the first few years of life, and preschoolers' emotional and behavioral problems and psychosocial functioning. Specifically, we focused on two proposed markers of child-level risk: physiological stress reactivity and temperamental negative emotionality (NE).

Child-Level Risk

Stress Reactivity One aspect of child-level physiological risk that has received increasing attention is stress reactivity. When humans are faced with stress, an integrated complex system of neurobiological processes, involving the hypothalamic-pituitary-adrenal (HPA) axis, is activated and results in the synthesis and release of the stress hormone cortisol. Abnormalities in the cortisol response to a psychosocial stressor have been linked concurrently (for a review see Gunnar and Vazquez 2006) and prospectively (e.g., Morris et al. 2012) to psychopathology in youth and adults. Recent work has begun to examine how HPA axis reactivity differentially influences associations between environmental factors and youths' emotional and behavioral problems, and findings suggest that dysregulated cortisol reactivity moderates associations between family stress and children's psychological functioning in both preschool-aged children (Hastings et al. 2011; Klitzing et al. 2012; Obradovic et al. 2010) and older youth (Badanes et al. 2011; Rudolph et al. 2011; Saxbe et al. 2012). However, findings have been mixed with studies showing both increased (Hastings et al. 2011; Obradovic et al. 2010; Rudolph et al. 2011; Saxbe et al. 2012) and blunted (Badanes et al. 2011; Klitzing et al. 2012) cortisol reactivity as potential child-level vulnerability markers. Although this work provides promising support for cortisol reactivity as a marker for increased risk, even in preschool-aged children, significant methodological limitations remain. First, assessments of preschoolers' cortisol reactivity have been limited by collecting cortisol samplings at two time points (Hastings et al. 2011; Klitzing et al. 2012; Obradovic et al. 2010) or three time points only (Badanes et al. 2011). Without multiple post-stress cortisol samples, these assessments limit the extent to which studies capture peak cortisol responses and the downregulation of the cortisol response, which may contribute to the conflicting findings. In addition, no study in preschoolers has examined whether the findings are consistent across measures of internalizing and externalizing symptoms and psychosocial functioning. Thus, the current study aimed to address these limitations by employing a rigorous stress assessment that includes the collection of one pre-stress cortisol sample followed by four post-stress cortisol samples and by including measures of preschoolers' internalizing and externalizing symptoms and psychosocial functioning.

Negative Emotionality (NE) Individual differences in children's temperament, particularly child NE, have been examined as early-emerging vulnerability markers. Temperament refers to patterns of behavioral and emotional reactivity and self-regulation processes that are relatively stable and are rooted in part in early developing biological systems (Rothbart and Bates 2006; Shiner et al. 2012). NE, one of the primary higher-order temperament dimensions identified in children and adults, reflects a tendency to respond to novel and frustrating situations with sadness, anger, fear, and distress (Rothbart and Bates 2006). NE has been linked concurrently and prospectively with children's internalizing and externalizing psychopathology and lower social competence and status (for reviews see Dougherty 2004 and Rothbart and Bates 2006). Findings suggest that children with temperaments characterized by high levels of NE are more likely to exhibit higher levels of psychological problems within the context of harsh parenting than children with easy temperaments (Belsky et al. 1998; Morris et al. 2002; van Aken et al. 2007). However, these studies generally have relied on parent-report measures of child temperament (e.g., Morris et al. 2002; van Aken et al. 2007), which are subject to informant biases and shared method variance with parent report measures of psychopathology. A limited number of studies have included observational measures of temperament. Observational laboratory measures, although time consuming and limited to a single context, provide a more objective and controlled approach to assessing temperament in young children. In addition, the majority of contextual-risk assessments have focused specifically on negative parenting behaviors, without

considering the child's broader exposure to multiple stressors in his or her environment. Furthermore, despite evidence linking maternal depression to increased risk in their offspring (Weissman et al. 2006) and evidence linking NE and depression (Kendler et al. 1993), no research to date has examined the moderating role of child NE on the association between exposure to maternal depression and children's adjustment.

Multiple Levels of Analysis

Much of the literature has examined one level of the hypothesized person-level moderator in associations between environmental risk and adjustment, which thwarts the opportunity to assess whether different levels of measurement (physiological vs. behavioral) reflect a common, shared sensitivity to the environment. Given that temperament is proposed to be at least partially biologically based and links, albeit small, between child temperament and neuroendocrine function have been observed (Gunnar and Vazquez 2006), it is possible that cortisol reactivity and temperament may reflect a common vulnerability to stressful environments (e.g., Boyce et al. 2006). Conversely, physiological and behavioral vulnerability markers may reflect distinct risks to adverse environmental experiences (e.g., Essex et al. 2011). Thus, it is important to examine early markers of risk across multiple levels of analysis.

Current Study

The current study examined the moderating role of young children's cortisol reactivity and temperamental NE on associations between negative features of the early rearing environment and preschoolers' internalizing and externalizing symptoms and psychosocial functioning. To minimize the effects of shared method variance, we used observational assessments of temperament, a comprehensive physiological stress assessment, and clinical interviews of parent and child psychopathology and life stress. Based on research demonstrating that child cortisol reactivity and child NE may render youth more vulnerable to negative outcomes in the face of stress, we hypothesized that preschoolers with abnormalities in cortisol reactivity and high NE would exhibit the greatest symptoms and impairment in high levels of early adversity, as measured by exposure to maternal depression and recent life stress. As both elevated and blunted cortisol reactivity have been linked to risk for psychopathology, we did not have an a priori hypothesis regarding whether increased or blunted cortisol reactivity would confer greater risk.

Method

Participants

The sample consisted of 175 children and parents recruited from the Washington, DC metropolitan area (Dougherty et al. 2013). Potential participants were identified using advertisements (73.1 %) and a commercial mailing list (26.9 %). In our recruitment efforts to obtain a high-risk sample of young offspring of depressed parents, we targeted a subsample of parents with a history of depression using advertisements. Included families had a child between 3 and 5 years of age, who had no significant medical condition or developmental disability, with no parental history of bipolar or psychotic disorder, and who lived with at least one English-speaking biological parent. Of the 175 children participating in the study, one child did not speak English well enough to understand the laboratory tasks and two children had a parent with a lifetime history of bipolar disorder-not otherwise specified and were excluded from the study. Sixteen (9.1 %) children did not return for the second laboratory visit that included the clinical interview on child psychopathology. Thus, the total sample for this study included 156 preschool-aged children (77 boys, 79 girls).

Children's mean age was 49.80 months (SD=9.57; range: 36–71). The sample was racially diverse: White (n=74, 48.4 %), Black/African-American (n=53, 34.6 %), Asian (n=3, 2.0 %), or multi-racial/other race (n=23, 15.0 %); 26 (17.1 %) children were of Hispanic/Latino descent. Mothers' mean age was 34.94 years (SD=6.15, range: 21–50), and fathers' mean age was 37.18 years (SD= 6.86; range=20–57). Participating families reported a range of family incomes: less than $20,000 (7.3 %), $20,001–$40,000 (8.7 %), $40,001–$70,000 (20.7 %), $70,001–$100,000 (28.7 %), and greater than $100,001 (34.7 %). The majority of children had at least one parent with a 4 year-college degree (n=108, 69.2 %) and lived in a two-parent household (n=112, 71.8 %).

Procedures

This study was approved by the human subjects review board, and informed consent was obtained from parents. At the first laboratory visit, children participated in an observational temperament assessment battery to assess child NE. Approximately 1 to 2 months later (M=33.74 days, SD= 37.88), families returned to the laboratory, and children participated in a psychological stressor paradigm, during which we collected salivary cortisol samples to assess child stress reactivity. At the second visit, parents were interviewed using a structured clinical interview to assess child psychopathology, stressful life events, and psychosocial functioning. In between laboratory sessions, mothers completed a structured clinical interview on the telephone to assess their psychiatric history.

Measures

Child NE All 156 children interacted with a female experimenter in eight standardized tasks from the Laboratory Temperament Assessment Battery (Lab-TAB; Goldsmith et al. 1995). In an independent sample, Lab-TAB ratings of temperamental NE demonstrated moderate stability from ages 3 to 7 (r=0.45) and moderate concurrent and longitudinal associations with home observations (Durbin et al. 2007). Episodes were ordered to prevent carry-over effects. Between episodes the child took brief play breaks with the experimenter to allow for return to a neutral state. Parents were present in the observation room for all episodes except *Pop-up Snakes* and *Box Empty* (see below). The episodes, in order of presentation, were*:* (1) *Make that Car Go* – child and experimenter raced two remote-controlled cars, (2) *Transparent Box* – child was given inoperable keys to open a transparent box that contained an appealing toy, (3) *Exploring New Objects* – child was presented with the opportunity to explore novel stimuli, (4) *Pop-up Snakes* – child surprised his/her parent with what appeared to be a can of potato chips, but actually contained coiled spring snakes, (5) *Impossibly Perfect Green Circles* – experimenter asked the child to draw several circles, mildly criticizing each one, *(6) Popping Bubbles* – child and experimenter played together with a bubble toy, (7) *Snack Delay* – child was instructed to wait for the experimenter to ring a bell before eating a small snack, and (8) *Box Empty* – child was given a wrapped empty box to open, under the assumption that a prize was inside.

Episodes were videotaped and scored using a detailed manual (e.g., Dougherty et al. 2011; Olino et al. 2010). Experienced coders who demonstrated reliability using the coding system on a previously coded independent sample (Dougherty et al. 2011; Olino et al. 2010) trained all novice coders. Novice coders first watched videos from a previously coded, independent sample to become familiar with children's behaviors in specific laboratory contexts. Second, experienced coders held weekly individual and group coding meetings to teach the coding system. When the coders demonstrated accurate coding compared to the previously coded sample and our experienced coders, the coders coded independently and continued to attend weekly coding meeting where every 10th participant of each coder was reviewed with an experienced coder to monitor rating drift. The coding system considered facial, bodily and vocal indicators of fear, sadness and anger. Each display of facial, bodily and vocal affect in each episode was rated on a three-point scale (low, moderate, high). Ratings were summed separately within each channel (facial, bodily, vocal) across the eight episodes for sadness and anger, standardized, and summed across the three channels to derive total scores for sadness and anger. The fear variable was created using the same procedure across three episodes (Exploring New Objects, Pop-up Snakes, and Box Empty). We calculated an aggregate NE variable, which consisted of averaging ratings of facial, bodily, and vocal anger, sadness, and fear. Internal consistency (Cronbach alpha = 0.79) and interrater reliability (intraclass correlation coefficient [ICC] = 0.83, n=15) for NE were good.

Cortisol Reactivity During the second laboratory session, we assessed children's cortisol reactivity using an acute psychological stressor paradigm that has been demonstrated to elicit a mean increase in cortisol in preschool-aged children (Kryski et al. 2011). The stressor paradigm consisted of a timed matching task consisting of three trials (M=8.1 min, SD= 1.8; for a complete description of the task see Tolep and Dougherty 2014). Children were told if they successfully completed the task, they would win their chosen prize. Children were told that they had 3 min to complete a matching trial, but the experimenter manipulated the timer such that children failed each trial. To elicit feelings of social evaluative threat, the experimenter maintained a neutral expression while sitting with a clipboard and pretending to take notes on the child's performance. Furthermore, children were told that the matching task was easy and that even little kids could finish in the allotted time. At the end of the third failed trial, the experimenter informed children that the timer was broken and provided children with a prize for their efforts. Cortisol samples were obtained prior to the start of the task following a 30-min acclimation period to the laboratory, and then at 20, 30, 40, and 50 min following the completion of the stressor task (i.e., after the third failed trial and before receipt of the prize). Parents were asked to refrain from feeding their child for 1 h and from giving their child caffeine for 2 h prior to the session. No children were taking corticosteroids.

Saliva samples were obtained by having children dip a cotton dental roll (Richmond Sterile Cotton Dental Roll) into a few grains (0.025 g) of Kool-Aid® mix. The children then placed the cotton roll in their mouths and were instructed to chew on the dental roll for 1 min or until saturated. The wet cotton was expressed into an Eppendorf Safe-Lock 1.5 ml micro tube. Tubes were kept frozen at −20 °C until assayed in duplicate using a time-resolved fluorescence immunoassay with flourometric end-point detection (DELFIA). Salivary cortisol samples were assayed at the Biochemical Laboratory at the University of Trier, Germany. The use of the oral stimulant was carefully monitored across all samples. The procedures employed here have been shown to yield little-to-no effect on cortisol concentrations (Talge et al. 2005). Inter- and intra-assay coefficients of variation were 7.1–9.0 % and 4.0–6.7 %, respectively. Of the 156 children, eight were excluded due to the following reasons: one child did not provide cortisol reactivity samples, four children had consistently extreme cortisol values (>3 *SD* above the mean; Gunnar

and White 2001), and three children were sick with a fever or currently taking antibiotic medication. Thus, 148 children's data were used in analyses involving cortisol.

To derive a measure of cortisol reactivity, we calculated the area under the curve with respect to the increase (AUCi) in cortisol using raw cortisol values, which estimates the total change in cortisol across the five cortisol samplings (Pruessner et al. 2003). As a common indicator of cortisol reactivity in psychoneuroendocrine research, the AUCi provides a summary statistic of change in cortisol responses without losing information gained from multiple measurements (Pruessner et al. 2003).

Child Exposure to Maternal Depression Children's biological mothers were interviewed using the Structured Clinical Interview for DSM-IV, Non-Patient version (SCID-NP; First et al. 1996). The SCID is among the most widely used diagnostic interviews, and its interrater reliability and procedural validity have been well documented (Williams et al. 1992). Interviews were conducted by telephone by a master's level rater with extensive training in the SCID. SCIDs were obtained from 154 (98.7 %) mothers. Major depressive disorder (MDD) and dysthymic disorder (DD) were collapsed into a single category reflecting depressive disorder. Based on audiotapes of 16 SCID interviews, the kappa for inter-rater reliability was 1.00 for lifetime depressive disorder. If a mother had a lifetime depressive disorder based on the SCID (n=76), the onset and offset dates of all episodes were recorded to determine whether the mother had depression during the child's life. A life event calendar approach was used to aid recall (Belli et al. 2001). A similar life event calendar approach yielded 92.5 % accurate recall of the timing of depressive episodes in a 1-month test-retest study of 10-year retrospective reporting of psychiatric symptoms (Kim-Cohen et al. 2005). The total number of months the child was exposed to maternal depression during his or her life was summed and divided by the child's age in months to yield the total proportion of offspring exposure across the child's life. Of the 47 children exposed to maternal depression, the average proportion of exposure was 0.49 (SD=0.36, range: 0.02–1.00).

Life Stressors Recent stressful life events were assessed using the Preschool Age Psychiatric Assessment (PAPA; Version 1.4; Egger et al. 1999) interview. Primary caregivers were asked whether 32 major life events (e.g., parental separation/divorce; parental arrest; moving to a new place; reductions in standard of living; loss of a home; hospitalization of parent; serious accident or hospitalization of child) occurred during the child's life, the extent to which the child was affected by the event, and the date of the event. The interviewer determined whether the parent's description matched the defined criteria. For the purposes of this study, we summed the total number of major stressful life events that occurred within the 12 months prior to the PAPA interview (M=2.38, SD=1.71; ICC=0.95, n=15).

Child Psychiatric Symptoms Children's emotional and behavioral symptoms were assessed using the PAPA, a structured psychiatric diagnostic interview with parents. The PAPA is a parent-reported interview that assesses a comprehensive set of symptoms from the DSM-IV-TR in young children (2–6 years-old) during the past 3 months. The PAPA follows a required set of questions and probes, but symptoms are only endorsed when they meet the criteria, as outlined in the extensive glossary. For information on the interview's psychometric properties, see Egger et al. (2006). Previous studies have supported the reliability and validity of the PAPA (e.g., Bufferd et al. 2011, 2012; Dougherty et al. 2011; Luby et al. 2009).

All interviews were conducted by trained graduate students. Primary caregivers (142 mothers, 9 fathers, 5 both parents) provided diagnostic information on all 156 children. Dimensional symptom scales for depression (MDD, DD, or depression-not otherwise specified [NOS]), anxiety (specific phobia, separation anxiety, social phobia, generalized anxiety disorder, agoraphobia, panic disorder, selective mutism), attention-deficit/hyperactivity disorder (ADHD), and oppositional defiant disorder (ODD) were created by summing items in each diagnostic category. We created total internalizing (M= 16.19, SD= 9.38) and externalizing (M= 7.00, SD= 5.20) symptom scales by summing the depression and anxiety symptom scales, and the ADHD and ODD symptom scales, respectively. Internal consistency and inter-rater reliability were good for the internalizing (α=0.83; ICC=0.96, n=15) and externalizing (α=0.83; ICC= 0.92, n=15) symptom scales.

Child Psychosocial Functioning Two measures of psychosocial functioning were assessed. First, following the completion of the PAPA, the interviewer rated children's overall level of psychosocial functioning using the Children's Global Assessment Scale (CGAS; Shaffer et al. 1983). CGAS scores range from 0 to 100, where 0 reflects the worst functioning and 100 reflects superior functioning (M= 72.33, SD= 13.95, ICC=0.78). CGAS scores demonstrate good convergent validity with clinician-rated child competence (Green et al. 1994) and differentiate treatment referred from non-referred children (Bird et al. 1987). Second, children's social competence was assessed using the Ratings of Children's Behaviors Scale (Eisenberg et al. 1993). Parents (n=149) rated children's general social skills on a

seven-item four-point response scale, with higher scores reflecting higher social competence (M=24.02, SD=3.02, α=0.68).

Data Analysis Plan

The study had four dependent variables: children's internalizing symptoms, externalizing symptoms, psychosocial functioning, and social competence. First, we examined correlations between demographic variables (child age, gender, race, family income, parental education, and single-parent household) and the dependent variables. Only child age and gender were associated with the dependent variables, and thus were included as covariates in all models. To test our hypothesis, we performed multiple regression analyses examining the main and interactive effects of early adverse environmental factors (recent life stress and child exposure to maternal depression) and child-level risk factors (cortisol reactivity and child NE). Separate models were run for each of the dependent variables. Significant interactions were probed using simple slopes tests according to Aiken and West (1991)'s guidelines. Furthermore, Hayes and Matthes' guidelines (Hayes and Matthes 2009) were used to test regions of significance according to the Johnson-Neyman technique (Johnson and Fay 1950). This approach uses the asymptotic variances, covariances, and other regression parameters to determine the upper and lower boundaries of the moderator at which the relation between independent and dependent variable is significant (p<0.05). In the current study, child-level risk factors were the moderators, and thus testing regions of significance revealed at which levels of child cortisol reactivity or child NE, early experiences were significantly associated with child outcomes.

Results

Means, standard deviations, and correlations among all study variables are presented in Table 1. Child age was positively associated with children's social competence, and child gender was significantly related to children's externalizing symptoms, psychosocial functioning, and social competence. Boys were reported to have more externalizing symptoms (M=8.00, SD=5.90) than girls (M=6.03, SD=4.22), t(137.45)=2.40, p=0.018; lower psychosocial functioning (M=69.84, SD=15.51) than girls (M=74.75, SD=11.85), t(142.22)=−2.21, p=0.028; and lower social competence (M=23.23, SD=3.22) than girls (M=24.77, SD=2.63), t(147)=−3.21, p=0.002. Thus, child gender and age were included as covariates. Child cortisol reactivity and NE were not significantly correlated.

Child-Level Risk, Early Experience, and Preschoolers' Symptoms and Functioning

Results of the multiple linear regression models are presented in Table 2. Main effects for child-level risk factors and early experience, and interactive effects between child-level risk factors and early experiences were examined for each of the outcomes. There were significant main effects for recent life stressors and child exposure to maternal depression. Specifically, children who experienced more stressful life events had higher internalizing and externalizing symptoms and worse psychosocial functioning. Child total exposure to maternal depression was associated with children's lower social competence.

As seen in Table 2, we observed a significant interaction between children's cortisol reactivity and recent stressors on children's externalizing symptoms. Figure 1a shows that for children with low levels of cortisol reactivity, there was a significant positive association between recent stressors and externalizing symptoms (B=2.13, SE=0.64, p=0.001), whereas for children with higher levels of cortisol reactivity there was no significant association between recent stressors and externalizing symptoms (B=0.01, SE=0.60, p=0.993). To determine the degree of children's cortisol reactivity at which the association between recent stressors and externalizing symptoms was statistically significant, we identified the regions of significance for children's cortisol reactivity. We found that for children with cortisol reactivity at levels less than −0.01 (standardized z-score), recent stressors were significantly positively associated with externalizing symptoms.

The interaction between children's cortisol reactivity and recent stressors was also significantly associated with children's psychosocial functioning. Figure 1b shows that for children with low cortisol reactivity, there was a significant negative association between recent stressors and psychosocial functioning (B=−6.56, SE=1.68, p<0.001), whereas for children with high cortisol reactivity, there was no significant association between recent stressors and psychosocial functioning (B=−1.65, SE=1.57, p=0.295). Regions of significance tests indicated that at levels of children's cortisol reactivity less than 1.02 (standardized z-score), recent stressors were significantly associated with poorer psychosocial functioning.

As seen in Table 2, we also observed a significant interaction between child NE and child cumulative exposure to maternal depression on children's social

Table 1 Correlations among all study variables

	1	2	3	4	5	6	7	8	9	10	11	12	13
1. Child cortisol reactivity	–												
2. Child NE	−0.05	–											
3. Cumulative maternal depression exposure	−0.04	−0.02	–										
4. Recent life stressors	0.04	−0.03	0.13	–									
5. Child internalizing symptoms	−0.03	−0.10	−0.03	**0.29****	–								
6. Child externalizing symptoms	−0.02	−0.03	0.15	**0.16***	**0.43****	–							
7. Child psychosocial functioning	0.02	−0.02	−0.20	**−0.27****	**−0.58****	**−0.68****	–						
8. Child social competence	0.03	**−0.17***	**−0.18***	0.04	−0.14	**−0.33****	**0.39****	–					
9. Child age	−0.07	**−0.17***	0.04	0.03	−0.08	0.01	0.02	**0.20***	–				
10. Child gender	−0.08	**−0.19***	−0.06	−0.05	0.12	**−0.19***	**0.18***	**0.26****	−0.02	–			
11. Child race	−0.08	**0.17***	0.08	**0.17***	0.16	0.05	−0.04	−0.08	−0.03	−0.01	–		
12. Parent education	−0.12	−0.16	−0.15	−0.14	0.01	−0.03	−0.02	0.05	0.08	0.14	**−0.33****	–	
13. Family income	0.01	**−0.18***	−0.12	−0.16	0.05	−0.03	0.00	0.00	−0.01	0.06	**−0.32****	**0.55****	–
14. Single parent	0.01	0.03	**0.27****	0.14	0.04	0.04	−0.09	0.11	0.06	−0.01	**0.32****	**−0.29****	**−0.60****
Mean	−1.50	−0.01	0.15	2.38	16.19	7.00	72.33	24.02	49.80	–	–	–	–
(SD)	(10.25)	(0.69)	(0.30)	(1.71)	(9.38)	(5.20)	(13.95)	(3.02)	9.57	–	–	–	–
N	148	148	147	148	148	148	148	142	148	148	145	148	142

NE = negative emotionality; child age reported in months; child gender: male = 1 (n=77), female = 2 (n=79); child race: White = 0 (n=71) and non-White = 1 (n=74); parent education: 0 = neither parent with 4 year college degree (n=44) and 1 = at least one parent with 4 year college degree (n=104); family income: 1 = < $20,000 ($n$=9), 2 = $20,001–$40,000 (n=13), 3 = $40,001–$70,000 (n=27), 4 = $70,001–$100,000 (n=41), 5 = > $100,000 ($n$=52); single parent household: 0 = two-parent household (n=115), 1 = single-parent household (n=33); * p<0.05, ** p<0.01

competence. Figure 2 shows that for children with high levels of NE, there was a significant negative association between child cumulative exposure to maternal depression and children's social competence (B=−1.19, SE=0.38, p=0.002), whereas for children with low levels of NE, there was no association between child cumulative exposure to maternal depression and social competence (B=0.03, SE=0.34, p=0.928). Regions of significance tests indicated that at levels of children's NE greater than −0.12 (standardized z-score), greater child cumulative exposure to maternal depression was significantly associated with lower social competence.

Table 2 Child-level risk, early experience, and preschoolers' symptoms and functioning

	Internalizing symptoms		Externalizing symptoms		Psychosocial functioning		Social competence	
	b (SE)	B	b (SE)	B	b (SE)	β	b (SE)	β
Child age	−0.87 (0.82)	−0.09	−0.06 (0.45)	−0.01	0.46 (1.18)	0.03	0.55 (0.25)	**0.17***
Child gender	2.23 (1.60)	0.12	−1.73 (0.88)	−0.17	3.61 (2.30)	0.13	1.11 (0.50)	**0.18***
Maternal depression exposure	−0.57 (0.80)	−0.06	0.63 (0.44)	0.12	−2.19 (1.16)	−0.15	−0.57 (0.25)	**−0.19***
Life stress	2.75 (0.81)	**0.29****	0.99 (0.44)	**0.19***	−3.87 (1.16)	**−0.27****	0.21 (0.25)	0.07
Child NE	−0.75 (0.79)	−0.08	−0.33 (0.44)	−0.06	0.14 (1.14)	0.01	−0.38 (0.24)	−0.13
Child CR	−0.46 (0.92)	−0.04	−0.41 (0.51)	−0.07	1.17 (1.33)	0.07	0.19 (0.28)	0.06
Child NE x maternal depression exposure	0.09 (0.92)	0.01	0.58 (0.50)	0.10	−2.00 (1.32)	−0.12	−0.62 (0.28)	**−0.18***
Child NE x stress	0.06 (0.76)	0.01	−0.33 (0.42)	−0.07	0.08 (1.01)	0.01	−0.22 (0.23)	−0.08
Child CR x maternal depression exposure	−0.67 (0.95)	−0.06	0.27 (0.52)	0.04	−1.12 (1.37)	−0.07	0.06 (0.30)	0.09
Child CR x stress	−0.53 (0.85)	−0.05	−1.06 (0.47)	**−0.19***	2.48 (1.22)	**0.17***	0.29 (0.26)	0.09
	R^2=0.12 F(10,136)=1.83†		R^2=0.13 F(10,136)=1.98*		R^2=0.17 F(10,136)=2.70**		R^2=0.19, F(10,131)=3.08**	

Child gender: male = 1, female = 2; CR = cortisol reactivity; NE = negative emotionality; † p<0.10, * p<0.05, ** p<0.01

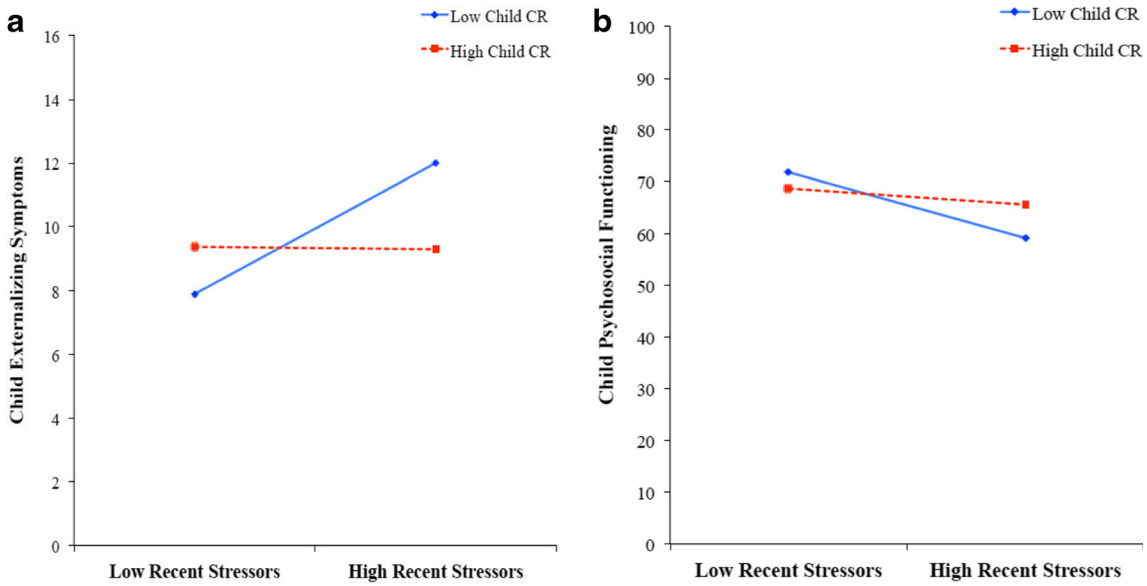

Fig. 1 **a**. The interaction between cortisol reactivity and recent stressors on preschoolers' externalizing symptoms. **b**. The interaction between cortisol reactivity and recent stressors on preschoolers' psychosocial functioning

No significant interactions were observed for preschoolers' internalizing symptoms.

Discussion

This study tested the moderating role of child physiological and behavioral risk factors on associations between early environmental stress and preschoolers' psychopathology and psychosocial functioning. Child physiological and behavioral risk factors were not significantly correlated, and distinct patterns of moderation emerged for child cortisol reactivity and observed NE. Preschool-aged children with low cortisol reactivity who experienced high stress in the past year evidenced more externalizing symptoms and greater functional impairment. In addition, children with high levels of observed NE who were exposed to maternal depression during the first few years of life evidenced poorer social competence. Although no moderated effects were observed for preschoolers' internalizing symptoms, higher levels of recent life stress were associated with preschoolers' greater internalizing symptoms. Our findings suggest that children's physiological and behavioral reactivity reflect separate pathways of risk to environmental influences rather than a shared, common system of sensitivity. Moreover, our findings are consistent with research in older youth and adults documenting that child-level risk factors increase risk for psychopathology in the face of stress, and highlight the critical importance of examining the joint interactive effects of person-level and context-level risks on young children's emotional and behavioral outcomes.

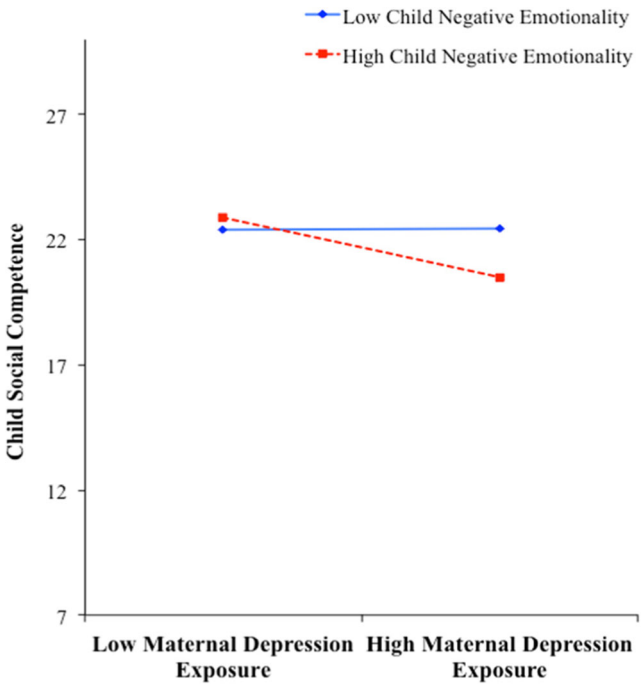

Fig. 2 The interaction between child negative emotionality and child cumulative exposure to maternal depression on preschoolers' social competence

Child Cortisol Reactivity

We found that for preschoolers with low cortisol reactivity, life stress in the past year was associated with greater externalizing symptoms and poorer psychosocial functioning, whereas for preschoolers with high cortisol reactivity, life stress was not associated with symptoms or impairment. Previous research has demonstrated links between blunted cortisol levels and preschoolers' externalizing behavior problems, although

the findings have been mixed (Alink et al. 2008). While we did not observe main effects of blunted cortisol reactivity in our sample of preschoolers, our findings are consistent with two studies that supported blunted cortisol as a vulnerability marker in the context of stressful environments. Badanes and colleagues (2011) found that in a sample of third, sixth, and ninth grade children, the combination of children's blunted cortisol reactivity and family stress predicted increases in depressive symptoms over time. Moreover, Klitzing and colleagues (2012) found that the combination of blunted cortisol reactivity and peer victimization in 5-year old children predicted greater emotional symptoms. While neither of these studies examined children's externalizing symptoms, they both support blunted cortisol reactivity as a physiological susceptibility marker rendering children more vulnerable to life stress.

A broader literature has suggested that blunted cortisol reactivity is a consequence of chronic exposure to life stress (Miller et al. 2007). It has been posited that after exposure to chronic sources of stress, the HPA axis down-regulates as an adaptive mechanism to protect the brain and body from the adverse effects of prolonged hyper-activation (Fries et al. 2005). Thus, a blunted pattern of cortisol reactivity in children may be a marker of allostatic load in response to repeated exposure to early life stressors (McEwen 1998). Interestingly, we did not find a significant association between exposure to major life stressors in the past 12 months and children's cortisol reactivity. Nevertheless, it is possible that the pattern of cortisol reactivity we observed was due to exposure to earlier chronic, rather than recent, life stressors, which may have rendered these young children more susceptible to recent life stress. Nevertheless, our results are cross-sectional and cannot directly test the directionality of these effects. Future research should examine whether blunted cortisol reactivity serves as a pre-existing vulnerability marker, and how early and concurrent life stress influence children's cortisol responses across development.

Consistent with previous research in preschoolers (Luby et al. 2006) and a large body of research in adults (Monroe and Reid 2009), stressful life event was associated with preschoolers' internalizing symptoms. However, no interactive effects between child-level and environmental-level risks were observed for preschoolers' internalizing symptoms. The specificity of our findings to externalizing symptomatology and psychosocial functioning, rather than internalizing symptoms, may be due in part to developmental factors. Externalizing problems are relatively common in early childhood and are the primary reason parents seek treatment for preschoolers (Dougherty et al. 2015). Furthermore, early externalizing problems are a potent predictor of later internalizing symptoms and disorders, even after accounting for early internalizing symptoms (e.g., Copeland et al. 2013; Mesman et al. 2001). Thus, the combination of blunted cortisol reactivity and exposure to life stress may be part of a developmental trajectory through which early externalizing problems lead to later internalizing problems. Longitudinal work should investigate these relations over time, particularly through adolescence, when risk for internalizing disorders significantly increases.

Child Negative Emotionality

We found that for children with high levels of observed NE, greater exposure to maternal depression was associated with preschoolers' poorer social competence. Our observed interaction effect is consistent with studies demonstrating that children high in NE are more susceptible to the negative effects of adverse parenting contexts (e.g., Belsky et al. 1998; Morris et al. 2002; van Aken et al. 2007). Children high in NE may be particularly vulnerable to repeated exposure to their mothers' depression and associated interpersonal deficits, such as disruptions in parenting. Although early exposure to maternal depression likely interferes with a child's opportunity to develop and engage successfully in appropriate social skills, it is also possible that a child's tendency to express high levels of NE contributes to or exacerbates a mother's depression.

Social competence and effective social skills hold great importance during the preschool period when early peer relationships emerge. Early negative peer interactions have been found to contribute to subsequent deficits in social competence and increase risk for later social rejection and depression (Cole et al. 1996). Interestingly, chronic social difficulties have been found to predict depression severity more strongly for the depressed adolescent offspring of depressed mothers in comparison to the depressed adolescents of non-depressed mothers (Hammen et al. 2003). Thus, it is possible that the social impairment we observed in young children, who were high in negative emotionality and exposed to maternal depression, reflects an early vulnerability to depression risk. It is also important to note that depression and negative emotionality/neuroticism share a common genetic liability (Kendler et al. 1993); thus, these early associations may reflect, at least partly, familial or shared genetic factors. As greater exposure to maternal depression is likely associated with greater genetic load for depression, future research should examine how genetic loading may also play a key role in the link between high NE and reduced social competence in children. Moreover, it will be important for future longitudinal research to test whether the early social functioning deficits in young children with high NE and who were exposed to maternal depression during early childhood predict the onset

of depression later in development, possibly identifying an ultra high risk group.

Clinical Implications

Our findings hold promise in informing the development of early prevention and intervention programs. Notably, we found that the combination of low cortisol reactivity and exposure to higher levels of life stress was associated with preschoolers' externalizing behavior problems and poorer psychosocial functioning. Early externalizing problems and functional impairment lay the foundation for subsequent deficits in peer and family relationships and increase risk for subsequent psychopathology. Thus, our findings suggest that blunted cortisol reactivity may identify a high-risk group of young children to target for early psychosocial interventions. Recent encouraging findings highlight how interventions that alter children's cortisol functioning may decrease risk for subsequent externalizing behavior problems (O'Neal et al. 2010). Treatment focused on helping parents manage family stress and increase effective parenting skills may modify young children's HPA axis response, and in turn reduce risk for externalizing psychopathology and promote improved psychosocial functioning. Moreover, children high in NE have been found to benefit most from parenting interventions (e.g., Klein-Velderman et al. 2006); therefore, the young offspring of depressed parents who also evidence high levels of NE may benefit from interventions focused on sensitive parenting and development of adaptive social skills.

Strengths, Limitations, and Future Directions

The present study had several strengths. We employed a multi-method approach, including an observational assessment of child temperament and clinical interviews assessing parental depression, child psychopathology, functional impairment, and life stress. In addition, we assessed children's stress reactivity using a developmentally appropriate, standardized laboratory stressor paradigm and collected five cortisol samples to capture individual differences in children's cortisol reactivity.

This study also had limitations. First, the study was cross-sectional. Thus, we cannot test the temporal precedence of child NE and cortisol reactivity as pre-existing vulnerability markers. Second, although we controlled for gender in our analyses, further work with larger samples is needed to examine whether gender moderates these associations. Third, most of our effects were relatively small in magnitude, and most of the analyses did not support the main and interactive effects of the child and environmental variables examined in this study, and thus require replication. Lastly, primary caregivers, typically mothers, were the sole informant for the outcome measures on children's psychopathology and psychosocial functioning. It will be important to assess whether our findings replicate using a multi-informant approach, including co-parent and teacher reports.

Our findings suggest several avenues for future research. Recent investigations of the biological sensitivity to context (Boyce and Ellis 2005) and differential susceptibility (Belsky and Pluess 2009) theories have demonstrated that children with certain vulnerability, or susceptibility, markers respond in a "for better or for worse" manner, experiencing the best outcomes when exposed to positive environments and the worst outcomes when exposed to negative outcomes. Thus, it will be important to test whether children with high NE and/or blunted cortisol reactivity thrive in the presence of a particularly supportive family context or peer relationship, or to interventions that promote increases in sensitive parenting. Future work should also examine the interactions between early environmental contexts and *multiple* biological and behavioral sources of vulnerability on child outcomes, including additional temperament constructs (e.g., child positive emotionality, effortful control), and genetic and neurobiological vulnerability markers. Furthermore, future research should investigate whether other environmental factors, such as high socioeconomic status and two-parent homes, buffer the negative effects of environmental stressors on child outcomes. Finally, it will be important to investigate how these relationships change across development. For example, recent work has found that the phenotypic and genetic structures of internalizing psychopathology are different in childhood, adolescence, and young adulthood (Waszczuk et al. 2014). Thus, it is likely that the moderated effects of biological and behavioral vulnerability markers also vary across development.

In sum, we observed that children with blunted cortisol reactivity and who experienced more life stress in the past year evidenced higher levels of externalizing behavior problems and poorer psychosocial functioning. In addition, children high in NE and who were exposed to maternal depression had the lowest social functioning. Our findings suggest that vulnerability-stress models play a role in the etiology of preschool mental health problems. Moreover, our findings underscore the need for early interventions targeting young children with vulnerability markers in adverse environments, particularly during early childhood, a period of development characterized by a high degree of neuroplasticity.

Acknowledgments This research was supported by the University of Maryland (UMD) College of Behavioral and Social Sciences Dean's Research Initiative Award (LRD) and the UMD Research and Scholars Award (LRD). We are indebted to the families and staff who made this

study possible. We are especially grateful to Caitlin Condit for all her efforts in recruiting families and running participants.

Conflict of interest Authors report no conflicts of interest.

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