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# Adrenocortical Responses to Strangers in Preschoolers: Relations With Parenting, Temperament, and Psychopathology

**ABSTRACT:** Previous research has provided inconsistent evidence for the relations between young children's hypothalamic–pituitary–adrenal (HPA) functioning and their temperament, parenting, and adjustment. Building biopsychosocial models of adjustment, we examined how temperamental inhibition and maternal punishment contributed to preschoolers' adrenocortical activity while interacting with adult strangers. We also examined whether HPA functioning moderated relations between dispositional and familial factors and children's internalizing and externalizing problems. A total of 402 preschool-aged children from three independent samples with parallel and overlapping measures were studied. Salivary cortisol levels were measured twice while interacting with adult strangers during testing protocols. Mothers reported on temperamental inhibition, maternal punishment and children's problems. Maternal punishment predicted higher cortisol levels 20 and 65 min after meeting adult strangers. Prolonged cortisol elevation was associated with having fewer externalizing problems. Boys who experienced more maternal punishment and had higher cortisol 20 min after meeting strangers manifested more externalizing problems. Girls who were more inhibited and had prolonged cortisol elevations had more internalizing problems. In accord with biopsychosocial models of psychopathology, HPA functioning in preschoolers was sensitive to variations in socialization experiences, and moderated children's risk for emotional and behavioral problems. © 2011 Wiley Periodicals, Inc. *Dev Psychobiol* 53: 694–710, 2011.

**Keywords:** cortisol; internalizing problems; externalizing problems; preschoolers; punishment; temperamental inhibition

## INTRODUCTION

The hypothalamic–pituitary–adrenal (HPA) axis is one of the principal stress response and regulation systems affecting children's social, emotional, and behavioral adjustment. A primary end-product of HPA axis activity is production of the glucocorticoid cortisol. The HPA axis follows a diurnal rhythm, producing

varying levels of circulating cortisol over the 24-hr cycle, and also produces acute elevations in cortisol levels in response to stressful events. Both animal (Meaney & Szyf, 2005) and human (Ouellet-Morin et al., 2009) studies have shown that disadvantageous environmental experiences contribute to the development of atypical adrenocortical functioning. Disruptions to normative adrenocortical functioning are evident both in elevated circulating cortisol over the diurnal period, and in exaggerated stress responses and prolonged elevations in cortisol levels, which can have neurotoxic effects (McEwen, 1999; Sapolsky, 2000). Disrupted HPA axis activity has also been associated with a variety of psychopathological conditions in children (Dorn et al., 2009; Feder et al., 2004; Lopez-

Received 7 July 2010; Accepted 11 February 2011

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Published online 22 March 2011 in Wiley Online Library  
(wileyonlinelibrary.com). DOI 10.1002/dev.20545

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Duran, Kovacs, & George, 2009). In accord with biopsychosocial models of psychopathology, we examined how dispositional and socialization risk factors were associated with children's salivary cortisol levels while interacting with adult strangers. Drawing specifically on models of biological sensitivity to context (Boyce & Ellis, 2005), we also examined whether adrenocortical activity moderated the links between other risk factors and the development of emotional and behavioral problems.

Upon experiencing a stressful event the HPA axis typically produces a cascade of hormones which stimulates the adrenal glands to secrete elevated cortisol levels which are detectable in saliva approximately 20–25 min after the event (Gunnar & Talge, 2008), although there are individual differences in the timing of peak secretion (Lopez-Duran, Hajal, Olson, Felt, & Vasquez, 2009). Elevated cortisol levels should activate a negative feedback loop which halts further production, resulting in a progressive return to preevent levels. Sampling cortisol after a stressful event can reveal the magnitude of response and the efficiency of regulation (i.e., duration of HPA activation). However, invoking adrenocortical reactivity in young children can be challenging (Gunnar & Fisher, 2006; Gunnar, Talge, & Herrera, 2009).

Age has a curvilinear relation with HPA regulation (Gunnar et al., 2009; Lupien, McEwen, Gunnar, & Heim, 2009). Although the normative pattern of diurnal cortisol production is evident in the first year of life, it is more difficult to elicit stress-induced increases in cortisol levels in preschool-aged children than it is in infants and toddlers, and stress responses become more reliably elicited through later childhood and into adolescence (Stroud et al., 2009). Further, associations between stress-responsive cortisol and indicators of emotional or behavioral functioning appear to be relatively muted in the preschool period (Gunnar & Adam, in press). Some success is eliciting a cortisol response has been obtained with procedures that involve interacting with adult strangers, a face-valid stressor for preschool-aged children (Fernald & Gunnar, 2009). Unfamiliar adult interactions, such as in a lab or field testing protocol, can involve uncontrollability, unpredictability, and social evaluation, which are the hallmarks of effective elicitors of HPA stress responses (Dickerson & Kemeny, 2004). Studies have shown an "arrival effect": about 20 min after initially meeting unfamiliar adults for testing in a laboratory or field study, children show cortisol elevations (Battaglia et al., 1997; Fernald & Gunnar, 2009; Klimes-Dougan, Hastings, Granger, Usher, & Zahn-Waxler, 2001).

For this study, we used the arrival effect to examine adrenocortical functioning in 402 preschool-aged

children. Salivary cortisol levels 20 min after meeting adult strangers were expected to be a reflection of individual differences in both basal levels of circulating cortisol and response to the visit. Thus, higher cortisol levels should reflect, in part, an exaggerated response to this naturalistic social challenge. In addition, children provided another sample approximately 45 min after the first. Given the normative time-course of HPA regulation, lower cortisol levels would be expected in the second sample. Children evidencing relatively higher cortisol levels in this second sample would be experiencing a prolonged response, or failure to effectively downregulate their arousal. Although time-matched samples on nontesting days, or a greater number of samples over the testing protocol, can be more effective for assessing the dynamic time course of cortisol reactivity, using two samples can be effective for demonstrating HPA regulation (Kirschbaum & Hellhammer, 1994; Obradović, Bush, Stamperdahl, Adler, & Boyce, 2010; Ouellet-Morin et al., 2010).

### Factors Associated With HPA Activity

We adopted a biopsychosocial perspective on factors that might impinge upon children's HPA function and adjustment, focusing on individual, dispositional, and parental influences. Considering children's individual characteristics first, gender is a characteristic with mixed associations with HPA regulation. Some research has suggested that women might have stronger stress responses than men (Weekes et al., 2008), or that women and men respond to different kinds of stressors (Stroud, Salovey, & Hellhammer, 2002). However, most have not shown gender differences in adults' stress responses (Dickerson & Kemeny, 2004; Kudielka & Kirschbaum, 2005). Similarly, studies have not identified consistent gender differences in children's HPA responses (Gunnar & Vazquez, 2006; Spinrad et al., 2009). Although they might not differ in cortisol levels, studies have suggested that HPA regulation might be differentially associated with boys' and girls' psychosocial adjustment (Gunnar, Kryzer, Van Ryzin, & Phillips, 2010; Klimes-Dougan et al., 2001). For example, Gunnar et al. (2010) found that increasing cortisol levels while at childcare was associated with anxious behavior in girls, but with disruptive behavior in boys. These differences mirror the often noted sex difference in children's developmental trajectories for internalizing and externalizing problems (Zahn-Waxler, Shirtcliff, & Marceau, 2008), and suggest that child gender is a possible moderator of associations between risk factors, cortisol and adjustment.

Children's temperament has been associated with cortisol reactivity and regulation. In particular, young

children who are more temperamentally inhibited, reacting to novelty with fear or distress, show greater cortisol elevations to stressful events (Blair et al., 2008; Kagan, Reznick, & Snidman, 1987; Kertes et al., 2009; Schmidt et al., 1997; Talge, Donzella, & Gunnar, 2008), although some studies have failed to support this (Dougherty, Klein, Olino, Dyson, & Rose, 2009; Gunnar, 2001; Zimmermann & Stansbury, 2004). Dysregulated physiological reactivity has been hypothesized as the basis for temperamental inhibition's links to later psychopathology, particularly anxiety, depression, and internalizing problems (Kagan & Snidman, 1999; Rubin & Burgess, 2001). Conversely, very low inhibition might be a risk factor for externalizing problems (Burgess, Marshall, Rubin & Fox, 2003), as uninhibited children could manifest patterns of physiological under-arousal in response to cues of potential danger (Miskovic & Schmidt, *in press*), contributing to poor behavioral control and maladaptive engagement in risky situations.

Considering influences external to the child, appropriate parental care supports healthy adrenocortical activity (Kertes et al., 2009; Meaney & Szyf, 2005; Pendry & Adam, 2007; Roisman et al., 2009). Conversely, extreme deviations in parental care, particularly abusive parenting, can result in chronically disrupted HPA axis functioning (Tarullo & Gunnar, 2006), such as hypocortisolism (Lupien et al., 2009), a pattern of diminished HPA reactivity. The evidence for associations between normative (e.g., nonabusive) punishment or parental overcontrol and young children's cortisol levels has been less conclusive. Some studies have found that parents who use more punishment have children who show increased elevated cortisol levels and stronger responses to stress (Azar, Paquette, Zoccolillo, Baltzer, & Tremblay, 2007; Flinn & England, 1995), but others have failed to replicate such links (Blair et al., 2008; Dougherty et al., 2009; Ouellet-Morin et al., 2009). Greater parental punitiveness is known to increase the risk of children's internalizing and externalizing problems (McLeod, Wood, & Weisz, 2007; Rothbaum & Weisz, 1994), particularly for children who have a dispositional vulnerability (Rubin, Burgess, Dwyer, & Hastings, 2003). Thus, explicating punishment's links with HPA axis functioning is warranted.

The inconsistency in the links between young children's HPA functioning and their temperament and parenting might suggest that any associations are quite modest in magnitude. Alternatively, in accord with biopsychosocial models, these dispositional and contextual factors might function in a synergistic fashion such that relations are manifest only under specific conditions or when factors are considered in combination. Few investigators have considered the joint

contributions of temperament and parenting to children's HPA activity, but positive parenting may buffer the tendency for inhibited children to show elevated stress responses (Gunnar & Donzella, 2002; Nachmias, Gunnar, Mangelsdorf, Parritz, & Buss, 1996). Similarly, Kertes et al. (2009) found that cortisol reactivity to social stress in highly inhibited toddlers was reduced when they experienced better maternal parenting (more sensitive and structuring, less hostile and intrusive). Conversely, Blair et al. (2008) reported that parenting and temperament were both associated with HPA reactivity to emotional stress, but there were no significant interactions between temperament and parenting in the prediction of cortisol levels. Given the growing evidence that children's psychosocial development is shaped by the joint contributions of their dispositional traits and socialization experiences (e.g., Bates & Pettit, 2007; Belsky, Bakersman-Kranenburg, & van IJzendoorn, 2007; Rubin, Burgess, & Hastings, 2002), further investigation of whether such interactive processes might also contribute to physiological regulation is warranted.

### HPA Activity and Children's Adjustment

HPA dysregulation has been linked with both internalizing and externalizing disorders in children. Most consistently, children with internalizing problems, anxiety or depression have been found to have higher levels of circulating cortisol and heightened and prolonged HPA responses to stressful events (Gunnar, 2001; Hastings, Fortier, Utendale, Simard, & Robaey, 2009; Lopez-Duran et al., 2009), whereas children with externalizing problems or disruptive behavior disorders have lower cortisol levels before and after challenging events (Fairchild, Van Goozen, Stollery, & Goodyer, 2008; Hastings et al., 2009; van Goozen, Fairchild, Snoek, & Harold, 2007). However, there have been discrepant reports, with relations between cortisol and problems that are nonsignificant or opposite to these patterns (Jansen et al., 1999; McBurnett et al., 2005; Murray-Close, Han, Cicchetti, Crick, & Rogosch, 2008), particularly in younger children (Alink et al., 2008; Dettling, Gunnar, & Donzella, 1999; Gunnar, Sebanc, Tout, Donzella, & van Dulmen, 2003; Spinrad et al., 2009). For example, Obradović et al. (2010) recently reported that greater HPA reactivity to a behavioral testing protocol was associated with more externalizing problems in kindergarten-aged children, while Ouellet-Morin et al. (2010) reported no associations between 3 year-olds' internalizing and externalizing problems and their cortisol levels while in child care.

It is possible that in the preschool years, deviations from normative HPA axis function might confer vulnerability towards problems, depending on other risk

factors. Less physiologically well-regulated children who are also temperamentally vulnerable, or who also experience punitive parenting, might be at greater risk of manifesting problems. Diathesis-stress models have most often been applied to this biopsychosocial hypothesis (Hankin & Abela, 2006). Children with elevated cortisol levels might only manifest more internalizing problems than children with lower cortisol levels if they also have highly punitive mothers. Conversely, children with very low cortisol levels might only manifest more externalizing problems in such an adverse socialization context. Few internalizing or externalizing problems would be expected for children with more moderate, normative cortisol responses, regardless of their socialization experiences.

Recently, Boyce and Ellis (2005) and Ellis and Boyce (2008) proposed a model of biological sensitivity to context to explain the interactive contributions of physiological regulation and environmental factors to adjustment. According to this model, rather than a vulnerability per se, poor physiological self-regulation denotes increased susceptibility to environmental influences, for better or for worse. In effect, these children are more developmentally flexible, or their trajectories of well-being are determined less by a dispositional maturational course than by the forces of nature. They might thrive and exceed expectations if they are raised in advantageous circumstances. Thus, children with poor adrenocortical regulation might manifest more problems than average should they have highly punitive mothers, but they would manifest fewer problems than average should their mothers avoid such aversive parenting practices. Children with more well-regulated HPA functioning would be expected to have average levels of functioning, regardless of their rearing conditions.

### The Current Investigation

Inconsistency in the documented relations between risk factors, cortisol and adjustment in early childhood might be partially attributable to small sample sizes in many studies. Combining samples provides many possible advantages (Curran, 2009), including a larger and more heterogeneous group of participants, and increased power to test multivariate models and detect small effects. This has been used effectively in previous studies of relations between children's cortisol and well-being (e.g., Chen & Paterson, 2006; Kertes et al., 2009), including one study of internalizing disorders in prepubertal children in which the combined sample revealed relations that had not been evident in the separate samples (Feder et al., 2004). We combined data from three independent studies that included measures of adrenocortical stress reactivity and regulation, maternal punishment, temperamental inhibition, and

internalizing and externalizing problems to produce a diverse sample of 402 preschool-aged children.

We hypothesized that children who were more inhibited or experienced more punishment would show higher cortisol levels after meeting unfamiliar adults. Cortisol levels were expected to be highest in inhibited children who also had punitive mothers. High cortisol levels were expected to be associated with increased internalizing problems, whereas children with very low cortisol levels were expected to have more externalizing problems. Relations between other risk factors and behavior problems were expected to be stronger for children with more less well-regulated HPA function. These children were expected to manifest the most behavior problems when they experienced highly punitive parenting, but the fewest problems if they had non-punitive parents. Similarly, temperamentally inhibited children who also had more elevated cortisol were expected to have more internalizing problems. Associations between cortisol and internalizing problems were expected to be stronger for girls, whereas cortisol was expected to be related to externalizing problems more strongly in boys.

## METHODS

### Participants

Participants were 196 girls and 206 boys ranging from 2.0 to 6.1 years ( $M = 4.01$  years;  $SD = .71$ ). Mothers' age ranged from 20 to 56 years ( $M = 34.36$ ,  $SD = 5.31$ ). The families were 88% two-parent and 83% Caucasian. Most mothers had college (38.3%) or advanced (40%) degrees, but 21.6% had not progressed beyond high school; comparable statistics for fathers were 36.8%, 34.3%, and 28.9%, respectively. The prestige of each family's most highest occupation was coded using the updated Standard International Occupational Prestige Scale (SIOPS; Hakim, 1998, Treiman, 1977); the average prestige was 51.06 ( $SD = 12.42$ ), representing mid-SES status, with ratings ranging from 16 (e.g., domestic laborers) to 78 (e.g., lawyers, physicians). This diverse sample was generated by integrating three independent samples from studies with overlapping physiological and behavioral measures, in order to increase power and diversity (e.g., Chen & Paterson, 2006; Feder et al., 2004; Kertes et al., 2009). Details on the three samples follow.

*Sample 1* ( $n = 63$ ): The Concordia Longitudinal Risk Project began in 1976–1978 as a community sample of over 1,770 French-speaking elementary-school children living in predominantly lower-income, urban neighborhoods of Montreal. As the original participants (now in their 30s and 40s) became parents, assessments of their children were conducted. In this study, 63 preschool-aged offspring of the original participants, and their mothers, were examined. Assessments were conducted in the home, at which time mothers completed instruments used in the present study.

**Sample 2 ( $n = 124$ ):** The Daycare and Preschool Adjustment Study used targeted recruitment to over-sample children in Montreal with elevated early anxiety problems. By mother report of behavior problems (Achenbach & Rescorla, 2001), 42 children were in the borderline-clinical to clinical range for internalizing problems for their age, while 48 were less than 1 *SD* above and 43 were at or below the norm for their age. Families were tested in English (80%) or French (20%). Assessments of cortisol were conducted in the home, and mothers completed questionnaires in the home and in a questionnaire packet returned by mail after the visit.

**Sample 3 ( $n = 215$ ):** The Shame in Childhood Study is a community sample of 3- to 4-year-old children and their parents, recruited through letters sent to a representative group of families with a child born between June 1, 1999 and May 31, 2000. Parents predominantly were Caucasian (94%), were of European ancestry (74%), and were at least second-generation Canadian (78%). All families were tested in English. Families who chose to participate were assessed in a laboratory visit where children's saliva was collected and mothers completed questionnaires.

## Procedures

**Saliva Collection.** In each of the three protocols, children and their mothers were either visited in their home by unfamiliar adult examiners, or they visited a university laboratory and interacted with unfamiliar adult examiners. Visits were conducted between 9 AM and 7 PM. All three subsamples tested children in both the mornings and the afternoons, and for each subsample, at least a third of children were seen earlier and later in the day (that is, no more than 66% of children in any one sample were tested either in the morning or in the afternoon). There is diurnal variation in baseline salivary cortisol levels across the day, and research with adults has shown greater adrenocortical reactivity to stressors in the afternoon compared to the morning (Dickerson & Kemeny, 2004), although this has not always been replicated with children (Hastings et al., 2009; Obradović et al., 2010). For this study, time of sample collection was controlled in all analyses, as has been done in previous studies with varying testing times (e.g., Ouellet-Morin et al., 2010). Exact procedures varied across the protocols,<sup>1</sup> but all involved a greeting and warm-up period, a saliva sample collected approximately 20 min after meeting the unfamiliar adults ("arrival" sample), a series of interactive tasks, and another saliva sample collected 40–

<sup>1</sup>Methodological standardization is usually a research goal, and variation in procedures across protocols should increase "noise" or error in the cortisol measurements. Thus, the current analyses should be considered *conservative*. Due to increased error, the magnitude of any relations identified between the predictor and outcome variables are likely to be under-estimated. The chance of Type I errors—false positives—is therefore minimized, and the power to detect small effects conferred by the large sample size should offset the possibility that protocol variability could increase Type II errors—failing to identify real associations. Dummy-coded variables for study sample were included as covariates in all analyses, and interaction effects that approached significance ( $.05 < p < .10$ ) but moderated significant main effects were examined to decrease the risk of Type II errors.

60 min after the first ("regulation" sample) ( $M = 45.87$  min,  $SD = 24.75$ ). The arrival and samples were used for the current analyses.

Saliva was collected by having children chew cotton dental rolls that were then placed into Salivettes (Sarstedt, Inc., Rommelsdorf, Germany). Samples collected at home were placed in refrigerated coolers, transported to the laboratory, then frozen. These were thawed and centrifuged to express saliva at the time of cortisol assay. Samples collected in the laboratory were centrifuged immediately following the visit, and the extracted saliva samples were immediately frozen and stored until thawing for assay. All samples were assayed using a high sensitivity enzyme immunoassay kit (High Sensitivity Salivary cortisol Catalog No. 1-0102/1-0112; Salimetrics, State College, PA).

## Measures

Mothers completed questionnaire packets to assess family demographic characteristics, parental punishment, children's inhibited temperament, and children's behavior problems. Parallel items were identified in the three protocols to create identical measures punishment and inhibition for each sample.

**Maternal Punishment.** Multiple measures of parenting were used in the three samples, including the Parenting Scale (Arnold, O'Leary, Wolff, & Acker, 1993) and Parenting Dimensions Inventory (Power, 1993) for Sample 1, Child Rearing Practices Report (Block, 1981) and Responses to Children's Emotions (Hastings & De, 2008) for Sample 2, and Parenting Styles & Dimensions Questionnaire (Robinson, Mandleco, Olsen, & Hart, 2001) for Sample 3. Across these measures, three parallel items were identified that reflected harsh maternal punishment (e.g., use physical punishment; yell or shout at my child; show when I'm angry), and had acceptable internal consistency,  $\alpha = .61$ .

**Child Temperament.** To assess child temperament parents completed the Children's Behavior Questionnaire (Rothbart, Ahadi, Hershey, & Fisher, 2001) in Samples 2 and 3, and the Emotionality Activity Sociability Temperament Survey for children (Buss & Plomin, 1984) in Sample 1. Six parallel items from the two scales were used to create the measure of inhibition (e.g., prefers to watch than join; at ease with almost anyone [reversed]; acts shy around new people),  $\alpha = .83$ .

**Behavior Problems.** In all three protocols, mothers completed the Child Behavior Checklist (CBCL; Achenbach and Rescorla, 2001) to report on children's internalizing problems (IP) and externalizing problems (EP). The CBCL is a widely used instrument with good internal reliability and 1-week test-retest reliability. For this study,  $\alpha \geq .70$  for IP and EP in all three protocols.

## Analyses

Usable arrival saliva samples were collected from 402 children, and regulation samples from 395 children. Raw cortisol data ( $\mu\text{g/dL}$ ) were leptokurtic and positively skewed. Log-transformations corrected the skews and eliminated outliers; thus, log-transformed data were used in analyses.

Untransformed data are reported for ease of interpretation. The arrival sample was used as the index of response to meeting adult strangers. Because cortisol levels in the arrival and regulation samples were significantly correlated, partial  $r = .45$ ,  $p < .001$ , controlling for time of visit and times of sample collections, the standardized residuals of the regulation cortisol predicted from arrival cortisol were used as the index of regulation (higher scores reflect higher regulation cortisol relative to arrival cortisol, hence, failure to regulate).

Analyses of variance (ANOVAs) and covariance (ANCOVAs) were used to conduct descriptive analyses. Dummy-coded variables indicating protocol group were computed to control for variation in procedures across the three groups of participants in subsequent analyses. Multiple hierarchical linear regressions were used to predict arrival and regulation cortisol from the risk variables (inhibition, punishment), and to predict IP and EP from cortisol and risk variables. The regression analyses predicting arrival and regulation cortisol included child age, times of visit and saliva collections, and dummy codes for protocol group at Step 1. Child gender, inhibition, and punishment were entered at Step 2, and the two-way and three-way interactions of these variables were entered in steps 3 and 4, respectively.

The relations between arrival cortisol and the two problem scores were examined in two separate analyses. Similarly, relations between regulation cortisol and problem scores were examined in separate analyses. The structure for these four regression analyses was similar to the preceding models. Regression analyses predicting IP and EP included the non-target problem score (EP or IP, respectively) in the first step, to control for overlap in scores,  $r = .53$ ,  $p < .001$ , and ensure specificity of relations between predictors and type of problem. Step 1 also included age, times of visit and saliva collections, and dummy codes for protocol group. Step 2 included gender, inhibition, punishment, and either arrival cortisol or regulation cortisol. All two-way interactions between cortisol and the child and parent factors were included in Step 3. The two-way interactions of gender, inhibition, and punishment were entered at Step 4, and finally the three-way interactions between cortisol and these two-way interactions were examined in Step 5.<sup>2,3</sup>

<sup>2</sup>Preliminary analyses revealed no significant interaction effects involving four or more variables. Therefore, these effects are not reported in this manuscript.

<sup>3</sup>To ensure that each sample equivalently predicted the dependent variables from the sets of independent variables (i.e., the statistical models for each sample were identical), we used a multilevel framework (Pinheiro & Bates, 2000; Raudenbush & Bryk, 2002) to model random components that were allowed to vary across samples for the independent variables. This allowed us to quantify the differences across the three samples by investigating the significance of the random components, and through the intraclass correlation coefficient (Cohen, Cohen, West, & Aiken, 2003). Statistically significant random components or ICCs with large magnitude indicate salient differences for the statistical model across the three samples. We ran the models with random components added to the variables to test ICCs for all variables. None of the variables had statistically significant random coefficients or ICCs with significant magnitudes. Therefore we can conclude that the statistical model is the same across the three subsamples, and the integrated sample allows for greater power to detect significant effects.

## RESULTS

### Descriptive Analyses

Descriptive statistics are presented in Table 1. For the broadband CBCL IP and EP scales,  $T$ -scores between 60 and 63 are considered "borderline clinical," and  $T$ -scores of 64 and greater are considered "clinical" (Achenbach and Rescorla, 2000). The sample included 57 children (14.2%) with at least one of their IP or EP  $T$ -scores in the borderline range, and 71 children (17.7%) with clinical IP and/or EP  $T$ -scores.

A mixed-design ANCOVA, with saliva sample as a within-subjects factor and testing in the morning versus afternoon as a between-subjects factor, controlling for study subsample and time between sample collections, showed that cortisol in the arrival samples was higher than in the regulation samples,  $F(1, 389) = 4.77$ ,  $p < .05$ , in accord with expectations that the first sample would reflect, in part, response to meeting strangers and the second a recovery from that response (regulation) (or a time-dependent decrease due to the normally expected diurnal rhythm). As would be expected given the diurnal pattern of HPA activity, cortisol levels in the arrival and regulation samples were higher in the 208 children tested in the morning than in the 194 children tested in the afternoon,  $F(1, 389) = 14.19$ ,  $p < .001$ . However, the interaction of sample and time of day was not significant,  $F(1, 389) = .21$ . Thus, the magnitude of the decrease in cortisol from the arrival to the regulation sample did not differ significantly for the children tested in the morning,  $M = .015 \mu\text{g/dL}$ , compared to those tested in the afternoon,  $M = .022 \mu\text{g/dL}$ . Despite the overall pattern of decreasing cortisol levels, almost one-third ( $n = 124$ ) of the children had unchanged or increased cortisol levels over the testing period, indicative of poor HPA regulation.<sup>4</sup>

A series of one-way ANOVAs showed that the three subsamples differed on inhibition, punishment, IP and EP, all  $F(2, 399) > 4.85$ ,  $p < .01$  (see Tab. 1), indicating that heterogeneity of the aggregated sample was increased by combining the three protocols. Controlling for time, the cortisol levels of the subsamples differed in the arrival sample,  $F(2, 397) = 10.97$ ,  $p < .001$ , and regulation sample,  $F(2, 389) = 5.98$ ,  $p < .01$ , but not in regulation cortisol (residualized change score),  $F(2, 389) = 1.07$ . To control for these differences, dummy

<sup>4</sup>To determine whether differences in subsample protocols contributed to which children experienced increasing or decreasing cortisol levels, we examined regulation cortisol (residual change scores) in a one-way ANCOVA, controlling for times of visit and saliva collections, with subsample as a between-subjects factor. The subsample effect was not significant,  $F(2, 389) = 1.07$ , ns.

**Table 1. Descriptive Statistics and Differences Between Samples ( $p < .05$ )**

Variable	Min	Max	Mean	SD	Sample Differences
Age	2.00	6.12	4.01	.71	1 > 3 > 2
Arrival cortisol	.01	2.13	.15	.24	1 > 2, 3
Regulation cortisol (raw)	.01	3.54	.13	.26	1 > 2 > 3
Regulation cortisol (SR)	-3.11	4.92	.00	1.00	ns
Punishment	1.00	6.50	2.81	.92	1, 2 > 3
Inhibition	1.00	6.83	3.36	1.35	2, 3 > 1
Internalizing problems	29.00	76.00	52.41	9.45	2 > 3
Externalizing problems	28.00	83.00	50.02	9.77	1 > 2

Note: Descriptive statistics for both the raw scores and standardized residual (SR) scores of the regulation cortisol sample are provided.

codes for subsample group were included in all subsequent analyses. In “Dummy Code: Sample 1,” Sample 1 (code 1) is contrasted with Samples 2 and 3 (code 0). In “Dummy Code: Sample 3,” Sample 3 (code 1) is contrasted with Samples 1 and 2 (code 0). Additional analyses indicated that language of testing (French or English) was not associated with children’s cortisol measures; therefore, language was not included as a covariate.

Partial correlations among the target variables, controlling for study sample, time of testing, and time of saliva sampling, are presented in Table 2a. Notably, more maternal punishment was significantly associated with higher cortisol levels in the arrival sample, and tended to be associated with higher regulation cortisol (higher residualized change scores), reflected prolonged elevation in HPA activity over the adult interaction period. Inhibition and punishment were associated with internalizing and externalizing problems consistently with the broader literature, and boys had more internalizing and externalizing problems than girls.

Because of the modest internal consistency of the punishment index, we also examined how each of the three punishment items was associated with children’s cortisol values and problem scores (see Tab. 2b). Mothers’ reports of more yelling and shouting, and showing anger, were associated with higher cortisol levels, and higher scores on all three items were associated with more internalizing and/or externalizing problems.

### Prediction of Arrival and Regulation Cortisol

**Arrival Cortisol.** After accounting for the control variables, only one predictor was significantly associated with children’s arrival cortisol. Children who received more maternal punishment had higher cortisol levels 20 min after meeting unfamiliar adults,  $\beta = .11$ ,  $t = 2.11$ ,  $p < .05$ . Overall, the regression model accounted for a significant portion of the variance in arrival cortisol,  $\text{adj. } R^2 = .112$ ,  $F(12, 385) = 5.15$ ,  $p < .001$ .

**Regulation Cortisol.** The variables that reached ( $p < .05$ ) or approached ( $p < .10$ ) significance in the prediction of regulation cortisol are summarized in Table 3. Punishment tended to predict higher cortisol levels in the regulation samples after accounting for arrival cortisol. This was significantly moderated by gender; punishment significantly predicted poorer regulation in girls,  $\beta = .25$ ,  $t = 3.25$ ,  $p < .01$ , but not in boys,  $\beta = -.06$ . The inhibition  $\times$  punishment interaction also approached significance (see Fig. 1). Maternal punishment predicted higher cortisol levels in the regulation sample more strongly in less inhibited preschoolers,  $\beta = .20$ ,  $t = 2.81$ ,  $p < .01$ , than in more inhibited preschoolers,  $\beta = .01$ . Finally, gender also tended to moderate the association between inhibition and regulation cortisol; however, the association between inhibition and regulation cortisol was not significant for girls,  $\beta = -.03$ , or for boys,  $\beta = .05$ .

### Prediction of Internalizing and Externalizing Problems from Cortisol and Risk Factors

**Arrival Cortisol.** There were no significant associations between children’s IP and their arrival cortisol, either on its own or in interaction with inhibition, punishment, or gender (see Tab. 4). Boys had more IP than girls, and more inhibited children had more IP.

In the prediction of EP, children had more EP if they received more punishment and if they were less inhibited. The effect of punishment was moderated by the three-way interaction of arrival cortisol  $\times$  punishment  $\times$  gender that approached significance (see Tab. 4). Considered separately by gender, the interaction of arrival cortisol and punishment was significant for boys,  $\beta = .12$ ,  $t = 2.20$ ,  $p < .05$ , but not for girls,  $\beta = -.06$ ,  $t = -.85$ . As Figure 2 shows, maternal punishment predicted EP more strongly in boys with higher arrival cortisol,  $\beta = .41$ ,  $t = 4.75$ ,  $p < .001$ , than in boys with lower cortisol levels,  $\beta = .17$ ,  $t = 2.20$ ,  $p < .05$ . Corresponding values in girls with higher and

**Table 2a. Partial Correlations Among Child Characteristics, Cortisol Values, Risk Factors and Behavior Problems (Controlling for Sample, Testing Time, and Saliva Sampling Times)**

	2.	3.	4.	5.	6.	7.	8.
1. Age	.07	-.01	-.06	.04	-.03	.00	-.01
2. Gender	—	.02	-.07	.08	.00	.15**	.10*
3. Arrival cortisol		—	-.09 <sup>+</sup>	.10*	.06	.00	.02
4. Regulation cortisol (SR)			—	.09 <sup>+</sup>	.00	-.02	-.07
5. Punishment				—	-.11*	.13*	.34***
6. Inhibition					—	.29***	-.03
7. Internalizing problems						—	.56***
8. Externalizing problems							—

<sup>+</sup>*p* < .10.\**p* < .05.\*\**p* < .01.\*\*\**p* < .001.**Table 2b. Partial Correlations Between Punishment Items and Cortisol Values and Behavior Problems (Controlling for Sample, Testing Time, and Saliva Sampling Times)**

	Arrival Cortisol	Regulation Cortisol	Internalizing Problems	Externalizing Problems
Item 1: Show anger	.03	.14**	.06	.20***
Item 2: Yell or shout	.15**	.04	.11*	.29***
Item 3: Physical punishment	.03	.01	.14**	.32***

Note: "Gender" is dummy coded for 0 = girls, 1 = boys.

\**p* < .05.\*\**p* < .01.\*\*\**p* < .001.

lower arrival cortisol were  $\beta = .24$  and  $.33$ ,  $t = 3.26$  and  $3.32$ , respectively, both  $p < .01$ .

**Regulation Cortisol.** In the prediction of IP (see Tab. 5), the significant links between gender and inhibition and children's IP were moderated by a three-way interaction of regulation cortisol  $\times$  inhibition  $\times$  gender that approached significance (see Fig. 3). Considered separately by gender, the interaction of regulation cortisol and inhibition was significant for girls,  $\beta = .14$ ,  $t = 2.31$ ,  $p < .05$ , but not for boys,  $\beta = .02$ ,  $t = .41$ . Temperamental inhibition predicted IP more strongly in girls with elevated cortisol levels in the regulation samples,  $\beta = .55$ ,  $t = 5.85$ ,  $p < .001$ , than in more well-regulated girls,  $\beta = .23$ ,  $t = 2.76$ ,  $p < .01$ . Corresponding values in less and more well-regulated boys were  $\beta = .27$  and  $.22$ ,  $t = 2.85$  and  $2.77$ , respectively, both  $p < .01$ . The interaction between gender and maternal punishment also approached significance, however, punishment did not significantly predict IP for girls,  $\beta = -.11$ , or for boys,  $\beta = -.03$ .

In the prediction of EP (see Tab. 5), children with higher regulation cortisol levels had fewer EP, over and above the significant associations between EP and

punishment and inhibition. Thus, children who maintained relatively higher cortisol levels over the testing period, after accounting for their levels of arrival cortisol, had fewer EP. The borderline interaction between sex and punishment indicated that the positive association between punishment and EP was slightly stronger for girls,  $\beta = .29$ ,  $t = 4.30$ ,  $p < .001$ , than for boys,  $\beta = .25$ ,  $t = 4.09$ ,  $p < .001$ .

## DISCUSSION

The objectives of this study were to examine the links between dispositional and socialization risk factors and children's adrenocortical functioning, and to determine whether cortisol levels and regulation moderated associations between those risk factors and children's adjustment. Children whose mothers used more punitive parenting practices showed higher cortisol levels after meeting strangers. Furthermore, daughters who experienced more harsh parenting were less able to regulate their high cortisol levels over the testing protocol. In contrast, those children who evidenced better HPA regulation, as shown by greater decreases in



**Table 3. Significant Effects in the Regression Model Predicting Standardized Residual Scores for Regulation Cortisol**

Step	Predictor	$\Delta R^2$	df	$F$	$\beta$	$t$	$p$
Regulation cortisol							
1. Control variables		.066	6, 384	4.49***			
	Time of visit				-.13	-2.34	.020
	Dummy code: sample 1				.11	1.65	.100
2. Main effects		.013	3, 381	1.85			
	Punish				.11	1.90	.058
3. Two-way interactions		.037	3, 378	5.35***			
	Gender $\times$ punish				-.15	-3.04	.003
	Gender $\times$ inhibition				.09	1.87	.063
	Punish $\times$ inhibition				-.10	-1.93	.054
4. Three-way interactions		.005	1, 377	1.99			

Notes: Model statistics: adj.  $R^2 = .091$ ,  $F(13, 377) = 4.00$ ,  $p < .001$ .

In "Dummy Code: Sample 1," Sample 1 (code 1) is contrasted with Samples 2 and 3 (code 0). "Gender" is dummy coded for 0 = girls, 1 = boys.

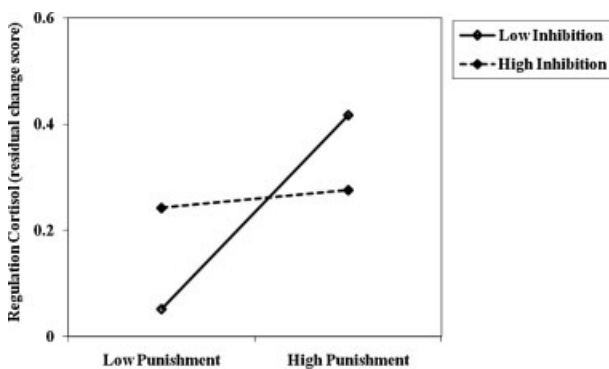
\*\*\* $p < .001$ .

cortisol over the testing protocols, were those who both experienced little maternal punishment and were less inhibited.

Salivary cortisol levels 20 and 65 min after meeting adult strangers also moderated the relations between dispositional and socialization risk factors and children's adjustment in ways that support gender models of developmental psychopathology (Zahn-Waxler et al., 2008). Maternal punishment predicted externalizing problems most strongly in boys who showed higher cortisol levels shortly after meeting strangers, whereas inhibition predicted internalizing problems most strongly in girls who showed poorer downregulation of HPA activity over a longer period. These associations have important implications for the specificity of links

between aspects of physiological risk and behavioral maladjustment.

Maternal care clearly emerged as an important contributor to HPA functioning and psychological adjustment. Appropriate care through parental relationships serves to modulate infants' and young children's stress physiology and, eventually, confer capacities for emotional self-regulation (Stansbury & Gunnar, 1994). Conversely, our results indicate that angry and punitive maternal care could constitute an adverse rearing condition that disrupts adaptive HPA functioning. The finding that more punitive mothers had children with elevated cortisol levels both 20 and 65 min after interacting with adult strangers echoes a recent report that more conflicted mother-child relationships are associated with higher morning cortisol levels while in child care (Rappolt-Schlichtmann, Willett, Ayoub, Lindsley, Hulette, & Fischer, 2010). More extreme, abusive parenting has been associated with HPA hypo-responsivity (e.g., Hart, Gunnar, & Cicchetti, 1995; see review by Gunnar & Quevedo, 2007). Conversely, it appears that punishment within the normal range of parenting represents modest, chronic stress that contributes to HPA hyper-responsivity, at least in young children. By focusing on adrenocortical functioning in preschoolers, we examined children during a period of rapid neurodevelopment and organization of emotion and stress regulation (Cicchetti & Rogosch, in press). The regular experience of punitive parenting might heighten young children's sensitivity to potential threat from adults. This would leave children vulnerable to maladaptive behavioral responses to acute events, such as coping



**FIGURE 1** Maternal punishment predicts less well-regulated cortisol 60 min after meeting strangers in less inhibited preschoolers.

**Table 4. Significant Effects in the Regression Models Predicting Internalizing and Externalizing Problems From Arrival Cortisol**

Step	Predictor	$\Delta R^2$	df	F	$\beta$	t	p
Prediction of internalizing problems from arrival cortisol <sup>a</sup>							
1. Control variables		.340	6, 391	33.56***			
	Externalizing problems				.54	12.93	.000
	Dummy code: Sample 1				-.23	-4.24	.000
	Dummy code: Sample 3				-.17	-2.27	.024
2. Main effects		.097	4, 387	16.60***			
	Gender				.09	2.29	.022
	Inhibition				.30	7.64	.000
3. Two-way cortisol interactions		.003	3, 384	.69			
4. Other two-way interactions		.007	3, 381	1.72			
5. Three-way cortisol interactions		.001	3, 378	.32			
Prediction of externalizing problems from arrival cortisol <sup>b</sup>							
1. Control variables		.326	6, 391	31.53***			
	Internalizing problems				.55	12.93	.000
	Dummy code: Sample 1				.22	4.01	.000
	Dummy code: Sample 3				.27	3.65	.000
2. Main effects		.098	4, 387	16.48***			
	Punish				.27	6.18	.000
	Inhibition				-.18	-4.21	.000
3. Two-way cortisol interactions		.002	3, 384	.39			
4. Other two-way interactions		.004	3, 381	.99			
5. Three-way cortisol interactions		.005	3, 378	1.21			
	Gender $\times$ punish $\times$ arrival cortisol				.08	1.69	.092

Note: In “Dummy Code: Sample 1,” Sample 1 (code 1) is contrasted with Samples 2 and 3 (code 0). In “Dummy Code: Sample 3,” Sample 3 (code 1) is contrasted with Samples 1 and 2 (code 0). “Gender” is dummy coded for 0 = girls, 1 = boys.

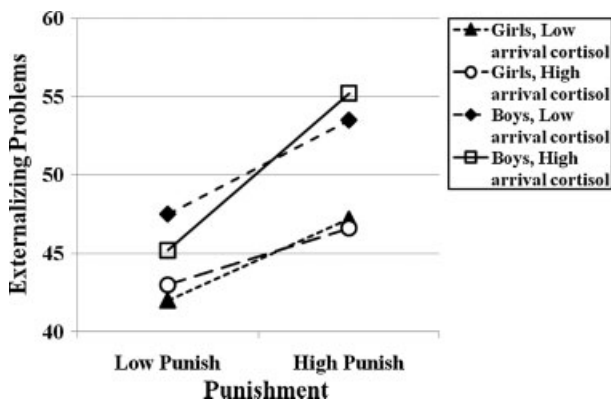
<sup>a</sup>Model statistics: adj.  $R^2 = .421$ ,  $F(19, 378) = 16.18$ ,  $p < .001$ .

<sup>b</sup>Model statistics: adj.  $R^2 = .407$ ,  $F(19, 378) = 15.37$ ,  $p < .001$ .

\*\*\* $p < .001$ .

with novel social challenges, and might increase their risk for poor adjustment.

The HPA axis is thought to contribute to the development of fear responsivity, and has been posited as a



**FIGURE 2** Maternal punishment predicts externalizing problems most strongly in boys with elevated cortisol 20 min after meeting strangers.

mechanism by which inhibited children develop anxiety problems (Rosen & Schulkin, 1998; Sapolsky, Romero, & Munck, 2000). Surprisingly, mother-reported temperamental inhibition was not directly associated with cortisol levels in this large sample of children. Although this was inconsistent with some past research (e.g., Blair et al., 2008), relations between inhibition and cortisol levels have been somewhat variable (Gunnar et al., 1997), suggesting that other factors might contribute to their association. Indeed, we found that parenting and inhibition together predicted cortisol regulation, but counter to our expectations, it was the less inhibited children for whom maternal punishment predicted more prolonged elevation in cortisol levels. This unanticipated finding does not seem to have precedence in the literature, although it might suggest that, as has been noted for temperamentally difficult children, temperamentally uninhibited children have enhanced susceptibility to environmental influence (Belsky et al., 2007). In the more supportive context of having nonpunitive mothers, they showed the pattern of

**Table 5. Significant Effects in the Regression Models Predicting Internalizing and Externalizing Problems From Standardized Residual Scores for Regulation Cortisol**

Step	Predictor	$\Delta R^2$	df	<i>F</i>	$\beta$	<i>t</i>	<i>p</i>
Prediction of internalizing problems from regulation cortisol <sup>a</sup>							
1. Control variables		.342	7, 383	28.49***			
	Externalizing problems				.54	12.85	.000
	Dummy code: Sample 1				-.22	-3.95	.000
2. Main effects		.095	4, 379	16.09***			
	Gender				.09	2.38	.018
	Inhibition				.30	7.54	.000
3. Two-way cortisol interactions		.004	3, 376	.89			
4. Other two-way interactions		.010	3, 373	2.21+			
	Gender × punish				.07	1.76	.079
5. Three-way cortisol interactions		.007	3, 370	1.61			
	Gender × inhibition × regulation cortisol				-.07	1.71	.089
Prediction of externalizing problems from regulation cortisol <sup>b</sup>							
1. Control variables		.329	7, 383	26.80***			
	Internalizing problems				.56	12.85	.000
	Dummy code: Sample 1				.20	3.47	.001
	Dummy code: Sample 3				.28	2.27	.024
	Time of arrival sample				.12	1.68	.093
2. Main effects		.097	4, 379	15.97***			
	Regulation cortisol				-.09	-2.12	.035
	Punish				.27	6.00	.000
	Inhibition				-.18	-4.14	.000
3. Two-way cortisol interactions		.001	3, 376	.26			
4. Other two-way interactions		.007	3, 373	1.54			
	Gender × punish				-.08	-1.92	.056
5. Three-way cortisol interactions		.001	3, 370	.27			

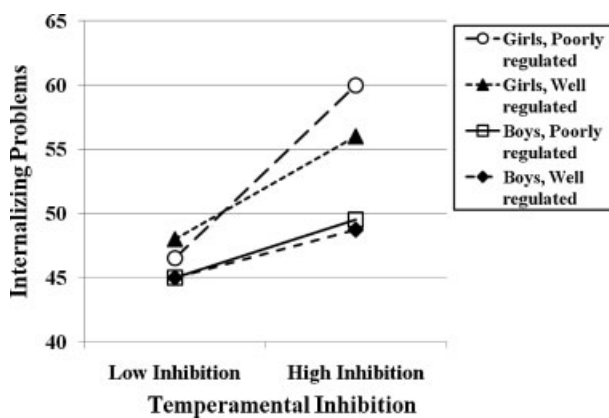
Note: In "Dummy Code: Sample 1," Sample 1 (code 1) is contrasted with Samples 2 and 3 (code 0). In "Dummy Code: Sample 3," Sample 3 (code 1) is contrasted with Samples 1 and 2 (code 0). "Gender" is dummy coded for 0 = girls, 1 = boys.

<sup>a</sup>Model statistics: adj.  $R^2 = .429$ ,  $F(20, 370) = 15.68$ ,  $p < .001$ .

<sup>b</sup>Model statistics: adj.  $R^2 = .404$ ,  $F(20, 370) = 14.25$ ,  $p < .001$ .

<sup>+</sup> $p < .10$ .

\*\*\* $p < .001$ .



**FIGURE 3** Temperamental inhibition predicts internalizing problems most strongly in girls with less well-regulated cortisol 60 min after meeting strangers.

stronger decreases in cortisol levels that was indicative of better HPA regulation, but in the adverse context of maternal punishment, they showed worse regulation and elevated cortisol for longer. Interesting, Gunnar, Tout, de Haan, Pierce, and Stansbury (1999) and Gunnar et al. (1997) have reported that temperamentally "surgent" children—those who are bold and extroverted, hence, low in temperamental inhibition—had elevated cortisol reactivity while interacting with unfamiliar peers in childcare and school settings. Thus, there appears to be something about the context of interacting with unfamiliar others that is physiologically provocative for uninhibited children. Further research on the associations between low inhibition, parenting and punishment and cortisol regulation is warranted.

Cortisol levels were associated with children's emotional and behavioral problems, both directly and by moderating associations between other risk factors and adjustment. Intriguingly, gender played an important role in these patterns. Although young girls tend to have somewhat higher temperamental inhibition than boys (Else-Quest, Hyde, Goldsmith, & Van Hulle, 2006), this is not paralleled by sex differences in adrenocortical reactivity or regulation (Gunnar & Vazquez, 2006). Still, a cascade of biological and social pressures is thought to make girls more prone to developing such internalizing difficulties as anxiety and depression (Zahn-Waxler et al., 2008). We found that girls who experienced angry and punitive maternal care manifested poorer HPA regulation, maintaining higher cortisol levels over a protracted period after meeting adult strangers. When girls with poor adrenocortical regulation also were highly temperamentally inhibited, they showed more internalizing problems. The link between inhibition and internalizing problems appears to be stronger in girls than boys (Letcher, Smart, Sanson, & Toumbourou, 2009), and HPA dysregulation stemming from suboptimal rearing conditions might function as one mechanism linking early inhibition to later maladjustment. Further, sex-typed socialization encourages girls to become overcontrolled, which in inhibited girls may facilitate the development of emotion regulation strategies that involve self-focused attention and rumination, leading to patterns of cognition and emotion (e.g., self-blame, worry, shame) that contribute to both protracted adrenocortical regulation and internalizing symptoms (Borkovec, Ray, & Stöber, 1998; Chaplin, Cole, & Zahn-Waxler, 2005; Mor & Winquist, 2002; Watkins, 2004).

Maternal punishment also was associated with elevated adrenocortical activity, as evidenced by higher cortisol levels 20 min after meeting adult strangers. Boys presented with more externalizing problems when they both experienced more punishment and manifested elevated cortisol at this time. A recent meta-analysis showed that, in the preschool period, basal cortisol levels were positively associated with externalizing problems (Alink et al., 2008). As well, a recent study of kindergarten-aged children showed that greater HPA reactivity to a behavioral testing protocol was associated with more externalizing problems (Obradović et al., 2010). These are consistent with our finding, as we expect that the saliva sample collected 20 min after meeting strangers reflected individual differences in both basal cortisol levels and response to this social challenge. The nature of the interaction also was consistent with the biological sensitivity to context model (Ellis & Boyce, 2008). At least for boys, having elevated cortisol levels was associated with the highest

levels of externalizing problems if mothers were highly punitive, but also with the lowest levels of problems if mothers avoided harsh punishment. The relation between punishment and externalizing problems was weaker for boys with lower cortisol levels. This could suggest that boys with stronger adrenocortical activity are more sensitive to, or more likely to be influenced by, the socialization cues they are receiving, mirroring their mothers' tendencies to be more or less aggressive in their own behavior. Further, our finding supports theories that boys' psychopathology is canalized toward externalizing trajectories (Zahn-Waxler et al., 2008). Compared to girls, young boys are relatively delayed in neurophysiological maturation supporting emotion regulation and inhibitory control, and more prone to anger. Coupled with poor parental support for effective regulation, which would likely be the case with highly punitive mothers, young boys who are more reactive to stressors may express their distress through aggressive and disruptive behavior problems.

At the same time, we found divergent associations between externalizing problems and different parameters of cortisol activity, suggestive of different implications for the relevance of HPA response versus regulation. There have been many studies documenting an inverse relation between cortisol levels and serious externalizing problems (Fairchild et al., 2008; Hastings et al., 2009; van Goozen et al., 2007). The meta-analysis by Alink et al. (2008) suggested that this relation might not be robust until middle-childhood, but support for this link was evident in this sample of preschoolers. Specifically, preschoolers had more externalizing problems if they had lower cortisol levels an hour after meeting strangers, suggesting that they found the ongoing interaction with the researchers to be less intimidating. One leading hypothesis to explain the inverse relation between cortisol and externalizing problems is that children with diminished fear responses are also unresponsive to cues to avoid risky behavior and socialization efforts aimed at curbing disruptive behavior (Raine, 1997). Certainly, the children in this study with the lowest cortisol levels after an hour also were the least inhibited children, if they had nonpunitive mothers. Together, our findings might suggest that there were two paths linking adrenocortical regulation and externalizing problems, and possibly two groups of children with externalizing problems stemming from different factors. Boys with high basal cortisol and/or strong acute reactivity were more sensitive to socialization contexts, and thus mirrored their mothers' harshly punitive parenting in their own aggressive and disruptive behavior. Children with steeper declines in cortisol levels over the testing protocols might have lacked normative and adaptive levels

of fear responses, and this absence of inhibitory cues to avoid risk or danger could contribute to under-controlled, disruptive behavior.

### Limitations

There were aspects of the study that constrain the conclusions that can be drawn. The lack of a true baseline measure of salivary cortisol, either a sample collected prior to meeting the adult strangers or a time-matched sample collected on another day, means that we cannot clearly determine the extent to which the “arrival sample” reflected baseline versus reactive cortisol. With salivary cortisol levels assessed 20 and 65 min after meeting adult strangers, decreasing cortisol over the testing protocol could reflect recovery from the stress of the arrival effect, or simply the time-dependent decrease to be expected given the normative diurnal course of cortisol. Although those two possibilities cannot be disentangled in this study, it is interesting that both mechanisms would convey less effective HPA regulation via increases, or smaller decreases, in cortisol levels. All data were collected contemporaneously, such that it cannot be determined whether risk factors preceded adrenocortical functioning, or whether cortisol reactivity and regulation contributed to the development of problems. Mothers provided all behavioral data; it would be important to replicate these findings with independent measures of parenting, temperament, and adjustment. For example, cortisol levels have been found to have stronger relations to behavioral measures of inhibition than to mother-reported temperament (Schmidt et al., 1997). Differences in the protocols across subsamples limited the ability to examine more fine-grained aspects of HPA activity. However, our findings emerged despite differences in tasks, times of day, and subsample characteristics, lending confidence to their robustness.

### Conclusions

Combining three independent subsamples to produce a large and heterogeneous sample was effective for identifying relations between HPA function, parenting, temperament, and psychopathology that have been elusive or inconsistent in past studies. Children’s adrenocortical reactivity and regulation appear to be responsive to individual differences in disposition and socialization, and in turn, shape how those factors affect young girls’ and boys’ problems. There are differences in the associations between behavior and emotion problems and cortisol levels at the points of HPA reactivity versus regulation, suggesting there are important distinctions in the functional significance of acute versus prolonged HPA activation. Drawing parents’ and

clinicians’ awareness to the roles of stress physiology and individual vulnerability in children’s adjustment could improve assessment and treatment of early-emerging problems.

### NOTES

This research was supported through funding by the Canadian Institutes of Health Research and the Social Sciences and Humanities Research Council of Canada. The authors would like to thank the participants, Dr. Elizabeth Shirtcliff for feedback on the manuscript, and Jonathan Helm for statistical assistance.

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