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# Salivary cortisol levels and infant temperament shape developmental trajectories in boys at risk for behavioral maladjustment

Koraly Pérez-Edgar [a,*], Louis A. Schmidt [b], Heather A. Henderson [c], Jay Schulkin [d], Nathan A. Fox [e]

[a] Department of Psychology, George Mason University, 4400 University Drive, MS 3F5, Fairfax, VA 22030, United States
[b] Department of Psychology, Neuroscience & Behavior, McMaster University, Hamilton, Ontario, Canada
[c] Department of Psychology, University of Miami, Coral Gables, FL, United States
[d] Departments of Physiology & Biophysics and Neuroscience, Center for Brain Basis of Cognition, Georgetown University, Washington, DC, United States
[e] Department of Human Development, University of Maryland, College Park, MD, United States

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**Summary** Behavioral problems in young children can take on a variety of forms, which are linked to distinct antecedents and co-occurring markers. Internalizing difficulties in young children, for example, have been linked to individual differences in infant temperament and cortisol levels. In addition, there is growing evidence that these biobehavioral mechanisms are also shaped by gender. Four-year-old children participated in a study examining the relations between salivary cortisol and behavioral maladjustment as a function of gender and temperament. Both longitudinal (maternal report of infant temperament at 9 months) and concurrent (morning salivary cortisol at age 4) data were used to predict two forms of maladjustment: 'Withdrawal' (maternal report of internalizing behavior and laboratory observation of social reticence) and 'Acting Out' (maternal report of externalizing behavior and laboratory observation of solitary active play). High basal cortisol levels were strongly associated with Withdrawal in male participants. However, the relation was significant only in boys who exhibited high levels of negative temperament in infancy. There were no comparable findings with 'Acting Out' beyond a main effect of gender reflecting greater difficulty in boys. The data suggested that there are unique biobehavioral mechanisms shaping specific patterns of maladjustment in childhood.


## 1. Introduction

The inability to adapt to evolving environmental demands can be linked to various forms of behavioral maladjustment ranging from the relatively benign (prolonged shyness when

* Corresponding author. Tel.: +1 703 993 9366; fax: +1 703 993 1359.
*E-mail address:* kperezed@gmu.edu (K. Pérez-Edgar).

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meeting new peers) to behaviors that mark a real impairment in functioning (as in the case of severe social anxiety). In addressing individual differences in adaptability, researchers have examined the forces that play a role in either hindering or facilitating the child's ability to respond to his or her environment's unique challenges. Throughout childhood, the environment and the demands it places on the individual are continuously evolving. As such, physiological systems involved in these transitions must be both pervasive and flexible in nature (Fox et al., 2006).

One such mechanism, centered on the glucocorticoid cortisol, works to sustain circadian energy levels (Rosen and Schulkin, 1998) and is sensitive to the ebb and flow of daily life. Although generally thought of as a stress hormone, cortisol coordinates adaptation to both environmental and internal conditions and plays a role in shaping both concurrent behavior and moderating subsequent patterns of development (see Shirtcliff et al., 2005 for a discussion). In terms of the daily events in the life of a young child, cortisol levels can increase when entering a new classroom (Tout et al., 1998) or social group (Granger et al., 1994), return to normal levels at a regularly scheduled naptime (Watamura et al., 2001), and then dip below baseline when actively engaged in a positive activity (Hertsgaard et al., 1992; Legendre and Trudel, 1996).

Cortisol levels reflect both individual differences in adjustment and the environmental factors that shape behavior. For example, Cicchetti and Rogosch (2001) found that maltreated children showed either depressed or elevated basal cortisol levels, depending on their behavioral symptomatology. O'Connor et al. (2005) suggested that prenatal anxiety can have long-lasting effects on cortisol secretion, potentially acting as a biological substrate for later psychopathology. Developmental work in normative samples has found that consistent individual differences in cortisol production are evident by 9 months of age (Lewis and Ramsay, 1995a,b). Questions remain regarding the specificity of the link between basal cortisol levels and behavioral maladjustment. For example, while many studies emphasize the link between cortisol and internalizing difficulties (see Gunnar, 2001 for a discussion), studies have linked increased cortisol levels in young children to both internalizing and externalizing difficulties (Essex et al., 2002).

An individual differences approach may help explain the previously observed pattern of behavior. The current study examined factors strongly implicated in shaping profiles of behavioral maladjustment in early childhood, namely infant temperament, basal cortisol levels, and gender. In doing so, the study also examined the specificity or interactive nature of these relations by predicting both internalizing and externalizing behaviors.

Temperament refers to a constellation of individual traits that bias a child to interpret and react to the environment in a predictable manner and is linked to stable patterns of physiological, emotional, and cognitive reactivity (Fox et al., 2001). Children with a 'difficult' or 'negative' temperament are viewed as less able to swiftly adjust to environmental demands and may be at greater risk for behavioral difficulties. Negative temperament or reactivity is marked by crying, fussing, and motoric agitation in infancy (e.g. Buss and Plomin, 1984) and often leads to behavioral inhibition in response to novel objects, people, and events in early childhood (Kagan and Snidman, 1991).

Behaviorally inhibited children appear to have higher baseline cortisol levels (Kagan et al., 1988b) and behavioral inhibition, in conjunction with social wariness, can predict baseline cortisol levels in early childhood (see Schmidt et al., 1997, but not Schmidt et al., 1999). Elevated cortisol levels may also help explain the documented links between early negative temperament and later anxiety (Biederman et al., 2001; Pérez-Edgar and Fox, 2005). In the same vein, cortisol levels have been linked to activation in the central nucleus of the amygdala (Rosen and Schulkin, 1998), a brain structure central to both behavioral inhibition (Kagan et al., 1989; Pérez-Edgar et al., 2007; Schwartz et al., 2003) and anxiety (Thomas et al., 2001a,b).

The literature suggests that temperament measured in infancy or early childhood is a moderately stable trait with stability measures ranging from 0.3 to 0.6 (Degnan and Fox, 2007). As expected, rates are higher when examining selected or at-risk samples rather than normative or randomly selected populations. In addition to issues of initial selection, a growing number of studies have found increased stability (as well as more extreme temperamental profiles) in boys (Fagan, 1990; Fox et al., 2001; Stevenson-Hinde and Glover, 1996). This is in turn linked to increased behavior problems in pre-school (Stevenson-Hinde and Glover, 1996), poor social skills in middle childhood (Rubin et al., 1993), and lower self-esteem in adolescence (Morison and Masten, 1991). In explaining this gender difference many have suggested that shyness or internalizing behaviors are less accepted in boys (Sadker and Sadker, 1994), as seen in social interactions with parents (Crockenberg and Smith, 1982; Radke-Yarrow et al., 1988).

Supplementing this work are findings suggesting that gender differences in developmental trajectories are not due solely to differences in the environment. Biological and psychophysiological markers generally linked to shyness and internalizing behaviors also play different roles across gender. For example, negative reactivity in infancy predicts social wariness at age 4 only if the infant is male and shows right frontal EEG asymmetry (Henderson et al., 2001). Interestingly, Buss et al. (2003) found that children with extreme right frontal asymmetry had higher levels of salivary cortisol than did peers with either extreme left frontal asymmetry or "average" levels of asymmetry.

The relations between cortisol and behavior may also differ as a function of gender (e.g., Jackson et al., 2006), although most of this work has focused on adults, with relatively little work in children (Gunnar et al., 1997; McBurnett et al., 1991; van Goozen et al., 1998). Four studies of note (Hatzinger et al., 2007; Shirtcliff et al., 2005; Smider et al., 2002; Tout et al., 1998) have directly examined gender differences in the relations between cortisol levels and behavioral adjustment in children, producing a complex pattern of findings.

Hatzinger et al. (2007) examined morning basal cortisol levels in a sample of kindergarten children. Their data indicate that increased levels of basal cortisol predicted increases in both internalizing and externalizing behaviors in boys, while decreased cortisol was linked to positive behavior in girls. In contrast, Smider et al. (2002) found no concurrent effects of *afternoon* cortisol levels in similarly aged children. Rather,

they found predictive strength to age 6 with increased levels predicting increased withdrawal in girls and low levels in boys linked to more externalizing difficulty. The inverse relation among boys was also evident in a longitudinal study of children (ages 6—16 years) by Shirtcliff et al. (2005). However, another study (Tout et al., 1998) found that decreased cortisol levels predicted *internalizing* difficulties in boys.

In contrast, studies with at-risk or clinical populations (Cicchetti and Rogosch, 2001; Granger et al., 1994, 1998; Kagan et al., 1988a,b; Schmidt et al., 1997) have generally found high levels of cortisol using both basal measures (e.g. Cicchetti and Rogosch, 2001) and acute responses to novel stimuli or stressors (e.g. Granger et al., 1998; Schmidt et al., 1997). However, studies of at-risk populations often either do not address potential gender differences or have a single-sex sample (e.g. van Goozen et al., 1998). Clearly, additional work will be needed to address the issue of potential gender differences in both normative and clinical populations.

As these data illustrate, questions remain concerning the specificity of the relations of interest. This is important as different forms of behavioral maladjustment are linked to differential outcomes into adolescence and adulthood. It is highly likely that different antecedent and underlying mechanisms may be at work with varying patterns of interrelation (Henderson et al., 2004). To aid the validity and stability of the outcome points, the current study employed both laboratory observation and maternal reports of two distinct forms of behavioral functioning. In particular, we created a measure of 'Withdrawal', by bringing together maternal report of internalizing on the Child Behavior Checklist (Achenbach, 1991) with laboratory observation of social reticence (Rubin, 1989) in the presence of same-age, same-sex unfamiliar peers.

Reticent behavior is marked by a conflict between approach and avoidance motivations (Asendorpf, 1990) in that the children do not engage in social interaction, although they persist in monitoring their peers. Reticence has been linked to behavioral inhibition, internalizing problems, and right frontal EEG asymmetry (Coplan, 2000; Fox et al., 2005; Henderson et al., 2004).

In parallel, we created a measure labeled 'Acting Out' that employed externalizing scores on the CBCL with solitary active play in the same laboratory paradigm noted above. Solitary active behavior is marked by boisterous, repetitive behaviors and dramatizing. It is important to note that solitary active play is defined as behaviors carried out in the presence of peers, as opposed to *with* peers, which is more normative. Solitary active play, while infrequent, is negatively salient to peers (Rubin, 1989) and has been linked to social immaturity, impulsivity, and externalizing behavior (Coplan and Rubin, 1998; Coplan et al., 2001b).

The current sample was selected for extreme negative reactivity at 4 months of age in preparation for a longitudinal study of behavioral inhibition and social withdrawal. Subsequently, the data in toddlerhood reflected this selection process finding high levels of withdrawal and very low levels of externalizing difficulties (Fox et al., 1996). In addition, psychophysiological measures were also found to chiefly moderate developmental trajectories linked to withdrawal. As such, the current 'Acting Out' measure is designed to act as a counter weight to our central interest in the emergence of 'Withdrawal' behaviors. It is for this reason that we have also chosen to focus on negative affect at 9 months, rather than the whole spectrum of temperament traits that may be assessed in infancy.

The present study examined the biological and behavioral factors that may shape developmental trajectories leading to behavioral maladjustment in young children. In doing so, the analyses brought together factors previously shown to influence developmental outcomes: Infant temperament, gender, and basal cortisol levels. To examine the specificity of these relations, two forms of maladjustment were used, 'Withdrawal' (internalizing and social reticence) and 'Acting Out' (externalizing and solitary active play).

Hypothesis one predicted that negative temperament in infancy would be associated with increased levels of Withdrawal at age 4, particularly when coupled with high levels of concurrent basal cortisol. The second hypothesis predicted that this relation would be particularly strong in boys. Hypothesis three further limited the scope of the mechanism by predicting that the model would not hold for Acting Out behaviors.

## 2. Methods

### 2.1. Participants

Children were drawn from two independent cohorts participating in longitudinal studies of temperament and affect regulation. Initially, 4-month-old infants were screened for motoric and emotional reactivity in response to the presentation of novel visual and auditory stimuli (for details see Calkins et al., 1996; Kagan and Snidman, 1991). Infants from both cohorts were selected based on the amount of motor reactivity as well as positive and negative affect expressed during the presentation of the novel sights and sounds. Of the 433 infants screened, 153 were selected for inclusion in the longitudinal studies. The families were Caucasian and of middle-class background, living in the greater Washington, DC area. Approximately 68% of the mothers and 72% of the fathers were college educated. One-third of the children were first born. Previous analyses involving these children can be found in Fox et al. (2001, 1995). After initial selection, the children returned to the laboratory at multiple age points (Calkins et al., 1996; Fox et al., 2001).

This study relied on four measures collected at two time points: 9 months and 4 years of age. As a result, the *N*'s varied across each of the measures (see below). In order to create a stable study population, the findings presented below are drawn solely from the 83 children (41 male) who had viable data on all measures.

Preliminary analyses found no significant differences between the children in this study and the remaining cohort with missing data (all $p$'s $> 0.12$). To ensure that the children included in this study reflect their relative position within the full cohort, any data manipulations (e.g., standardization, composite creation) were completed using the full cohort before removing the children with missing data.

### 2.2. Infant temperament

At 9 months of age, maternal reports of temperament were gathered using the Infant Behavior Questionnaire (IBQ; Rothbart, 1981). The IBQ is an 87-item parent rating form in which

parents are asked to rate the frequency of specific infant behaviors as they occurred in the previous week. Scaled scores are derived from the measure by taking the mean ratings on all items in the particular scale, omitting the items marked as 'Does not apply'. The composite measure 'Negative Affect' was created by summing the children's scores on two scales, 'Distress to Limitations' and 'Distress to Novelty'. Data were available for 141 infants.

## 2.3. Morning salivary cortisol

During a laboratory visit for the larger longitudinal study, parents were instructed in how to collect salivary cortisol samples. They were then given a saliva collection kit and written instructions. At collection, the children were asked to chew for approximately 1 min on a dental cotton roll saturated with Kool-Aid© crystals. Schwartz et al. (1998) has cautioned that citrus-based substances may interfere with some assays. However, Talge et al. (2005) recently completed a series of experiments showing that, when used consistently within a sample, the introduction of this oral stimulant does not affect the rank ordering of participants and does not compromise the quality of the salivary cortisol data. In our experience, these stimulants are important for maximizing compliance in children and have been previously used successfully in multiple laboratories (Schmidt et al., 1997; Tout et al., 1998).

The saliva absorbed by the cotton was squeezed into a cryogenic tube with a needleless syringe. The procedure was repeated, if necessary, until at least 500 μl were collected. Saliva samples were then frozen. Collection took place within 30 min of waking over the course of 3 consecutive days. Parents kept a written log of wake-up and sampling times. The three frozen saliva samples were then returned to the laboratory at a subsequent visit. Similar procedures have been previously used (Gunnar et al., 1989; Schmidt et al., 1997) with good results.

The saliva samples were assayed by the Clinical Neuroendocrinology Branch of the National Institute of Mental Health, Bethesda, MD. Samples were thawed, vortexed, and centrifuged for 30 min at 2250 × *g*. Concentrations were determined using a solid phase radioimmunoassay (125I) (Coat-A-Count, Diagnostic Products Corporation, Los Angeles, CA), using 200 μl of saliva. Samples incubated for 3 h at room temperature. Following aspiration, tubes were counted using an ICN Micromedic Systems, Apex Automatic Gamma Counter. Both samples and standards were determined in duplicate. Samples were performed simultaneously in order to eliminate interassay variability. The lower detection limit assay was 0.1 μg/dl.

A single composite measure of mean morning cortisol level (expressed in μg/dl) was computed by averaging across all useable morning samples. The average collection time (see Table 1) was 7:53 a.m. and reliable data were available for 111 4-year-olds (51 males).

Two children (both male) were removed from analysis due to extreme scores (*Z*'s > 5). As such, the analyses reported below were completed with a sample of 81 children (39 male).

Initial analyses examined the range of collection times for the cortisol samples in relation to the measures of interest.

**Table 1** Cortisol sample collection times

| | Mean | Minimum | Maximum | S.D. |
|---|---|---|---|---|
| (A) Mean collection times | | | | |
| Overall | 7:53 a.m. | 5:53 a.m. | 10:10 a.m. | 0:48 |
| Boys | 7:45 a.m. | 5:53 a.m. | 10:10 a.m. | 0:54 |
| Girls | 8:04 a.m. | 6:46 a.m. | 9:15 a.m. | 0:37 |
| (B) Elapsed collection times | | | | |
| Overall | 0:31 | 0:20 | 0:50 | 0:06 |
| Boys | 0:30 | 0:20 | 0:45 | 0:05 |
| Girls | 0:32 | 0:20 | 0:50 | 0:07 |

Samples were collected within 30 min of rising for three consecutive mornings. A presents the data for the mean collection times for the three samples. B presents the elapsed time between the earliest and latest cortisol sample for each child.

There were no significant gender differences for mean collection time and mean time range, *t*'s < 1.05, *p*'s > 0.30. In addition, there were no relations between collection time and mean time range and the other measures of interest, *r*'s < 0.18, *p*'s > 0.30. For the regression analyses presented below, collection data were included as predictors. However, there were no significant findings and will therefore not be presented here.

## 2.4. Maternal reports of behavioral maladjustment

Maternal reports of the children's behavioral adjustment were assessed using the Child Behavior Checklist (CBCL; Achenbach, 1991) at age 4. The CBCL is a 113-item checklist in which parents use a three-point scale to rate how descriptive a series of behavior problems are of their own child. Information concerning the reliability and validity of the CBCL can be found in Achenbach (1991). The CBCL yields eight narrow-band factors: social withdrawal, somatic problems, anxiety/depression, social problems, thought problems, attention problems, delinquency, and aggressive behavior. These factors can be further reduced to two broad-band factors, internalizing and externalizing behavior problems. Data were available from 112 children.

All analyses relied on standardized *T*-scores for internalizing (mean = 46.96, S.D. = 8.44) and externalizing (mean = 50.51, S.D. = 8.35) problems. Initial analyses found that ten children met the threshold cutoffs (i.e., *T*'s greater than or equal to 60) for internalizing problems. Of these, nine were boys, $\chi^2 = 7.50$, $p = 0.01$. For externalizing problems, again 10 children met the threshold cutoff. However, here they were evenly divided between boys and girls, $\chi^2 = 0.002$, $p = 0.97$.

## 2.5. Laboratory observations of social behavior

As part of the larger experimental battery, the children also participated in a group play session with three unfamiliar, same sex, same age peers. Each quartet consisted of one socially inhibited child, one non-inhibited child, and two average children. The children were assigned to quartets based on levels of behavioral inhibition noted at 24 months of age.

The four children were led into a playroom where several age-appropriate toys were accessible. The visit was split into several episodes, a complete description of which may be found in Fox et al. (1995). Behaviors were coded with Rubin's (1989) play observation scale (POS), scoring 10-s intervals for social participation and the cognitive quality of play. Three independent observers, with reliability greater than 0.8, coded the POS. For purposes of this study, data from two 15-min free play sessions were used to calculate an index of reticent (the sum of onlooking and unoccupied behavior; Coplan et al., 1994) and solitary active (solitary-functional and solitary dramatic play; Coplan et al., 2001a) behavior. As expected, reticence behaviors (mean = 0.163, S.D. = 0.133) were more evident in this sample than solitary active behaviors (mean = 0.066, S.D. = 0.084). While boys and girls did not differ in their levels of reticence, $t(79) = 0.586$, $p = 0.56$, boys were more likely to display solitary active behavior, $t(79) = 5.70$, $p < 0.001$.

### 2.6. Composite measures

In order to create a stable measure of adjustment at age 4, we created two composite scores bringing together maternal report and laboratory observations. Standardized laboratory reticence scores and standardized CBCL internalizing *T* scores were meaned to create a 'Withdrawal' score. In parallel, standardized laboratory scores of solitary active behavior and standardized CBCL externalizing T scores were meaned to create an 'Acting Out' measure.

## 3. Results

### 3.1. Preliminary data analysis

Simple correlations were conducted to examine the relations between the four central measures of the study (Table 2). Withdrawal was significantly correlated with both negative affect, $r(81) = 0.29$, $p = 0.01$, and home cortisol, $r(81) = 0.30$, $p = 0.01$. The two measures were not significantly correlated with Acting Out, $r$'s $< 0.07$. In addition, there was a trend linking Withdrawal and Acting Out, $r(81) = 0.19$, $p = 0.08$.

Initial analyses found no gender-linked differences in negative affect at 9 months, cortisol levels at age 4, and Withdrawal scores, $t$'s $< 1.45$, $p$'s $> 0.15$ (Table 3). In contrast, boys had significantly higher scores on the Acting Out measure, $t(79) = 4.89$, $p < 0.001$.

**Table 2** Zero-order correlations between negative affect, home cortisol levels, and maladjustment composite scores

| | Negative affect | Cortisol | Withdrawal | Acting Out |
|---|---|---|---|---|
| Negative affect | 1.00 | | | |
| Cortisol | 0.116 | 1.00 | | |
| Withdrawal | 0.287** | 0.300** | 1.00 | |
| Acting Out | 0.067 | 0.074 | 0.194+ | 1.00 |

Negative affect was measured at 9 months of age, while the other measures were each collected at 4 years of age. *N*'s = 81.
*$p < 0.05$, **$p < 0.01$.

**Table 3** Cortisol levels, temperament measures, and maladjustment scores for boys and girls in the sample

| | Overall | Boys | Girls |
|---|---|---|---|
| Negative affect | 6.29 (1.38) | 6.52 (1.28) | 6.08 (1.45) |
| Basal cortisol | 0.41 (0.18) | 0.43 (0.17) | 0.39 (0.18) |
| Withdrawal | −0.05 (0.80) | 0.06 (0.94) | −0.15 (0.63) |
| Acting Out | 0.00 (0.68) | 0.34** (0.66) | −0.31** (0.54) |

Negative affect was measured at 9 months of age, while the other measures were each collected at 4 years of age. Standard deviations are noted in parentheses.
**$p < 0.01$.

### 3.2. Longitudinal analyses

Two hierarchical multiple regression analyses were conducted to examine the full model. Regressions were run using data at age 9 months (negative affect) and age 4 (cortisol), along with gender, to predict behavioral maladjustment (Withdrawal and Acting Out) at age 4.

For each analysis, the predictors were entered into the regression equation in the following order: (1) negative affect at 9 months, (2) home cortisol levels, (3) gender, (4) negative affect × home cortisol levels, (5) negative affect × gender, (6) home cortisol × gender, and (7) negative affect × home cortisol × gender. The dependent measures were (a) Withdrawal and (b) Acting Out. Predictive measures were mean centered before use in the regressions. The results of the hierarchical regression analyses are presented in Table 4.

### 3.3. Withdrawal

When predicting Withdrawal behavior at age 4, the full model accounted for 36.1% of the total variance, $F(7,73) = 5.97$, $p < 0.001$. Negative affect significantly predicted Withdrawal, accounting for 9.0% of the variance, $\Delta F(1,73) = 7.95$, $p = 0.006$. The main effect of home cortisol levels was also significant, accounting for 4.6% of the overall variance, $\Delta F(1,73) = 4.25$, $p = 0.04$. These reflected the significant zero-order correlations noted above. The main effect of gender was not significant.

The interaction between negative affect and cortisol levels accounted for an additional 14.1% of the variance in Withdrawal, $\Delta F(1,73) = 15.01$, $p < 0.001$. In order to interpret this interaction, the children were divided into two groups based on negative affect at 9 months and the correlations between the concurrent cortisol levels and Withdrawal were then calculated separately for each group. For the children high in negative affect, there was a significant positive correlation between cortisol and Withdrawal, $r(39) = 0.44$, $p < 0.01$. In contrast, the children low in negative affect showed no relation, $r(42) = 0.20$, $p = 0.22$.

The interaction between negative affect and gender also accounted for 3.8% of the variance, $\Delta F(1,73) = 4.17$, $p = 0.05$. To examine this finding, separate zero-order correlations between negative affect and Withdrawal were calculated for boys and girls. Boys showed a significant positive relation, $r(39) = 0.46$, $p < 0.01$, while girls showed no relation, $r(42) = 0.15$, $p = 0.33$.

**Table 4** Predicting Withdrawal and Acting Out scores at age 4 using measures of infant temperament (negative affect at 9 months), concurrent cortisol levels, and gender

| | Withdrawal[a] | | | Acting Out[b] | | |
|---|---|---|---|---|---|---|
| Predictor | ß | $\Delta R^2$ | $\Delta F$ | ß | $\Delta R^2$ | $\Delta F$ |
| Negative affect | 0.92** | 0.09 | 7.95** | −0.35 | 0.00 | 0.33 |
| Cortisol | 1.47** | 0.05 | 4.25* | −0.01 | 0.00 | 0.28 |
| Gender | −0.02 | 0.00 | 0.02 | −0.49** | 0.23 | 23.34** |
| Negative affect × cortisol | 1.03* | 0.14 | 15.01** | 0.17 | 0.00 | 0.01 |
| Negative affect × gender | −0.69* | 0.04 | 4.17* | 0.35 | 0.01 | 0.91 |
| Cortisol × gender | −0.82* | 0.03 | 3.59+ | 0.01 | 0.00 | 0.02 |
| Negative affect × cortisol × gender | −0.47 | 0.01 | 1.62 | −0.11 | 0.00 | 0.08 |

*Note*: Standardized ß are presented.
+$p < 0.10$, *$p < 0.05$, **$p < 0.01$.
[a] $F(7,73) = 5.97$, $p < 0.001$.
[b] $F(7,73) = 3.46$, $p < 0.001$.

**Table 5** Predicting Withdrawal scores at age 4 as a function of negative affect at 9 months and cortisol levels at age 4

| | Boys[a] | | | Girls[b] | | |
|---|---|---|---|---|---|---|
| Predictor | ß | $\Delta R^2$ | $\Delta F$ | ß | $\Delta R^2$ | $\Delta F$ |
| Negative affect | 0.33* | 0.18 | 8.57** | 0.18 | 0.02 | 0.96 |
| Cortisol | 0.93** | 0.10 | 5.14* | 0.05 | 0.00 | 0.03 |
| Negative affect × cortisol | 0.81** | 0.23 | 16.92** | 0.18 | 0.03 | 1.16 |

Analyses were conducted separately for boys and girls.
*Note*: Standardized ß are presented.
*$p < 0.05$, **$p < 0.01$.
[a] $F(3,35) = 12.62$, $p < 0.001$.
[b] $F(3,38) = 0.71$, $p = 0.55$.

There was an additional trend for an interaction between home cortisol levels and gender, accounting for 3.1% of the variance, $\Delta F(1,73) = 3.59$, $p = 0.06$. As in the previous analyses, separate zero-order correlations found a significant relation between cortisol and Withdrawal for boys, $r(39) = 0.55$, $p < 0.01$, but not girls, $r(42) = 0.03$, $p = 0.84$.

### 3.3.1. Exploratory analyses

Although the three-way interaction between negative affect, cortisol, and gender was not significant, $\Delta F(1,73) = 1.64$, $p = 0.21$, our *a priori* hypotheses led us to further explore gender-linked differences in the relations of interest. Two separate regressions were completed for boys and girls using the following predictors: (1) negative affect at 9 months, (2) home cortisol levels at age 4, and (3) negative affect × home cortisol levels (see Table 5).

When predicting Withdrawal in boys, the full model accounted for 51.3% of the variance, $F(3,35) = 12.62$, $p < 0.001$. Both the main effects of negative affect, $\Delta F(1,35) = 8.57$, $p < 0.01$, 18.4% of variance, and home cortisol, $\Delta F(1,35) = 5.14$, $p = 0.03$, 10.0% of variance, were significant. In addition, there was a significant interaction between negative affect and cortisol, $\Delta F(1,35) = 16.92$, $p < 0.001$, 22.9% of variance.

To examine this interaction, children were placed into two groups based on levels of negative affect in infancy (Fig. 1). Simple correlations found that for boys high in early negative affect, home cortisol levels positively correlated with Withdrawal at age 4, $r(24) = 0.63$, $p = 0.001$. Boys with low levels of negative affect showed no relation, $r(15) = 0.32$, $p = 0.24$.

The equivalent analysis with girls found a non-significant model, $F(3,38) = 0.71$, $p = 0.55$, accounting for only 5.3% of the variance.

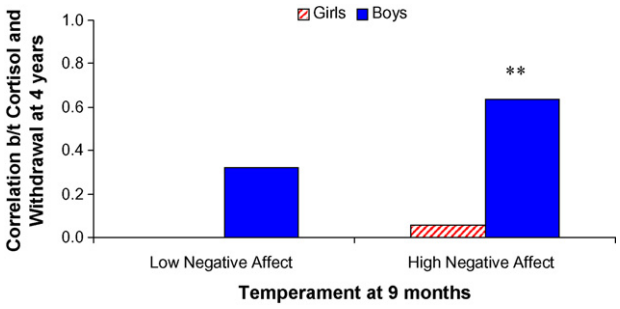


**Figure 1** Correlations between cortisol levels and Withdrawal as a function of infant temperament. The data are presented separately for boys and girls (**$p < 0.01$).

## 3.4. Acting Out

When predicting Acting Out, the full model accounted for 24.6% of the total variance, $F(7,73) = 3.46$, $p < 0.001$. This was driven entirely by the main effect of gender, $\Delta F(1,73) = 23.34$, $p < 0.001$, which accounted for 22.9% of

variance. This reflected the higher Acting Out scores in boys (0.34) than girls (−0.31), $t(79) = 4.89$, $p < 0.001$.

## 4. Discussion

The current study assessed the relations between infant temperament, gender, and basal cortisol levels in shaping internalizing and externalizing forms of behavioral maladjustment in 4-year-old children. The data indicated that while gender-linked differences were evident for both outcome measures, the underlying mechanisms were quite distinct. As expected, infant temperament was linked to later Withdrawal. However, the relation was carried by children who also showed high levels of basal cortisol at age 4. Exploratory analyses also indicated that the mechanism was only evident in boys. In contrast, analyses using the Acting Out measure found only a main effect of gender, with boys showing more difficulties than girls.

The ability to effectively regulate cortisol levels to meet environmental and internal challenges is of great importance given that both chronic hypo- and hyper-reactivity have been linked to behavioral maladjustment (Gunnar and Donzella, 2002), reflecting cortisol's role as a central nervous system regulator, shaping basic mechanisms of arousal, attention, perception, and memory (Erickson et al., 2003).

The current findings suggested that elevated cortisol may help sustain early appearing biases from infancy into early childhood. This is in line with Gunnar's (1999) contention that cortisol production is rooted in the child's ability to cope with life stressors. A failure in the overall coping mechanism can, therefore, be displayed in a variety of ways, depending on individual, environmental, and experiential factors.

The accumulating data suggest that early temperamental biases, when coupled with a psychophysiological marker (cortisol in the current study, EEG asymmetry in Henderson et al., 2001), will shape later development. Recent data indicating that differences in neural functioning can be detected decades after initial observations of temperamental inhibition (Guyer et al., 2006; Pérez-Edgar et al., 2007; Schwartz et al., 2003) serve to underscore the stability and importance of these biobehavioral mechanisms.

The current study joins recent work (Hatzinger et al., 2007; Shirtcliff et al., 2005; Smider et al., 2002; Tout et al., 1998) examining potential gender-linked differences in the relations between cortisol levels and behavior. In the current sample, boys were at increased risk for both forms of behavioral maladjustment. This may in part reflect the high levels of co-morbidity often found in early childhood (e.g., Essex et al., 2002). In addition, the children in this study were part of a selected sample at risk for behavioral inhibition (Fox et al., 1995, 2001). In these samples, boys often show increased temperamental stability and poorer outcomes. These data were therefore in line with other studies noting that the relation between cortisol and internalizing behaviors is most evident in selected or at-risk samples (e.g., Cicchetti and Rogosch, 2001; Shirtcliff et al., 2005). These data further illustrated the specificity of these findings in that the underlying mechanisms implicated in the overall patterns of behavior were quite distinct between Withdrawal and Acting Out.

The EEG asymmetry findings from Henderson et al. (2001) suggest that the current data reflect a broad biobehavioral system at work. In that study, EEG asymmetry and negative affect at 9 months predicted social withdrawal at age 4 only in boys. Building on these data, recent work (Martin McDermott, Pérez-Edgar, Henderson, Pine, & Fox, under review) finds an analogous pattern with the error-related negativity (ERN), a marker for error detection and performance monitoring, in adolescents who were behaviorally inhibited as young children. Here, a large ERN predicted clinical anxiety levels only in boys who were inhibited as children. Neither Henderson et al. (2001), Martin McDermott et al. (under review), nor the current study found a significant main effect of gender for any of the central psychophysiological measures (EEG Asymmetry, ERN, basal cortisol). This suggests that the findings were not an artifact of undifferentiated levels of reactivity in boys and girls, but rather reflected unique patterns of functioning linking biology to behavior.

The current study's limitations should be noted when reviewing the findings. First, the current study was unable to address potential differences in the diurnal pattern of cortisol. While morning levels of cortisol were linked to differing patterns of maladjustment as a function of gender, we could not say if this measure is part of a larger pattern of hormonal secretion that may go beyond the relations noted here (Goodyer et al., 1996).

Second, collection times for the cortisol samples varied among participants. Given the cyclical nature of cortisol secretions, there was a risk that this affected the findings presented here. Analyses, however, found no differences linked to cortisol levels, gender, or behavior problems. Future work would benefit from a more tightly regulated collection schedule.

Third, assessing cortisol in both early childhood and infancy may be of particular importance when considering long term adjustment given that stress early in life, such as maltreatment and abuse in the extreme, may alter the set point for the stress response well into adulthood (Heim et al., 2001; King et al., 2001). While there are some indications that individual patterns in cortisol levels may be evident as early as 6 months of age (Lewis and Ramsay, 1995a,b), concurrent cortisol measures in infancy would help extend the observed data.

The current study spanned a time period marked by important changes in the functioning and regulation of cortisol. Shirtcliff et al. (2005) noted that most studies focus on children ages 6–12 (e.g. Smider et al., 2002) due to the fact that major behavioral difficulties often first emerge at this time (Graber and Brooks-Gunn, 1996). The functional and developmental significance of this system is closely tied to the context under which it is observed.

As such, rather than serving as an independent test of successful adaptation, cortisol may better serve our empirical and theoretical concerns when placed within a larger developmental profile that takes into account individual variations in biology, environment, and behavior. For example, previous studies have found that the cortisol wakening response and evening cortisol levels reflect independent characteristics of functioning (Netherton et al., 2004; Rosmalen et al., 2005), potentially leading to inconsistent findings across studies that employ different methodologies, populations, and measures. Work of this nature must take into account the (1) initiating stimulus, (2) the objective and subjective components of stimulus processing, and (3) the

resulting response, which may incorporate both physiology and behavior. As such, it is difficult to *a priori* define and quantify the ''load'' or ''burden'' cortisol levels place on the individual (Levine and Ursin, 1991; Steptoe, 2000).

Indeed, the findings from the current study may be best understood in the context of a larger pattern of research showing a consistent link between underlying biological mechanisms (e.g., cortisol levels, frontal EEG asymmetry, limbic reactivity) and broad psychological and behavioral profiles (e.g., temperament, anxiety, externalizing difficulties) that is moderated by a number of factors that include an individual's gender, environment, and genetic profile. In this sense, cortisol is one mechanism within a larger web of interconnected systems that may act independently or in tandem to shape developmental trajectories.

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## Conflict of interest

None declared.

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