

Salivary Cortisol as a Predictor of Socioemotional Adjustment during Kindergarten: A Prospective Study

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This study, based on a sample of 172 children, examined the relation between average afternoon salivary cortisol levels measured at home at age 4.5 years and socioemotional adjustment a year and a half later, as reported by mothers, fathers, and teachers. Cortisol levels were hypothesized to be positively associated with withdrawal-type behaviors (e.g., internalizing, social wariness) and inversely related to approach-type behaviors, both negative and positive (e.g., externalizing, school engagement). Higher cortisol levels at age 4.5 predicted more internalizing behavior and social wariness as reported by teachers and mothers, although child gender moderated the relation between cortisol and mother report measures. An inverse relation was found between boys' cortisol levels and father report of externalizing behavior. A marginal inverse relation was found between child cortisol levels and teacher report of school engagement. Behavior assessed concurrently with cortisol collection did not account for the prospective relations observed, suggesting that cortisol adds uniquely to an understanding of behavioral development.

INTRODUCTION

Over the past decade, there has been a rapid proliferation of studies on socioemotional development that include the assessment of various facets of physiological systems related to emotional reactivity and regulation (Dawson, Hessler, & Frey, 1994; Fox, 1994; Kagan, 1998). Of particular interest has been the identification of predictors and sequelae of individual differences in biobehavioral processes, from infancy through adulthood, especially as they relate to the tendency to withdraw or approach in novel or challenging circumstances (Davidson & Rickman, 1999; Gunnar, 1994; Harmon-Jones & Allen, 1998; Kagan, Reznick, & Snidman, 1987; Sutton & Davidson, 1997). More recent consideration has been given to how these processes may be manifest in ecologically valid situations such as interactions with peers or parents (Granger, Stansbury, & Henker, 1994; Granger, Weisz, McCracken, Ikeda, & Douglas, 1996; Schmidt et al., 1997) or social and behavioral adjustment in school settings (Granger, Stansbury, & Henker, 1994; Gunnar, Tout, de Haan, Pierce, & Stansbury, 1997; Tout, de Haan, Campbell, & Gunnar, 1998).

The assessment of cortisol, in particular, is increasingly prevalent in studies of children's socioemotional development. Cortisol is the primary glucocorticoid produced by the hypothalamic-pituitary-adrenocortical (HPA) axis, which is part of the human stress-response system. A well-established relation between the activity of the HPA axis (as marked by cortisol) and psychological processes and emotional states makes this system particularly relevant in the study of socioemotional adjustment (de Kloet & de Wied, 1980; Mason, 1980; Rose, 1980). Furthermore, there are

several reasons why the measurement of cortisol is especially advantageous when investigating associations between biological systems and behavior in children. First, the general physiology of the HPA axis is clearly understood in both humans and animals. Cortisol, more specifically, has been studied extensively and its functional role has been detailed (e.g., de Kloet, 1991). Second, because salivary levels are highly correlated with plasma concentrations (Kirschbaum & Hellhammer, 1989, 1994), cortisol can be collected via noninvasive methods. Third, cortisol sampled from saliva is hardy and robust with respect to a range of processing conditions and it can be analyzed using widely available laboratory radioimmunoassay (RIA) kits with well-established modifications for use with saliva (Kirschbaum & Hellhammer, 1989, 1994; Schwartz, Granger, Susman, Gunnar, & Laird, 1998).

Under normal, nonstressed conditions, the HPA axis shows a circadian pattern of activation, releasing cortisol in greater concentrations and with greater frequency in the morning hours, with a steep decrease in concentration toward midday, a relatively quiescent period through the late afternoon hours, and slowly decreasing levels throughout the evening. Receptors for cortisol are found throughout the brain, and cortisol affects a number of brain regions (e.g., prefrontal cortex, hypothalamus, hippocampus, amygdala) involved in emotion, learning, and memory (de Kloet & de Wied, 1980; Sapolsky, Krey, & McEwen, 1986). Animal studies indicate that chronically elevated levels

of cortisol can have deleterious effects on the brain, including shrinkage of the hippocampus (McEwen, 1995; Sapolsky, 1996), as well as deficits in memory and learning processes (Kirschbaum, Wolf, May, Wiprich, & Hellhammer, 1996). Similar associations have been reported in human studies of adult depressives (Sapolsky, 1996). Prolonged cortisol elevations have been associated with poor memory performance and hippocampal atrophy in the elderly (Lupien et al., 1998). There is no research, however, on whether such relations exist in child populations.

Research in child populations has focused predominantly on the relation of HPA activity to social and emotional adjustment. Much of this research has looked at reactivity of the HPA system as indexed by changes in cortisol concentrations in pre- to post-stressor designs. Individual differences in HPA reactivity have been associated with social and emotional difficulties during early childhood (e.g., Granger, Stansbury, & Henker, 1994; Gunnar, 1994; Gunnar et al., 1997; Stansbury & Gunnar, 1994)—behavioral patterns that may persist through the early school years and into later childhood and even adolescence (e.g., Rubin, Hymel, Mills, & Rose-Krasnor, 1991). Pre-post studies are advantageous for investigating how individuals respond under acutely stressful specific situations. It is not clear, however, whether they reveal much about more general day-to-day activity of the HPA system and its relation to emotional adjustment and behavioral styles. On the other hand, there are both theoretical and empirical reasons to believe that individual differences in *average levels* of activity of the HPA system, and cortisol in particular, may more appropriately reflect general, non-specific, behavioral tendencies in response to the day-to-day challenges of life. Although a single measurement of cortisol is likely to reflect reactivity of the HPA system to recent stressors, samples self-collected over a number of days at home can provide a useful index of what is typical for a person across days in an ecologically valid context. In the past, some studies reported single samples (often pre-experiment samples taken at the beginning of a laboratory session) as reflecting an “unstressed” level. Not only are such single-sample measures probably inadequate representations of average levels of activation (given the dynamic nature of the system), but they may, in fact, be misleading in that substantial anxiety may be aroused in anticipation of what is going to happen during the session. Recently, investigators interested in assessing individual differences in average levels of activation of the HPA system have addressed this issue with indices derived from repeated sampling over a number of days at the same time

across days (e.g., Gunnar et al., 1997; Schmidt et al., 1997; Tout et al., 1998).

Approach, Withdrawal, and the HPA Axis

Two prominent lines of research suggest that an approach-withdrawal conceptual framework is a promising perspective from which to study the relation between physiological systems and emotional states and behavioral tendencies. EEG studies demonstrate that asymmetric left frontal brain activation is associated with approach-related emotions (positive emotions and some negative emotions such as anger), whereas emotions associated with withdrawal behavior (negative emotions such as sadness and fear) are characterized by asymmetric right frontal EEG activity (e.g., Davidson, 1994; Harmon-Jones & Allen, 1998; Sutton & Davidson, 1997). Furthermore, complementing the findings that individual differences in activation of the HPA axis are related to depressive symptoms and mood disorders (e.g., Gold, Goodwin, & Chrousos, 1988), Davidson and colleagues reported that depressed patients exhibited greater relative right-sided prefrontal activation (e.g., Davidson & Irwin, 1999). In addition, Kalin and colleagues (Kalin, Larson, Shelton, & Davidson, 1998) reported that in rhesus monkeys, those with greater relative right-sided activation had higher levels of basal cortisol. Other evidence in support of asymmetric hemispheric control of the HPA axis comes from the work of Wittling (1995), who reported that affective stimuli presented directly to the right hemisphere provoked a significantly larger increase in cortisol compared with the identical stimuli presented directly to the left hemisphere. Collectively, these findings point to some of the potentially important central contributions to individual differences in HPA activation and provide additional rationale for the search for behavioral correlates of such differences.

A second line of research highlighting the potential of an approach-withdrawal conceptual framework comes from studies of the associations of cortisol levels and behavior. In studies of children, behaviors that can be broadly classified as internalizing behaviors have received the most attention, although externalizing behaviors have also been considered. It has been hypothesized that children with internalizing behaviors have lower thresholds for activation of stress-sensitive physiological systems (e.g., Kagan et al., 1987), whereas children with externalizing behaviors have higher thresholds for physiological arousal (e.g., Rogness, Javors, Maas, & Macedo, 1990). These hypotheses have been examined in numerous studies, with mixed results that might be accounted for, in

part, by differences in measures, reporters, time of day, samples (e.g., clinical versus community), and research settings (e.g., laboratory, home, nursery school; or familiar versus novel). Nevertheless, there is a body of evidence, summarized below, that is consistent with these hypotheses.

Over a decade ago, Kagan reported that extremely inhibited young children had high levels of cortisol, suggesting that basal or average levels might be an important component in understanding the relation between biology and child behavioral tendencies (Kagan et al., 1987). Since then, studies examining acute HPA reactivity have been favored, reflecting a belief that HPA-behavior associations are more likely to be observed during HPA reactivity to stress than during HPA basal activity (Granger, Weisz, & Kauneckis, 1994). Evidence has also accrued slowly, however, to lend empirical credibility to the view that average cortisol levels (as opposed to stress-induced changes) may be an important individual difference factor related to behavioral withdrawal and inversely related to behavioral approach. In an extensive study, Schmidt and colleagues (1997) found that maternal report of behavioral inhibition of their children at 14 months of age was related to baseline cortisol levels at age 4 years. Baseline cortisol was also associated with concurrently assessed social wariness. Consistent with Kagan's report, children who were extremely socially wary at 4 years of age showed significantly higher baseline cortisol levels than children who were classified as low in social wariness. As in the Kagan study, the method of assessment consisted of multiple sampling of cortisol over several days at the same time each day. Others have reported associations between measures of average levels of cortisol and concurrent parental reports (which parent was unspecified) of internalizing behavior (e.g., Scerbo & Kolko, 1994) and shyness (de Haan, Gunnar, Tout, Hart, & Stansbury, 1998).

Studies of adults and studies of nonhuman primates provide corroborating evidence of the positive association of withdrawal types of behaviors with basal cortisol. Chronically lonely college students have been found to have elevated mean levels of cortisol across the course of the day (Cacioppo et al., 2000). Further, elevated cortisol levels have been associated with depression, occurring in up to 50% of depressed patients (Hansen-Grant, Pariante, Kalin, & Miller, 1998). A subgroup of patients with particularly pronounced melancholic features has also been found to show elevations in cortisol (Gold et al., 1988). Finally, in rhesus monkeys, freezing behavior (the monkey analog to extreme behavioral inhibition in humans) has been shown to be positively correlated with basal cortisol levels (Kalin, Shelton, Rickman, & Davidson, 1998).

An inverse relation between cortisol and negative approach behaviors, such as aggressiveness and conduct difficulties, has also been reported, although the results are more mixed for externalizing behaviors than for internalizing behaviors. As early as 1985, Tennes and Kreye showed that 45% of the variance in a 7-day baseline measure of school-aged children's cortisol could be explained by observed hostility (largely oppositional behavior) toward the teacher. Specifically, higher observed hostility was associated with lower baseline levels of cortisol. Subsequent studies of children with conduct disorder without comorbid anxiety (McBurnett et al., 1991) and of oppositional defiant children (van Goozen et al., 1998) have found similar inverse associations between externalizing behaviors and cortisol. In these latter two studies, however, only males were studied.

Summary

Taken together, a picture begins to emerge of a relation between cortisol levels as a reflection of the HPA axis and individual differences in behavioral and emotional dispositions. Higher activity of the HPA axis may reflect an underlying biobehavioral tendency to withdraw in the face of normative social challenges of day-to-day life, whereas lower levels of activation may reflect the tendency to approach, even if it is associated with negative affect. Few if any studies, however, have examined long-term prospective relations between average daily cortisol levels in children and adaptation to normative stresses and behavioral tendencies at some later point in time. In addition, the question of the extent to which cortisol-behavior relations found in recent studies are evident in larger community samples of children (i.e., a sample not preselected on any behavioral dimension) and consistent between genders has gone largely unaddressed (for a recent exception, see Tout et al., 1998).

The Present Study

In the present study, we addressed a number of the issues raised above. Based on a large community sample, we investigated the extent to which children's average afternoon cortisol levels at age 4.5 years, measured across days at home (i.e., in a non-stress-induced situation), predicted mother, father, and teacher reports of child adjustment over a year and a half later at the end of kindergarten. Parallel mother-report measures of child adjustment that were available at the 4.5-year assessment were also included to investigate whether the associations of children's average afternoon cortisol levels at that time

predicted later child adjustment independent of its association with concurrent adjustment.

For young children, the kindergarten year presents an array of adaptational challenges. Over the course of the year, children are faced with numerous developmental tasks, including cognitive, behavioral, and social challenges, and the necessity to adhere to the structure imposed by the classroom and school setting. Individual differences in adaptation to school have been shown to predict children's attitudes about school, academic performance, relationships with teachers and peers (Birch & Ladd, 1997; Ladd, 1990; Pianta, Steinberg, & Rollins, 1995), and the propensity to develop respiratory illnesses (e.g., Boyce et al., 1993). These findings suggest that behavior during the kindergarten year may be indicative of children's adaptation to relatively mild but chronic stressful conditions, making it a useful time to study individual differences in cortisol levels (assessed at a prior point in time) and socioemotional adjustment.

Based on current hypotheses and literature, we expected that average cortisol levels at age 4.5 years would be positively correlated with children's propensity to engage in withdrawal behaviors during their kindergarten year. Conversely, we expected that the tendency to engage in approach behaviors during kindergarten would be inversely associated with children's cortisol levels at age 4.5 years.

METHOD

Sample

The 172 children in the present study were a subsample of children from families participating in a large-scale longitudinal study of families, work, and child development. A total of 570 women in the second trimester of pregnancy were recruited in 1989/1990 for participation in the Wisconsin Study of Families and Work (WSFW, see Essex, Klein, Miech, & Smider, 2001) from obstetrics or family practice clinics in two midwestern cities. Prospective participants were required to meet the following inclusion criteria: (1) between weeks 21 and 25 of pregnancy at first contact (second trimester) and not a high-risk pregnancy; (2) over age 18; (3) not disabled in a way that would significantly alter functioning as a parent, for example, in a wheelchair; (4) living within a specified Standard Metropolitan Statistical Area; (5) living with the father of their child, although not necessarily married; (6) at least one member of the couple was working for pay or profit and the woman either employed or a full-time homemaker, that is, not self-reported as unemployed or a student; (7) had a tele-

phone to be contacted for appointments for interviews; (8) spoke English well enough to understand the interviewer; and (9) sufficiently literate to complete paper-and-pencil questionnaires. Seventy-five percent of women who met these original criteria consented to participate.

At the time of recruitment, of the 570 women in the sample, 95% were married, 39% were first-time mothers to be, 81% were employed, 16% were homemakers, and their ages ranged from 20 to 43 years, with a median of 29. Educational experience ranged from 2% who had not completed high school to 11% who had postgraduate degrees; the median level of education was graduation from college. Family income ranged from \$7,500 to \$200,000, with a median of \$45,000. Five hundred and fifty of the women's spouses/partners also agreed to participate. Ten percent of families had at least one ethnic minority member. Eligible women who refused participation had fewer years of education (participants: $M = 15.0$, $SD = 2.2$; refusers: $M = 14.6$, $SD = 2.2$), $t(742) = 2.26$, $p = .02$, but higher family incomes (participants: $M = \$47.8$, $SD = \$23.2$; refusers: $M = \$53.7$, $SD = \$35.4$), $t(721) = 2.00$, $p = .05$. There were no differences between participants and refusers in the original sample for mother's age or minority status.

Prior to the 4.5-year assessment (T7, the first wave reported in this article), women and their families were interviewed once during pregnancy, three times in the postpartum year (1, 4, and 12 months), and when children were 2 and 3.5 years old. There were no differences, with respect to mother's or father's ages, educational levels, family income, ethnicity, or parity, between the 473 women and families who participated at Time 7 (83%) and those who did not. Cortisol was collected only during the 4.5-year (T7) assessment. Eighty percent of the 473 families ($N = 370$) participating at Time 7 agreed to participate in the cortisol component of the study. A total of 333 children provided at least 1 home sample.

Inclusion Criteria for Current Study

To be included in the current analyses, the following specific criteria had to be met: at least two of three Time 7 home cortisol samples were necessary (i.e., children providing only one sample were excluded), all samples needed to be taken within a 2-week window, all samples needed to be taken within a 90-minute window across days, and all samples needed to be taken in the afternoon or early evening. Finally, data from all three adult reporters were required to be included. The resulting sample that met these additional criteria were 172 children (85 males, 87 females).

Demographic Characteristics

The ethnic backgrounds of the 172 children in the current study were 88% European American, 3.5% African American, 3.5% American Indian or Native Alaskan, 2% Hispanic American, 2% Asian American or Pacific Islander, and 1% other. The mean age of the children at the time of cortisol collection was 4 years, 7 months. At the 4.5-year data collection point (in 1995 and 1996) mean annual family income of participating families was \$68,000 (median = \$62,000). The children were not preselected on the basis of any behavioral or temperamental characteristics. There were no differences with respect to mother's or father's ages, educational levels, Time 1 family income, ethnicity, or parity between the 172 families whose children met the inclusion criteria for the present study and those of the original sample of 570, although there was a nonsignificant trend for the participating mothers to be somewhat better educated, $\chi^2(570) = 11.27, p = .08$.

Design and Procedures

Collection of Salivary Cortisol

Cortisol samples used to assess average levels were collected on 3 consecutive days prior to a home assessment when the children were 4.5 years of age. Pre-, mid-, and postassessment cortisol samples were also collected the day of the home visit. We did not, however, include these reactive samples in this study because (1) the focus was on individual differences in average "nonstressed" levels of cortisol, and (2) the time of day for the home visits varied, making it difficult to consider these samples simultaneously with average afternoon levels. Subsequent studies from these data will investigate the home-reactive samples.

A target collection time was agreed to by the families prior to collection. Families were encouraged to select a time between the hours of 3:00 PM and 7:00 PM, but prior to dinner, and to collect all three samples as close to that target time as possible on each of the 3 days. The afternoon was targeted because it reflects a more quiescent period of the circadian cycle of cortisol release compared with morning hours. Not only does the adrenal gland have fewer secretory episodes at this time of day compared with morning, but cortisol levels change less drastically as a function of time during this period of the day. Consequently, the extent of within-person variability due to these factors can be minimized by collecting samples in the afternoon.

Collection tubes (BD Falcon® Blue Max 15 ml polypropylene, BD Biosciences, Bedford, MA) were supplied to families. Each tube was pre-labeled for day

of collection and marked at the 2 ml line for amount of saliva to collect. Candy (SweetTarts®) was provided to participants to stimulate saliva production. Recently, there has been concern that the use of some types of salivary stimulants might influence the cortisol values obtained, especially when using the Pantex RIA kit (Schwartz et al., 1998). A methods check, described below, was conducted to examine any possible interference effects of the candy stimulant used.

Using a within-subjects design, samples were collected from 14 adult volunteers. Our goal was to examine any possible dose-response effects or pH effects on the cortisol results of the specific saliva stimulant (SweetTarts®) used in the study. Saliva was collected on 2 days at noon, prior to eating lunch. Condition (SweetTarts® versus nothing) was counter-balanced across days. On the first day, half of the individuals used SweetTarts® (ST) to stimulate saliva production and the other half used nothing (N). On the second day, the groups were reversed. The experiment was designed to look for any dose-response effects of the stimulant as well. For the ST group, four samples were collected for each individual, separated by 5 min. The first sample (ST0) was collected without any stimulant, the second sample, taken 5 min later (ST5) was collected using one SweetTarts®, the third sample (ST10) was collected using three SweetTarts®, and the fourth sample (ST15) used six SweetTarts®. For the no-candy group (N), four samples were taken at five-min intervals (N0, N5, N10, N15) without the use of any stimulant.

No significant differences in mean cortisol levels were found either between conditions or between dose levels within the ST condition. Furthermore, intraclass correlations (ICR) were computed for each individual's four samples in each condition. The mean ICR for the ST condition samples was .93 and for the N condition was .95, indicating high reliability of the samples in both conditions. We examined whether the variance was greater among the ST group samples than the N group samples. For each individual, the *SD* of their four ST samples and of their four N samples were calculated. The average *SD* for ST values ($M = .037$) was not significantly different than the average *SD* for N values ($M = .034$). Finally, we examined whether SweetTarts® affected pH levels of the cortisol samples. There was no significant difference in pH between the ST samples and the N samples, $pH_{ST} = 6.32$ ($SD = 1.04$), $pH_N = 6.57$ ($SD = .37$), $t = 1.41, p = .16$. Other stimulants such as Kool-Aid® drink mix crystals and Crystal Light® drink mix crystals have been shown to affect the pH of samples with implications for cortisol assay results (Schwartz et al., 1998). It is noteworthy

that both Kool-Aid® and Crystal Light® contain citric acid as one of the primary ingredients and citric acid is not an ingredient in SweetTarts®. One possibility is that the interference effects reported by Schwartz may reflect a sensitivity of the Pantex assay to citric acid. Our results suggested that the assay results obtained using the Pantex (Santa Monica, CA) ¹²⁵I Cortisol RIA Kit modified for saliva would not be significantly or systematically affected by the use of SweetTarts® as a stimulant for saliva production. This should not, however, be taken as a signal that this stimulant is appropriate for all studies in which saliva samples are collected from children. Effects can vary by assay kit, and effects (or lack thereof) on estimates of other hormone levels have not been documented.

Sample Storage

Once a child's sample was collected at home, it was immediately stored in the family's freezer. The research team subsequently collected all samples, which were transported back to the laboratory on ice. Samples were temporarily stored at -20°C for prelab accounting and then transferred to the wet lab where they were stored at -70°C until assayed.

Cortisol Assay

Processing of saliva samples occurred in two stages. First, samples were thawed and centrifuged at 7500 rpm for 10 min. The supernatant was transferred to 2-ml tubes for storage (-70°C). Then, when a complete batch of samples accumulated, a large assay was conducted using the Pantex ¹²⁵I Cortisol RIA Kit modified for saliva. All samples were assayed in duplicate within the same assay. Twenty-six assays were conducted over the course of 18 months.

Results were considered acceptable only if they met the following criteria: For cortisol concentrations ($\mu\text{g}/\text{dl}$) $\leq .055$, required coefficient of variation (CV%) of the duplicate samples was $\leq 25\%$. For cortisol concentrations ($\mu\text{g}/\text{dl}$) $> .055$, required CV% of the duplicates was $\leq 15\%$. Repeat assays were performed on all samples not meeting these criteria. Reliable results, using these criteria, were obtained for 97% of all samples assayed (for the remaining 3% of the samples, either there was not enough sample to assay again, or the repeat assay also was not acceptable). The detection limit of the assay (ED_{80}) was $.03 \mu\text{g}/\text{dl}$. The mean interassay CV% was 7.4%; mean intraassay CV% was 3.8%.

Cortisol and Related Measures

Average Cortisol Level

A majority of the 172 children (83%) provided three usable samples. The remainder provided two samples. Average values were computed as the mean of the 3 days (or of 2, when only two samples were provided). The average day-to-day variability, as indexed by a coefficient of variability in each individual's samples, was .45 for children who provided three samples and .33 for children who provided two samples. In 97% of the cases, all samples were collected within 7 days of each other (generally on 3 consecutive days, as targeted); for the remaining 3%, all samples were collected within a 2-week period.

Average Time of Collection

The time each individual sample was collected was marked on a "game card" provided to each family. Average time of collection values were computed as the mean of the recorded collection times. The average collection time for a large majority of the 172 children (87%) was between the suggested target time of 3:00 and 6:59 PM. Two percent of the children ($N = 3$) had an average collection time between 2 and 2:59 PM. The remaining 11% had an average collection time between the hours of 7:00 and 9:59 PM. The individual variability in collection times was quite low. For 91% of the cases, at least two of three samples were collected within 15 min of the same time across days. For the remaining 9% of cases, collection times for two of the three samples varied by no more than 30 min. For 93% of the cases, all three samples were collected within a 1-hr period across days. The window was 90 min for the remaining 7% of cases. Because the association of time with cortisol is nonlinear and the afternoon hours are relatively quiescent, average time of collection was categorized as being within the targeted window (3:00 to 6:59 PM, the reference category for later analyses), earlier (2:00 to 2:59 PM), or later (7:00 to 9:59 PM).

Concurrent Medications

Maternal reports of child medications were assessed and children were classified into one of three categories: (1) no medications except vitamins (the reference category for later analyses); (2) over-the-counter aspirins, or nonnarcotic/nonsteroidal anti-inflammatories, or antacids; and (3) antibiotics, or cold/flu/sinus medications containing antihistamines, or steroid-based asthma medications administered nasally or via inhaler. In this sample of 172 children,

70% were not taking any medications, 10% were in Category 2, and 20% were in Category 3.

Adult Reports of Child Behaviors and Adjustment

Mothers, fathers, and kindergarten teachers were asked to report on child socioemotional adjustment at the end of the kindergarten year, approximately a year and a half after the home cortisol collection. All kindergarten measures included in the present analyses asked about the adult's general perception of the child's behavior in the preceding 6 months. Two socioemotional withdrawal behaviors and two approach behaviors, one negative (externalizing) and one positive (school engagement) were considered. In addition, two parallel, but not identical, mother report measures of earlier socioemotional withdrawal behaviors from the 4.5-year assessment were included. Parallel measures of earlier approach behaviors and reports from fathers and teachers were not available.

Internalizing Behaviors (Kindergarten)

The internalizing subscale of the MacArthur Health and Behavior Questionnaire (HBQ-internalizing; Boyce et al., in press; Essex et al., in press) was used. The HBQ is a parent- and teacher-report instrument, developed specifically for use in samples of 4- to 8-year-old children. The HBQ mental health symptom subscales (internalizing and externalizing) were drawn primarily from the well-established Ontario Child Health Study Scales (OCHS-Revised; Boyle et al., 1987; Boyle, O'ford, Racine, Szatmari, & Sanford, 1993). Evidence of their validity and reliability has recently been reported (Ablow et al., 1999). The internalizing subscale consists of 29 items for parents that assess a broad spectrum of symptoms related to depression, generalized anxiety, and separation anxiety (the teacher version includes 14 items that tap depression and generalized anxiety only). Using a 3-point scale (0 = never or not true, 1 = sometimes or somewhat true, 2 = often or very true), reporters were asked to select the statement that best described how often or true the behavior was of the particular child over the past 6 months. The internal consistencies (coefficient α) for mother, father, and teacher report on this measure were $\alpha = .87$, $\alpha = .81$, and $\alpha = .83$, respectively. Scores reflected the mean of the items.

Social Wariness (Kindergarten)

A composite score of social wariness was created. The seven items used in this composite came from two sources, the HBQ and the Child Adaptive Behav-

ior Inventory (CABI; Cowan, Cowan, Heming, & Miller, 1995). Three questions that ask about shyness with other children, shyness with unfamiliar adults, and fear of strangers were taken from the HBQ. Ratings were made on a 0 to 2 scale. Four items that assess self-isolating behavior (difficulty initiating play, isolates self from peer group, plays/works with only one child, plays or works alone) were taken from the CABI. These items were rated on a 1 (not at all like child) to 4 (very much like child) scale. A mean of items on each subscale was computed for each child, and the two means then were converted to z scores. Each child's two z scores were averaged to form a single social wariness composite score. The internal consistencies (coefficient α) for mother, father, and teacher report of child social wariness were $\alpha = .84$, $\alpha = .79$, and $\alpha = .85$, respectively.

Earlier Socioemotional Withdrawal Behaviors (Age 4.5)

Anxious-fearful behavior. Mother report of child anxious-fearful behavior at age 4.5 was assessed with the anxious-fearful subscale of the Preschool Behavior Questionnaire (Behar & Stringfield, 1974). The subscale consists of nine items that assess a broad spectrum of behaviors indicative of anxiety and fearfulness (e.g., "is worried," "tends to be fearful or afraid of new things or new situations," "cries easily"). Using a 3-point scale (0 = doesn't apply, 1 = applies sometimes, 2 = certainly applies), mothers were asked to select the scale point that best described how often or how much each behavior applied to their child. The internal consistency (coefficient α) on this measure was $\alpha = .70$. Scores reflected the mean of the items.

Shyness. Mother report of child shyness at age 4.5 was assessed with a shortened version of the shyness subscale of the Child Behavior Questionnaire (Rothbart, Ahadi, & Hershey, 1994). The subscale consists of six items that assess child shyness with adults and other children (e.g., reverse of "seems to be at ease with almost any person," reverse of "acts very friendly and outgoing with new children"). Using a 7-point scale (1 = extremely untrue, 7 = extremely true), mothers were asked to select the scale point that best described how true each behavior was of their child over the past 6 months. The internal consistency (coefficient α) on this measure was $\alpha = .91$. Scores reflected the mean of the items.

Externalizing Behaviors (Kindergarten)

The externalizing subscale of the HBQ (HBQ-externalizing) consists of 24 items that assess a broad spectrum of symptoms related to conduct difficulties,

oppositional behaviors, and aggressive behaviors (e.g., “argues a lot with adults,” “taunts and teases other children,” “destroys others’ belongings”). These questions were rated on a 3-point scale (0 = never or not true, 1 = sometimes or somewhat true, 2 = often or very true). The internal consistencies (coefficient α) for mother, father, and teacher report on this measure were $\alpha = .88$, and $\alpha = .88$, $\alpha = .92$, respectively. Scores reflected the mean rating for the items.

School Engagement (Kindergarten)

Parent report (SchEmot). Parent report of child emotional engagement in school consisted of an eight-item scale that asked each parent to rate, on a scale of 1 (not at all) to 4 (quite a bit), how excited, interested, eager, happy, upset (reversed), distressed (reversed), frustrated (reversed), and irritable (reversed) about school he or she perceived the child to be. Scores represented the mean of the eight items. Internal consistencies (coefficient α) for this scale were $\alpha = .86$ for mothers and $\alpha = .84$ for fathers.

Teacher report (SchEng). The teacher report of child engagement in school was based on a different, but conceptually parallel, set of items obtained from the Teacher Rating of School and Social Adjustment (Ladd, Kochenderfer, & Coleman, 1996). Items such as “approaches new activities with enthusiasm” and “likes being in school” were rated on a 3-point scale from 0 (doesn’t apply) to 2 (certainly applies). The internal consistency (coefficient α) for this measure was $\alpha = .69$.

Multiple Reporters and Information on Child Behavior

It is well established that the correspondence between different reporters of child behavior is modest, at best. Although low correlations could indicate low reliability of instruments or reporters, they also may reflect situational and contextual differences; “. . . informants differ in their opportunities for observing children, their effects on the children, and their stan-

dards of judgement” (Achenbach, McConaughy, & Howell, 1987, p. 214).

Agreement among parent–teacher pairs is typically lower than agreement between mother–father pairs, a pattern that was confirmed in a meta-analysis of 269 studies (Achenbach et al., 1987) as well as in subsequent studies (e.g., Stanger & Lewis, 1993). Furthermore, greater agreement is observed among different reporters with respect to externalizing difficulties than internalizing difficulties (Achenbach et al., 1987; Hinshaw, Han, Erhardt, & Huber, 1992; Stanger & Lewis, 1993). Different adults have different relationships with children and have opportunities to observe children in different contexts. As such, each reporter’s view of the child was considered independently with respect to the biobehavioral processes under investigation.

RESULTS

Cortisol Levels

Average afternoon cortisol values for this study ranged from .007 $\mu\text{g}/\text{dl}$ to 1.66 $\mu\text{g}/\text{dl}$ ($M = .15 \mu\text{g}/\text{dl}$, $SD = .19 \mu\text{g}/\text{dl}$, median = .10 $\mu\text{g}/\text{dl}$, mode = .14 $\mu\text{g}/\text{dl}$). Typical of distributions of cortisol values, the distribution of cortisol values in the present study was highly skewed (skewness = 4.84). Cortisol values were transformed for subsequent analyses using a \log_{10} transformation. Following transformation, the skewness of the distribution was .24. Transformed cortisol values were used in all analyses.

Table 1 shows the distribution of cases across the medication and time categories, by gender. A 3 (medications) \times 3 (time of day) \times 2 (gender) ANOVA examined the unique effects of these factors on cortisol values. The combined effect of all three factors was significant, $F(5, 166) = 8.02$, $p < .001$. There was a significant main effect of medication type, $F(2, 171) = 3.04$, $p = .05$; time of day, $F(2, 166) = 14.13$, $p < .001$; and child gender, $F(1, 166) = 4.97$, $p = .03$. Girls had significantly higher cortisol values than boys, samples taken between

Table 1 Distribution of Boys and Girls across Medication and Time of Day Categories

Medication Category	Time of Day Category		
	Noon to 2:59 PM	3:00 to 6:59 PM	7:00 to 9:59 PM
None/vitamins	1 boy/1 girl	51 boys/50 girls	8 boys/9 girls
Aspirins/anti-inflammatories/ antacids	—/—	8 boys/9 girls	1 boy/—
Antibiotics/antihistamines/ asthma medications	—/1 girl	15 boys/16 girls	1 boy/1 girl

Table 2 Intercorrelations among Outcome Measures

	Mother				Father				Teacher			
	1	2	3	4	5	6	7	8	9	10	11	12
1. Mother report, HBQ-internalizing												
2. Mother report, social wariness	.47**											
3. Mother report, HBQ-externalizing	.50**	.20**										
4. Mother report, school emotional engagement	-.32**	-.16*	-.26**									
5. Father report, HBQ-internalizing	.34**	.11	.19*	-.23**								
6. Father report, social wariness	.11	.44**	-.04	-.01	.40**							
7. Father report, HBQ-externalizing	.13	-.05	.46**	-.17*	.45**	.14						
8. Father report, school emotional engagement	-.19*	-.10	-.22**	.47**	-.47**	-.25**	-.33**					
9. Teacher report, HBQ-internalizing	.19*	.28**	-.05	-.23**	.09	.14	-.10	-.19*				
10. Teacher report, social wariness	.09	.37**	-.14	-.13	.04	.26**	-.13	-.14	.61**			
11. Teacher report, HBQ-externalizing	-.08	-.15*	.22**	-.22**	.05	-.06	.30**	-.08	.12	-.13		
12. Teacher report, school engagement	-.01	-.02	-.05	.22**	-.09	-.10	-.10	.18*	-.43**	-.42**	-.35**	

Note: HBQ = MacArthur Health and Behavior Questionnaire. Boldface values are cross-rater correlations.

* $p \leq .05$; ** $p \leq .01$.

7:00 and 10:00 PM were significantly lower in cortisol than those taken between 3:00 to 6:59 PM (Bonferroni correction), and children taking medications in the antibiotics/asthma medications category had marginally, $p = .053$, higher cortisol levels than children taking no medications (Bonferroni correction).

Kindergarten Adjustment Measures

Correlations Among Kindergarten Adjustment Measures

Table 2 shows the bivariate Pearson correlation coefficients between measures from the three reporters (mother, father, and teacher). Consistent with patterns previously reported in the literature (e.g., Stanger

& Lewis, 1993), the coefficients on the diagonals show that the agreement between parents on the same measures, mean $r = .43$, tended to be greater than the agreement between parents and teachers on the same measures, mean $r = .30$ for mothers and teachers; mean $r = .28$ for fathers and teachers. Because the measures of internalizing and school engagement were not identical for parents and teachers, these measures were excluded from the computation of the mean interreporter correlations.

Effects of Reporter and Child Gender

Means and SDs of all outcome variables by reporter and child gender are shown in Table 3. A doubly multivariate repeated-measures ANOVA was

Table 3 Means and Standard Deviations of Outcome Measures by Reporter

	Mother Report		Father Report		Teacher Report		Significant Effects
	M	SD	M	SD	M	SD	
Internalizing	.29 (.29/.30)	.22 (.22/.21)	.31 (.31/.31)	.19 (.20/.17)	.26 (.24/.28)	.25 (.24/.27)	
Social wariness (z score based)	0 (-.11/.10)	.88 (.85/.90)	0 (-.04/.04)	.85 (.87/.84)	0 (-.21/.20)	.89 (.71/1.00)	G*
Externalizing	.39 (.45/.34)	.26 (.26/.24)	.38 (.42/.34)	.25 (.27/.22)	.14 (.17/.11)	.23 (.26/.18)	R***, G**
School engagement	3.75 (3.73/3.78)	.36 (.42/.29)	3.72 (3.61/3.83)	.36 (.42/.24)	1.89 (1.90/1.89)	.18 (.18/.18)	G**, G × R** (excluding teacher report)

Note: $N = 172$. Numbers in parentheses represent boys/girls. R = reporter; G = gender.

* $p \leq .05$; ** $p \leq .01$; *** $p \leq .001$.

conducted to examine the effects of reporter (within-subjects factor) and child gender (between-subjects factor) on the three outcome measures for which mothers, fathers, and teachers all provided reports. The overall multivariate tests (using Wilks' λ criterion) for child gender and reporter were significant, $F_{\text{gender}}(3, 168) = 4.69, p < .01$; $F_{\text{reporter}}(6, 165) = 29.3, p < .001$. The multivariate F test for the interaction of child gender and reporter was not significant. Follow-up univariate analyses indicated that across reporters, girls were rated as higher in social wariness than boys, $F(1, 170) = 5.35, p < .05$, and boys were rated as showing more externalizing behaviors than girls, $F(1, 170) = 9.06, p < .01$. A significant effect of reporter was found for externalizing behavior $F(2, 340) = 88.60, p < .001$. Multiple pairwise comparisons, using Bonferroni correction, indicated that teachers reported less externalizing behavior than did mothers or fathers.

A second repeated-measures ANOVA was conducted to examine the effects of reporter (within-subjects factor) and child gender (between-subjects

factor) on parent report of child emotional engagement in school. Significant effects were found for child gender, $F(1, 170) = 8.74, p < .01$ and the interaction of child gender and reporter, $F(1, 170) = 8.23, p < .01$. Overall, girls were rated as more positively engaged in school than boys. Mothers, however, tended to rate boys higher in engagement than fathers, but they rated girls lower in engagement than fathers.

A one-way ANOVA compared teacher reports of girls and boys on school engagement. There was no significant effect of child gender on teacher report of school engagement.

Child Cortisol Levels and Adult Reports of Child Behavior

To examine the association of cortisol with adult reports of child behavior, three separate sets of regression analyses were conducted. First, the relation of child cortisol to mother report of child behavior was examined, then father report of child behavior, and fi-

Table 4 Regression Analyses of the Effect of Child Gender and Cortisol^a on Mother Report of Child Behavior during the Kindergarten Year

	Model					
	1		2		3	
	<i>b</i>	β	<i>b</i>	β	<i>b</i>	β
A. Dependent Variable = mother report internalizing behavior						
(1) Child gender	.01	.02	-.003	-.01	-.004	-.01
Medications dummy 1	.05	.08	.06	.09	.06	.09
Medications dummy 2	-.04	-.08	-.06	-.11	-.06	-.11
Time of day dummy 1	.28*	.17*	.33**	.20**	.29*	.18*
Time of day dummy 2	.09	.14	.13*	.20*	.14**	.21**
(2) Cortisol			.10*	.16*	-.004	-.01
(3) Interaction:						
Gender \times Cortisol					.25**	.27**
R^2	.06		.08		.12	
R^2 change	.06		.02*		.04**	
B. Dependent Variable = mother report social wariness						
(1) Child gender	.21	.12	.14	.08	.13	.08
Medications dummy 1	.39	.14	.44*	.15*	.43*	.15*
Medications dummy 2	.06	.03	-.01	-.01	-.02	-.01
Time of day dummy 1	.25	.04	.53	.08	.35	.05
Time of day dummy 2	.28	.10	.51*	.18*	.55*	.20*
(2) Cortisol			.61**	.24**	.12	.05
(3) Interaction:						
Gender \times Cortisol					1.18**	.30**
R^2	.04		.09		.14	
R^2 change	.04		.05**		.05**	

Note: $N = 172$.

^aThree-day average afternoon cortisol level measured at home.

* $p \leq .05$; ** $p \leq .01$.

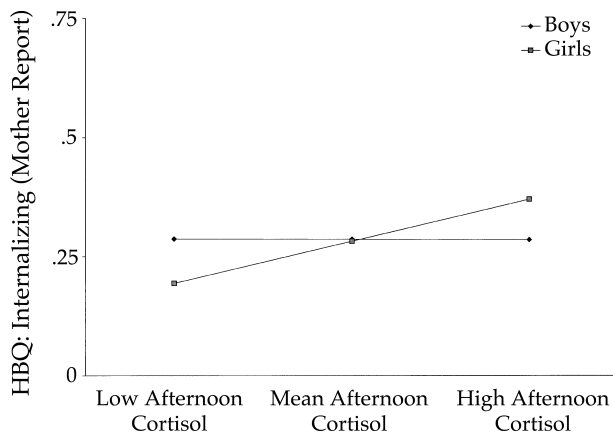


Figure 1 Cortisol \times Child Gender interaction predicting mother report of internalizing behavior. HBQ = MacArthur Health and Behavior Questionnaire.

nally teacher report of child behavior. Due to the moderate correlation of the four dependent variables, the standard p value of .05 was corrected by a factor of 4, resulting in a protected p value of .0125. Models for which the omnibus F met this criterion are reported; one marginally significant model is also reported.

Each regression analysis consisted of three nested models. The first model (Model 1) in each analysis accounted for differences in outcome due to child gender, medications, and time of afternoon/evening sample collection. The second model (Model 2) added average cortisol level. The third model (Model 3) examined whether cortisol and child gender had an in-

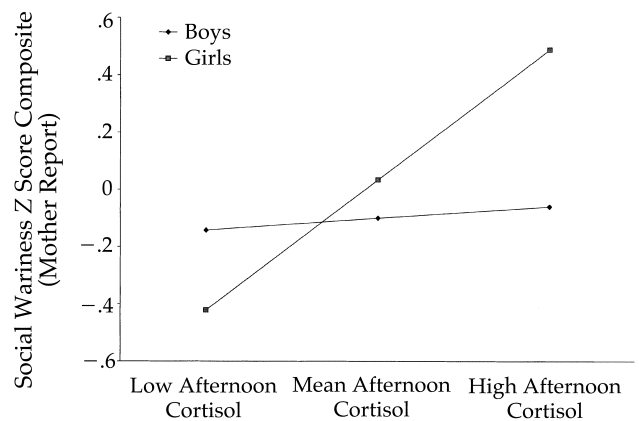


Figure 2 Cortisol \times Child Gender interaction predicting mother report of social warmth.

teractive effect. In these analyses, the continuous first-order component of the interaction term (\log_{10} cortisol value) was centered prior to analysis. Centering of the binary categorical gender factor is not necessary. In the presence of an interaction, centering provides a meaningful representation of the first-order component term as the weighted average effect of one component across all values of the other (Aiken & West, 1991; Jaccard, Turrisi, & Wan, 1990).

Mother Report Data

The series of regression analyses examining mother report of child adjustment yielded two significant in-

Table 5 Regression Analysis of the Effect of Child Gender and Cortisol^a on Father Report of Child Externalizing Behavior during the Kindergarten Year

	Model					
	1		2		3	
	<i>b</i>	β	<i>b</i>	β	<i>b</i>	β
Dependent variable = father report externalizing behavior						
(1) Child gender	-.09*	-.18*	-.08*	-.16*	-.08*	-.16*
Medications dummy 1	-.02	-.02	-.03	-.03	-.03	-.04
Medications dummy 2	.01	.02	.02	.04	.02	.03
Time of day dummy 1	.19	.10	.15	.08	.12	.06
Time of day dummy 2	.15*	.19*	.11	.15	.12*	.16*
(2) Cortisol			-.09	-.13	-.18**	-.26**
(3) Interaction:						
Gender \times Cortisol					.22*	.20*
R^2	.08		.09		.11	
R^2 change	.08*		.01		.02*	

Note: $N = 172$.

^a Three-day average afternoon cortisol level measured at home.

* $p \leq .05$; ** $p \leq .01$.

teractions. For Model 3 predicting internalizing, the overall $F(7,171) = 3.23, p = .003$. For Model 3 predicting social wariness, the overall $F(7,171) = 3.83, p = .001$. The two panels of Table 4 show the full results of the regression analyses predicting these two outcomes. Both outcomes were predicted by cortisol, but these effects were qualified by gender of the child (significant Cortisol \times Gender interactions). Each incremental change in variance accounted for by the nested models is indicated from left to right in the rows of the table showing "Change in R^2 ." The significant Cortisol \times Gender interactions are illustrated in Figures 1 and 2. In each figure, one line represents the predicted values of the dependent variable for boys and the other line represents the predicted values for girls as a function of level of cortisol. "High" and "low" on cortisol level is defined as $\pm 1 SD$ of the mean. As can be seen in the figures, the positive association between cortisol and mother report of child behavior was stronger for girls than for boys. Cortisol was not significantly associated with mothers' reports of child externalizing behavior. There was a marginal inverse relation between cortisol and maternal report of emotional engagement in school, Model 2, overall $F(6,171) = 2.32, p = .03$.

Father Report Data

Fathers' reports of children's externalizing behavior were significantly and inversely related to child cortisol. This relation, however, was conditional on the gender of the child, Model 3, overall $F(7,171) = 2.99, p = .006$. Table 5 shows the results of the regression analysis. The significant interaction is depicted in Figure 3, which illustrates that it was the

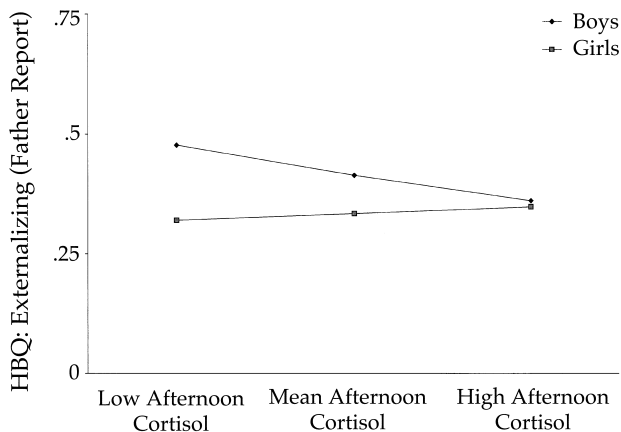


Figure 3 Cortisol \times Child Gender interaction predicting father report of externalizing behavior. HBQ = MacArthur Health and Behavior Questionnaire.

lower levels of baseline cortisol that distinguished boys and girls on externalizing behavior. Specifically, boys with lower cortisol levels were predicted to show more externalizing behaviors, according to the report of fathers. Child cortisol was not significantly associated with fathers' reports of internalizing behavior, social wariness, or emotional engagement in school.

Teacher Report Data

The two panels of Table 6 show the results of Models 1 and 2 predicting teacher report of internalizing behavior and social wariness, respectively. Because there were no significant interactions with respect to teacher report of child adjustment, Model 3 (the addition of the interaction term) was excluded. Overall, regardless of child gender, higher average afternoon home cortisol levels at age 4.5 positively predicted later teacher reports of internalizing behavior, Model 2, overall $F(6,171) = 2.91, p = .010$, and social wariness, Model 2, overall $F(6,171) = 4.20, p = .001$. No significant relation was found between child cortisol and teacher report of externalizing behavior.

Table 6 Regression Analyses of the Effect of Child Gender and Cortisol^a on Teacher Report of Child Behavior during the Kindergarten Year

	Model			
	1		2	
	<i>b</i>	β	<i>b</i>	β
A. Dependent variable = teacher report internalizing behavior				
(1) Child gender	.04	.08	.02	.04
Medications dummy 1	.03	.04	.05	.06
Medications dummy 2	-.004	-.01	-.03	-.04
Time of day dummy 1	.09	.05	.18	.09
Time of day dummy 2	.14*	.17*	.21**	.26**
(2) Cortisol			.19**	.27**
R^2	.04		.10	
R^2 change	.04		.06**	
B. Dependent variable = teacher report social wariness				
(1) Child gender	.41**	.23**	.35**	.20**
Medications dummy 1	.35	.12	.39	.14
Medications dummy 2	.02	.01	-.05	-.02
Time of day dummy 1	-.18	-.03	.07	.01
Time of day dummy 2	.46*	.17*	.67**	.24**
(2) Cortisol			.56**	.22**
R^2	.09		.13	
R^2 change	.09**		.04**	

Note: $N = 172$.

^a Three-day average afternoon cortisol level measured at home.

* $p \leq .05$; ** $p \leq .01$.

Higher cortisol levels marginally predicted lower school engagement as reported by teachers, Model 2, overall $F(6,171) = 2.91, p = .065$; Model 2, $b_{\text{cort}} = -.30, p = .02$.

Earlier Behavior

It is possible that the associations found between average afternoon home levels of cortisol measured at age 4.5 and adult report of child behavior a year and a half later can be explained away by the associations of these measures with behavioral measures obtained at age 4.5. To examine this possibility, additional analyses were conducted that included the most parallel 4.5-year behavior measures (identical behavioral measures were not collected at that time) reported by mothers (fathers and teachers were not asked as extensive a set of measures at that time).

Two additional sets of regression analyses were conducted, identical to those detailed above (three model series) with one exception: for the first series, with mother report of child internalizing at kindergarten as the outcome, mother report of child's anxious-fearful behavior at age 4.6 was added to Model 2. Anxious-fearful behavior at age 4.5 was a strong predictor of internalizing behavior during kindergarten, Model 2, $\beta_{\text{anx/fear}} = 6.38, p = .000$, and remained so, $(\beta = 6.22_{\text{anx/fear}}, p = .000)$, in Model 3. In spite of this, the interaction between child gender and 4.5-year average cortisol level also remained a significant predictor of child internalizing during kindergarten, $(\beta = 2.4, p = .017)$. For the second set of analyses, mother report of social wariness at kindergarten was examined as the outcome, and mother report of child shyness at age 4.5 was included. Shyness at age 4.5 was a strong predictor of subsequently measured social wariness, Model 2, $\beta_{\text{shy}} = 7.12, p = .000$. It remained so; $\beta = 6.22_{\text{shy}}, p = .000$, in Model 3, in which the interaction between child gender and cortisol was also significant, $\beta = 3.41, p = .001$. Both 4.5-year behavior measures (anxious-fearful and shyness) were not significantly correlated to cortisol, $r = .049$ and $r = .052$, respectively, indicating that cortisol was an independent predictor of later behavior.

DISCUSSION

The purpose of this study was to examine whether activation of the HPA system, as indexed by average home afternoon levels of cortisol at 4.5 years, would significantly predict individual differences in children's tendency to show withdrawal-related behaviors (internalizing symptoms, social wariness) or approach-related behaviors (externalizing symptoms, school engagement) during kindergarten, a year and a half

later. Specifically, we expected that higher average cortisol levels would be related to withdrawal behaviors and lower cortisol levels would be inversely related to approach behaviors. Overall, consistent with an approach-withdrawal framework, the findings suggest that individual differences in average activity level of the HPA axis, when assessed in the home context, may reflect an underlying general behavioral tendency to withdraw or approach in the face of the daily challenges of life.

Boys and girls who were reported by teachers as being more withdrawn (i.e., more internalizing behaviors, more social wariness) were characterized by higher levels of cortisol a year and a half earlier. Furthermore, there was also the suggestion (marginal result) of an inverse relation between cortisol and teacher report of school engagement. These findings are generally consistent with the reports of a positive association between baseline cortisol and social wariness and inhibition in children around the age of 4 to 5 years (Kagan et al., 1987; Schmidt et al., 1997). Whereas others have suggested that associations between cortisol and inhibition may only be apparent at the behavioral extremes (Gunnar et al., 1997), such as the groups identified in the Kagan and Schmidt studies, our findings extend the generalizability of such a relation because the children in this study were not preselected on the basis of any temperamental or behavioral characteristics.

Mother report of child withdrawal behaviors were also related to child cortisol levels. There was a significant Gender \times Cortisol interaction, however. Higher average levels of cortisol predicted higher levels of withdrawal behaviors (maternal report) for girls compared with boys. This significant association between withdrawal behaviors and cortisol for girls is a provocative finding developmentally in light of the gender difference in the prevalence of depression that emerges during adolescence. Other researchers have suggested that inhibition may be more stable for girls than for boys from childhood to adolescence (Kerr, Lambert, Stattin, & Klackenberg-Larsson, 1994), and extreme inhibition may be a risk factor for the later development of internalizing disorders and depression (Biederman et al., 1990).

The positive association between elevated average cortisol levels and internalizing during childhood might be considered a developmental analog to studies that have reported a relation between cortisol and depression in adolescent samples (Dahl et al., 1991), although others have not found such an association (Dorn et al., 1996; Puig-Antich et al., 1989). Although Dorn et al. did not find evidence of differences in cortisol levels or reactivity as a function of gender or depres-

sion status in a small sample of adolescents, she cautioned against dismissing the possibility that gender differences in neurophysiological functioning may play an important role in females' heightened vulnerability to depression. At adolescence, other emerging endocrine factors may mask the relation between cortisol and depression. For example, one recent study suggested the possibility that dehydroepiandrosterone may be particularly relevant to the relation between cortisol and depression in adolescents (Goodyer, Herbert, & Altham, 1998).

In spite of the consistency of the present study's general findings with existing research and theory, few studies have reported gender differences in associations between cortisol and internalizing tendencies during childhood. Notably, the one other study that looked specifically at gender differences in cortisol-behavior relations in children (Tout et al., 1998) reported significant associations between median levels of cortisol for boys with respect to internalizing behavior, but not for girls; additionally, the association for boys was negative, not positive. Taken together, the findings suggest at the very least that the examination of gender differences in biobehavioral processes in childhood, especially as they relate to internalizing difficulties and disorders, merits further investigation.

Our findings with respect to externalizing behavior suggest that boys characterized by low afternoon home levels of cortisol may be more susceptible to aggressive and angry behavior, especially according to their fathers. This negative association is consistent with reports of inverse associations between externalizing behaviors and baseline cortisol in other studies (McBurnett et al., 1991; van Goozen et al., 1998), although overall, the results of other studies are mixed (e.g., see Gunnar et al., 1997, for the opposite results). In addition, the samples in prior studies have been almost exclusively male. The findings from the present study suggest that although boys who are characterized by lower levels of cortisol are at heightened risk for demonstrating externalizing types of behaviors, the same degree of association does not hold for girls. Future studies of children that look at externalizing difficulties should include girls, so that possible differential development of underlying neurophysiological mechanisms related to this type of behavior might be better understood. Although only marginal, there was also a trend for school engagement, another approach-related behavior, to be inversely related to afternoon levels of cortisol measured at home. This trend, if corroborated with other evidence, would complement the findings of Gunnar and colleagues (1997) showing that outgoing and socially engaged children (in this case,

preschoolers) are characterized by lower cortisol reactivity later in the school year, whereas children exhibiting solitary behaviors later in the school year are characterized by higher cortisol reactivity.

Given the community-based nature of our sample and the sample size, a reasonable question is whether the differences in child behaviors during kindergarten between those with high and low average levels of cortisol at age 4.5 years are meaningful beyond statistical significance—that is, what are the differences in terms of behavioral phenomenology? To address this question, the differences in child behaviors in this sample of children were compared with those of young children (ages 4 to 8) in a case-control study (Ablow et al., 1999) based on the same measures of internalizing and externalizing behaviors used in the present study (measures of social wariness and school engagement were not available). Because of the way in which cases were identified in the case-control study (i.e., children referred for treatment), we expected that they would have higher internalizing and externalizing scores than would those children with high average levels of cortisol in the present study. The mean internalizing scores (mother report) of the cases and controls were .55 and .31, respectively, compared with a mean score of .47 for girls with high (upper 10%) average levels of cortisol in the present study (mean internalizing score for total sample of girls = .30; mean for lower 20% of cortisol levels = .25). The mean externalizing scores (mother report; father report not available) of the cases and controls were .75 and .33, respectively, compared with a mean score of .66 for boys with low (lower 10%) average levels of cortisol in this study (mean externalizing score for total sample of boys = .42; mean for upper 20% of cortisol levels = .40). These comparisons show that although the scores, as expected, tended to be lower in the present study than in the case-control study, the internalizing and externalizing behaviors of the children with high average cortisol levels were considerably more congruent with the problem behaviors expressed by children referred for treatment than with the behaviors expressed by the controls.

Reconciling the existence of Gender \times Cortisol interactions for parent report and a lack of interactions for teacher report presents a challenge. As expected, and consistent with existing literature, agreement between reporters, although significant, was not high. Mothers, fathers, and teachers may indeed have different phenomenological experiences of children. Furthermore, given the well-established phenomenon that teachers report less difficulties overall, it is possible that children who are identified as having difficulties may really only be those at the behavioral

extremes, relative to all the children the teachers have worked with. That is, the subjective anchor points of the scale that teachers use may be different than that of parents. If this were the case, and one were to assume that those children who are toward the extreme end of the spectrum, male and female, have a stronger underlying biological vulnerability, then gender differences in the biobehavioral relations may not be apparent with respect to teacher report of child difficulties. In fact, in studies looking at extremely inhibited children (Kagan et al., 1987; Schmidt et al., 1997), no gender differences were reported, although both genders were represented in those extreme groups. Another possibility is that there is some other factor that heightens mothers' sensitivity to (and therefore reports of) their daughters' withdrawal behaviors and fathers' reports of their sons' negative approach (i.e., oppositional and aggressive) behaviors. Studies that simply average across multiple reporters do so at the risk of masking or suppressing the unique insights that these reporters may have on the children being studied.

On a similar note, different environments (e.g., home versus school) may have different effects on the "typical" activity of the HPA axis, even when acute stressors are not experienced. It is important to specify the context of saliva collection, for it may be no less important methodologically than time of day of collection, or whether stimulants are used to produce the saliva itself (although context effects may not be as predictable). A study by de Haan et al. (1998) underscores this point. In that study, cortisol levels measured in the home produced associations with shyness in toddlers, but cortisol measures obtained in nursery school did not. Rather, increases in cortisol measures obtained in the nursery school setting across the start of the preschool year were positively associated with externalizing types of behaviors. Throughout this article, we have referred to average afternoon levels collected at home to emphasize the context of cortisol collection.

Perhaps what is most notable about the findings of this study is the predictive nature of the relations: cortisol measured at 4.5 years predicted behavioral tendencies at age 6 years. Although prior studies have shown concurrent relations between baseline levels and behavior as well as early behavioral styles predicting later HPA activity (e.g., Kagan et al., 1987; Schmidt et al., 1997), this study extends those findings by providing evidence that individual differences in cortisol levels can contribute to the prediction of individual differences in behavioral styles over a year later. These findings are robust: Even when behavior measured at the same time as cortisol collection was included in the equation, the predictive relations of prior cortisol levels and later behaviors remained.

The predictive relations identified in the current study lend further support to the notion that certain children may be at greater risk of developing social and emotional difficulties from an early age as a function of underlying biopsychological processes and characteristics. This may be particularly important for identifying children at risk for internalizing disorders. Because of the inward and nondisruptive nature of the symptoms, internalizing difficulties are more likely to go undetected in children. Preclinical rodent and nonhuman primate data suggest that early stress and differences in maternal–infant interactions can determine the later heightened physiological and behavioral vulnerability to stress, including increased HPA reactivity (Coplan et al., 1996; Plotsky & Meaney, 1993). There is a paucity of research, however, regarding developmental physiological precursors evident in early childhood that might indicate vulnerability to experience social and emotional adjustment difficulties when faced with normative stresses and challenges such as those experienced in the early school years.

Although the findings of this study are consistent with the suggestion that early individual differences in average afternoon measures of cortisol taken at home may reflect part of an underlying physiological vulnerability for the development of certain socioemotional adjustment difficulties, this is undoubtedly premature. Little is known about either the long-term stability of individual differences in functioning of the HPA axis, nor how early physiological differences relate to different developmental trajectories. For example, investigating the extent to which cortisol levels measured in early childhood predict stability or instability of social and emotional adjustment from kindergarten to first grade and throughout grade school would be informative. Exploration of social–contextual factors that influence these long-term biological–behavioral associations might help identify possible early intervention strategies as well. Finally, it seems worthwhile to pursue the possibility of a gender-based differential effect of cortisol on the vulnerability to depression in adolescence. The findings of this study, in addition to providing data consistent with some of the existing research, have generated a number of important avenues for future research on the relation between socioemotional development and cortisol levels measured in childhood.

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ADDRESSES AND AFFILIATIONS

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