ORIGINAL ARTICLE

Alterations in Stress Cortisol Reactivity in Depressed Preschoolers Relative to Psychiatric and No-Disorder Comparison Groups

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Background: Despite the robust and widely replicated finding of elevated hypothalamic-pituitary-adrenal (HPA) axis reactivity in depressed adults, studies of depressed children have yielded ambiguous findings. Animal models of early depression and studies of children experiencing early psychosocial deprivation have suggested that alterations in HPA axis reactivity are evident in early "depressive-like" conditions. The current study is, to our knowledge, the first investigation of HPA axis reactivity in very young children with a clinical depressive syndrome for which content validity has been established.

Methods: Depressed, psychiatric, and no-disorder comparison children aged 3 through 5.6 years were studied for HPA axis reactivity in response to experimental psychosocial stressors. The children were diagnosed using a developmentally appropriate, structured psychiatric interview. Salivary cortisol was obtained at 3 time points during a laboratory assessment before and after stressors involving separation from the parent and frustrating tasks.

Results: Repeated measures of multivariate analysis of variance revealed a significant interaction between the diagnostic group and 2 cortisol percent change scores. Depressed preschoolers displayed a pattern of increasing cortisol levels throughout the assessment in response to both separation and frustration stressors. In contrast, both comparison groups showed decreasing cortisol levels in response to the separation stressor. All groups displayed increasing cortisol levels in response to frustrating tasks. Preschoolers with a presumptive melancholic depressive subtype displayed these alterations at a greater magnitude relative to comparison groups.

Conclusions: To our knowledge, these findings are the first to demonstrate altered HPA axis reactivity in depressed preschoolers. These alterations are consistent with those described in depressed adults and in animal models of early depression. These findings provide evidence for possible continuity of HPA axis alterations in depressive disorders across the lifespan and are discussed in the context of prior studies of HPA axis reactivity in clinically depressed children and adolescents, suggesting that younger age and inpatient status are features associated with altered HPA axis reactivity.

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THE FINDING of alterations in the reactivity of the hypothalamic-pituitary-adrenal (HPA) axis in adults with major depressive disorders (MDD) is perhaps the most consistent and robust biological finding in psychiatry to date.[1-5] Numerous investigations have consistently reported elevated levels of the adrenal corticosteriod cortisol and nonsuppression of this hormone in response to administration of the synthetic corticosteriod dexamethasone (the dexamethasone suppression test or DST) among depressed adults.[6] Further, the finding that these alterations of HPA axis reactivity were state and severity dependent (eg, they were reversed during recovery and enhanced in more severe melancholic or psychotic subtypes) increased enthusiasm for the possibility that the DST might have utility as a diagnostic laboratory test in psychiatry.[7] However, this early enthusiasm for the DST was sharply diminished by the finding of DST nonsuppression in many other serious psychiatric conditions, including anxiety and eating disorders, which demonstrated a lack of specificity of these findings to MDD.

While hypersecretion and nonsuppression of cortisol are well established in depressed adults, investigations of HPA axis reactivity in depressed children have yielded a more ambiguous array of findings. In contrast to findings in depressed adults, investigations of 24-hour cortisol secretion have demonstrated no differences between depressed child and adolescent outpatients and control groups.[8-10] Numerous studies of the DST have been

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done in depressed child and adolescent populations using varying methodologies and study samples. Despite this wide variety of methods and findings, there has been a general consensus that the sensitivity of the DST is higher in children than adolescents and higher in inpatients compared with outpatients.[11,12] Although there has been much investigation of pharmacologic challenges, such as the DST in depressed child and adolescent samples, experimental psychosocial stress paradigms widely used in developmental studies have not been reported, to our knowledge, in clinical samples of depressed children to date.

There has been much interest in the implications of HPA alterations to the developmental neurobiology of MDD. Nemeroff[13] and others have proposed a developmental model of depression as "stress response gone awry" in which corticotropin-releasing factor and related HPA alterations are hypothesized to be central to the developmental neurobiology of MDD.[14] In keeping with this theory, rodent models of early adversity induced by postnatal mother-infant separations at key time points during early development display an endophenotype characterized by increased HPA axis responsiveness and stress-induced glucocorticoid secretion with enduring increases in HPA activity.[15-17] Longitudinal follow-up studies of such maternally deprived rats that resume normal maternal contact have found persistent elevations in cortisol secretion in response to stress. Postmortem examination reveals reduced corticotropin-releasing factor receptor density in the anterior pituitary of these animals, further suggesting that there are long-lasting corticotropin-releasing hormone alterations associated with this model of early adversity at developmentally specific time points.[18]

Despite a large body of compelling data on alterations in HPA response subsequent to early adversity in rodents, whether similar HPA alterations can also be demonstrated in nonhuman primates remains less clear.[19] Fewer data are available on the long-term effects of early adverse life events on nonhuman primates. Differences in the early neural development of primates and the absence of a stress hyporesponsive period as occurs in rodents[20] may be among many factors explaining why replication of rodent findings in nonhuman primates has not been straightforward. While the data paint a mixed picture, evidence is available demonstrating that intermittent early separations between infant primates and their mothers result in blunted cortisol reactivity and associated behavioral changes.[17,21,22]

The potential relevance of these findings to forms of psychopathology in humans early in life is suggested by studies of children who have experienced early adversity in the form of psychosocial deprivation, maltreatment, malnutrition, or exposure to toxins. Both elevations and blunting of HPA axis reactivity have been reported, with findings varying by sample and design characteristics. Blunting of diurnal variation in cortisol secretion among depressed, maltreated school-aged children and among Romanian orphans has been reported.[23-25] Alternately, Gunnar et al[26] have also reported cortisol elevations in deprived Romanian orphans compared with control groups of nondeprived adoptees. These studies suggest that early and profound emotional deprivation, conditions similar to those observed to be associated with hospitalism or anaclitic depression described by pediatrician Renee Spitz[27] almost 50 years ago, gives rise to HPA alterations, some of which are similar to those known to exist in depressed adults.

Studies of human and animal infants under conditions of postnatal separation from their mothers have yielded similar HPA alterations and have been helpful in the development of models of early adversity as a proxy for depressive syndromes. However, such models, while informative, are imperfect approximations of early depression. Specifically, these adversity models do not address the development of early-onset depression in the absence of adversity, which is known to occur in older child and adult populations. Alterations in cortisol reactivity as a result of depression may differ qualitatively from those arising as a result of adversity. This notion is underscored by the findings that alterations in cortisol secretion in response to corticotropin-releasing hormone challenge can be found only in depressed children when maltreatment was present.[23,24] These studies had relatively small samples of depressed children and thus power to detect changes as a result of depression alone may have been limited.

While numerous studies using experimental physiological stress conditions have been done in depressed child and adolescent populations, the effects of experimental psychological stressors have not been widely studied in clinical populations. Ashman et al[28] have provided the first data demonstrating that children at high risk for depression (offspring of depressed mothers) exhibited greater elevations in cortisol in response to a psychosocial stressor compared with control children. Although findings in this group at high risk for depression are suggestive, to our knowledge there have been no data to date addressing changes in psychosocial, stress-induced cortisol secretion, or other aspects of HPA function, in very early clinical depressive states in humans.

Basic developmental studies have established that the high-risk offspring of depressed mothers demonstrate alterations in affect expression, behavior, and psychophysiology very early in life.[29-37] Despite these highly suggestive findings, until recently there have been insufficient data to determine whether clinically significant depressive disorders could occur in children younger than 6 years.[38-40] Luby et al[41,42] have now demonstrated that children aged 3 to 5.6 years can manifest a clinically significant depressive syndrome characterized by typical *DSM-IV* MDD symptoms. The *DSM-IV* MDD criteria were modified by setting aside the 2-week duration criterion and developmentally translating a number of symptom states in the structured psychiatric interview (described later) when indicated to capture the pertinent life experiences of the young child.

Validation of this preschool depressive syndrome has been established using the methods first described by Robins and Guze[43] and used in the development of the *DSM* system. Preschool children with this depressive syndrome displayed a specific and stable symptom constellation, increased family history of related disorders, and significantly greater depression severity compared with both psychiatric and no-disorder comparison groups.[41] This depressed group also displayed social impairment

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and self-reported more negative affect compared with the no-disorder comparison group.[41] Further investigation, now underway in our laboratory, of the predictive validity of this preschool syndrome will be essential to its clinical definition.

The finding of a biological correlate provides a key validator for a psychiatric syndrome according to the Robins and Guze model.[43] To investigate a possible early biological marker of MDD, the current study used an experimental stress paradigm to explore HPA axis reactivity in depressed preschool children compared with psychiatric and no-disorder comparison groups. The primary hypothesis of this investigation was that the depressed preschoolers would show greater elevations in salivary cortisol in response to a psychosocial stressor compared with the no-disorder comparison group, similar to findings in depressed adults. We also hypothesized, consistent with the adult literature, that anhedonic, depressed preschoolers (n=21), a hypothesized melancholic subtype, would show greater elevations in stress cortisol reactivity than nonmelancholically depressed children.

METHODS

STUDY POPULATION

The Early Emotional Development Program at the Washington University School of Medicine, St Louis, Mo, assessed 174 preschoolers aged 3 to 5.6 years as part of a study of the nosology of preschool depression. Children were recruited from community pediatricians' offices using a checklist designed to screen for early-onset behavior problems[44] and by consecutive case ascertainment from a specialty mental health clinic exclusively serving young children. Three study groups were compared in the following analyses: (1) those who met *DSM-IV* MDD criteria (with the exception of the duration criterion); (2) a psychiatric comparison group who met *DSM-IV* criteria for attention-deficit/hyperactivity disorder or oppositional defiant disorder; and (3) those who did not meet criteria for any *DSM-IV* psychiatric disorder (no-disorder group). Excluded were children with chronic medical illnesses or neurologic problems and those with pervasive developmental disorders or language and cognitive delays that would prohibit their ability to understand the study questions. After complete description of the study and procedures, written informed consent was obtained from the child's guardian. Assent was not obtained because of the very young age of the study subjects.

ASSESSMENTS

Preschoolers and their primary caregivers underwent a comprehensive 2- to 3-hour assessment at Washington University School of Medicine's Early Emotional Development Program during which time 3 salivary cortisol samples were obtained. Parents were given materials for the collection of salivary cortisol samples at home on the evening after the assessment and for the following 2 consecutive nights. They were also given instructions about the handling of the saliva samples and appropriate packaging and postage to mail them back to the Early Emotional Development Program.

A comprehensive, structured diagnostic interview, a version of the Diagnostic Interview Schedule for Children, Version IV[45] modified for young children,[46] was administered to caregivers about their children. To develop this interview, several Diagnostic Interview Schedule for Children, Version IV items were modified to account for their age-appropriate developmental manifestations. This was deemed necessary at face value, because some items as they were described in the Diagnostic Interview Schedule for Children, Version IV did not apply to the life experiences of preschool children. Those diagnostic modules that were developmentally inapplicable to young children (eg, schizophrenia and substance abuse modules) were not administered. All anxiety disorders with the exception of posttraumatic stress disorder and panic disorder were administered. These disorders were excluded because of either uncertainty about their application to young children or the presumption that they would occur in very low frequency in the study population.

While child report and observational measures of symptom states were used in the assessment, diagnostic group status was determined solely by parent ratings from the Diagnostic Interview Schedule for Children, Version IV modified for young children using *DSM-IV* criteria. Children who met *DSM-IV* MDD criteria (with the exception of the duration criterion) were included in the depressed group regardless of comorbid status. Consistent with findings in older depressed children, high levels of comorbidity with other *DSM-IV* disorders were found.[42] Those with the diagnoses of attention-deficit/hyperactivity disorder or oppositional defiant disorder (could be comorbid for both disorders but could not have any affective disorder) were included in the psychiatric comparison group. To be included in the no-disorder comparison group, the child could not meet criteria for any psychiatric disorder. From the 174 subjects studied at baseline, 155 fell into 1 of the 3 comparison groups (n=55 depressed, n=43 psychiatric comparison, n=57 no disorder). Among the 55 depressed preschoolers, 31 (56%) were characterized by anhedonia and had a number of other characteristics suggesting they constituted a melancholic subgroup (J. L. Luby, MD, unpublished data, 2003). Therefore, in some of the analyses that follow, the depressed group was divided into anhedonic or melancholic vs nonmelancholic or hedonic subgroups so that differences between these 2 depressive subtypes could be investigated.

Children were observed in interaction with their caregivers and were interviewed directly about their internal experience of depression and anxiety states using an age-appropriate puppet interview, the Berkeley Puppet Interview-Symptom Scales.[47] Children also independently underwent an observational assessment of emotional reactivity as well as cognitive and neuropsychological testing. Parents filled out a number of additional standardized developmental, behavioral, and family history questionnaires. All assessments and all coding were done by raters blind to the diagnostic status of the child.

SALIVARY CORTISOL COLLECTION METHODS

Salivary cortisol was collected 3 times at specifically designated intervals during the assessment by having the children place a sterile dental cotton roll in their mouthes. To avoid any potential contamination of the assay, no salivary stimulant was used. Guided-imagery techniques to visualize various preferred foods were used to stimulate salivation after which the dental roll was spit out into the examiner's sterile gloved hand. Out of the child's sight, the dental roll was then placed in a needleless syringe, the saliva was extracted into a vial, and the vial was sealed, labeled, and frozen. The timing of the cortisol samples was determined based on the stressful events of the assessment and the principle that cortisol levels are believed to measure the level of stress experienced about 30 minutes prior. Based on this, the first level would represent preassessment time with a parent, which was not hypothesized to be stressful. The second was collected 30 minutes following the separation from a parent (a relational stressor), and the third was 60 minutes

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later after qualitatively different frustration-inducing stressors. This second set of stressful events were structured play tasks designed to produce transient and mild frustration or anger in the child (such as not being able to unlock a transparent box with a desirable toy inside). Prior to salivary cortisol collection, an ear temperature was taken to verify afebrile status. To control for nonstress related elevations of cortisol, assessments were conducted either at 9 AM (50% of the sample) or 1 PM. Time of day (assessment time) was then considered as a potential confounding variable in all analyses.

The first saliva sample was taken at least 1 hour after a meal, and children were provided with a snack of water and crackers no later than 30 minutes prior to the prestress cortisol sample. Information pertinent to conditions known to alter salivary cortisol values was obtained. This included current medication history (particularly use of any form of steroid medication), any recent tooth loss, and the time since the child's last meal (since food or blood in saliva are known to alter cortisol values). Families were asked to obtain and mail back to the laboratory 1 saliva sample on the evening after the laboratory assessment and for the 2 consecutive nights thereafter. Cortisol levels in the saliva sample were assayed through the Washington University General Clinical Research Center, St Louis, Mo, using the Gamma Coat Cortisol Radioimmunoassay kit procedure (DiaSovin, Stillwater, Minn). Appropriate adjustments for the assay of cortisol in saliva were made.

To be included in these analyses, it was necessary for subjects to have interpretable data on all 3 cortisol samples. Eleven subjects were excluded because they had taken a steroid medication or used a sympathomimetic inhaler, both of which are known to increase levels of cortisol. An additional 27 subjects were excluded because of insufficient amounts of saliva for assay, child refusal or inability to cooperate with saliva collection, and laboratory error (eg, failure to obtain a sample or lost samples) in 1 or more of the 3 samples. Based on these exclusions, 40 depressed subjects (21 anhedonics and 19 hedonics), 33 psychiatric control subjects, and 44 no-disorder control subjects were included in the following analyses.

The findings of samples with insufficient amounts of saliva raised the possibility that the child was too anxious to produce enough saliva; therefore we investigated whether these insufficient samples came more often from the depressed group. We found that 4 were from children in the depressed group and 3 were from the psychiatric control group. To determine if subjects who failed to give all 3 cortisol samples differed from the included group on any demographic variables or diagnoses, *t* tests or χ^2 analyses were performed as appropriate. No significant differences were found for any measures.

DATA ANALYSIS

Differences in mean raw cortisol values between groups were investigated using repeated-measures analysis of variance. To investigate differences in cortisol stress reactivity between diagnostic groups, cortisol change variables were calculated. The percent change between the first (baseline) and second (first post stress) cortisol values (percent change 1 to 2) and between the second (post stress) and third (second post stress) cortisol values (percent change 2 to 3) were calculated as a method of representing reactivity. This transformation allowed for standardization of the baseline and stress response values between subjects' comparisons of change values. Percent change 1 to 2 was (cortisol 2 – cortisol 1/cortisol 1 × 100) and percent change 2 to 3 was (cortisol 3 – cortisol 2/cortisol 2 × 100).

Differences in the raw cortisol values between groups were investigated using a repeated-measures multivariate analysis of variance (MANOVA) with group as the between-subjects variable and cortisol values in microgram per deciliter as the within-subjects variable. To investigate whether the depressed group had a different response pattern to the stressor than the 2 comparison groups, a repeated-measures MANOVA was completed with the diagnostic group as the fixed variable and cortisol percent change 1 to 2 and cortisol percent change 2 to 3 as the repeated measures. As opposed to ordinary repeated measures of analysis of variance, MANOVA makes no assumption about the correlation of the error term. Therefore, it retains the desired type I error probability (α) even though the second cortisol value is common to both measures, owing to their being constructed as changes. To test the hypothesis that cortisol change values would differ as a function of diagnostic group, the interactions were of primary interest. To further explore the significant interactions, the repeated measures were rerun between the depressed group and each control group. Post hoc *t* tests were computed to determine which groups differed for the interaction effects. Finally, another repeated-measures analysis of variance was conducted with 4 diagnostic groups, the 2 nondepressed comparison groups and 2 subgroups of depressed children (anhedonic and hedonic), to explore whether anhedonically depressed preschoolers (a presumptive melancholic subtype) displayed a significantly more elevated cortisol response to stress than did hedonically depressed preschoolers.

Table 1. Demographic Characteristics of the Study Sample*

Demographic Variable	Depressed Group (n = 40)	Psychiatric Group (n = 33)	No-Disorder Group (n = 44)
Age, mean (SD), mo	57.18 (7.11)	53.82 (9.24)	56.8 (7.30)
Female	55.0	45.5	59.1
Family income, $			
<30 000	25.0	9.1	11.3
<60 000	30.0	36.4	27.3
≥60 000	45.0	54.5	59.1
Refused to answer	0	0	2.3
Ethnicity			
White	80.0	91.0	84.1
African American	10.0	3.0	9.1
Hispanic	2.5	0	2.3
Other	7.5	6.0	4.5
Marital status			
Married	75.0	87.8	86.0
Separated/divorced/ single	25.0	12.2	14.0

*Data are presented as percentage unless otherwise indicated.

RESULTS

DEMOGRAPHIC CHARACTERISTICS OF THE STUDY SAMPLE

No significant differences were found in any demographic variable, such as age in months, sex, household income per year, ethnicity, marital status, and parental education, between the depressed, psychiatric, and no-disorder control groups (**Table 1**).

To assess any effect that stressful life events could have had on cortisol reactivity on the day of assessment, we investigated group differences in parents' report of stressful life events according to the Coddington Life Events Scale.[48] No differences were found in the frequency of any recent or major stressful life event be-

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Table 2. Medians, Means, and Standard Deviations of Raw Cortisol Values by Study Group

	Laboratory Assessment Cortisol Samples			Nighttime Cortisol Samples		
	1	2	3	4	5	6
			No-Disorder Group			
Mean (SD)	0.31 (0.40)	0.16 (0.10)	0.19 (0.10)	0.27 (0.62)	0.26 (0.51)	0.13 (0.12)
Median	0.19	0.15	0.19	0.10	0.12	0.10
			Psychiatric Group			
Mean (SD)	0.31 (0.33)	0.18 (0.10)	0.21 (0.09)	0.37 (1.00)	0.42 (1.10)	0.40 (0.89)
Median	0.21	0.15	0.21	0.12	0.10	0.12
			Depressed Group			
Mean (SD)	0.32 (0.63)	0.24 (0.37)	0.23 (0.18)	0.47 (0.17)	0.32 (0.89)	0.33 (0.95)
Median	0.16	0.17	0.19	0.09	0.09	0.10

tween study groups. To address any effect that daycare or preschool participation could have on cortisol reactivity, we investigated whether there were differences in daycare or preschool participation between groups and whether daycare or preschool participation had an impact on cortisol values. We found no differences in daycare or preschool participation between diagnostic groups ($\chi^2_{2,144}=5.57$; $P=.06$). Participation in daycare or preschool had no significant relationship to cortisol 1 values ($F_{1,135}=0.494$; $P=.48$) or to cortisol percent change 1 to 2 ($F_{1,73}=0.013$; $P=.91$). There was no significant interaction between participation in daycare or preschool and clinical group membership on cortisol 1 values ($F_{2,135}=0.078$; $P=.93$).

CORTISOL FINDINGS

Cortisol values obtained in this investigation were similar to those obtained in several other studies of children of the same age despite variation in saliva sampling techniques and assay procedures.[49,50] Because cortisol values were highly skewed, means and standard deviations were determined to be poorly representative of group characteristics. Qualitative investigation of the median scores of the groups at the various assessment points demonstrated that the groups differed in the fluctuations of cortisol levels across the assessment. **Table 2** displays medians, means, and standard deviations of all cortisol samples obtained in each study group. **Figure 1** displays the median cortisol value for each study group of interest at the 3 time points in the context of the overall assessment and timing of the stressful events.

Because of the limitations of statistical procedures available for nonparametric repeated measures in multiple groups, a repeated-measures MANOVA was completed even though cortisol is not normally distributed. A main effect for salivary cortisol values was present across time of samples ($F_{2,114}=5.458$; $P<.01$) suggesting that regardless of group, salivary cortisol levels fluctuated over the assessment. There was no significant interaction between group and sample time found when raw data were explored ($F_4=0.249$; $P>.05$).

When both percent change variables (percent change 1 to 2 and percent change 2 to 3) by diagnostic group were investigated with a repeated-measures MANOVA, a statistically significant interaction was found ($F_{2,114}=3.49$; $P=.03$). The repeated-measures MANOVA was recomputed to compare 2 groups at a time to determine the sources of the interaction. There was a significant interaction between the no-disorder and the depressed group ($F_{1,82}=4.09$; $P=.05$), but no significant differences were found between the depressed or the no-disorder groups in comparison with the psychiatric group in these analyses. Groups did not significantly differ in their percent change in cortisol between cortisol sample 2 and 3. When only percent change cortisol 1 to 2 was entered into an analysis of variance, a significant difference was found between the 3 diagnostic groups ($F_{2,117}=4.46$; $P=.02$). Post hoc Bonferroni-corrected comparisons demonstrated significant differences between the depressed and no-disorder groups ($P<.05$) and the depressed and psychiatric comparison groups ($P<.05$), with the depressed group showing greater elevations in cortisol in response to stress than the other 2 groups. There was no difference between the psychiatric and no-disorder groups (**Figure 2**).

There were no significant differences in the distribution of morning vs afternoon time assessments between diagnostic groups. The pattern of alterations in cortisol among the diagnostic groups was not different in the morning vs the afternoon; however, as would be expected based on known diurnal variations, there was a greater decline in cortisol levels between time 1 and time 2 in the morning compared with the afternoon across groups. When time of assessment (morning vs afternoon) was considered as a covariate, the MANOVA remained significant for percent change cortisol 1 to 2 ($F_{2,116}=3.77$; $P<.05$). The depressed group had greater elevations than the no-disorder ($P<.05$) and the psychiatric ($P<.05$) comparison groups.

HEDONIC VS ANHEDONIC DEPRESSED PRESCHOOLERS

When the depressed group was further divided into hedonic and anhedonic subgroups and a repeated-measures MANOVA was completed, there was a trend for a diagnostic group by cortisol percent change interaction ($F_{3,113}=2.33$; $P<.08$). To test the hypothesis that group differences would be a result of alterations in anhedonic depressed children primarily, post hoc Bonferroni-corrected comparisons were made. For cortisol per-

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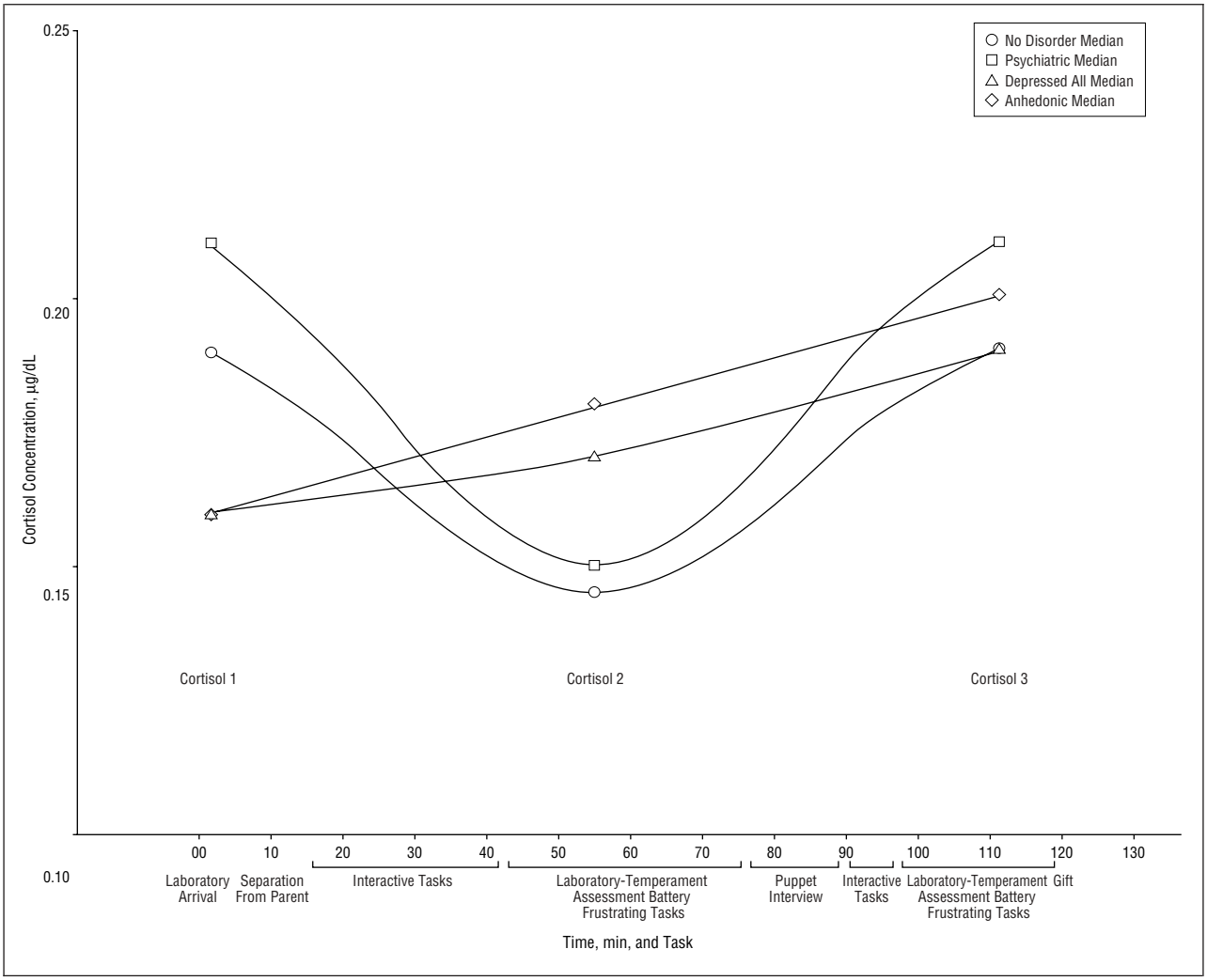

Figure 1. Median cortisol value by study group.

cent change 1 to 2, the no-disorder group differed from the anhedonic group ($P<.05$) but not the hedonic group (**Figure 3**). In addition, there was a trend for a statistical difference between the anhedonic group and the psychiatric comparison group ($P<.07$), but there were no significant differences between the hedonically depressed subgroup and the psychiatric controls. There were no group differences in cortisol percent change 2 to 3.

COMMENT

These data are the first, to our knowledge, demonstrating that clinically depressed preschool children display a unique pattern of stress cortisol reactivity. While the study hypothesis that depressed preschoolers would display elevations in cortisol reactivity was not supported by the data, the findings do provide the first evidence for a unique and altered pattern of stress cortisol reactivity in the youngest population of clinically depressed humans investigated to date. Based on the methods of validation proposed by Robins and Guze[43] and used in the development of the *DSM-IV* system, associations with biological markers provide an important component in establishing the content validity of a clinical syndrome. Because these depressed preschoolers met *DSM-IV* MDD criteria (except the duration criterion), the findings provide further validation for *DSM-IV* MDD criteria for use in these very young children. Other associated markers of a valid psychiatric disorder, such as increased family history of related disorders, a specific and stable symptoms constellation, and impairment, have been estab-

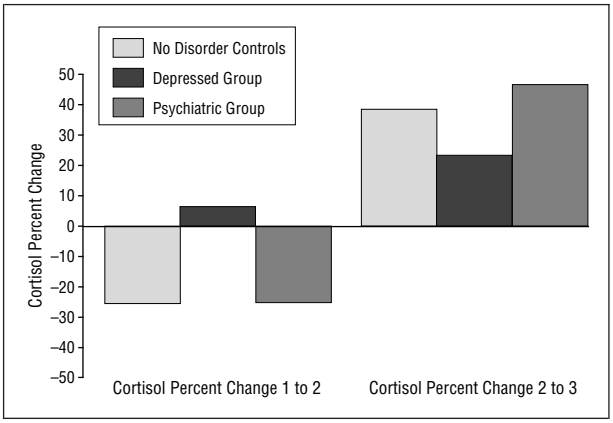

Figure 2. Cortisol percent change 1 to 2 and 2 to 3.

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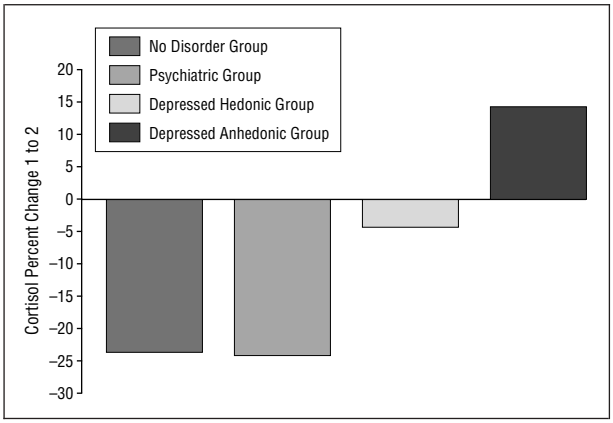

Figure 3. Cortisol percent change 1 to 2 for all groups including hedonic and anhedonic subgroups.

lished for preschool MDD.[41] Investigations of the longitudinal course of this syndrome, now underway in our laboratory, will provide needed data on predictive validity and will inform the accurate diagnostic definition of this early-onset clinical syndrome. Valid diagnostic criteria for MDD in preschool children are needed to facilitate the earliest possible identification of MDD for future treatment trials.

The finding that the experimental psychosocial stressors used in this study served effectively as a physiological stressor to the preschool study subjects has been previously established (A. H. Heffelfinger, PhD, unpublished data, 2003). However, based on the relatively high level of the first cortisol sample (collected at baseline on entry into the laboratory), it would appear that the circumstances of coming into the lab itself may also have served as a significant stressor for these very young children (suggestive of anticipatory anxiety). While there were no statistical differences between diagnostic groups in this first cortisol value alone, nor in the second cortisol value alone after the separation stressor, differences were found in the percent change between these 2 values, suggesting that the pattern of reactivity to the combination of these stressors differed between groups (as demonstrated by Figure 1). Further, post hoc analyses of the repeated-measures MANOVA revealed that the first cortisol change score was the source of the significant findings rather than the second change score.

These data demonstrated that in contrast to both no-disorder and psychiatric comparison groups, depressed preschoolers displayed increasing rather than decreasing cortisol in response to the stress of arrival at the laboratory and subsequent separation from the caregiver. All 3 groups displayed increasing cortisol levels in response to the frustrating stressful events occurring in the second half of the assessment. These findings suggest that depressed preschoolers failed to recover from the stress of laboratory entry and that in this context are further stressed by a separation from the caregiver, an event that did not appear stressful to the 2 age-matched comparison groups. While these findings do not replicate those known in depressed adults, they are consistent with adult findings in that a pattern of sustained elevations in cortisol level in response to stressful events is evident. These findings suggest continuity of HPA axis alterations in MDD across the lifespan. As such, findings provide support for the hypothesis that HPA axis alterations could play a role in the developmental neurobiology of MDD.

Discrepancies between the findings presented in depressed preschoolers and those reported in older depressed children and adolescents might be understood by considering the methodological differences in these studies. In particular, physiological stress paradigms (eg, DST) or measures of diurnal cortisol variation were used in studies of older depressed children, in contrast to the experimental psychosocial stress paradigm used in the study presented. Despite these methodological differences, the general findings that altered HPA axis reactivity was most evident in children rather than adolescents and inpatients rather than outpatients might be seen as consistent with the data presented here. Alterations of the HPA axis in child and adolescent populations were more robust with younger age, and by virtue of being inpatient, in those children who had experienced the stress of an extended separation from a parent. These features approximate the conditions under which elevated cortisol reactivity in the study sample were found as subjects were very young and had experienced the stress of separation from a primary caregiver. This brief separation from the caregiver for a 3- to 5-year-old represents a significant stress that might be developmentally comparable to the extended separation that an inpatient hospital stay would represent for an older child.

The fact that some children were assessed in the morning and some were assessed in the afternoon was a limitation to the study design owing to the known diurnal variation in cortisol. However, the use of change scores may have served to minimize this discrepancy, as no effects based on time of day were found in the analyses. Future studies should consider using a standard morning or afternoon assessment time if feasible. In addition, the collection of awakening morning and daytime cortisol values at home at the same time of day that the assessment values were obtained would allow a better approximation of baseline cortisol values and diurnal variation patterns. This would be important as significant differences in diurnal variation among children experiencing early and chronic deprivation have recently been reported.[51]

Future studies that use psychosocial, in lieu of the previously used physiological (eg, DST), stress paradigms in populations of older clinically depressed children and adolescents are now indicated. The use of age-appropriate stressors would be critical to the successful design of these studies, and paradigms have been developed for school-aged children.[28] These investigations would provide useful data on whether similar stress-induced HPA alterations are evident in older depressed children and adolescents. Along these lines, longitudinal follow-up of these depressed preschool children to investigate the relationship between course and severity and their relationship to the stability or change in HPA axis alterations is now needed. These findings could further address the question of continuity and change in HPA axis alterations in MDD across childhood.

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