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Right Frontal Brain Activity, Cortisol, and Withdrawal Behavior in 6-Month-Old Infants

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Although several studies have examined anterior asymmetric brain electrical activity and cortisol in infants, children, and adults, the direct association between asymmetry and cortisol has not systematically been reported. In nonhuman primates, greater relative right anterior activation has been associated with higher cortisol levels. The current study examines the relation between frontal electroencephalographic (EEG) asymmetry and cortisol (basal and reactive) and withdrawal-related behaviors (fear and sadness) in 6-month-old infants. As predicted, the authors found that higher basal and reactive cortisol levels were associated with extreme right EEG asymmetry. EEG during the withdrawal–negative affect task was associated with fear and sadness behaviors. Results are interpreted in the context of the previous primate work, and some putative mechanisms are discussed.

Asymmetry in activation of the prefrontal cortex (PFC) and levels of cortisol or cortisol responses have independently been implicated in fearful or withdrawal-related behavior. Until recently, the association between these two putative physiological correlates of fear and withdrawal has not been investigated. Three studies with nonhuman primates reported that right PFC activity was associated with higher cortisol levels (Kalin, Larson, Shelton, & Davidson, 1998; Kalin, Shelton, & Davidson, 2000; Rilling et al., 2001). Our study attempts a conceptual replication of the Kalin et al. (1998) primate study with human infants.

Considering the three primate studies in more detail, in rhesus monkeys with trait-like fear phenotypes, higher cortisol levels were associated with greater relative right frontal electroencephalographic (EEG) asymmetry across 3 years of measurement (Kalin et al., 1998). In a longitudinal study, monkeys with extreme right frontal brain activity had increased cerebrospinal fluid corticotropin-releasing hormone (CRH) concentrations at 4, 8, 14, 40, and 52 months of age (Kalin et al., 2000). Similar findings were noted in response to the stress of maternal separation (Rilling et al., 2001). With [^{18}F]-FDG positron emission tomography, increased activity in the right dorsolateral PFC during the separation correlated positively with cortisol levels, and decreased activity in the left dorsolateral PFC correlated with separation distress behaviors and cortisol. This last finding has a parallel in the human literature: Affective stimuli presented directly to the right hemisphere resulted in a greater increase in cortisol than did affective stimuli presented directly to the left hemisphere (Wittling & Pfluger, 1990).

In humans, both infants and adults, the left and right hemispheres of the frontal cortex are differentially activated during certain behaviors. Greater left frontal hemisphere activation has been associated with approach-related behaviors and positive affect, and greater right frontal activation has been associated with withdrawal-related behaviors and negative affect (see Davidson, 1992, 1995, for reviews). Children with an extremely fearful or shy temperament have greater relative right frontal EEG activity at baseline (Calkins, Fox, & Marshall, 1996; Davidson & Rickman, 1999; Finman, Davidson, Colton, Straus, & Kagan, 1989; Fox et al., 1995) and during stressful tasks (Schmidt, Fox, Schulkin, & Gold, 1999).

The relation between activity in the hypothalamic–pituitary–adrenal (HPA) axis and withdrawal behaviors, particularly behavioral inhibition, has received a great deal of attention in the developmental literature. Extremely inhibited children have higher cortisol levels (Kagan, Reznick, Snidman, 1987). Novelty elevates cortisol, but only for inhibited toddlers with nonoptimal maternal support (Nachmias, Gunnar, Mangelsdorf, Parritz, & Buss, 1996). Maternal report of behavioral inhibition during infancy and concurrent social wariness predicted baseline cortisol levels at age 4

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years (Schmidt et al., 1997). However, not all studies have reported the predicted relation between cortisol and behavioral inhibition in infants and children (e.g., Schmidt et al., 1999).

With regard to behavioral problems, higher cortisol levels are associated with internalizing behaviors and shyness (e.g., de Haan, Gunnar, Tout, Hart, & Stansbury, 1998; Scerbo & Kolko, 1994; Tout, de Haan, Campbell, & Gunnar; 1998). Furthermore, baseline cortisol levels taken during preschool predict maternal and teacher ratings of internalizing and social wariness 1.5 years later, during the transition into kindergarten (Smider et al., 2002).

Although several studies have examined asymmetric brain activity and cortisol in infants, children, and adults, the direct association between asymmetry and cortisol has not systematically been reported. The current study examines the relation between frontal EEG asymmetry across two conditions or episodes (resting, withdrawal–negative affect task) and cortisol (basal and reactive) and withdrawal behaviors (fear and sadness) in infants at 6 months of age. On the basis of Kalin et al.'s (1998) findings with rhesus monkeys, we made the following predictions: (a) resting relative right frontal EEG activation would be associated with higher baseline and reactive cortisol and more observed withdrawal-related behaviors, and (b) greater relative right frontal EEG activation during the withdrawal task would be associated with higher baseline and reactive cortisol and a greater number of observed withdrawal-related behaviors.

Method

Participants and Design

As part of a larger twin project, eighty-five 6-month-old infants, with a family history of right-handedness, participated in a physiology session that involved EEG recording during three conditions: baseline/resting, a stranger approach, and a peek-a-boo game.[1] Salivary cortisol samples were collected from the infants after the physiology session and on 3 consecutive days at home. The bulk of the analyses focus on data from the baseline and stranger approach, because the peek-a-boo game did not contain fear/withdrawal incentives.

Procedure

The infants were seated in a highchair for the entire session. For EEG baseline recording, the infants were kept alert and quiet by being shown several different objects for approximately 5 min. During the stranger approach, a male stranger entered the room, slowly approached the infant with a neutral expression, and stared at the infant for up to 2 min. During the peek-a-boo episode, the mother played a structured peek-a-boo game with her infant. The mother would hide behind a wooden structure; the experimenter would say, "Where's mommy?" and open a door; and the mother would smile and say "Peek-a-boo." This sequence was repeated 6 times and lasted approximately 2 min.

EEG recording and data reduction. Immediately after electrode placement, EEG was recorded for five 1-min baseline trials, during the entire stranger approach episode, and during the peek-a-boo episode. EEG was recorded with a Lycra electrode cap (Electro-Cap International, Eaton, OH), positioned on the infant's head according to known anatomical landmarks. EEG was recorded from the following sites in the standard 10/20 system (Electrode Position Nomenclature Committee, 1994): Fp1, Fp2, F3, F4, F7, F8, T3, T4, T5, T6, C3, C4, P3, P4, O1, O2, PZ, and FZ referred to CZ. Data were referenced offline by using a whole-head average reference, with a minimum of 12 sites evenly distributed across the head (Bertrand, Perrin, & Oernier, 1985; Hagemann, Naumann, & Thayer, 2001). Electrode impedances were below 20 kΩ, and impedances at homologous sites were kept within 5 kΩ of each other. EEG data were amplified[2] with a bandpass of 1–300 Hz and collected with a 60-Hz notch filter. Antialiasing filters were set at 200 Hz, and the signal was sampled at 500 Hz.

Cortisol collection and analysis. Salivary samples were collected after the physiology visit, approximately 1 hr after the session began and on time-matched occasions on 3 consecutive days at home.[3] The postvisit saliva sample occurred approximately 15–20 min after the end of the stranger approach episode. Saliva was collected with a suction catheter. Cortisol was assayed with the Pantex ^{125}I Cortisol (CORT) RIA Kit modified for saliva.

Data Reduction

Stranger behavioral coding and data reduction. We coded the stranger approach episode for facial fear, facial sadness, bodily fear, bodily sadness, vocal distress (crying), and escape behaviors. Coder reliability was calculated for 20% of the cases. Cohen's kappas exceeded .65 for all behaviors of interest. Correlations among clusters of these variables justified the creation of three behavioral composites: a fear composite (the average of facial and bodily fear), sadness composite (the average of facial and bodily sadness), and a distress composite (the average of all behaviors).

EEG asymmetry. EEG data were visually scored and edited to remove artifact caused by eye movement, muscle activity, and gross muscle movement. A fast Hartley transform (FHT; Bracewell, 1984) was applied to all artifact-free chunks of data that were at least 1.024 s in duration. Power spectra in the 5–9 Hz band were computed, which approximates the alpha band in adults (Bell, 1998; Marshall, 2001). Asymmetry scores were computed by subtracting log left power from log right power. Power in this band is inversely related to cortical activation, so negative scores on this metric reflect greater activity on the right side compared with the left; and positive scores reflect greater relative left cortical activity.

Cortisol. The three home samples were averaged for a measure of baseline cortisol (home cortisol). The home and lab cortisol distributions were skewed and $\log_{10}$ transformed. The difference between the lab cortisol and the home cortisol was calculated and referred to as delta cortisol. Samples were excluded from the analyses if the infant was ill with an infection (e.g., ear infection), if they were taking antibiotics, and/or if the sample was taken within 1 hr of eating. There was some variability in the time of visit and thus the saliva sampling. Because some research suggests that the magnitude of a stress response may vary with the circadian rhythm (Dallman et al., 1992), the effects of time-of-day on cortisol levels were examined. Time-of-day was correlated with the lab ($r = -.31$, $p < .01$) and home cortisol values ($r = -.25$, $p < .05$), but not with delta cortisol. Cortisol values were significantly lower if taken in the evening (5–8 p.m.) compared with morning (8–11 a.m.) or afternoon (12–4 p.m.) for home cortisol, $F(2, 73) = 5.76$, $p < .01$, and for visit cortisol, $F(2, 70) = 2.86$, $p < .07$. So, time-of-day effects were regressed out of the lab and home cortisol variables prior to analyses.

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[1] Electrocardiogram and respiration were also recorded during this visit but are not reported in this paper.

[2] EEG was amplified with either a Grass Model 12 Neurodata System amplifier or James Long amplifier. The amplifiers were switched approximately halfway through the study as a result of laboratory remodeling.

[3] A previsit saliva sample was not collected because the infants and parents were already being asked to do a considerable amount at this visit. In particular, the sensor placement for ECG and the capping for EEG were somewhat intrusive for the infants. The method of saliva collection periodically caused distress for the infants, and we needed to avoid an early visit stressor.

Data Analysis

To replicate the Kalin et al. (1998) results, we used an extreme-group design with a third, intermediate group. The use of an extreme-group design is consistent with the approach often adopted in the behavioral inhibition and temperament literatures (e.g., Kagan et al., 1987; Schmidt et al., 1997). In addition, such an approach is similar to that adopted in previous work from this laboratory (e.g., Tomarken, Davidson, Wheeler, & Doss, 1992). A series of one-way analyses of variance (ANOVAs) with asymmetry group as the independent variable tested the hypothesis that greater relative right frontal asymmetry would be associated with higher cortisol levels and more fear and sadness. There was only a moderate association in EEG asymmetry between baseline and stranger ($r = .42$, $p < .01$; $r = .75$, $p < .001$; $r = .33$, $p < .02$, for Fp1/2, F3/4, and F7/8, respectively), so EEG groups were created separately for baseline and stranger. EEG asymmetry groups were formed for each frontal asymmetry score (Fp1/2, F3/4, F7/8) for both conditions (baseline, stranger) analysis, for a total of six asymmetry group variables. Extreme groups were created from the distribution using a 0.5-*SD* cutoff. The *right* group included children who were 0.5-*SD* below the mean and the *left* group were children who were 0.5-*SD* above the mean. The middle asymmetry group was added in order to determine whether either or both of the extreme groups differed from the average asymmetry score. There was only moderate overlap between baseline and stranger EEG for group membership ($\lambda = .37$, $\lambda = .00$, $\lambda = .40$ for Fp1/2, F3/4, and F7/8, respectively). Finally, sample sizes for the different analyses vary because of the inherent difficulty in collecting psychophysiological data in infants. In particular, the sizes of the groups from baseline EEG to stranger EEG differ as a result of attrition across the visit (see Tables 1 and 2). In addition to the extreme-group analyses, we also conducted correlational analyses across the entire sample. Finally, EEG asymmetry from the peek-a-boo episode was used to support the hypotheses that EEG from only the baseline and stranger tasks would be associated with cortisol and withdrawal behaviors.

Prior to analyses, we determined that gender did not affect EEG asymmetry, cortisol, or behavior. Although the participants were twins, no genetic analyses were feasible because of small sample size (Neale & Cardon, 1992). Given that the participants were twins (some paired and others where only one twin from a pair contributed usable data), the data might violate rules of statistical independence of observations. We addressed this issue in three ways. First, we estimated the level of dependence between members of twin pairs by calculating intraclass correlations for all variables. The average intraclass correlations for the variables was near zero and not significant ($r = .14$), thus suggesting that any violations of independence were not grave. Second, we checked the actual degree of representation of both twins in a pair in all analyses. In the group analyses for cortisol, there were 17 pairs represented (5 monozygotic [MZ]); however, only 5 pairs (1 MZ) shared the same group status (e.g., both right frontal EEG). For the ANOVA with behavior as the dependent variable, 19 pairs were represented (5 MZ); however, only 6 pairs (2 MZ) shared the same group. This modest degree of members of the same pair being represented in the extreme groups also diminishes concern about statistical dependence in the data. Finally, to be conservative, degrees of freedom for statistical tests were decreased by 1 for every MZ pair in the analysis and by 0.5 for every dizygotic pair in the analyses when the pairs were represented in the same EEG group.

Results

Extreme EEG Frontal Asymmetry, Cortisol, and Withdrawal Behavior

The baseline EEG results are summarized in Table 1, and stranger EEG results are summarized in Table 2. First, we consider the Cortisol × Asymmetry Group analyses. These analyses revealed that the EEG groups differed in lab cortisol and delta

Table 1
Descriptive Statistics and ANOVA Results for Baseline EEG Asymmetry Groups

	Left			Middle			Right			
Variable	*n*	*M*	*SD*	*n*	*M*	*SD*	*n*	*M*	*SD*	*F*
					Fp1/2 asymmetry groups					
Lab cortisol	22	−.1680	0.40	47	−.1547	0.32	16	.0820	0.24	3.400*
Home cortisol	10	−.1372	1.09	37	.1634	0.93	12	−.0536	0.82	0.529
Delta cortisol	10	−.0544	0.26	35	.0021	0.12	11	.1500	0.20	4.530*
Sadness	21	−.0298	0.82	39	.0247	0.92	12	.3188	1.32	0.542
Fear	21	.0858	0.54	39	.0618	0.98	12	−.0270	0.90	0.060
					F3/4 asymmetry groups					
Lab cortisol	18	−.1968	0.37	52	−.1053	0.35	18	−.0290	0.27	1.090
Home cortisol	13	−.1171	0.95	35	−.0319	0.94	12	.2974	0.79	0.709
Delta cortisol	12	.0149	0.11	33	.0316	0.14	12	−.0700	0.28	0.349
Sadness	15	.0769	0.94	44	−.0368	0.95	16	.3066	0.99	0.754
Fear	15	.2356	1.21	44	−.0360	0.71	16	.0308	0.82	0.573
					F7/8 asymmetry groups					
Lab cortisol	26	−.1576	0.32	27	−.1213	0.29	34	−.0370	0.40	0.960
Home cortisol	20	.1939	1.16	14	−.1743	0.76	26	.1013	0.80	0.678
Delta cortisol	17	−.0224	0.19	14	−.0267	0.09	26	.0771	0.19	2.480†
Sadness	26	−.0723	0.64	24	.2224	1.15	24	.0627	1.04	0.580
Fear	26	−.2275	0.40	24	.0571	1.16	24	.2866	0.78	2.390†

Note. Sadness and fear refer to the composite variables. ANOVA = analysis of variance; EEG = electroencephalographic; Fp = frontopolar; F = frontal.
† $p < .10$. * $p < .05$.

Table 2
Descriptive Statistics and ANOVA Results for Stranger EEG Asymmetry Groups

	Left			Middle			Right			
Variable	*n*	*M*	*SD*	*n*	*M*	*SD*	*n*	*M*	*SD*	*F*
					Fp1/2 asymmetry groups					
Lab cortisol	19	−.1511	0.41	20	−.2744	0.37	20	−.0563	0.23	1.200
Home cortisol	13	−.0180	0.86	17	−.1851	1.09	14	.8941	0.96	5.050**
Delta cortisol	12	.0353	0.13	12	.0186	0.21	14	−.0349	0.09	0.755
Sadness	20	−.1747	0.47	25	−.2109	0.45	22	.5894	1.36	6.050**
Fear	20	.1615	1.00	25	−.0980	0.57	22	.1446	0.98	0.667
					F3/4 asymmetry groups					
Lab cortisol	13	−.3234	0.35	37	−.0992	0.35	13	−.2008	0.30	2.210
Home cortisol	8	.0746	1.07	30	.1081	1.09	9	.5181	0.98	0.554
Delta cortisol	9	−.0162	0.08	24	.0263	0.18	8	−.0771	0.07	1.560
Sadness	13	−.0982	0.58	46	.0637	0.97	12	.1958	1.04	0.317
Fear	13	.1607	1.21	46	−.0172	0.70	12	.0403	0.94	0.223
					F7/8 asymmetry groups					
Lab cortisol	15	−.2630	0.37	28	−.0900	0.33	17	−.2286	0.34	1.550
Home cortisol	7	.0705	0.74	26	.1203	0.94	11	.3223	1.20	0.197
Delta cortisol	6	.0558	0.26	22	−.0907	0.10	11	−.0356	0.16	0.711
Sadness	17	.0448	0.82	34	.0277	0.84	17	.2250	1.22	0.265
Fear	17	−.0953	0.82	34	−.0556	0.83	17	.2343	0.82	0.866

Note. Sadness and fear refer to the composite variables. ANOVA = analysis of variance; EEG = electroencephalographic; Fp = frontopolar; F = frontal.
** $p < .01$.

cortisol such that infants with greater right frontal activation had higher lab cortisol values. The analyses for lab cortisol and delta cortisol comparing baseline EEG asymmetry groups at Fp1/2 revealed significant main effects for group (see Figure 1). Post hoc comparisons revealed that the groups with greater right-sided activation had significantly ($p < .05$) higher cortisol values than both the middle and left groups. The middle and left groups were not significantly different. The ANOVA with home cortisol as the dependent variable and EEG asymmetry groups at Fp1/2 from the stranger approach task revealed a significant main effect for group (see Figure 2). Again, post hoc comparisons revealed that home cortisol for the group with greater right frontal activation was significantly higher than both the middle ($p < .05$) and left ($p < .05$) groups. Again, there was no significant difference between the middle and left groups. In sum, infants in the right asymmetry groups during baseline had the highest lab cortisol and the greatest increase in delta cortisol, whereas infants in the right asymmetry group during stranger approach had higher home cortisol levels. As predicted, right frontal EEG asymmetry was associated with higher cortisol levels.

There were no main effects for baseline asymmetry group on the fear and sadness behaviors. However, for EEG measured during the stranger approach task, frontal asymmetry was associated with fear or sadness behaviors during the same task. More sadness (see Figure 2) and fear were observed for infants in the right EEG asymmetry groups than for infants in the other two groups. The ANOVA for the sadness behavior composite comparing stranger EEG asymmetry groups at Fp1/2 revealed a significant main effect for group. The right-activated group scored higher on the broad sadness composite than the middle ($p < .05$) or left ($p = .06$) groups in post hoc comparisons. We found similar effects when we examined the two components of the sadness composite, facial and bodily sadness behaviors. Finally, the ANOVA for the overall fear composite was not significant. However, there were differences among groups on some of the discrete behaviors that made up the fear composite. The ANOVA for facial fear comparing stranger EEG asymmetry groups at F7/8 revealed a significant main effect for group, $F(2, 61) = 4.60$, $p < .02$. There were higher levels of facial fear for the right group versus the left group ($p < .01$) in post hoc comparisons.

Two questions that might arise in interpreting our results are whether we might have capitalized on chance in selecting among anterior EEG leads and, relatedly, how specific our results are to frontal regions. We address both of these issues by turning to measures of whole-head asymmetry.

Whole-Head Asymmetry, Cortisol, and Withdrawal Behavior

Asymmetry correlations. Figure 3 summarizes the pattern of correlations between asymmetry at all sites and cortisol (top two panels) and withdrawal behaviors (bottom panel). These topographic maps depict the magnitude of correlation between EEG asymmetry and cortisol or withdrawal behavior for each homologous pair of electrodes across the entire scalp. Recall that log band power is inversely related to activation, so greater relative right frontal activity is reflected in negative values. The color scale represents the magnitude and direction of the correlations, with larger negative correlations being represented as red. As visual inspection would suggest, frontal correlations (i.e., Fp1/2 or F7/8)

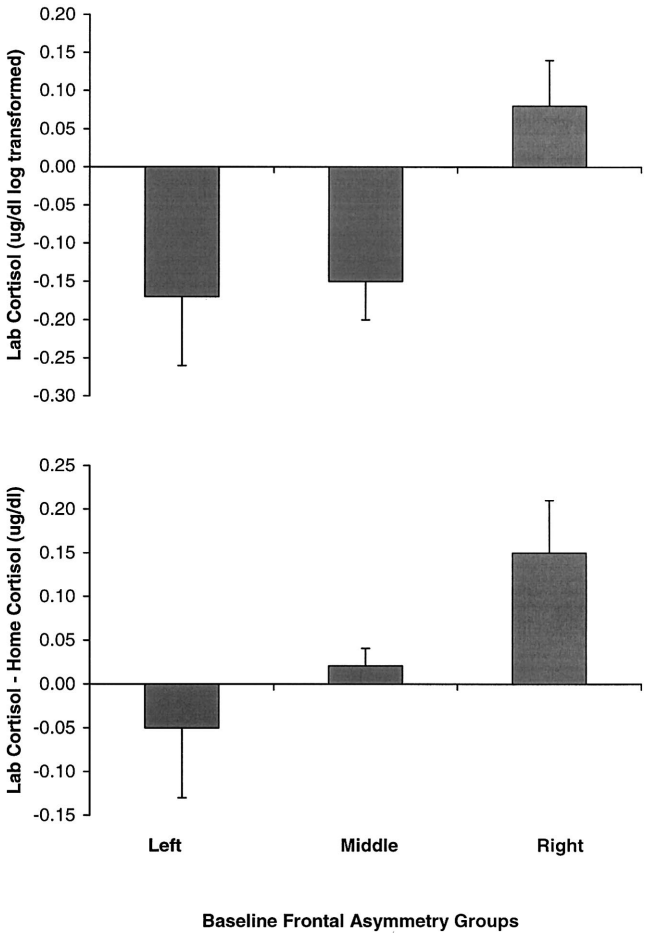

Figure 1. Lab cortisol in left, middle, and right frontal asymmetry groups during baseline. Top: Lab cortisol at frontopolar leads (Fp1/2; $p < .05$). Bottom: Delta (lab minus home) cortisol at Fp1/2 ($p < .02$). Error bars represent *SE*.

were significantly greater than correlations in the posterior sites (i.e., P3/4) for baseline EEG asymmetry and delta cortisol ($z = -2.89$, $p < .01$), for stranger approach EEG asymmetry and home cortisol ($z = -1.98$, $p < .05$), and for stranger approach EEG asymmetry and sadness behaviors ($z = -2.51$, $p < .01$). As a set, these three topographic maps confirm that the predicted association between asymmetry and cortisol or behavior is restricted to anterior scalp regions.

Left and right power regressions. We next examined which hemisphere within a homologous pair of frontal electrodes uniquely contributed to these findings. Whole-head power residualized scores were calculated for each frontal lead (Fp1, Fp2, F3, F4, F7, and F8) for baseline and stranger EEG separately. This score was calculated by first taking an average of all leads on the head, then regressing each individual lead (e.g., baseline Fp1) on the average. The residual score was saved as the new variable. Using a homologous set of residualized scores, we performed a series of hierarchical regressions on the whole-head residualized individual-site power values only for those effects that were statistically significant with the EEG asymmetry variable to guard against Type I errors. The following regressions were performed: baseline Fp1 and Fp2, F3 and F4, and F7 and F8 predicting lab cortisol and delta cortisol; and stranger approach Fp1 and Fp2, F3 and F4, and F7 and F8 predicting home cortisol and composite sadness behavior.

The results of all regressions are presented in Table 3. The regression predicting lab cortisol from baseline Fp1 and Fp2 revealed that it was only power from the left frontal lead (Fp1) that accounted for a significant amount of variance in the positive direction, indicative of decreased left prefrontal activation predicting higher levels of lab cortisol, as higher EEG power values in the 5–9 Hz band in this age group indicate decreased activation. Regressions predicting delta cortisol from baseline EEG leads failed to reach conventional levels of statistical significance for the full model. However, there was a trend for the more posterior frontal leads, suggesting that power from the right lead (F8) predicted delta cortisol in the expected negative direction (i.e., increased right prefrontal activation predicting greater increases in cortisol).

Power from both frontopolar leads (Fp1, Fp2) during stranger approach accounted for a significant amount of variance in the

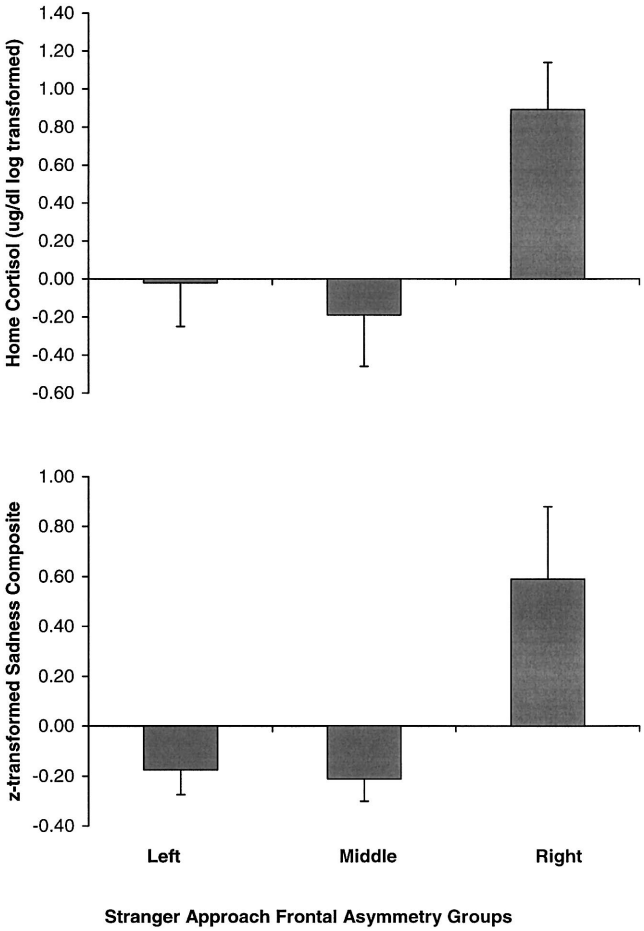

Figure 2. Home cortisol and sadness in left, middle, and right frontal asymmetry groups during stranger approach. Top: Home cortisol at frontopolar leads (Fp1/2; $p < .05$). Bottom: Sadness behavior at Fp1/2 ($p < .01$). Error bars represent *SE*.

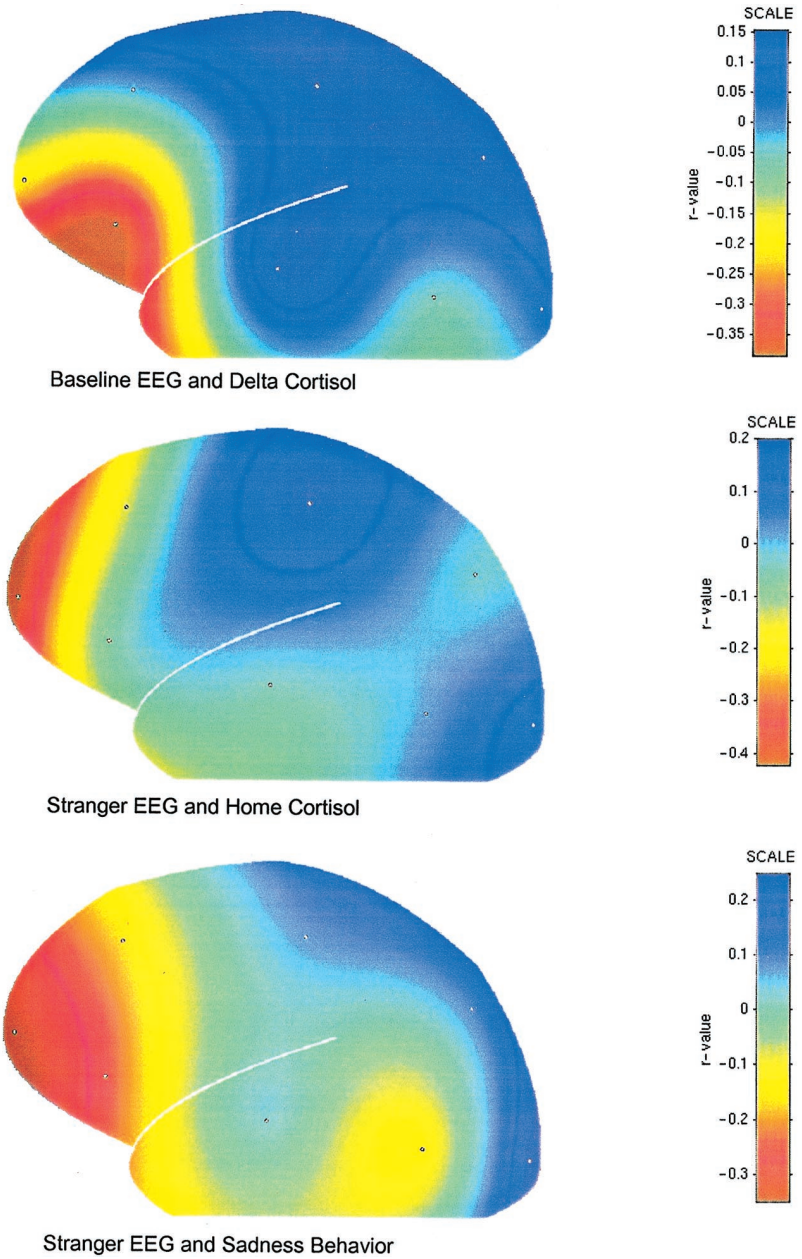

Figure 3. Topographic maps of the correlations between anterior and posterior electroencephalographic (EEG) asymmetry, cortisol, and sadness behavior. The asymmetry score for each homologous electrode pair (represented by the small white dots) was correlated to the cortisol measures and withdrawal measures. These correlations were used to generate spline-interpolated maps across a left lateral view of the head. Top: Correlations between delta cortisol and whole-head asymmetry during baseline. Middle: Correlations between home cortisol and whole-head asymmetry during stranger approach. Bottom: Correlations between sadness behavior and whole-head asymmetry during stranger approach.

home cortisol. Power from the left lead was positively associated with cortisol, whereas power from the right lead was inversely related with cortisol. These data indicate that left hemisphere hypoactivation and right hemisphere activation were both associated with higher levels of cortisol. Although the full model failed to reach statistical significance, a trend in the positive direction for power from the left lead (F3) was predictive of home cortisol, indicative of a decrease in left frontal activation.

Power from both frontopolar leads (Fp1, Fp2) and more posterior leads (F7, F8) accounted for a significant amount of variance in the composite sadness variable for EEG derived from the stranger approach condition. Power from the left lead was posi-

Table 3
Predicting Cortisol and Sadness from Left and Right Frontal Leads

	Left leads			Right leads		
Dependent measure	Predictor	β	ΔR^2	Predictor	β	ΔR^2
Lab cortisol	bFp1	.261*	.07	**bFp2**	**−.176**	—
	bF3	.159	.03	bF4	−.188	.03
	bF7	**.102**	—	**bF8**	**−.128**	—
Delta cortisol	**bFp1**	**.333**	—	**bFp2**	**−.189**	—
	bF3	**.016**	—	**bF4**	**.032**	—
	bF7	.211	.04	bF8	−.253†	.04
Home cortisol	sFp1	.914**	.19	sFp2	−1.095†	.09
	sF3	.302†	.09	**sF4**	**.033**	—
	sF7	**.054**	—	**sF8**	**−.154**	—
Sadness	sFp1	.580*	.03	sFp2	−.502*	.09
	sF3	**.103**	—	**sF4**	**−.136**	—
	sF7	.224	.03	sF8	−.305*	.04

Note. Predictors in boldface were dropped from the final regression equation. Dashes refer to statistics that were not computed. b = baseline electroencephalogram (EEG); Fp = frontopolar, F = frontal; s = stranger EEG.
† $p < .10$. * $p < .05$. ** $p < .01$.

tively associated with sadness. In contrast, power from the right lead was inversely related to sadness. This indicated that left hemisphere hypoactivation (more alpha power) and right hemisphere activation (less alpha power) were each associated with higher levels of sadness.

In sum, there was evidence that both right PFC activation and left PFC hypoactivation (i.e., greater alpha) during the stranger approach task accounted for significant variance in sadness behavior and home cortisol; and baseline left PFC hypoactivation only accounted for significant variance in laboratory cortisol.

Extreme EEG Frontal Asymmetry During Peek-A-Boo

One potential question that emerged is whether cortisol reactivity for the right frontal group was a result of the stranger approach or the experience of the physiology session as a whole. To address this issue, EEG asymmetry data from the peek-a-boo task was used. Recall that after the stranger approach episode, infants participated in the peek-a-boo episode ($n = 50$). EEG asymmetry extreme groups were formed by using the same method described above. There were no significant differences between the right, middle, and left asymmetry groups for peek-a-boo EEG asymmetry and any of the cortisol measures. Moreover, peek-a-boo asymmetry was not associated with distress during the stranger approach.

Discussion

We predicted that resting and task frontal EEG asymmetry favoring the right side would be associated with higher baseline and reactive cortisol and a greater frequency of withdrawal-related behaviors. The findings largely supported our predictions. Using an extreme-group analysis, we found that infants with extreme right frontal EEG at rest had higher stress cortisol levels and that infants with extreme right frontal EEG during a withdrawal task had higher basal cortisol than infants with average and extreme left frontal EEG activation asymmetries. Despite our prediction that baseline EEG would be associated with withdrawal behaviors during the stranger approach, only task EEG was associated with withdrawal behaviors. Specifically, infants with an extreme right frontal EEG asymmetry during the stranger approach showed the most concurrent sadness behaviors.

Despite reports of an association between withdrawal behaviors and cortisol levels, we failed to find a significant association between cortisol and withdrawal behaviors in this study. Although there are data consistent with the expectation of such an association (e.g., Kagan et al., 1987; Kalin et al., 1998), there are also recent findings that have failed to detect such an association (e.g., Schmidt et al., 1999).

We also extended our analysis of extreme groups in two important ways. First, we provided evidence that the associations between asymmetric activation in brain electrical signals and cortisol and behavior are localized to frontal scalp regions. Second, we investigated the separate contributions of left- and right-sided activation to the EEG asymmetry effects. One of three possible patterns can account for an association with an asymmetry metric—variation in the left hemisphere; variation in the right hemisphere; or a combination of both. For example, in the adult literature, specifically left prefrontal hypoactivation has been responsible for the difference in EEG asymmetry between depressed patients and normal controls (e.g., Henriques & Davidson, 1991). We found evidence in the current study for both left hemisphere hypoactivation and right hemisphere activation influences on asymmetry scores. Specifically, we found that cortisol levels were largely predicted by left frontal hypoactivation and that sadness was predicted by both left frontal hypoactivation and right frontal hyperactivation.

The data replicate and extend in human infants the major finding of the Kalin et al. (1998) primate study—that basal cortisol levels differ in extreme EEG asymmetry groups, with higher levels in individuals who have greater right-sided prefrontal activation. Our findings differed in two main ways from earlier findings. Only EEG asymmetry during the stranger task was associated with withdrawal behaviors, despite our prediction that baseline EEG asymmetry would be associated with withdrawal behaviors. The

second difference from Kalin et al.'s (1998) primate findings was that the association between frontal EEG asymmetry and cortisol was found for both basal and stress (delta) measures of cortisol. In the Kalin et al. (1998) report, only basal cortisol was examined.

Our findings were also strengthened by the analyses using EEG from the peek-a-boo episode. Recall that we failed to find an association between peek-a-boo EEG and cortisol and withdrawal behaviors. Thus, we are more confident that cortisol reactivity in the right frontal baseline asymmetry group was specific to stress during the stranger approach and not from stress related to the visit as a whole.

The increase in cortisol during the lab visit for infants in the right frontal group is even more dramatic when put in the context of the developmental HPA literature, particularly the data suggesting that there is a stress-hyporesponsive period in infants around this age (Gunnar, Broderson, Krueger, & Rigatuso, 1996; Larson, White, Cochran, Donzella, & Gunnar, 1998; Ramsay & Lewis, 1994). Despite a highly reactive adrenocortical response to stressors in healthy newborn infants (Gunnar, 1989), across the first year of life there appears to be difficulty in eliciting a cortisol stress response, notwithstanding the presence of behavioral distress (for a review, see Gunnar & Donzella, 2001). However, failure to elicit a stress response (i.e., hyporesponsivity) has not been demonstrated in infants as young as 6 months. Studies that have reported stress elevations in cortisol often focus on a small subgroup of infants who show extreme fearful behavior and have limited coping resources available to them (Gunnar, Larson, Hertsgaard, Harris, & Broderson, 1992; Nachmias et al., 1996). These findings fit with the extreme-group nature of the current study; only infants with extreme right frontal EEG asymmetry show a cortisol stress response.

Neural Circuitry

The association between right frontal electrical brain activity and cortisol is important because both have independently been implicated as biological components of withdrawal-related emotional behavior and fearful temperament styles (e.g., Davidson & Rickman, 1999; Kagan et al., 1987). For instance, Kagan suggests that inhibited children are physiologically biased to react with fear and withdrawal (Kagan, Snidman, & Arcus, 1992). In particular, these children may be sympathetically overreactive. Moreover, theory and research suggests that these children may also have a lower threshold for reactivity in the amygdala (Kagan & Snidman, 1999).

Although the neural circuitry of cortisol is well established (see De Kloet, 1991), less is known about the circuitry regulating PFC asymmetry. Moreover, the mechanisms by which prefrontal EEG asymmetry and activity of the HPA system are related need to be established. We suggest that the circuitry relating these systems is the same as the neural circuitry controlling emotions more generally (see Davidson, Pizzagalli, Nitschke, & Kalin, 2003). Extensive evidence is now available indicating that the neurocircuitry of emotion and the neural substrates of HPA regulation overlap (Davidson, Jackson, & Kalin, 2000). CRH is a key molecule that stimulates the pituitary to secrete adrenocorticotropin hormone (ACTH), which acts on the adrenal gland to secrete cortisol. Kalin et al. (2000) recently demonstrated that animals with extreme right-sided prefrontal activation had higher levels of CRH in cerebrospinal fluid measurements. Other evidence suggests that various territories of the PFC modulate subcortical activity elsewhere in the circuitry (e.g., Quirk, Russo, Barron, & Lebron, 2000). It should be noted that although the amygdala may play an important role in the initial learning of the affective styles examined in this report, other data suggest that the amygdala is not required for the expression of these styles (Kalin, Shelton, Davidson, & Kelley, 2001). It is likely that after the initial learning required to establish the consistency of an affective style, the PFC assumes importance in its representation and expression. On the other hand, given the age of the subjects tested here, it may well be that the amygdala is still required for the expression of the individual differences featured. Resolution of this issue will require the use of neuroimaging procedures that have sufficient spatial resolution to resolve the circuitry discussed.

Caveats

Although the predictions were the same for all three frontal sites, findings were strongest for the most anterior site (Fp1/2). It must be emphasized that these labels refer to scalp, not brain, locations and that better localization can only be achieved with the use of source localization methods that permit tomographic localization of the intracerebral sources of brain electrical signals. Such methods require higher density recordings but offer considerable promise for achieving good spatial resolution without the constraints imposed by MRI scanning (see e.g., Pizzagalli et al., 2001; Pizzagalli et al., 2002).

Some caution in interpreting elevations in cortisol is warranted. First, there was no pretest measure of cortisol, so we cannot be sure that postvisit cortisol actually reflects an increase from a previsit baseline. However, pre- to postvisit levels are often highly correlated (Ramsay & Lewis, 1994), possibly as a result of stress related to traveling to the lab and initial reactions to the new environment. In addition, there is some evidence suggesting that prestress samples collected in the lab are often lower than baseline levels collected at the same time of day in the home (e.g., Larson, Gunnar, & Hertsgaard, 1991). Therefore, we argue that the home measure of baseline, used in this study, may better reflect true baseline activity, at least nonstress measures of basal activity. Second, the timing of the postvisit cortisol measure may not reflect reactivity to stressor but differential negative feedback regulation of the HPA system. Recall that the postvisit saliva sample was collected approximately 15–20 min after the end of the stranger approach. Given the brief duration ($\sim$ 2 min) of the stranger episode itself, we cannot be sure that the peak of the cortisol response to that stressor corresponds to our sample time. It is likely that the sample was timed such that it reflects the peak following the EEG capping procedure. It is also likely that this sample reflects differential negative feedback of the HPA system rather than individual differences in the stress response itself. Repeated sampling across the visit would be necessary to tease this apart.

Conclusion

The findings from this study conceptually replicate and extend previous findings in nonhuman primates showing an association between asymmetric prefrontal activation and cortisol in human infants. Our data indicate that both baseline and reactive cortisol

are higher in infants with greater relative right-sided prefrontal activation. These findings add additional weight to the use of prefrontal activation asymmetry as a central variable in the definition of a fearful endophenotype.

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