Asymmetric Frontal Brain Activity, Cortisol, and Behavior Associated With Fearful Temperament in Rhesus Monkeys

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The authors examined the hypothesis that rhesus monkeys with extreme right frontal electroencephalographic activity would have higher cortisol levels and would be more fearful compared with monkeys with extreme left frontal activity. The authors first showed that individual differences in asymmetric frontal electrical activity are a stable characteristic. Next, the authors demonstrated that relative right asymmetric frontal activity and cortisol levels are correlated in animals 1 year of age. Additionally, extreme right frontal animals had elevated cortisol concentrations and more intense defensive responses. At 3 years of age, extreme right frontal animals continued to have elevated cortisol concentrations. These findings demonstrate important relations among extreme asymmetric frontal electrical activity, cortisol levels, and trait-like fear-related behaviors in young rhesus monkeys.

Considerable evidence demonstrates that individual differences in temperament are associated with differences in brain and peripheral physiological functioning (e.g., Davidson & Tomarken, 1989; Kagan, Reznick, & Snidman, 1988). Thus, temperament can no longer be viewed simply as a stable trait-like behavioral and emotional style but should be considered as a constellation of stable behavioral, emotional, and physiological characteristics. How these different characteristics interact and the mechanisms underlying the formation of individual differences in temperament are important questions that remain to be answered. To begin to unravel these issues, we have used rhesus monkeys to more completely characterize the physiological concomitants of behavioral responses associated with fearful temperaments. Eventually, this information will guide studies aimed at elucidating the mechanisms underlying the development of individual differences in fear-related temperaments.

Findings from human studies suggest that asymmetric electrical activity of frontal brain regions is associated with different emotional and temperamental styles (see Davidson, 1995, for review). For example, individuals with accentuated activation in right prefrontal regions report more dispositional negative affect (Tomarken, Davidson, Wheeler, & Kinney, 1992) and a larger increase in negative affect in response to negative laboratory elicitors (Wheeler, Davidson, & Tomarken, 1993) compared with individuals displaying left prefrontal activation. Studies in young children demonstrate that the trait of behavioral inhibition, which in its extreme form is thought to be an early marker of fearful temperament, is associated with relative right prefrontal asymmetric brain activation (Davidson, 1992). Other studies have demonstrated that increased sympathetic activation occurs in adults and children with fearful traits. In some, but not all studies, extremely inhibited children have been shown to have increased sympathetic activity as well as increased levels of the stress-related hormone cortisol (Kagan et al., 1988).

Studies from our laboratory have demonstrated that rhesus monkeys display stable fear-related behaviors that are similar to those observed in humans with fearful temperaments. Using a paradigm that reliably elicits these responses, we have shown an association between individual differences in nonstressed levels of cortisol and extreme behavioral inhibition (Kalin, Shelton, Rickman, & Davidson, 1998). Our studies of asymmetric frontal electrical activity in rhesus monkeys also have demonstrated similarities in this measure between rhesus monkeys and humans. As in humans, rhesus monkeys display asymmetric anterior brain electrical activity. Both monkeys (Davidson, Kalin, & Shelton, 1993) and humans (Tomarken, Davidson, Wheeler, & Doss, 1992) exhibit marked individual differences in the degree of relative right and left frontal electrical activity, which in both species have been demonstrated to be a stable characteristic of an individual. These behavioral and electrophysiological similarities between rhesus monkeys and humans support the feasibility of using rhesus monkeys to understand the behavioral correlates and neurobiological substrates of individual differences in asymmetric frontal activation (Kalin, 1993). We previously demonstrated in monkeys that the anxiolytic benzodiazepine, diazepam, increases relative left frontal electrical activity while decreasing the occurrence of fear-related behaviors (Davidson,
Kalin, & Shelton, 1992; Kalin & Shelton, 1989). In addition, diazepam had its greatest effect on shifting frontal activity to the left in monkeys that were dispositionally more fearful (Davidson et al., 1993). This finding suggests the possibility that individual differences in fearful temperament and asymmetric frontal brain electrical activity may be related to asymmetric functioning of endogenous benzodiazepine systems.

In both humans and monkeys, asymmetric brain electrical activity is recorded noninvasively from the scalp surface. The methods used to process the brain electrical activity data are identical in both species. In adult humans, power in the alpha band (8–13 Hz) has been used as the primary dependent measure because it has been shown to vary inversely with cortical activation (e.g., Davidson, Chapman, Chapman, & Henriquez, 1990). In our studies with human infants and children (e.g., Davidson & Fox, 1989), we have used power in a theta frequency band (4–8 Hz) in view of the well-known developmental changes in the frequency distribution of the electroencephalogram (e.g., Niedermeyer, 1993). In humans, the frequency of the dominant background rhythm gradually increases until it attains its maximum value (approximately 10 Hz) at about 10 years of age. Similar developmental changes are likely to occur in the brain electrical activity of rhesus monkeys. In previous work, we determined that the peak of the power spectrum in monkeys 1 year of age was between 4 and 8 Hz (Davidson et al., 1992). Accordingly, we used the same frequency band in the present study, given that the age of the monkeys studied was similar to those tested previously (Davidson et al., 1992).

Because both asymmetric frontal electrical activity and cortisol have been independently demonstrated to be associated with a fearful temperamental style, the current study was designed to examine the relation between individual differences in asymmetric frontal electrical activity and circulating levels of cortisol in a large sample of rhesus monkeys. We also studied the extent to which individual differences in these parameters are associated with stable, fear-related behavioral traits. We hypothesized that rhesus monkeys with extreme relative right frontal activity would have higher nonstressed cortisol levels and would be more fearful when compared with monkeys with extreme left frontal activity. The relation between brain electrical measures and cortisol was examined both contemporaneously as well as predictively. For the latter, we hypothesized that measures of asymmetric prefrontal activation would predict basal cortisol levels when assessed 2 years after the collection of the initial brain electrical data.

Method

Experimental Design

Subjects were 50 rhesus monkeys (Macaca mulatta; 29 male, 21 female). All monkeys were maintained on a 12-hr light-dark cycle (lights on 0600 to 1800 hr) at the Wisconsin Regional Primate Center and the Harlow Primate Laboratory (Madison, WI). Animal housing and experimental procedures were in accordance with institutional guidelines.

At 8 months of age, 15 monkeys were manually restrained and the electroencephalogram (EEG) was recorded. Restraint was achieved by holding monkeys on a table in a darkened room. At 1 year of age, EEG measurements were collected from 50 monkeys, which included the 15 monkeys that were assessed at 8 months. In addition, blood was sampled for cortisol and behavior was assessed using the human intruder paradigm (Kalin & Shelton, 1989). Animals received four human intruder tests at weekly intervals, which assessed defensive responses in three different contexts, each lasting 9 min. First, monkeys were separated from their mothers and placed in a cage alone (A), during which animals respond with distress-related coo calls. Next, freezing duration was assessed as elicited by a human entering the room and presenting his or her profile to the monkey, ensuring never to engage the monkey in eye contact (NEC). Finally, defensive aggressive responses, such as barking and hostility, were elicited by having the human stare directly at the animal (ST; Kalin & Shelton, 1989; Kalin, Shelton, & Takahashi, 1991). Behavior and vocalizations were scored from videotapes using a scoring system developed in our laboratory (Kalin & Shelton, 1989). Vocalization data were collected as the frequency of occurrence, and other behavior was scored as duration of occurrence during 9-min periods of either A, NEC, or ST. Based on the EEG data collected from the 50 monkeys at this age, extreme left and right frontal groups and a middle group were selected. Monkeys with EEG asymmetry scores more than .7 standard deviations greater than the mean were classified as extreme left frontals (n = 12; 5 males, 7 females), and those with asymmetry scores more than .7 standard deviations less than the mean were classified as right frontal (n = 11; 3 males, 8 females). The middle group was formed using −.35 standard deviation and .35 standard deviation as the cutoffs (n = 16; 8 males, 8 females). These cutoffs are similar to those used in related human research (Tomarken, Davidson, Wheeler, & Kinney, 1992). After selection, the extreme left and right frontal animals were maintained for follow-up at 3 years of age when blood was resampled for cortisol determinations.

EEG Recording and Quantification

Left and right frontal and parietal and mastoid gold-cup electrodes were placed according to the standard 10/20 system. The five active leads (left and right frontal, and parietal and right mastoid) were referenced to the left mastoid, and the linked mastoids were mathematically derived. All electrode impedances were below 5K ohms. We used a mathematically derived averaged mastoid reference rather than a physically linked mastoid reference because of the possible contribution of slight impedance differences between the mastoids affecting the effective spatial location of the reference when they are physically linked. When an averaged mastoid reference is mathematically derived, the data are recorded referenced to a single mastoid. The other mastoid is then recorded as an active channel. In this way, the high input impedance of the amplifiers prevents any slight difference in electrode impedances from affecting the recorded signal at the scalp surface. This is the preferred method for quantitative EEG analysis when asymmetric effects are the subject of analysis (Pivik et al., 1993). EEG was amplified using Grass Model 12 EEG amplifiers (Grass Instrument Company, Quincy, MA), with a gain of 20,000 and a band-pass filter of 0.1 to 200 Hz. A minimum of 20 artifact-free s of EEG was required per condition, per monkey. There was a mean of 70.95 artifact-free s of EEG per condition.

EEG signals were passed through active antialiasing, low-pass filters set at 210 Hz with a 36 db/octave roll-off. The output of the filters was digitized on-line at 500 Hz with an 80486 PC-clone (Diversified Systems, Madison, WI), equipped with a 12-bit,
32-channel A/D board and signal-acquisition software and stored on magnetic tape cartridges.

All data were edited for artifact on a high-resolution graphics monitor. EEGs from each of the scalp leads during all artifact-free periods were analyzed. Epochs of EEG 2 s in duration were extracted through a Hamming window. A Fast Fourier Transform (FFT) was applied to each chunk of EEG, with epochs overlapping by 50%. The FFT output was in µV^2. Data were aggregated into periods were analyzed. Epochs of EEG 2 s in duration were

Blood Sampling and Cortisol Radioimmunoassay

Blood was sampled at 1 and 3 years of age. At 1 year, one sample was obtained, whereas at 3 years, two samples were obtained on different days. To control for diurnal variation as well as potential environmental disturbance, all samples were collected during the circadian peak between 0830 and 1030 hr and under nonstressed conditions. Blood was collected (2 ml) by femoral venipuncture into glass tubes containing 4.5 mg ethylenediaminetetraacetate (EDTA) within 5 min (M = 2.22 min) after the monkey was removed from its cage. Previous studies from our laboratory demonstrated that sampling within this time frame resulted in levels that were significantly lower than those induced by stress (Kalin, Shelton, & Turner, 1992). Blood was immediately placed on ice until plasma was separated by centrifugation at 4 ° C for 10 min at 4,000 rpm and stored at −70°C until assayed. Plasma cortisol concentration was determined using a cortisol RIA kit (Pantex Corp., Santa Ana, CA). The intra-assay variability was 10.4% with an interassay variability of 11.3% and a detection limit (ED90) of 1.0 µg/dl.

Data Analysis

First, we hypothesized that individual differences in asymmetric frontal activity would be stable within monkeys. To test this, we performed intraclass correlations on the frontal asymmetry scores (log-right minus log-left power) collected from the 15 monkeys sampled both at 8 months and 1 year of age.

We hypothesized that increased right frontal asymmetry scores would be correlated with higher levels of cortisol and defensive behaviors and that cortisol levels also would be directly related to defensive behaviors. Because the experiment contained male and female monkeys, we established that gender did not affect cortisol levels. Spearman rank-order correlations were performed on data collected from the 50 animals at 1 year of age among (a) frontal asymmetry scores and plasma cortisol levels, (b) frontal asymmetry scores and defensive behaviors (average NEC-induced freezing duration, A-induced cooing, ST-induced barking, and ST-induced hostility), and (c) cortisol and the defensive behaviors.

Next, an extreme-groups analysis was used to further understand the differences between left and right frontally activated animals. Between the extreme left and right frontal groups, t tests were performed on measures of cortisol and individual defensive behaviors. The middle group was also used in a one-way analysis of variance (ANOVA) to determine the extent to which the two extreme groups differed from the middle asymmetry group. The inclusion of this group allowed for the specification of whether either or both extreme groups differed from the middle group control. One-way ANOVAs were computed on both the cortisol levels and defensive behaviors. Post hoc contrasts were performed when appropriate.

At the Year 3 resampling time, the sample size of the left and right frontal groups had decreased to 10 and 9 monkeys, respectively. Based on the Year 1 data, we expected that right frontal monkeys would continue to have cortisol levels greater than those in the left frontal group. First, we tested the stability of cortisol values within monkeys between 1 and 3 years of age by correlating the Year 1 value with the mean of the Year 3 cortisol values (an intraclass correlation was not computed because the Year 3 cortisols were significantly higher than those at 3 years of age). Next, one-tailed t tests were performed between the laterality groups for the two separately collected cortisol values as well as on their mean values.

Results

Stability of Individual Differences in Frontal Asymmetry and Relations Among EEG, Behavior, and Cortisol

As can be seen in Figure 1, frontal asymmetry scores (log right minus log left, 4–8 Hz power) derived from the 15 monkeys at 8 months and 1 year of age showed stability over time as reflected in a significant intraclass correlation (ICC = .58, p < .05).

The data collected from the entire group of 50 monkeys at 1 year of age revealed a significant inverse relation between frontal asymmetry scores and plasma cortisol levels (r = −.41, p < .003; Figure 2). Animals with greater relative right-sided prefrontal activation (i.e., lower asymmetry scores) had higher levels of plasma cortisol.

Correlations between the frontal asymmetry score and measures of freezing duration, defensive aggression (barking and hostility), and cooing were not significant. However, cortisol concentrations were correlated with ST-induced barking (r = .46, p < .01; Figure 3A) and ST-induced hostility (r = .37, p < .01; Figure 3B). Neither A-induced cooing nor NEC-induced freezing were significantly correlated to cortisol.

Figure 1. The stability of individual differences in asymmetric brain activity in 15 monkeys assessed by intraclass correlation (ICC = .58, p < .05) repeatedly at 8 and 12 months of age. Spearman rank = order correlation is shown.
BRAIN ACTIVITY, CORTISOL, AND BEHAVIOR IN MONKEYS

Figure 2. The relation between asymmetric frontal activity (lower scores indicate greater relative right-sided activity) and plasma cortisol concentrations in 50 monkeys at 1 year of age. EEG = electroencephalogram.

Extreme EEG Asymmetry, Cortisol, and Behavior Assessed at 1 Year

The analyses comparing the extreme left and right frontal groups revealed that, compared to left frontal monkeys, the right frontal monkeys had significantly higher cortisol concentrations: 58.02 ± SE 3.17 ug/dl vs. 40.60 ± SE 3.36 ug/dl; t(21) = -3.76, p < .0006; see Figure 4. In addition, right frontal monkeys were more hostile during ST: right = 81.23 ± SE 13.54 s, left = 43.28 ± SE 9.81 s; t(21) = -2.30, p < .04; see Figure 5, and froze for longer durations during A and NEC conditions. The only statistically significant difference in freezing occurred during the A period: right = 24.1 ± SE 7.28 s, left = 7.1 ± SE 4.05 s; t(21) = -2.08, p < .05; see Figure 5.

The data also were analyzed using the middle group to compare with the two extreme groups. The one-way ANOVA for cortisol comparing the left, middle, and right frontal asymmetry groups revealed a significant main effect for group, F(2, 36) = 5.70, p < .007; Figure 4. Post hoc comparisons revealed that the middle and right frontal groups did not significantly differ from each other. However, the extreme left frontal monkeys had cortisol concentrations that were significantly lower than both the middle (p < .02) and the right frontal groups (p < .01). Among the three frontal asymmetry groups, significant differences were not found for freezing duration, cooing, barking, and hostility.

Cortisol Concentrations in Left and Right Extreme Laterality Groups Assessed 2 Years Later

Cortisol values measured at 1 year of age were significantly correlated with the mean of the two values measured at 3 years (r = .51, p < .05). At 3 years of age, cortisol levels continued to be significantly different between the left and right frontal groups on Day 1 of sampling, 22.76 ± SE = 1.78 ug/dl and 32.80 ± SE = 1.78 ug/dl, respectively; t(17) = -2.26, p < .02. On Day 2 of sampling, cortisol differences between the groups were in the same direction (left frontal = 25.59 ug/dl vs. right frontal = 31.86 ug/dl).

Figure 3. The relation between plasma cortisol concentrations and ST-induced barking (A), and cortisol and ST-induced hostility (B) in 50 monkeys at 1 year of age.
but were not statistically different, $t(17) = -1.16$, $p = .13$. When comparing the means of the two samples, significant differences were found between the left and right frontal groups: left = 24.17 ± SE 2.73 μg/dl vs. right = 32.33 ± SE 3.44 μg/dl; $t(17) = -1.87$, $p < .04$; see Figure 6.

Discussion

The findings from this study demonstrate important relations among extreme asymmetric frontal brain electrical activity, nonstressed levels of cortisol, and traitlike, fear-related behaviors in young rhesus monkeys. The data underscore the similarities between humans and monkeys in these dimensions and further strengthen the rationale for using rhesus monkeys to explore mechanisms underlying the development of individual differences in fearful temperamental styles in humans.

In earlier studies, we demonstrated that fear-related behavioral responses, such as freezing and defensive aggression, are relatively stable characteristics of individual animals (Kalin & Shelton, 1989). In another study using a small group of animals, we found that individual differences in patterns of frontal asymmetric activity were stable over time (Davidson et al., 1992). The data from this study replicate the earlier finding and confirm the stability of individual differences in asymmetric frontal brain electrical activity. A similar pattern of stability in frontal brain electrical asymmetry has also been demonstrated in humans (Tomarken, Davidson, Wheeler, & Doss, 1992).

Correlational analyses performed at 1 year of age revealed that this stable characteristic of brain activity was associated with nonstressed plasma cortisol concentrations. Monkeys with greater left frontal activation had lower cortisol levels, whereas higher cortisol levels tended to occur in monkeys with greater right frontal activation. When the monkeys were divided into extreme left and right frontal asymmetric groups, the same finding was apparent, with left frontal monkeys having cortisol levels that were significantly lower than right frontal monkeys. Additional analyses of the data comparing the middle asymmetry group with the two extreme groups demonstrated that the difference in cortisol between the two extreme groups was due to reduced levels in the left frontal group. This finding underscores the importance of including a middle group and suggests a different interpretation than the traditional view that increased fearfulness is necessarily associated with increased cortisol levels. Perhaps humans who are less fearful and have greater left frontal asymmetry also have lower levels of cortisol.

When the extreme left and right frontal groups were resampled approximately 2 years later, similar group differences in cortisol concentrations were found. Taken together, these findings support a linkage between indexes of brain activity that have been linked to temperament and circulating levels of the stress-related hormone cortisol. The data from the follow-up measures of cortisol, obtained 2 years after the measures of brain electrical activity were acquired, indicated that the relation between asymmetric frontal electrical activity and nonstressed cortisol levels is long lasting.

Though robust differences in nonstressed cortisol were obtained between monkeys with extreme left versus right frontal activation, a comparison of plasma cortisol levels in a small sample of left- and right-frontally activated humans in an earlier study did not reveal a reliable group difference (Kang et al., 1991). The lack of consistent findings between this and the current study could be due to a number of factors. In the human study, only women were studied and they were considerably older than the relative age of the monkeys studied in the present study. In addition, many of the women, especially those in the extreme left frontal group, were taking oral contraceptives, which are known to affect cortisol concentrations (Coenen, Thomas, Borm, & Rolland, 1995; Kirschbaum, Pirke, & Hellhammer, 1995; Reinberg et al., 1996). In the present study, monkeys were manually restrained for the recording of brain electrical activity. This is likely a more stressful situation than that used in the recording of brain activity in humans and also could have contributed to differences between the two studies. Future studies in monkeys that use chared animals habituated to the chairing procedure for EEG recording would help to determine if the stress of manual restraint is a key compo-

**Figure 5.** Differences in A-induced freezing and ST-induced hostility in animals in the left compared to the right frontal asymmetry groups. A = monkeys in a cage alone; ST = monkeys stared at by the humans.

**Figure 6.** The mean cortisol concentration of two samples collected on different days at 3 years of age for animals in the extreme left ($n = 10$) and extreme right ($n = 9$) frontal asymmetry groups ($p < .04$).
nent in uncovering relations between frontal brain electrical asymmetries and cortisol.

Based on human studies, we hypothesized that defensive behavioral traits indicative of fear and anxiety would be associated with extreme right frontal brain activity. Correlational analyses between EEG asymmetry and these behaviors, at 1 year of age, were not significant. We also failed to find this relation in an earlier study that used a smaller group of monkeys (Davidson et al., 1993). However, the extreme-groups analytic approach demonstrated that, compared with humans with left frontal activity, those monkeys with extreme right frontal activity engaged in greater amounts of freezing and defensive aggression. When these data were analyzed with the additional middle asymmetry group, the differences were no longer significant. This was due to the large range of variability in the behavioral responses of the middle group and should not diminish the importance of the findings from the two-way extreme group comparison. In general, the finding that extreme right frontal monkeys display more intense defensive responses is consistent with findings in human children and adults, demonstrating that right frontal asymmetry is associated with increased behavioral and emotional distress (see Davidson, 1995, for review).

From the data collected at 1 year of age, we also examined the relations between cortisol concentrations and the various defensive behaviors. Based on earlier work in behaviorally inhibited children (Kagan et al., 1988) and monkeys with a propensity to freeze (Kalin et al., 1998), we expected that individual differences in freezing behavior would be positively correlated with cortisol levels. In the present study, nonstressed cortisol concentrations were positively correlated with the amount of hostility, or defensive aggression, displayed toward the startling human intruder. However, cortisol was not correlated with freezing duration. A possible explanation for the differences found between these studies could be that a single cortisol measurement can only grossly reflect a system that has a dynamic pattern of change. Alternatively, the relation between nonstressed levels of cortisol and defensive behaviors may be a general one that is not specifically linked to one type of defensive behavior. Despite these variations in the specific type of defensive behavior associated with cortisol, together the human and monkey studies suggest that nonstressed cortisol concentrations are generally related to individual differences in a constellation of behavioral responses all associated with fearful temperaments.

The findings from the current study are the first to link individual differences in asymmetric frontal electrical activity with circulating levels of cortisol. This is of importance because both parameters have been independently associated with fear-related temperamental styles. However, the extent to which the relation between extreme right frontal asymmetry and nonstressed cortisol levels is associational or causally linked remains to be determined. The finding that the differences in cortisol were attributable to extreme left frontal activity is interesting and should be pursued. Although the brain neural circuitry that ultimately regulates the release of cortisol has been established, little is known regarding the mechanisms underlying frontal asymmetric electrical activity. Consistent with the findings from the current study, a study in humans suggests a preferential role for the right hemisphere in emotion-induced release of cortisol (Wittling & Pfuger, 1990; see review in Wittling, 1995). Future studies in monkeys will be aimed at elucidating the mechanisms underlying this relation as well as those regulating emotion-related asymmetric frontal electrical activity.

References


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