

Nonhuman Primate Studies of Fear, Anxiety, and Temperament and the Role of Benzodiazepine Receptors and GABA Systems

Ned H. Kalin, M.D.

Studies of nonhuman primate models have been useful in defining anxious temperament as an individual's stable set of physiologic and behavioral responses and in providing insights regarding human anxiety. Anxious temperament in rhesus monkeys (*Macaca mulatta*) is marked by excessive anxiety, exaggerated defensive behavioral responses, extreme asymmetric right frontal brain electrical activity, and elevated cerebrospinal fluid levels of corticotropin-releasing hormone and plasma cortisol. In the human brain, extreme asymmetric right frontal activation is likewise associated with negative affect and anxious disposition. Our studies of infant rhesus monkeys using the human intruder paradigm allow us to investigate individual differences in fear-related defensive behavioral responses and suggest that responses to threatening stimuli are mediated by γ -aminobutyric acid and benzodiazepine receptors. Ongoing studies with nonhuman primates in our laboratory are further examining the neurochemistry underlying individual differences in anxious temperament. We believe that these studies will provide insights into the adaptive and maladaptive responses of humans as they relate to psychopathology as associated with anxiety.

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Behavioral studies of nonhuman primates yield valuable information regarding the pathophysiology of human neuropsychiatric illnesses. In particular, rhesus monkeys (*Macaca mulatta*) provide excellent models for human behavior because rhesus and humans have similarities in brain structure, behavior, and physiologic responses to stress, and in social behavior. Studies of rhesus monkeys performed by my colleagues and me present ethologically relevant models of fear and anxiety that are reasonably extrapolated to humans. Among our findings are the existence and stability of anxious temperamental traits that are associated with asymmetric electrical activity in the right frontal region of the brain and the integral involvement of benzodiazepine systems in adaptive responses to threat.

THE ANXIOUS TEMPERAMENT ENDOPHENOTYPE IN RHESUS MONKEYS: BIOLOGICAL AND BEHAVIORAL CHARACTERISTICS

Temperament can be defined as an individual's set of stable emotional, behavioral, and physiologic characteristics that are present from early in life. Behavioral differences in individual temperaments result from genetic influences in conjunction with early experience. Considerable animal research has demonstrated that developmental stress such as maternal deprivation can alter an offspring's propensity for fear-related behavioral and hormonal responses and produce an anxious adult.¹

We have suggested that in rhesus monkeys the anxious temperament endophenotype is marked by excessive fearful or defensive responses and a number of physiologic parameters including increased electrical activity in the right frontal region of the brain relative to the left frontal region.^{2,3} Extreme asymmetric right frontal activation in humans, as in monkeys, is associated with negative affect and anxious disposition and is stable over time.⁴ Temperamentally anxious individuals can be identified in childhood and may be at increased risk to develop anxiety and depressive disorders later in life.

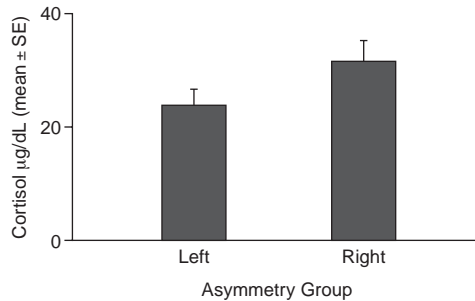
In examining the hormonal responses of the monkeys, we found that plasma cortisol levels were significantly higher in monkeys with extreme right frontal activation compared with monkeys with extreme left frontal activa-

From the Department of Psychiatry, University of Wisconsin, Madison.

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Corresponding author and reprint requests: Ned H. Kalin, M.D., University of Wisconsin, Department of Psychiatry, 6001 Research Park Blvd., Madison, WI 53719 (e-mail: nkalin@facstaff.wisc.edu).

Figure 1. Basal Cortisol Levels in Rhesus Monkeys With Extreme Left Frontal Brain Activity Versus Those With Extreme Right Frontal Brain Activity^a



^aReprinted with permission from Kalin et al.²
Abbreviation: SE = standard error.

tion (Figure 1).² At 1 year of age, the monkeys with extreme right frontal electrical activity and with higher cortisol levels demonstrated a propensity toward fearful behaviors when confronted with threatening stimuli. When the monkeys with extreme left or extreme right asymmetric activity were resampled 2 years later, the differences in plasma cortisol levels remained, indicating that the relationship between electroencephalograph (EEG) asymmetry and cortisol is long term. These findings are interesting because cortisol is known to modulate numerous physiologic systems and, when chronically elevated, is associated with metabolic and behavioral/emotional disturbances. Also, human children who are excessively shy have been demonstrated to have elevated cortisol.⁵

Numerous preclinical and clinical studies have demonstrated that corticotropin-releasing hormone (CRH) in the brain contributes to behavioral, autonomic, and neuroendocrine responses to stress, culminating in the release of the major stress hormone cortisol. Overactivity of the CRH system has been implicated in depressions and anxiety; elevated CRH levels occur in the cerebrospinal fluid (CSF) of some depressed or anxious individuals.³ We measured CSF CRH in rhesus monkeys with extreme right versus extreme left frontal brain activation.³ The monkeys were tested at 4, 8, 14, 40, and 52 months of age. Across all ages, monkeys with extreme right frontal brain activity had elevated concentrations of CRH. Furthermore, individual concentrations remained relatively stable across the entire range of ages tested, suggesting that the nonstressed level of CSF CRH, like asymmetric brain activation, is a trait characteristic. These electrophysiologic and hormonal studies reveal important links between asymmetric frontal brain activity, nonstressed levels of CSF CRH and cortisol, and trait-like fearful behaviors as indicators of temperament in young rhesus monkeys.

To begin to understand the neural circuits underlying the behavioral and biological characteristics associated

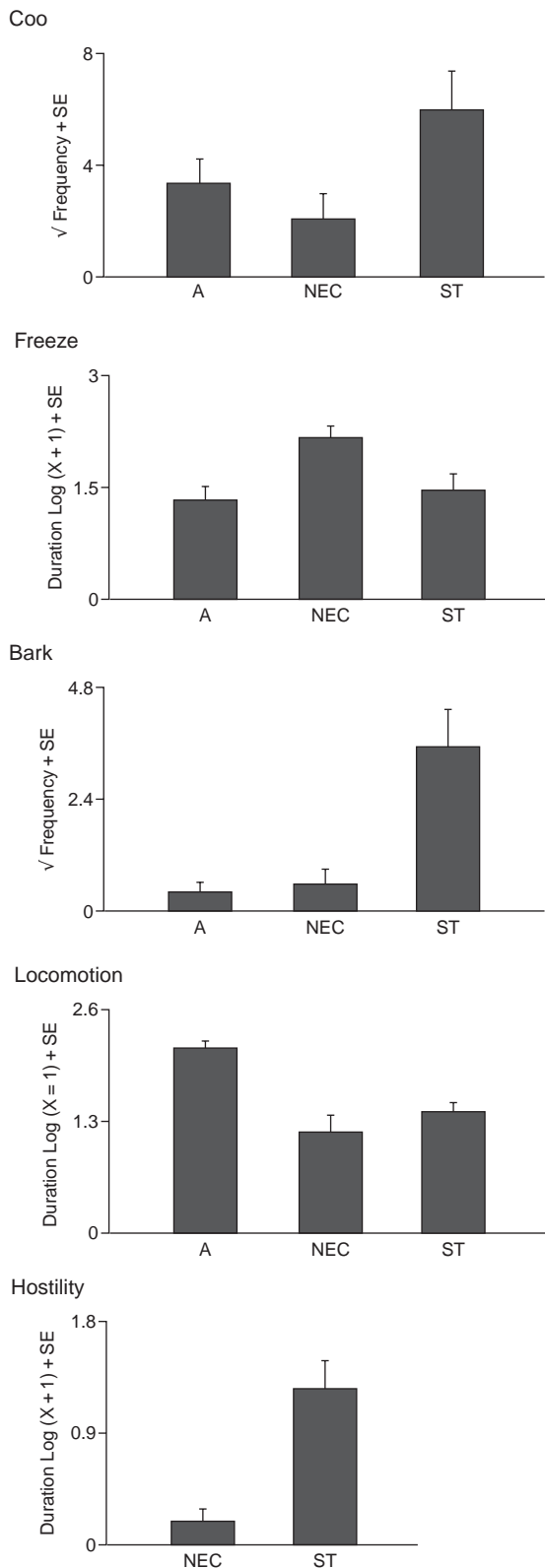
with fearful temperament, we assessed these parameters in monkeys that had undergone procedures to lesion the amygdala.⁶ The amygdala is of interest since numerous other animal and human imaging studies implicate its involvement in the processing of negative emotion and fearful stimuli. In addition, overactivity of the amygdala may be involved in mediating anxiety and depressive psychopathology. In the monkeys, lesions were made by administering ibotenic acid directly into the amygdala, which caused cell death in this structure without affecting communicating fibers passing through the amygdala. We hypothesized that, in the absence of an intact amygdala, the monkeys would display a reduction of threat-related behavioral responses, a leftward shift in asymmetric frontal EEG activity, and a decrease in basal cortisol and CSF CRH concentrations. To our surprise, we found that these parameters were not significantly affected by the lesions.² However, other responses associated with acute fear responses were blunted. While unexpected, these findings led us to speculate that the amygdala is not critical for the expression of fear-related behavioral and physiologic responses that have been stable for long periods of time and are associated with temperament. Our current investigations are focused on understanding the role of the orbitofrontal cortex in mediating features of the fearful temperament, as this brain region is associated with emotion regulation and may be involved in maintaining responses over the long term.

THE ROLE OF BENZODIAZEPINE AND GAMMA-AMINO BUTYRIC ACID SYSTEMS IN MEDIATING FEATURES OF THE ANXIOUS TEMPERAMENT

To study behavioral responses associated with fear and anxiety, my colleagues and I developed the human intruder paradigm.⁷ Exposure to this paradigm evokes responses associated with attachment bond disruption (separation distress) and threat in infant rhesus monkeys and also allows us to study the monkey's ability to adaptively regulate its defensive responses in relation to changing contexts. In a developmental study, we found that prior to 3 months of age, rhesus infants did not effectively regulate their defensive responses; however, after the first 3 months of age, the monkeys displayed adaptive fear-related responses similar to those of adults.⁸

In the human intruder paradigm, the monkey is exposed to 3 different conditions. In the alone (A) condition the monkey is separated from its mother or other conspecific and is placed in a cage alone.⁸ Monkeys typically respond to this separation with increased locomotion and coo vocalizations. Coos, which are rising and falling melodious sounds produced with pursed lips, are thought to be analogous to human crying. Both of these responses may function to attract the mother's attention so that she can re-

Figure 2. Behaviors Elicited by the Conditions of the Human Intruder Paradigm^a



^aReprinted with permission from Kalin et al.⁹
Abbreviations: A = alone condition; NEC = no eye contact condition; ST = stare condition.

trieve her separated infant. In the no eye contact (NEC) condition, a human enters the room and presents her profile to the monkey without making eye contact. This condition normally elicits a period of freezing—tense, motionless posture associated with silence—in which the monkey seeks to escape the intruder's notice. It is critical that the intruder has no eye contact with the monkey in this condition. We believe that the freezing behavior functions to keep the monkey inconspicuous in the face of a potential predator. Individual differences in freezing behavior exist and are generally stable over time. In the stare (ST) condition, a human intruder stares directly at the monkey while maintaining a neutral expression. The ST condition normally provokes defensively hostile, aggressive, and submissive behaviors including barking, reciprocated staring, lip smacking, and tooth grinding. Increased vocalization in the ST condition may also include cooing—here, most likely expressing fearfulness as opposed to separation distress. Individual responses to the different conditions of the human intruder paradigm are clear and stable over time (Figure 2). Although protective and defensive behaviors are adaptive, overexpression of these behaviors may indicate the presence of anxious temperament.

Using the human intruder paradigm, we demonstrated involvement of benzodiazepine and GABA systems in mediating the threat-induced responses observed in the NEC and ST conditions.⁷ For example, administration of diazepam selectively decreased the threat-related behaviors. Interestingly, the administration of benzodiazepines had little effect on the behaviors associated with attachment bond disruption in the A condition. In contrast, administration of low doses of morphine selectively reduced A-induced distress vocalizations without affecting the threat-related behaviors occurring in the other conditions. Not only did these studies implicate benzodiazepine systems in modulating threat-related behaviors, but they also indicated a relative lack of benzodiazepine involvement in modulating attachment distress.¹⁰ Young rhesus have also been tested with the triazolobenzodiazepine alprazolam in the human intruder paradigm.¹⁰ As expected, alprazolam decreased the threat-induced behavioral responses occurring during the NEC and ST conditions. Additionally, a reduction in threat and distress vocalizations was noted in the ST condition. At the highest dose, distress vocalizations were also decreased in the A condition. This finding was different from our studies with diazepam. Whether the differences in the effects between alprazolam and diazepam in relation to distress vocalizations are due to differences in the drugs or other factors remains to be determined.

In this study¹⁰ it was also demonstrated that the stressful conditions of the human intruder paradigm increased plasma adrenocorticotrophic hormone (ACTH), cortisol, and growth hormone (GH) levels. At doses that had no effect on baseline levels of ACTH and cortisol, alprazolam

attenuated the human intruder paradigm–induced ACTH and cortisol increases. In contrast to the effects on ACTH and cortisol, alprazolam further increased the stress-induced elevations in GH. Neither test conditions nor pretreatment with alprazolam affected CSF CRH concentrations.

My colleagues and I have also examined the effects of the anxiogenic agent β -carboline, an inverse benzodiazepine agonist.⁹ Administered prior to exposure to the human intruder paradigm, β -carboline evoked a dose-dependent increase in threat-induced behavior such as freezing and a decrease in environmental exploration. In a second trial administering a higher dose (1000 μ /kg) of β -carboline, more prominent behavioral effects were observed. Taken together, these data demonstrated a role for benzodiazepine and GABA systems in mediating threat-related behavioral and hormonal responses in primates and suggest that these effects are relatively selective for threat-related responses.

We also examined the extent to which benzodiazepine systems can modulate brain activity associated with the anxious temperament. As discussed above, we had previously determined that monkeys with increased fear-related defensive responses were more likely to have asymmetric right frontal brain activity. Using rhesus monkeys undergoing the mild stress of restraint, we explored the hypothesis that benzodiazepines would shift the monkeys' frontal brain electrical activity from right to left frontal asymmetry. As predicted, the stress of restraint resulted in asymmetric right frontal activity that shifted to the left in response to diazepam treatment. This effect was selective to the frontal brain regions; patterns of parietal activity were unchanged.¹¹ In a later study,¹² we demonstrated that those monkeys that showed the largest frontal EEG changes in responses to diazepam tended to be those that displayed the most intense threat-related fear responses in the human intruder paradigm. These studies suggest that benzodiazepine receptor and GABA systems in frontal brain regions can modulate patterns of brain activity associated with stress and threat-related responses. In addition, the results suggest that individual differences in threat-related responses may be related to individual differences in frontal cortical benzodiazepine receptor function.

CONCLUSION

Rhesus monkeys provide relevant models for studying the mechanisms underlying human fear, anxiety, and temperament. Our studies of rhesus monkeys have revealed the biobehavioral characteristics of anxious temperament—an individual's stable set of physiologic, emotional, and behavioral responses. Monkeys with extreme anxious

temperament display excessive threat-related behavioral responses, have extreme asymmetric right frontal brain activity, and have increased basal levels of cortisol and CSF CRH. Studies are underway to establish the neural circuits underlying these characteristics and suggest that the amygdala is not critical for the maintenance of these responses once they have become stable and trait-like. However, the benzodiazepine and GABA systems may be important in modulating the expression of anxious temperament. Our data demonstrate that benzodiazepines reduce threat-related behavioral responses and also promote a shift in brain electrical activity from right to left frontal asymmetry. By combining pharmacologic with behavioral and functional brain imaging approaches, future studies will attempt to elucidate the sites in the brain at which benzodiazepine receptor and GABA systems mediate these effects.

Drug names: alprazolam (Xanax and others), diazepam (Valium and others), morphine (Avinza, MS Contin, and others).

Disclosure of off-label usage: The author has determined that, to the best of his knowledge, no investigational information about pharmaceutical agents has been presented in this article that is outside U.S. Food and Drug Administration–approved labeling.

REFERENCES

1. Bakshi VP, Kalin NH. Corticotropin-releasing hormone and animal models of anxiety: gene-environment interactions. *Biol Psychiatry* 2000;48:1175–1198
2. Kalin NH, Larson C, Shelton SE. Asymmetric frontal brain activity, cortisol, and behavior associated with fearful temperament in rhesus monkeys. *Behav Neurosci* 1998;112:286–292
3. Kalin NH, Shelton SE, Davidson RJ. Cerebrospinal fluid corticotropin-releasing hormone levels are elevated in monkeys with patterns of brain activity associated with fearful temperament. *Biol Psychiatry* 2000;47:579–585
4. Tomarken AJ, Davidson RJ, Wheeler RE, et al. Individual differences in anterior brain asymmetry and fundamental dimensions of emotion. *J Pers Soc Psychology* 1992;62:676–687
5. Kagan J, Reznick JS, Snidman N. Biological bases of childhood shyness. *Science* 1988;240:167–171
6. Kalin NH, Shelton SE, Davidson RJ, et al. The primate amygdala mediates acute fear but not the behavioral and physiological components of anxious temperament. *J Neuroscience* 2001;21:2067–2074
7. Kalin NH, Shelton SE. Defensive behaviors in infant rhesus monkeys: environmental cues and neurochemical regulation. *Science* 1989;243:1718–1721
8. Kalin NH, Shelton SE. Ontogeny and stability of separation and threat-induced defensive behaviors in rhesus monkeys during the first year of life. *Am J Primatol* 1998;44:125–135
9. Kalin NH, Shelton SE, Turner JG. Effects of beta-carboline on fear-related behavioral and neurohormonal responses in infant rhesus monkeys. *Biol Psychiatry* 1992;31:1008–1019
10. Kalin NH, Shelton SE, Turner JG. Effects of alprazolam on fear-related behavioral, hormonal, and catecholamine responses in infant rhesus monkeys. *Life Sci* 1991;49:2031–2044
11. Davidson RJ, Kalin NH, Shelton SE. Lateralized effects of diazepam on frontal brain electrical asymmetries in rhesus monkeys. *Biol Psychiatry* 1992;32:438–451
12. Davidson RJ, Kalin NH, Shelton SE. Lateralized effects to diazepam predicts temperamental style in rhesus monkeys. *Behav Neurosci* 1993;107:1106–1110