Behavioral Neuroscience
1998, Vol. 112, No. 1, 251–254

0735-7044/98/$3.00

Individual Differences in Freezing and Cortisol in Infant and Mother Rhesus Monkeys

Ned H. Kalin, Steven E. Shelton, Maureen Rickman, and Richard J. Davidson
University of Wisconsin

Freezing is an adaptive defensive behavior that is expressed in response to an imminent threat. In prior studies with rhesus monkeys, stable individual differences in animals' propensities to freeze have been demonstrated. To understand the factors associated with these individual differences, freezing behavior was examined in infant rhesus monkeys and their mothers, in conjunction with levels of the stress-related hormone cortisol. In both mothers and infants, basal cortisol levels were positively correlated with freezing duration. Additionally, the number of offspring a mother had was negatively correlated with her infant's cortisol level. These findings suggest a link between basal cortisol levels and an animal's propensity to freeze, as well as a mechanism by which maternal experience may affect infants' cortisol levels.

Freezing in response to threat is used by many species as an adaptive–defensive strategy and functions to reduce the likelihood of predatory attack (Blanchard, Flannelly & Blanchard, 1986; Eible-Eibesfeldt, 1961; Kalin, 1993; Kalin & Shelton, 1989). When freezing, animals remain motionless, however activation of physiological systems occurs that prepares animals to engage in fight or flight behavior (Kalin, 1993). The study of defensive behaviors in rhesus monkeys has shown marked differences in individuals' propensities to freeze. These individual differences were stable over time (Kalin & Shelton, 1989). Additionally, infant monkeys were shown to develop the ability to adaptively regulate their freezing behavior around 2 months of age, and benzodiazepine- and corticotropin-releasing hormone systems were shown to modulate the expression of monkey freezing (Kalin, 1993; Kalin & Shelton, 1989; Kalin, Shelton, & Takahashi, 1991).

Because freezing is an extreme form of behavioral inhibition, it has been suggested that freezing in rhesus monkeys is analogous to fearful responses displayed by extremely inhibited children (Kalin & Shelton, 1989; Kalin et al., 1991). Extreme behavioral inhibition in human children is thought to mark an increased vulnerability to develop anxiety disorders later in life (Hirshfeld et al., 1993). Elevated levels of salivary cortisol (Kagan, Reznick, & Snidman, 1987), increased sympathetic nervous system activity, and relative right frontal brain activity are physiological correlates associated with extreme behavioral inhibition (Davidson, 1992). Because of the analogy between freezing in monkeys and behavioral inhibition in children, we have used rhesus monkeys to focus our efforts on understanding the psychobiological factors related to the development and expression of individual differences in freezing.

The current study examined hormonal and maternal factors hypothesized to be associated with individual differences in infant monkey freezing. Cortisol was of interest because in rat pups its analogue corticosterone is critical for the development of freezing (Takahashi & Rubin, 1993); in human children an association exists between the degree of behavioral inhibition and cortisol levels (Kagan et al., 1987, 1988). We hypothesized that infant monkeys with longer freezing durations would also have higher levels of cortisol. Parent–child similarities related to anxiety and behavioral inhibition have been found in humans (Rosenbaum et al., 1988), therefore we expected to find a similar relation between mother and infant monkeys as assessed by freezing duration.

Method

Subjects

The subjects were 28 (13 females and 15 males) infant rhesus monkeys (*Macaca mulatta*) and their mothers. During the entire period of the study, infants lived exclusively with their mothers at the Harlow Primate Laboratory and the Wisconsin Regional Primate Research Center. Animals were maintained on a 12-hr light–dark cycle (lights on 0600 hr) in cages 71.5 cm wide by 84 cm high by 71 cm deep. Animal housing and experimental procedures were in accordance with institutional guidelines. At the time of behavioral testing, the infants' mean age was 7.5 ± 0.61 ($M \pm SE$) months and the mothers' mean age was 11.0 ± 0.93 ($M \pm SE$) years. Differences in maternal parity ranged from 1 to 8 offspring ($M \pm SE = 3.43 \pm 0.37$).

Ned H. Kalin and Richard J. Davidson, Departments of Psychiatry and Psychology, University of Wisconsin; Steven E. Shelton, Department of Psychiatry, University of Wisconsin; and Maureen Rickman, Department of Psychology, University of Wisconsin.

We gratefully acknowledge the technical assistance of Joel Turner, Kathy Feil, and Helen Van Valkenberg. This research was supported by National Institute of Mental Health Grants MH46729, MH5234, and NIMH P50-MH52354, and by the Wisconsin Center for Affective Science, The HealthEmotions Research Institute, and Meriter Hospital.

Correspondence concerning this article should be addressed to Ned H. Kalin, Department of Psychiatry, University of Wisconsin, 6001 Research Park Boulevard, Madison, Wisconsin 53719-1179.

Test Conditions

Each mother and her infant were behaviorally tested on separate occasions 1 week apart, at the same time of day, with a counterbalanced design. Immediately following separation, either mother or infant was placed in another room in a test cage (79 cm wide by 76 cm high by 71 cm deep). For the first 10 min the test animal remained alone (condition A), followed by a 10-min period in which a human entered the room and stood 2.5 m from the cage, presenting his profile to the subject and making sure not to engage the monkey in eye contact (condition NEC). This method reliably elicits freezing behavior (Kalin & Shelton, 1989). All test sessions were recorded on videotape for later behavioral scoring.

Blood Sampling and Radioimmunoassay (RIA) of Cortisol

Infant blood samples were obtained approximately 2.5 months after behavioral testing when infants were on average 10.0 ± 0.97 months ($M \pm SE$). Blood samples from mothers were collected when mothers were 11.18 ± 0.93 years old ($M \pm SE$), which was 2.54 ± 0.38 months ($M \pm SE$) after they had been behaviorally tested. Samples were collected between 9:30 a.m. and 1:30 p.m. by femoral venipuncture into glass tubes containing 4.5 mg EDTA, within 4 min (M = 1.9 min) after mother and infant were separated. Previous studies from our laboratory demonstrated that sampling within this time frame results in nonstressed cortisol levels (Kalin, Shelton, & Turner, 1992). Plasma was separated by centrifugation at 4 °C (×1000 *g*) for 10 min and was stored at −70 °C until assayed. Plasma cortisol concentration was determined using a cortisol RIA kit (NEN/DuPont, Billerica, MA) with a detection limit (ED_{90}) of 0.2 mg/dl, and an intra-assay variability of 2.5–5.7%, with high- and low-cortisol concentration plasma pools, respectively.

Behavioral and Statistical Analysis

Freezing duration was rated from videotapes by two expert observers using methods previously described. The Pearson product correlation for each behavior was determined to be ≥.95 between the two raters. Freezing was defined as a period of immobility, lasting for at least 3 s, that was associated with a tense body posture in the absence of any vocalizations (Kalin & Shelton, 1989). We performed *t* tests for cortisol and freezing between males and females. Because no significant differences were found, data from both sexes were analyzed together. The data were analyzed separately for A and NEC conditions. Inspection of the data revealed nonnormal distributions, therefore nonparametric statistics were used. We used Spearman rank correlations to assess the relation between variables.

Results

Effects of Alone and NEC Conditions on Infant and Mother Freezing

In both mothers and infants, the NEC condition effectively elicited freezing. The Wilcoxon two-sample test showed that infants ($Z = -3.45$, $p < .001$) and mothers ($Z = -2.28$, $p < .02$) froze significantly longer during NEC than during A conditions (Figure 1). The small amount of freezing that occurred during A significantly correlated with the freezing duration during NEC (infants, $r_S = .65$, $p < .001$, and mothers, $r_S = .79$, $p < .001$).

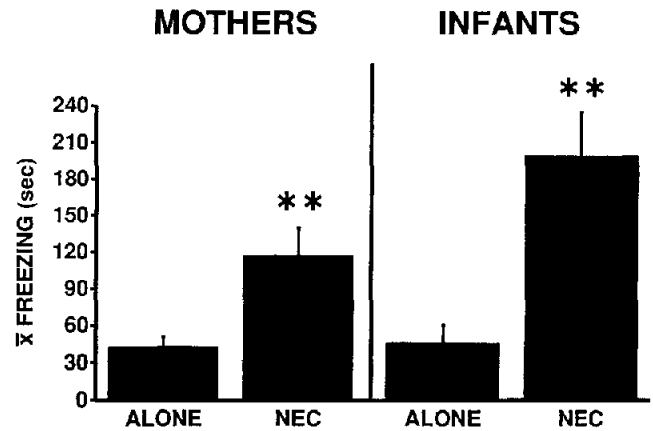

Figure 1. Mean seconds of freezing elicited by the alone and no eye contact (NEC) conditions in 28 mothers and their offspring. $^{**}p < .01$.

Factors Associated With Freezing Duration and Cortisol in Infants

The degree to which maternal freezing duration, maternal cortisol, and infant cortisol were associated with individual differences in infants' freezing was explored. Among these variables, only infant cortisol significantly correlated with infant freezing during NEC ($r_S = .62$, $p < .001$; Figure 2a).[1] Additionally, maternal parity was significantly negatively correlated with infant cortisol levels ($r_S = -.55$, $p < .01$; Figure 3). Although maternal age and parity were positively correlated, maternal age did not significantly correlate with infant cortisol levels. Maternal cortisol was moderately correlated with infant cortisol ($r = .34$, $p < .05$).

Factors Predicting Maternal Freezing

As in the infants, maternal cortisol levels were positively correlated with maternal freezing duration during NEC ($r_S = .53$, $p < .01$; Figure 2b). Additionally, cortisol was positively correlated with freezing during A ($r = .38$, $p < .05$). Neither maternal age, nor maternal parity, correlated with maternal freezing.

Discussion

It was previously suggested that freezing in the rhesus monkey could be used as a model to investigate mechanisms underlying human behavioral inhibition (Kalin, 1993; Kalin & Shelton, 1989; Kalin et al., 1991). This cross-species comparison between rhesus monkeys and humans is valid because freezing in monkeys and behavioral inhibition in children are elicited by similar threat-related cues and have

[1] Because cortisol follows a circadian rhythm and was sampled over a 4-hr period, we assessed whether the time of day in which samples were collected correlated with cortisol levels. In mothers, but not in infants, time of day and cortisol were significantly correlated ($r = .53$). Partialing out the effect of time of day did not significantly alter the relation between cortisol and freezing.

similar behavioral characteristics. When freezing, young rhesus monkeys are immobile, stop calling for their mothers, and decrease their environmental exploration. Similarly, when extremely inhibited children are confronted with novelty or a stranger, they are quiet and passive and display a long latency to talk and to approach desirable objects (Kagan et al., 1987, 1988).

The findings from the current study show that basal cortisol levels are associated with the magnitude of a monkey's freezing response elicited by threatening cues. This relation was found in both infants and mothers demonstrating that the association between cortisol and freezing is evident early in life and maintained into adulthood. These data are consistent with human studies demonstrating that extremely inhibited children, in contrast to uninhibited children, have higher levels of salivary cortisol (Kagan et al., 1988).

We did not find that individual differences in maternal freezing predicted individual differences in infant freezing. This implies that parental modeling of freezing behavior is unlikely to be a satisfactory explanation for determining individual differences in the duration of infants' freezing. Maternal cortisol was moderately related to infant cortisol levels; however, the data suggest that maternal rearing experience is a stronger factor associated with infants' cortisol levels. Less experienced mothers (lower parity) raised offspring with higher cortisol levels. This finding is consistent with rodent studies demonstrating that prenatal and early postnatal environmental experience influences the development of the tone of infants' pituitary–adrenal system (Takahashi & Kalin, 1991; Takahashi, Kalin, Barksdale, & Vanden Burgt, 1988). The design of the current study, in which mothers and infants lived exclusively as dyads, allowed us to isolate the effects of parity on infant cortisol. However, the generality of these results to more complex rearing conditions, such as those in human families, remains untested. While not directly addressing the issue related to infant cortisol, it has been reported that extremely behaviorally inhibited human children are more likely to be later born. Uninhibited children are reported to be predominantly first born (Kagan et al., 1988).

In summary, we found that basal cortisol concentrations were positively correlated with freezing in mothers and infants and that maternal parity was negatively associated with infant cortisol levels. Since studies in rat pups suggest that early in life glucocorticoids are important in modulating the development of individual differences in freezing (Takahashi & Rubin, 1993), it is tempting to speculate that the relation between cortisol and freezing in infant monkeys is not merely associational. However, further developmental studies must be performed to establish such a causal link in primates.

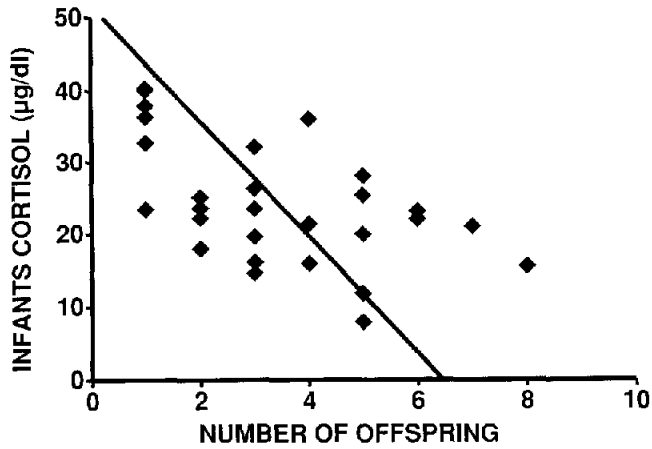

Figure 3. Correlation between parity level (number of offspring) and infant plasma cortisol concentrations ($r_S = -.55$).

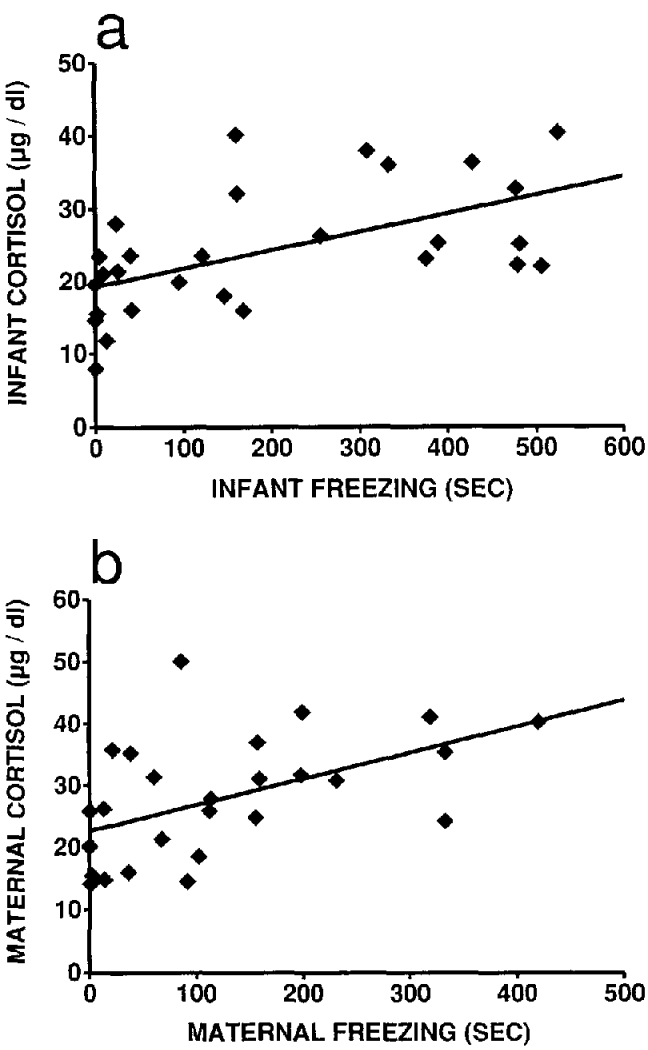

Figure 2. Correlation between absolute values for freezing duration and plasma cortisol concentrations in (a) infants ($r_S = .62$) and (b) between maternal cortisol and maternal freezing duration during no eye contact ($r_S = .53$).

References

Blanchard, R. J., Flannelly, K. J., & Blanchard, D. C. (1986). Defensive behaviors of laboratory and wild *Rattus norvegicus*. *Journal of Comparative Psychology, 100,* 101–107.

Davidson, R. J. (1992). Anterior cerebral asymmetry and the nature of emotion. *Brain and Cognition, 20,* 125–151.

Eible-Eibesfeldt, I. (1961). Brain mechanisms and learning. In E. A. Fessard, R. W. Gerard, & J. Konorski (Eds.), *The ethology of predation* (pp. 53–73). Oxford, England: Blackwell Scientific.

Hirshfeld, D. R., Rosenbaum, J. F., Biederman, J., Bolduc, E. A., Faraone, S. V., Snidman, N., Reznick, J. S., & Kagan J. (1993). A 3-year follow-up of children with and without behavioral inhibition. *Journal of the American Academy of Child and Adolescent Psychiatry, 32(4),* 814–821.

Kagan, J., Reznick, J. S., & Snidman, N. (1987). The physiology and psychology of behavioral inhibition in children. *Child Development, 58,* 1459–1473.

Kagan, J., Reznick, J. S., & Snidman, N. (1988, April 8). Biological bases of childhood shyness. *Science, 240,* 167–171.

Kalin, N. H. (1993). The neurobiology of fear. *Scientific American, 268(5),* 94–101.

Kalin, N. H., & Shelton, S. E. (1989, March 31). Defensive behaviors in infant rhesus monkeys: Environmental cues and neurochemical regulation. *Science, 243,* 1718–1721.

Kalin, N. H., Shelton, S. E., & Takahashi, L. K. (1991). Defensive behaviors in infant rhesus monkeys: Ontogeny and context-dependent selective expression. *Child Development, 62,* 1175–1183.

Kalin, N. H., Shelton, S. E., & Turner, J. G. (1992). Effects of b-carboline on fear-related behavioral and neurohormonal responses in infant rhesus monkeys. *Biological Psychiatry, 31,* 1008–1019.

Rosenbaum, J. F., Biederman, J., Gersten, M., Hirshfeld, D. R., Meminger, S. R., Herman, J. B., Kagan, J., Reznick, J. S., & Snidman, M. (1988). Behavioral inhibition in children of parents with panic disorder and agoraphobia. A controlled study. *Archives of General Psychiatry, 45(5),* 463–470.

Takahashi, L. K., & Kalin, N. H. (1991). Early developmental and temporal characteristics of stress-induced secretion of pituitary–adrenal hormones in prenatally stressed rat pups. *Brain Research, 558,* 75–78.

Takahashi, L. K., Kalin, N. H., Barksdale, C. M., & Vanden Burgt, J. A. (1988). Stressor controllability during pregnancy influences pituitary–adrenal hormone concentrations and analgesic responsiveness in offspring. *Physiology & Behavior, 42,* 323–329.

Takahashi, L. K., & Rubin, W. W. (1993). Corticosteroid induction of threat-induced behavioral inhibition in preweanling rats. *Behavioral Neuroscience, 107,* 860–866.

Received January 14, 1997
Revision received June 16, 1997
Accepted June 17, 1997 ■