Alterations in Diurnal Cortisol Rhythm and Acoustic Startle Response in Nonhuman Primates with Adverse Rearing

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Background: *Early adverse experiences represent risk factors for the development of anxiety and mood disorders. Studies in nonhuman primates have largely focused on the impact of protracted maternal and social deprivation, but such intense manipulations also result in severe social and emotional deficits very difficult to remediate. This study attempts to model more subtle developmental perturbations that may increase the vulnerability for anxiety/mood disorders but lack the severe deficits associated with motherless rearing.*

Methods: *We investigated the consequences of repeated maternal separations between 3 to 6 months of age on rhesus monkeys' hypothalamic-pituitary-adrenal (HPA) axis function and acoustic startle reactivity.*

Results: *Repetitive maternal separation led to increased cortisol reactivity to the separation protocol in female infants and alterations in mother-infant interaction. It also resulted in a flattened diurnal rhythm of cortisol secretion and increased acoustic startle reactivity at later ages.*

Conclusions: *Macaques with adverse rearing exhibited short-term and long-term alterations in HPA axis function and increased acoustic startle response comparable with changes associated with mood/anxiety disorders. The magnitude of HPA axis reactivity to the separations and the alterations in mother-infant relationship detected during the separation protocol predicted some of the alterations in HPA axis and emotionality exhibited later in life.*

Key Words: HPA axis, fear, rhesus monkeys, maternal separation, amygdala, behavioral inhibition

Substantial evidence supports the view that early life stress is a significant risk factor for the development of mood and anxiety disorders later in life (Glaser 2000; Heim and Nemeroff 2001; Sánchez et al 2001). Indeed, early adverse experiences result in persistent neurobiological effects critical to normal emotional and neuroendocrine development in different species. One of the systems affected is the hypothalamic-pituitary-adrenal (HPA) axis, long implicated in the etiology of affective disorders (Caldji et al 2001; Gold et al 1998; Nemeroff 1996; Plotsky and Meaney 1993; Sánchez et al 2001).

Repeated maternal separation has been used in rats to scrutinize the short-term and long-term effects of maternal-infant relationship disruption as an early adverse experience. When compared with appropriate control subjects (early handled animals), maternal separation is associated with increased anxiety-like behavior, increased HPA axis response to stress, and increased activity of corticotropin-releasing factor (CRF) pathways in the central nervous system (CNS) (Caldji et al 2001; Sánchez et al 2001).

Primate infants are in continuous physical contact with their mother early in life (Pryce 1996), and maternal separation for even brief periods provokes robust behavioral, neuroendocrine, and neurochemical activation (McKinney 1985; Rilling et al 2001; Sánchez et al 2001). Studies of the impact of early adverse experiences in nonhuman primates have largely focused on the effects of the permanent removal of the mother and some level of further social deprivation (Harlow et al 1965, 1971; see Sánchez et al 2001). Although these models have heuristic value in highlighting the devastating consequences of maternal deprivation, they represent intense manipulations associated with severe social and emotional deficits very difficult to remediate. And they do not appear to represent the more subtle developmental alterations that may increase the vulnerability for adult-onset anxiety/mood disorders, evident in the clinical population in response to maternal neglect and other early life stresses.

In contrast to the permanent removal of the mother, prolonged maternal separations (e.g., 6 days, 2 to 3 weeks) followed by mother-infant reunion produce more subtle developmental alterations in the nonhuman primate infant without the severe social and emotional deficits associated with motherless rearing. Although some of these studies have been performed in rhesus monkeys (Hinde et al 1966; McKinney 1985; Spencer-Booth and Hinde 1971a, 1971b), most of the long-term physiological consequences of repeated, brief maternal separations have been reported in New World monkeys (squirrel monkeys and marmosets). These long-term effects include alterations in HPA axis and sympathetic functions, as well as in stress reactivity (Dettling et al 2002; Levine and Mody 2003; Lyons et al 2000; Pryce et al 2004).

The present study investigated the immediate and long-term consequences of repeated, brief mother-infant separations during 3 to 6 months of age in an Old World primate, the rhesus monkey (*Macaca mulatta*). Separations were achieved by removing the mother but not the infant from their social group. Similar disruptions of maternal contact in this age range have previously been associated with persistent changes in emotional behavior and/or stress-related endocrine systems without the marked social deficits typical of nursery-rearing (Andrews and Rosenblum 1991, 1994; Coplan et al 1996; Hinde et al 1966;

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Spencer-Booth and Hinde 1971a). We examined acute and long-term effects of this rearing protocol on HPA axis function, including reactivity and diurnal rhythm, as well as on acoustic startle reactivity (Davis 1998; Parr et al 2002; Winslow et al 2002).

Methods and Materials

Subjects

Thirty-nine rhesus monkeys were born in March through May of 2000 (11 male monkeys, 8 female monkeys) and 2001 (13 male, 7 female) to 23 adult female monkeys living in six stable social groups (4 to 7 adult female monkeys and 1 adult male sire per social group). The groups were maintained in indoor/outdoor run-type facilities at the Yerkes National Primate Research Center Field Station. Animals were randomly assigned to experimental (EXP) or control (CON) rearing conditions to yield 20 CON (12 M, 8 F) and 19 EXP (11 M, 8 F) animals. Each EXP animal had at least one half-sib in the CON condition, although we were unable to match half-sibs for sex. For the 2001 cohort, mothers were pseudo-randomly assigned to rearing groups to achieve a similar distribution of mothers who were CON in both years 1 and 2 (CC; $n = 8$), CON in year 1 and EXP in year 2 (CE; $n = 6$), or EXP in both years 1 and 2 (EE; $n = 6$). Our purpose was to examine the potential role of the prior experience of the mother on infant outcome measures in the second cohort. An EC group could not be included in year 2 due to small group sizes and the priority given to more extreme groups. Three females (two CON and one EXP) did not give birth in year 2.

All experiments described in this study were performed in accordance with the *NIH Guide for the Care and Use of Laboratory Animals* and approved by the Emory University Institutional Animal Care and Use Committee (IACUC).

Rearing Conditions

All adult subjects were familiar with box capture techniques due to standard husbandry/veterinary practices. Nevertheless, 2 to 3 weeks prior to the beginning of the separation protocol (it starts when infants are 3 months old), all mothers were transport box retrained to be captured within 5 minutes of first contact with research staff, during two to three biweekly refreshing sessions. Both CON and EXP female animals participated in all retraining sessions.

The adverse rearing protocol was imposed between 3 to 6 months postpartum. Adverse rearing was comprised of repeated separations of systematically varied durations (.5, 3.0, and 6.0 hours) following a counterbalanced design. Separations were scheduled once per day between 8:00 AM and 11:00 AM (onset of separation was variable/unpredictable within this time window), two to three times per week to yield a total of 36 separations per infant in 90 days. Separations were achieved by herding the entire social group into a small precapture area and then releasing individuals back into their home run through a capture tunnel. Consequently, every member of the social group was disturbed during a separation, though only one EXP female animal was removed from each social group at a time. Initially, mother and infant were captured as pairs and transported to a restraint cage where the infant was removed from his/her mother. Infants were then immediately returned to their social group. Mothers were transported to a separate facility in an adjacent building where they remained for the duration of the separation and were provided with food and water. However, within several separations, all EXP mothers routinely separated from infants in the precapture area and entered the transport cages alone with minor prompting. In this case, infants were not captured and remained with their social group. At the end of each separation, mothers were returned to their social group and reunited with their infants and social group.

At the completion of the separation protocol, social groups remained undisturbed by research staff for a minimum of 6 months. All offspring were then assessed in a study of diurnal rhythm of cortisol secretion. At the completion of this study, infants were transferred to pair-housing at the Yerkes Main Station facility where additional studies were conducted.

Behavioral Video Recording

Infant behavior during separation and reunion was video recorded during nine 1-hour sessions for every monkey, at approximately weekly intervals. Within each session, we recorded the final 30 minutes of a separation and the first 30 minutes of the mother-infant reunion. One-hour recording sessions were collected from each of the three separation intervals (.5, 3, and 6 hours) per month in counterbalanced order for each subject. Thus, across the entire 3 months, a total of three recording sessions were obtained per each separation interval per subject. Two additional 1-hour sessions were videotaped from each mother-infant pair at the beginning and end of the 3-month separation protocol, during days when no separation was scheduled (undisturbed mother-infant interaction).

Behavioral Scoring

A comprehensive ethogram was adapted (Altman 1962; Hinde and Spencer-Booth 1967) to provide an exhaustive description of social and nonsocial behaviors. Trained observers viewed videotaped recordings and used an interval sampling (60-second) strategy to blindly code elements of behavior (mother-infant interactions; social behaviors; infant vocalizations (coo calls), screams; latency to infant retrieval following reunion; motor and stationary activity) on a computer using the Noldus Observer software (Noldus Information Technology Inc., Leesburg, Virginia). Motor and social behavior categories were not mutually exclusive. Incidence of each behavioral element was compiled as appropriate and expressed as frequency or proportion of observation.

Multiple observers were trained to achieve intraobserver and interobserver reliabilities of ≥80% for frequency and duration of social and nonsocial behaviors. Reliability was determined for individual behaviors and was retested periodically throughout the project. Training consisted of real-time scoring of monkey behavior, as well as selected training videos. Interobserver and intraobserver reliability was tested following independent review and scoring of fresh recordings of rhesus monkey behavior in groups.

Plasma Cortisol Samples

Basal Versus Separation Stress Cortisol Concentrations at 5.5 Months of Age (During the Separation Protocol). Blood samples were collected from CON and EXP infants at 5.5 months of age either immediately after capture (basal) or following a 30-minute mother-infant separation as detailed above (separation stress). The order of treatment was counterbalanced between subjects. For basal concentrations, blood samples were collected within 10 minutes of first contact with research staff and only one group was accessed per day. Blood was collected from the saphenous or femoral veins between 9:30 AM and 10:30 AM, following ketamine anesthesia (5 mg/kg, intramuscular [IM]). In