Pituitary-adrenal response to weaning in infant squirrel monkeys

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This paper examines the pituitary-adrenal response of infant squirrel monkeys during the 2 weeks following the permanent removal from their mothers at the time of weaning in the laboratory. The influence of three major factors on the pituitary-adrenal response to weaning was studied: (1) preweaning experience with maternal separations; (2) familiarity with the postweaning environment; (3) preweaning social experience. The results indicated that prior separations lead to greater cortisol elevations than those observed in infants experiencing their first maternal separation at weaning. Those infants permitted to remain in their familiar social group displayed lower cortisol than did those in the novel cage alone. This was due to the social interactions and not to familiarity with the postweaning environment, since infants reared only with their mothers did not show this reduced cortisol elevation in their home environment.

There is now an extensive literature dealing with the biological and behavioral responses to disturbances in mother–infant relationships (Reite & Field, 1985). The primary technique used to study these effects has been separation of the mother–infant dyad. In separation studies, which have been performed on avian, rodent, canine, and primate species, there are probably as many variations in experimental paradigms as there are investigators. Nonhuman primate studies have used a variety of species and have investigated many physiological responses as well as behavior. It would be difficult to catalog all of the differences between experimental paradigms. In addition to the obvious diversity among species, the separation environment, length of separation, and age at separation have been markedly different. The studies on mother–infant separation in macaques (rhesus, bonnet, and pigtail) have tended to use long periods of time (i.e., days to weeks) to observe the biobehavioral responses of the infant to separation from the mother (see review by Mineka & Suomi, 1978). Although there have been studies that have examined the behavioral response to separations in squirrel monkeys lasting 7 days (Jones & Clark, 1973; Kaplan, 1970), there is only one experiment that has studied the physiological responses to separation in this species for longer than 24 h. Coe, Wiener, Rosenberg, and Levine (1985b) demonstrated that, following 14 days of separation, infant squirrel monkeys (6–7 months old) showed a persistent elevation of cortisol if they were separated and isolated at the time of weaning. Group-housed separated infants also showed increases in cortisol levels following separation, but the magnitude of the response was reduced and returned to basal levels at the end of the 2 weeks of maternal separation.

The experiments to be reported in this paper were an extension of the original observations. In these experiments, we examined the influence of social factors on the endocrine response to weaning, which, during the initial phase of the weaning process, represents the first prolonged separation for these lab-born monkeys. Furthermore, we investigated the influence of prior brief separations on the response to a separation of considerably longer duration. As a standard practice in this laboratory, we remove the infant from the mother when the infant is between 6 and 7 months of age in order to maximize the subsequent breeding of the mother. At this age, the infant is spending little time in dorsal contact with the mother and is capable of independent maintenance functions (i.e., eating solid food, drinking, thermoregulation, etc.; see Coe, Wiener, Rosenberg, & Levine, 1985a). Thus, the process of weaning (as defined by independence from the mother) has already been started prior to our permanent removal of the mother. Although the infants used in the following studies are several months older than those examined previously during brief periods of separation (i.e., 6.0–7.0 vs. 3.5–5.5 months), there is evidence, at least for rhesus macaques, that age is not a critical variable in determining the response of infants to maternal separation (Levine, Johnson, & Gonzalez, 1985; Mineka & Suomi, 1978).

There now exists a body of data that clearly indicates that the activation of the hypothalamic-pituitary-adrenal (HPA) system is highly sensitive to subtle differences in environmental events and is capable of responding differentially to seemingly minor changes in events that result in activation of this neuroendocrine system (Hennessy & Levine, 1979). This property of the HPA system renders it extremely useful for studying the effects of both differ-
ent environmental stimuli and different prior rearing conditions on the level of arousal in response to the environmental challenge of permanent separation from the mother. The HPA axis is activated under conditions that are considered stressful. Although there is some controversy as to whether levels of cortisol can be used as an index of the magnitude of the stress response, the fact remains that HPA activation has been one of the most reliable and predictive responses to stress, and, as such, it has been used by many investigators using both animals and humans as an indicator of the degree of stress the organism is experiencing. The pituitary-adrenal response following weaning was chosen because this physiological system has been demonstrated to be sensitive to differences in the environmental situation in which the infant finds itself following separation from the mother (Coe et al., 1985b; Wiener, Bayart, Faull, & Levine, 1990; Wiener, Johnson, & Levine, 1987). Thus, the infant’s plasma cortisol elevations, following separation from the mother, may indicate the degree of stressfulness of the procedure. Golub and associates (Golub, Anderson, Goo, & Sassenrath, 1981) have used this hormonal measure to determine the stressfulness of the laboratory weaning procedure in rhesus macaques. They also found that the pituitary-adrenal response was sensitive to the environment into which the rhesus infant was placed following weaning.

In the rhesus macaque, the pituitary-adrenal infant’s response to separation from its mother is relatively acute; after a period of several days, the infant stabilizes at near basal levels (Golub et al., 1981; Gunnar, Gonzalez, Goodlin, & Levine, 1981; Levine et al., 1985). In contrast, infant squirrel monkeys display a much more pronounced endocrine response to maternal separation so that by the end of a 24-h period of separation from the mother, plasma cortisol levels have risen to approximately 10 times that of the basal level (Coe et al., 1985b; Wiener et al., 1990). In the present study, whether older infants at the time of weaning display levels equivalent to those of younger infants was investigated by the use of a similar 24-h post-separation time.

In the following two experiments, we examined the prior experience with mother-infant separations during the preweaning period (previously separated vs. nonseparated infants), the type of postweaning housing condition (weaned individually into a novel cage vs. remaining in the familiar home cage following mother removal), and preweaning rearing conditions (mother-infant dyads raised in social groups with other mother-infant dyads vs. dyads reared without social partners). The first experiment examined the time course of the cortisol response during the first 14 days following weaning in infants reared with other mother-infant dyads that were either maternally separated or nonseparated prior to weaning and placed in either a novel cage alone or allowed to remain in their familiar home cage with age-matched conspecifics. The second experiment examined this time course during the first 14 days postweaning in infants raised only with their mothers to determine whether familiarity with the postweaning environment per se, rather than the interactions with familiar peers, influenced the pituitary-adrenal response following weaning.

**GENERAL METHOD**

**Breeding Conditions**

Adult female squirrel monkeys of Guyanese origin (*Saimiri sciureus*) were bred in our laboratory in large wire-mesh cages (1.8 x 1.2 x 1.8 m). The monkeys were bred in groups of 5-8 females with 1-2 males; once pregnancy was noted, the males were removed and stable social groups of 5-8 pregnant females were formed. Following the end of the birth season, females that did not have viable offspring were removed and groups of 3-5 mother-infant dyads were established. Each cage contained three sets of perches extending the length of the cage at varying heights. Solid metal partitions divided adjacent cages.

The cages were located in a temperature-controlled colony building with natural lighting conditions. Wayne 25% protein Monkey Diet (Allied Mills, Inc., Chicago, IL) and water were available ad lib. Vitamin supplements in a cherry-flavored liquid and fresh fruit were provided twice a week.

**Blood Collection and Hormonal Assay**

Blood samples were collected for plasma cortisol determination following rapid anesthetization with ether. A 0.5-ml sample was collected in a heparinized syringe via cardiac puncture. This technique was utilized because it permits the routine collection of samples within 2 min, minimizing hormonal responses to capture. The blood was centrifuged for 20 min at 2,000 rpm, and the plasma was frozen at −20°C until assayed. Cortisol was assayed by the radioimmunoassay method described by Klemm and Gupta (1975) using antiseraum F3-314 from Endocrine Sciences (Tarzana, CA). Further details of blood sampling and radioimmunoassay procedures can be found in Coe et al. (1978).

**Data Analysis**

Results were analyzed by analysis of variance (ANOVA) procedures as described by Winer (1971), with the level of significance set at *p* < .05. When appropriate, post hoc analysis for simple main effects and comparisons by Newman–Keuls procedures were performed. Initially, the plasma cortisol levels were analyzed with sex as a factor; however, since the sex of the infant was not a significant variable in either experiment, it was eliminated as a factor.

**EXPERIMENT 1**

Experiment 1 was designed to determine whether weaning into a familiar social group influenced the pituitary-adrenal response compared with being weaned into a novel cage alone. This experiment also addressed the question of whether or not experience with separations from the mother prior to weaning influenced this physiological measure.

**Method**

**Subjects.** Forty-eight Guyanese infant squirrel monkeys served as subjects in Experiment 1. Twenty-four infants (12 males and 12 females) were permitted to remain in their home social group, without being separated from their mothers during the preweaning period. Twenty-four additional infants (12 males and 12 females) were separated from their mothers four to seven times (for 4 h on each
occasion) when the infants were between 3.5 and 5.5 months of age. All monkeys in the group cages were treated identically. Thus, all members of a single social group were either separated or non-separated. During this phase of the experiment, all animals were blood sampled. For the nonseparated animals, blood samples were obtained to determine basal levels. The separated infants were sampled on the first and last separation to assess the response to separation. At no time were order effects observed when the animals in the social groups were blood sampled.

Procedure. An initial blood sample was obtained from each infant at 23 weeks of age while still with the mother under nondisturbed conditions, to determine basal plasma cortisol levels prior to weaning. One week later, the infants were removed from their mothers (Day 0). Twelve of the previously nonseparated infants (6 males and 6 females) and 12 of the previously separated infants (6 males and 6 females) were allowed to remain in their familiar home social group with other infants from the same conditions weaned at the same time. Twelve of the previously nonseparated infants (6 males and 6 females) and 12 of the previously separated infants (6 males and 6 females) were transferred individually to novel Wahmann primate cages (61 x 61 x 91 cm) that contained two perches.

Blood samples were obtained on Days 1, 7, and 14. The cortisol levels postweaning but did not differ in basal levels from infants weaned with familiar conspecifics. Thus, the results of Experiment 1 indicate that experience with separations from the mother prior to weaning accentuates the pituitary-adrenal response following weaning and that weaning into the familiar home cage with familiar conspecifics attenuates the adrenocortical response relative to weaning without conspecifics in a novel cage.

Despite these modulations in the magnitude of the plasma cortisol, it should be noted that all infants responded to the maternal separation associated with weaning. For that matter, statistically none of the infants returned to basal levels by Day 14. Although it appears in Figure 1 (left) that those previously nonseparated infants allowed to remain with their familiar peers had returned to basal levels by Day 14, the three-way interaction of preweaning history x postweaning condition x days did not reach significance and thus could not be tested.

EXPERIMENT 2

Experiment 2 examined the pituitary-adrenal response following weaning of infants reared only with their mothers from 2 months of age. Similar to Experiment 1, both previously maternally separated and nonseparated infants were examined. Following weaning, the infants were either allowed to remain in their home cage following removal of the mother or placed in a novel cage alone in order to determine whether familiarity per se with the weaning environment was responsible for the attenuated pituitary-adrenal response observed in the infants weaned in their home cage in Experiment 1 or whether social interactions with the familiar conspecifics was the important factor.

Method

Subjects. Twenty-four mother-infant dyads served as subjects in Experiment 2. All dyads were removed from their natal social groups when the infants were 8–10 weeks of age and placed as a
dyad in a Wahmann primate cage (61×61×91 cm) that contained two perches. They remained in these cages until weaned at 24 weeks postnatal. Twelve infants (7 males and 5 females) were separated from their mothers four times (for 4 h on each occasion) when the infants were between 3.5 and 5.5 months of age. The remaining 12 (6 males and 6 females) infants were left undisturbed until weaning.

Procedure. An initial blood sample was obtained from each infant at 23 weeks of age while still with its mother under nondisturbed conditions, to determine basal plasma cortisol levels prior to weaning. One week later, the infants were removed from their mothers (Day 0). Six of the previously separated infants (3 males and 3 females) and six of the previously nonseparated infants (3 males and 3 females) were allowed to remain in their home cages following removal of the mother. The remaining six previously separated infants (4 males and 2 females) and six previously nonseparated infants (3 males and 3 females) were placed alone in a novel cage (46×46×51 cm) that contained a single perch.

Blood samples were collected on Days 1, 7, and 14 postweaning. The plasma cortisol levels were analyzed by a 2 (preweaning history: nonseparated, separated) × 2 (postweaning housing: home, novel) × 4 (days: base, Days 1, 7, 14) ANOVA with the last factor considered a repeated measure.

Results

The analysis of cortisol levels indicated a significant preweaning history × days interaction [F(3,60) = 13.65, \(p < .001\); see Figure 2]. Similar to the results of Experiment 1, previously separated infants displayed higher cortisol levels than did nonseparated infants following weaning but did not differ in their preweaning basal levels. However, in contrast to the findings of Experiment 1, the postweaning housing condition did not play a significant role in modulating the adrenocortical response in Experiment 2. The infants that were permitted to remain in their familiar home cage following weaning did not differ from those placed in a novel cage postweaning. Thus, familiarity per se with the weaning environment did not attenuate the pituitary–adrenal response, suggesting that the social interactions possible in the familiar home group cage was responsible for the lower plasma cortisol elevations observed in Experiment 1.

**GENERAL DISCUSSION**

The results of these experiments indicate that experience with maternal separations prior to weaning accentuated the pituitary–adrenal response observed following the permanent removal of the mother in infants reared either in a social group with other mother–infant dyads or only with their mothers. Although there was no difference in their basal corticoid levels prior to weaning, previously separated infants displayed higher plasma cortisol levels throughout the 14 days following weaning. One hypothesis to explain this sensitization of the pituitary–adrenal response following permanent removal of the mother may lie in the nature of the preweaning separations. All of the previous separations ended with the return of the mother. However, the separation at weaning was permanent, with no reunion possible with the mother. The infants may have learned to expect a return of the mother following these acute preweaning separations and, thus, the failure of return of the mother following the permanent removal from the mother at weaning would then violate the infant's expectancy of the mother's return. It has been demonstrated that violation of expectancy is a potent activator of the pituitary–adrenal axis (Hennessy & Levine, 1979).

A second finding from these studies is that both previously separated and nonseparated infants displayed a lower
corticoid response when they were allowed to remain with their familiar peers following weaning from the mother. The observation that infants reared only with their mothers did not show a lower plasma cortisol response when allowed to remain in their familiar home cages following weaning, relative to that of infants placed into novel cages, indicates that social interactions are the important factor in this attenuated pituitary-adrenal response. Thus, familiarity with the postweaning environment per se does not contribute to a lower corticoid response. These results are in agreement with our results comparing preweaning separations in the home social environment versus a novel nonsocial environment in the squirrel monkey (Wiener et al., 1990; Wiener et al., 1987) and those of Golub et al. (1981) on the response of the rhesus infant weaned under these two different environments. It should be noted that although previously separated infants showed an overall higher cortisol response in both experiments, the presence of familiar social partners was still capable of reducing this response, relative to the response of previously separated infants that were weaned into a novel environment and were socially isolated. The ability to interact with familiar conspecifics during stressful stimuli has also been observed to reduce or completely eliminate a pituitary-adrenal elevation in the adult squirrel monkey following exposure to stressful stimuli (Stanton, Patterson, & Levine, 1985). Although social interactions reduced the magnitude of the initial plasma cortisol elevation, all infants displayed marked cortisol elevations that did not return to basal levels over the 14-day period postweaning. Thus, unlike the rhesus macaque infant, the squirrel monkey weanling shows a prolonged corticoid elevation following permanent removal of the mother. Although acute activation of the pituitary-adrenal system is necessary for survival, prolonged elevations of glucocorticoids can have negative consequences (Munck, Guyre, & Holbrook, 1984). Coe and associates (Coe, Cassayre, Levine, & Rosenberg, 1988; Coe, Rosenberg, Fischer, & Levine, 1987; Coe, Rosenberg, & Levine, 1988; Coe et al., 1985a) have documented suppression in the immune system that is associated with different magnitudes of cortisol elevations. For example, infants weaned with familiar conspecifics that have lower postweaning cortisol levels show less immune suppression than do infants weaned into a novel cage that display higher cortisol levels.

Finally, unlike the rhesus, the cortisol response 24 h following separation from the mother at the age of weaning (6.0–7.0 months) is less than that observed between 3.5 to 5.0 months. Although the levels achieved by younger infants have exceeded 1,000 μg/dl, the highest levels observed in these experiments is 500–600 μg/dl. The pituitary-adrenal system appears to be undergoing a maturation process, probably in either synthesis or, more likely, catabolism or clearance, which would account for the lower steroid levels at the older ages.

In conclusion, the least stressful weaning procedure is to allow the infant to remain with familiar conspecifics following the removal of the mother. The higher plasma corticoid response following other weaning procedures places the infant at risk due to chronic high cortisol levels. Thus, as a husbandry practice, it is recommended that infants be reared in social groups and placed with familiar conspecifics following weaning whenever possible.

REFERENCES

Coe, C. L., Cassayre, P., Levine, S., & Rosenberg, L. T. (1988). Effects of age, sex, and psychological disturbance on immunoglobulin levels in the squirrel monkey. Developmental Psychobiology, 21, 161-175.

Coe, C. L., Mendoza, S. P., Davidson, J. M., Smith, E. R., Dallman, M. F., & Levine, S. (1978). Hormonal response to stress in the squirrel monkey (Saimiri sciureus). Neuroendocrinology, 26, 367-377.

Coe, C. L., Rosenberg, L. T., Fischer, M., & Levine, S. (1987). Psychological factors capable of preventing the inhibition of antibody responses in separated infant monkeys. Child Development, 58, 1420-1430.

Coe, C. L., Rosenberg, L. T., & Levine, S. (1988). Effect of maternal separation on the complement system and antibody responses in infant primates. International Journal of Neuroscience, 40, 299-302.

Coe, C. L., Wiener, S. G., Rosenberg, L. T., & Levine, S. (1985a). Endocrine and immune responses to separation and maternal loss in nonhuman primates. In M. Reite & T. Field (Eds.), The psychobiology of attachment and separation (pp. 163-199). New York: Academic Press.

Coe, C. L., Wiener, S. G., Rosenberg, L. T., & Levine, S. (1985b). Physiological consequences of maternal separation and loss in the squirrel monkey. In L. A. Rosenblum & C. L. Coe (Eds.), The handbook of squirrel monkey research (pp. 127-148). New York: Plenum Press.

Golub, M. S., Anderson, J. H., Goo, G. P., & Sassenrath, E. N. (1981). Plasma cortisol response to different methods of weaning in rhesus monkey (Macaca mulatta) infants. Laboratory Animal Science, 31, 400-402.

Gunnar, M. R., Gonzalez, C. A., Goodlin, B. L., & Levine, S. (1981). Behavioral and pituitary-adrenal responses during a prolonged separation period in infant rhesus macaques. Psychoneuroendocrinology, 6, 65-75.

Hennessy, J. W., & Levine, S. (1979). Stress, arousal and the pituitary-adrenal system: A psychoendocrine model. In J. M. Sprague & A. N. Epstein (Eds.), Progress in psychobiology and physiological psychology (Vol. 8, pp. 133-178). New York: Academic Press.

Jones, B. C., & Clark, D. L. (1973). Mother-infant separations in squirrel monkeys living in a group. Developmental Psychology, 6, 259-269.

Kaplan, J. (1970). The effects of separation and reunion on the behavior of mother and infant squirrel monkeys. Developmental Psychology, 3, 43-52.

Kleemann, W., & Gupta, D. (1975). A routine method for the radioimmunoassay of plasma cortisol without chromatography. In D. Gupta (Ed.), Radioimmunoassay of steroid hormones (pp. 143-151). Weinheim, Germany: Verlag Chemie.

Levine, S., Johnson, D. F., & Gonzalez, C. A. (1985). Behavioral and hormonal responses to separation in infant rhesus monkeys and mothers. Behavioral Neuroscience, 99, 399-410.

Mintz, J., & Suomi, S. J. (1978). Social separation in monkeys. Psychological Bulletin, 85, 1376-1400.

Munck, A., Guyre, P. M., & Holbrook, N. J. (1984). Physiological functions of glucocorticoids in stress and their relation to pharmacological actions. Endocrine Reviews, 5, 25-44.

Reite, M., & Field, T. (Eds.) (1985). The psychobiology of attachment and separation. New York: Academic Press.

Stanton, M. E., Patterson, J. M., & Levine, S. (1985). Social influences on conditioned cortisol secretion in the squirrel monkey. Psychoneuroendocrinology, 10, 125-134.
I. N. E N E R, L O W E, A N D L E V I N E

WINER, S. G., BAYART, F. E. S., FAULL, K. F., & LEVINE, S. (1990). Behavioral and physiological responses to maternal separation in the squirrel monkey (Saimiri sciureus). Behavioral Neuroscience, 104, 108-115.

WINER, S. G., JOHNSON, D. F., & LEVINE, S. (1987). Influence of postnatal rearing conditions on the response of squirrel monkey infants to brief perturbations in mother-infant relationships. Physiology & Behavior, 39, 21-26.

WINER, B. J. (1971). Statistical principles in experimental design (2nd ed.). New York: McGraw-Hill.

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