Total Cortisol, Free Cortisol, and Growth Hormone Associated with Brief Social Separation Experiences in Young Macaques

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Many behavioral, immunological, and physiological consequences of brief maternal separation in bonnet (*Macaca radiata*) and pigtail monkeys (*Macaca nemistrina*) have been documented. However, the impact of social separation on plasma cortisol and growth hormone is unknown for these particular species. In the present study, the behavioral and endocrinological consequences of a 2-week maternal separation in socially housed infant bonnet and pigtail monkeys were followed. In seven pairs (separated and matched control) of bonnet and six pairs of pigtail infants, plasma was obtained under baseline, separated, and reunion conditions twice weekly for the duration of the study. Blood samples were obtained from both infants of the pair in approximately 10 min. Plasma total cortisol, free cortisol, and growth hormone were measured in these samples. Focal animal behavioral observations were made on all subjects twice daily throughout the study period. In both species, total cortisol and free cortisol rose immediately following maternal separation in comparison to the matched nonseparated controls and returned to basal levels (e.g., that of matched non-separated controls) following reunion with the mother. In contrast, plasma growth hormone rose only in the pigtail infants over a time course that peaked around the time of reunion. Multiple regression techniques indicated for the first week of separation, in the separated but not control subjects, that mean plasma free and total cortisol was positively related to distress behaviors (vocalization and postural slouch) observed during this week and negatively related to social behaviors (play and proximity to others) noted during the same period. In contrast, plasma growth hormone was related to both species and sex of the subjects but unrelated to behavioral variables. © 1995 by John Wiley & Sons, Inc.

Introduction

The behavioral and biological consequences of brief maternal separation in young macaques have been observed to vary both across species and with the conditions of separation (Coe, 1993; Laudenslager & Fleshner, 1994; Mineka & Suomi, 1978; Reite, Short, Seiller, & Pauley, 1981). Our experience in *socially housed* macaques has indicated that the response of an infant pigtail macaque (*Macaca nemistrina*) reflects a protest–despair or agitation–depression response associated with an initial increase in agitated behaviors (vocalization, activity, searching behavior) followed by a withdrawal phase (reduced activity and play, postural collapse, altered ingestive patterns) (Reite et al., 1981). These behavioral changes are associated with alterations in the regulation of a number of autonomic systems including the cardiovascular system, sleep–wake cycles, body-temperature regulation, and immunoregulation (Laudenslager, Held, Boccia, Reite, & Cohen, 1990; Reite et al., 1981). In contrast, the bonnet macaque (*Macaca radiata*) shows a less-dramatic behavioral and autonomic response to brief maternal separation presumably due to the presence of allomaternal care present under the conditions of social housing (Boccia, Reite, & Laudenslager, 1992; Laudenslager et al., 1990; Reite, Kaemingk, & Boccia, 1989). Pigtail mothers show restrictive mothering styles leading to the formation of few alliances with older members of the social group by the infant, whereas in bonnet groups, the infant experiences interactions with many members of the social group including older siblings, other juveniles, and unrelated adult females. Thus, when a pigtail mother is removed from the social group, it is not surprising to observe a greater behavioral response in pigtail infants in the absence of the allomaternal interactions available to the bonnet infants.

The hypothalamic–pituitary—adrenal axis (HPA) is activated under these conditions of social separation and stress for both human and nonhuman primates (see Gunnar, 1992 and Levine & Wiener, 1988 for reviews). In nonhuman primates, the magnitude and duration of these effects are quite different depending on a variety of conditions, including the number of previous separation experiences, the presence of a conspecific, the visual or tactile availability of the mother, the age of the infant, environmental novelty, and, not surprisingly, species (see also Clarke, 1991). The relationship between behaviors associated with social separation and HPA activation has been investigated in several of these studies and the relationship is not always direct as one might assume. For example, the relationship between rates of vocalization and plasma cortisol can dissociate in infant monkeys experiencing social separation (Levine, Franklin, & Gonzalez, 1984). Rather than vocalization reflecting a behavioral measure of distress, it has been viewed as a coping behavior subject to both reinforcement and extinction processes (Levine & Wiener, 1988). Thus, it would not be surprising to observe a reduction in vocalization frequency without a fall in plasma cortisol levels when vocalization has failed to result in the return of the mother. In support of this assertion, these behaviors habituate or extinguish over repeated separations without a diminution in plasma cortisol (Coe, Glass, Wiener, & Levine, 1983).

The relationship between behavioral responses associated with maternal separation and changes in glucocorticoid levels in bonnet and pigtail macaques has not been addressed to date. Previous observations have suggested that changes in immune regulation are associated with changes in behavioral response in separated infants (Laudenslager et al., 1990). As the behavioral changes are greatest in the pigtail infant, one might expect a greater hormonal response in that species in comparison to the bonnet macaque. However, other observations (Levine & Wiener, 1988) would suggest that these relationships might be more complicated.

The preceding studies of the consequences of maternal separation in nonhuman primates have examined only total cortisol in plasma. However, cortisol in the plasma exists in one of two forms: free and bound. Cortisol is bound to one of several factors in the plasma, including corticosteroid binding globulin (CBG or transcortin), albumin, and erythrocytes (see Kirschbaum & Hellhammer, 1989 for review). The free fraction, roughly 10–15% of total plasma cortisol in humans, is assumed to be the biologically active component (Mendel, 1989). Recent developments have made it possible to assess free corticosteroids in plasma using ultrafiltration techniques (MacMahon, Thompson, Bowers, & Sgoutas, 1983) in small volumes of plasma (Richardson, Rhyne, Kumar, Antonacci, & Calvano, 1988). This procedure correlates quite well with procedures in which CBG and other binding factors are directly measured and from which free levels were determined. Ultrafiltration is well suited for the study of free cortisol in nonhuman primates.

Growth hormone (GH) has also been noted to change in association with both acute and chronic stressors (see Wheeler & Styne, 1988 for review). There are significant species differences between rodents and primates in the GH response associated with stress. Nonhuman primates typically show increased GH levels following an acute stressor whereas rodents show a reduction in plasma GH. Changes in plasma GH have not been as extensively documented as glucocorticoids in the context of maternal separation. This is surprising given the role of GH in growth, development, and immunoregulation (Gala, 1991; Kelley & Dantzer, 1991; Wheeler & Styne, 1988). Growth hormone has been observed to show an increase following a 30-min maternal separation in 30-day-old rhesus infants (Champoux, Coe, Schanberg, Kuhn, & Suomi, 1989). A similar effect has also been documented by Kalin, Shelton, and Turner (1991) in 6- to 8-month-old rhesus monkeys separated from their mothers in a novel environment. However, long-term changes in GH following maternal separation have not been observed in infant macaques separated for more than a few hours.

The present study compared cortisol (both total and free), growth hormone, and behavioral responses in two species of macaque infants, bonnet and pigtail, to a 2-week maternal separation experience while the infants remained in their natal social group. It was predicted that pigtail macaque infants would show a greater endocrine response to maternal separation and the behavioral and endocrine responses of the bonnet infant would be mitigated in the presence of more opportunities for receiving allomaternal care. It was assumed that free levels of cortisol would follow the rise in total cortisol.

Methods

Subjects

A total of 26 monkeys, 14 bonnet (*Macaca radiata*) in seven separated/matched control pairs and 12 pigtail (*Macaca nemistrina*) in six separated/matched control pairs were studied. The bonnet subjects ranged in age from 100 to 216 days at the time the mother was removed from the social group with an average (±*SD*) age of 159 ± 34 days for the control condition (3 males and 4 females) and 157 ± 34 days for the separated condition (4 males and 3 females). The pigtail subjects ranged in age from 176 to 295 days at separation with an average age of 217 ± 54 days for the control condition (4 males and 2 females) and 212 ± 32 days for the separated condition (3 males and 3 females). All subjects were reared by their mothers in social groups consisting of an adult male, 6–8 adult females, and a number of juveniles. Each separated infant was

studied with a concurrent matched control of approximately the same age but not necessarily the same sex. All subjects were housed in a 2.1 × 2.5 × 4 m pen with cinderblock walls, shelving for sitting, PVC pipes for climbing, and sawdust distributed over an epoxy-coated floor. Subjects could be observed from outside the pen through one-way mirrors without disturbing the group. Lights were on between 0700 and 2000 hr. Feeding occurred at approximately 0900 hr and consisted of standard monkey chow (Purina) and fresh fruit that was spread uniformly across the floor. Water was available ad lib through a water spout in the pen. At 1300 hr each day, sunflower seeds or millet was spread over the floor as foraging enrichment for the group. Subjects were tested between 1988 and 1993. This protocol was approved by the Institutional Animal Care and Use Committee of the University of Colorado Health Sciences Center.

Procedure

Behavioral observations were collected using 5-min focal animal sampling (Altmann, 1974) twice daily, in the morning (between 1000 and 1200 hr) and afternoon (between 1400 and 1600 hr), through all phases of the experiment. Behavior was recorded 5 days/week using a commercial behavioral observation system, The Observer (Noldus, 1991), implemented on a PC using a behavioral taxonomy for mother and infant behaviors previously described in detail (Laudenslager et al., 1990). On the day of separation and the day of reunion, six behavioral observations were recorded, three times each in the morning and afternoon.

Separation took place at an average age of 5 months for the bonnets and 7 months for the pigtails, following a 2-week period of observation of the infant and mother in their social group (baseline period). The differences in age at separation for the two species and within the species were uncontrollable, representing the influences of both timing of births in the colony and experimental scheduling conflicts. Separations and reunions took place on Monday mornings prior to 0900 hr. On the morning of the separation the mother and infant were removed from the social group: The infant was removed from the lightly restrained mother and the mother was removed to a location out of the sight, smell, and hearing of the infant, and the infant was returned to its natal social group. Behavioral observation began immediately and continued for 2 weeks (separation period). On the day of reunion 2 weeks following separation, the mother was returned to the social group at 0900 hr, after blood was obtained from both experimental subjects (see below), and behavioral observations were begun immediately. Behavioral observations continued for an additional 2 weeks (reunion period).

Blood was obtained for endocrine parameters by venipuncture without anesthesia from both the separated and matched control infants. Both subjects were bled simultaneously after entry into the social group to retrieve the designated pairs of subjects. Average time required for collecting blood was 9.4 ± 4.1 min for the bonnet infants and 12.4 ± 5.3 min for the pigtail infants. Many samples were obtained in less than 6–7 min as the study progressed and the animals became familiar with the procedures. There was no difference between separated and control conditions in the time required to collect the blood samples, although the time required to collect blood from the pigtail infants was longer than for the bonnet infants, $p < .05$. The longer time noted for the pigtail subjects generally tended to be associated with greater difficulty in sorting the study pairs from the social group in this more aggressive species. Blood samples were obtained on Monday and Friday of each week of baseline, separation, and reunion phases of the project, except on the day of separation (always a Monday) when blood

was obtained either 2 hr or 24 hr after separation. It was noted that reentering the social group 2 hr after removal of the mother on the day of separation was associated with significant disturbance in the social group, particularly in the male. We elected to discontinue this procedure and the remaining subjects were bled 24 hr following separation instead. Subjects, with their mothers present except during the separation phase, were lightly restrained in a transport cage and blood (1.5–2.0 ml) was removed from the antecubital vein. Both the mother and infant were given fruit rewards after blood samples were collected. The subjects were immediately returned to their natal group after this procedure was completed.

From each heparinized blood sample, plasma was removed and frozen at $-20°C$ in o-ring sealed tubes for subsequent radioimmunoassay. Samples were assayed by standard radioimmunoassay (RIA) for total cortisol and growth hormone. Growth hormone was assayed through the use of a commercial kit (hGH human growth hormone double antibody, Diagnostic Products). Plasma was diluted 1:200 and run in a RIA against rabbit antihuman cortisol antisera (kindly supplied by Dr. Steven Calvano, Cornell Medical Center) using previously described procedures (Keith, Winslow, & Reynolds, 1978). Samples were tested in duplicate and the mean of each determination was used in all subsequent calculations. Standard curves (run in triplicate at each concentration) were fit by a weighted regression analysis (Rodbard & Leward, 1970) from which all sample concentrations were determined. All samples were tested in the same assay to reduce variability. The intraassay and interassay coefficients of variability for these assays in our laboratory is less than 5 and 10%, respectively.

Free cortisol was determined using an ultrafiltration method (MacMahon et al., 1983) modified for small sample volumes (Richardson et al., 1988). Briefly, 200 μl of serum was placed in the upper chamber of a microconcentrator tube (10,000 molecular weight cutoff, low-binding membrane filter, Millipore Corporation) to which 0.25 μCi (approximately 500,000 dpm) of tritiated cortisol (1,2,6,7 ^3H-cortisol, New England Nuclear) was added and allowed to incubate at 37°C for 60 min to permit equilibrium between the tracer and binding proteins to be established. The tubes were spun at 1200 g for 20 min in centrifuge buckets warmed to 37°C at the beginning of the spin in a room temperature (22–25°C) centrifuge. Equal aliquots (10 μl) of the retentate (approximately 25,000 cpm) and filtrate (approximately 3,000 cpm) were counted on a liquid scintillation counter. The ratio of filtrate to retentate cpm represents the percent of free cortisol in the plasma. Multiplying the total cortisol determined from the RIA by this ratio provides an estimate of biologically active free cortisol. Pilot studies in which plasma was thawed and refrozen up to six times indicated that the percent free determined in the above manner was unaffected by repeated freezing (CV < 1%).

Data Analysis

Plasma total cortisol, free cortisol, and growth hormone levels were analyzed by a repeated measures analysis of variance (ANOVA) including species, separation experience, and sex as between subjects factors and phase (baseline, separation, reunion) and replication (four samples obtained within each phase) as the within subjects factor. A similar repeated measures ANOVA was also performed on the behavioral observations collected during baseline [mean of observations collected during baseline Week 1(B1), mean of baseline Week 2(B2)], separation [mean of six observations collected on separation Day 1(SD1), mean of remaining observations collected during separation Week 1(S1), mean of separation Week 2 (S2)], and reunion [mean of six observations col-

lected on reunion Day 1 (RD1), mean of remaining observations collected during the first week of reunion (R1), mean of reunion week 2(R2)], treated as within subjects variables.

The relationship between mean plasma hormone levels for the first and second week of separation and behaviors observed during the corresponding week of separation was tested by multiple regression techniques. An independent multiple regression analysis was run for each group, separated and matched controls, with sex, age at separation, and species entered in the first step of the regression and behaviors observed entered in the second step. Thus, the contributions of sex, age, and species were removed before the influences of behavior were entered and tested for each condition. The specific behaviors selected for entry into the second step of the regression analysis were those behaviors found to change significantly as a function of separation experience by ANOVA as described earlier. Behaviors reflecting (a) distress, which included locomotion, vocalization, and withdrawn huddled postures, (b) self maintenance, which included rest and ingestive behaviors, and (c) social interactions, which included play and proximity with others and receive groom from others, were entered.

Results

Behavioral Responses Associated With Maternal Separation

There were many parallels to our previous observations of separation responses to maternal separation in macaques (Laudenslager et al., 1990). However, the behavioral changes noted in association with maternal separation were less dependent on species than previously noted. Activity patterns of the infant changed in association with removal of the mother from the social group. For example, time spent sleeping and quiet (rest) declined in association with separation, Separation Experience × Phase interaction; $F(7,154) = 2.38$, $p = .024$, and this was associated with a concomitant rise in locomotion, Separation Experience × Phase interaction, $F(7,154) = 2.42$, $p = .023$. Species differences and interactions with gender were not noted for either rest or locomotion. Ingestive behaviors (eating and drinking) increased dramatically during maternal separation in separated subjects of both species, $F(7,154) = 4.14$, $p < .001$. Self-play and play with others declined during separation and remained low following reunion in separated subjects, Self-play: Separation Experience × Phase × Sex interaction, $F(7,105) = 2.28$, $p = .033$ with males returning to baseline prior to females; Play with others: Separation Experience × Phase interaction, $F(7,154) = 2.15$, $p = .041$. Proximity to others was highest in the bonnet monkeys, Species main effect, $F(1, 18) = 9.52$, $p = .006$, and increased in separated subjects of both species following separation, Separation Experience × Phase interaction, $F(7,126) = 2.08$, $p = .051$. Vocalization increased in association with separation, Separation Experience × Phase interaction, $F(7,126) = 6.78$, $p < .001$, with the increase in vocalization greatest in the separated male bonnet infants, Species × Separation Experience × Sex × Phase interaction, $F(7,126) = 4.97$, $p < .001$. In the pigtails, both the separated and matched controls evidenced an increase in vocalization on the day of separation and the day of reunion (see Figure 1). Withdrawn huddled postures were seen primarily in the separated male pigtail infants and only rarely in the separated bonnet infants, Species × Separation Experience × Sex × Phase interaction, $F(7,126) = 9.94$, $p < .001$. Grooming received from others, particularly separated female pigtail infants, increased during the separation phase, Species × Separation Experience × Sex × Phase interaction, $F(7,126) = 2.09$, $p = .049$. Thus, many of the behaviors (distress, self-maintenance, and social)
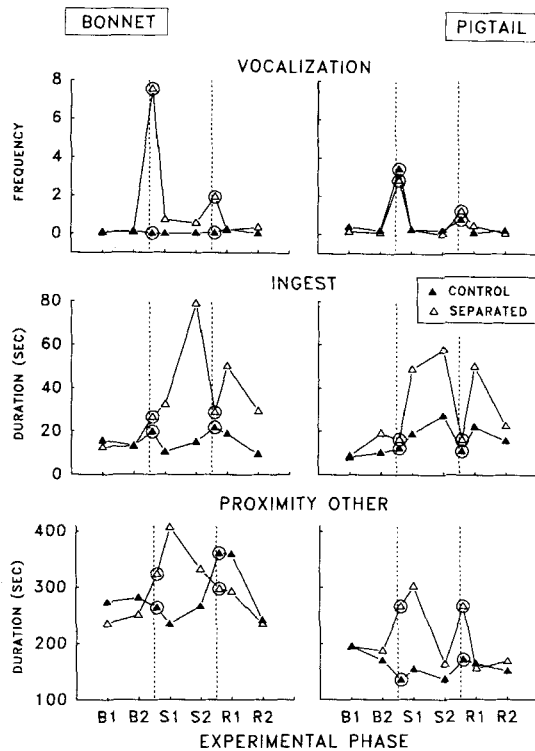
Fig. 1.   Examples of distress (Vocalization), maintenance (Ingest), and social (Proximity Other) behaviors noted in control and separated bonnet and pigtail subjects. Symbols are the mean of observations noted for each respective week during baseline, separation, and reunion. The mean of observations made on the first day of separation and reunion are enclosed by a circle.

noted previously in association with separation (Laudenslager et al., 1990) were observed in the present cohort of subjects. These effects often interacted with gender and species. Separated males seemed to reflect greater distress such as increased vocalization (bonnets) and huddled postures (pigtail), and female pigtail infants received greater grooming from others. Examples of distress (vocalization), maintenance (ingest), and social (proximity other) behaviors are shown graphically in Figure 1.

Cortisol and GH During Maternal Separation

Both free and total cortisol rose in subjects experiencing a 2-week maternal separation with comparable patterns observed in both species. Plasma total and free cortisol levels rose initially at separation relative to matched control subjects and returned to levels observed in matched controls by the end of the 2-week separation interval, Separation Experience × Phase × Replication interaction for total cortisol, $F(6,132) = 3.28, p = .005$, and $F(6,132) = 2.03, p = .066$, for free cortisol, as shown in Figure 2a and b. Some of the study group were bled either 2 hr (4 bonnet and 4 pigtail pairs) or 24 hr (2 bonnet pairs and 1 pigtail pair) following separation. Total cortisol observed in the samples taken at 2 and 24 hr are given in Table 1. There were no differences in cortisol between species with regard to separation experience, $p < .05$, nor significant interactions with gender or species, $p > .05$. Although it appears that for the separated pigtail
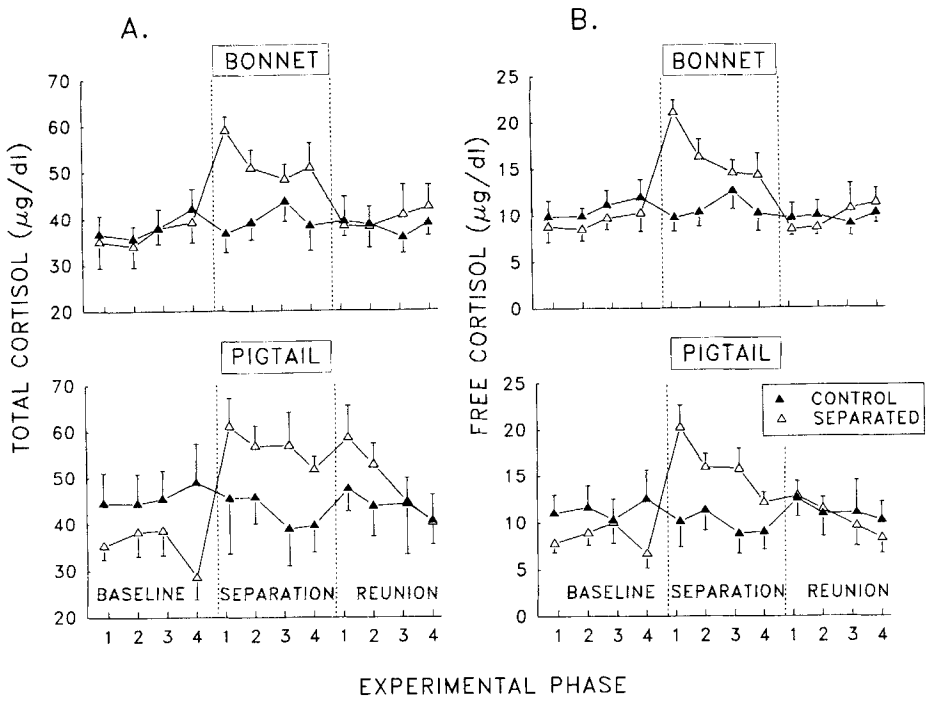
Fig. 2.　Total plasma cortisol (μg/dl) and free cortisol (μg/dl) are indicated by symbols for each phase of the experiment. Standard errors are indicated by brackets to one side of the mean.

subjects, total plasma cortisol remained elevated above matched control levels until the sample obtained 11 days following maternal reunion (R3), these differences were not significant, $p > .05$ by Bonferroni t-tests. The average percent free cortisol during baseline for the bonnet and pigtail subjects was 26.3% ± 3.6 (SD) and 23.3% ± 5.8, respectively.

In contrast to the changes noted in cortisol associated with a 2-week maternal separation, plasma GH rose during the separation phase in pigtail monkeys but not the bonnet infants as shown in Figure 3. There was a significant effect of species on plasma GH, with the pigtail infants exhibiting higher plasma levels of GH, species main effect, $F(1,22) = 15.72$, $p = .001$. There also appeared to be more variability in plasma GH in pigtail infants than noted in the bonnet infants. Plasma GH rose slowly in the pigtail

Table 1

Plasma Total Cortisol (μg/dl) at 2 and 24 hr After Separation

Time Since Separation	Bonnet		Pigtail	
	Control	Separated	Control	Separated
2 Hr	38.2 ± 11.9[1]	35.2 ± 5.3[1]	36.3 ± 11.0[1]	48.8 ± 14.6[1]
24 Hr	46.6 ± 9.4[2]	62.2 ± 2.2[2]	37.5[3]	102.0[3]

Note.
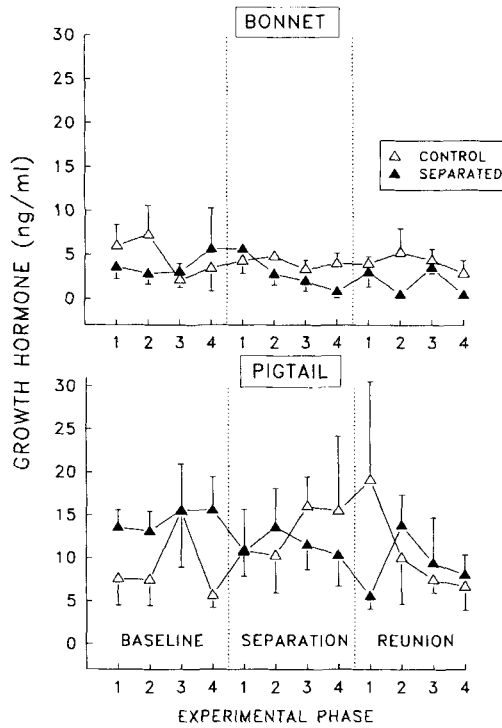[1] $n = 4$ (±SEM). [2] $n = 2$ (±SEM). [3] $n = 1$.

Fig. 3. Plasma GH (ng/ml) is indicated by the symbols for each phase of the experiment. Standard errors are indicated by brackets to one side of the mean.

infants during the separation phase and fell to baseline levels by 7 days following reunion (R2). The apparent increase in pigtail separated subjects but not bonnet separated subjects was evidenced as a marginally significant Species × Separation Experience × Replication interaction, $F(3,66) = 2.42$, $p = .074$. Table 2 indicates the plasma GH levels noted 2 and 24 hr following maternal separation. Visual inspection, as statistical analysis was not possible with the small and unbalanced samples, of Table 2 would suggest that small increases were noted at these immediate time points. The more robust trend, however, is the slow increase noted in the separated pigtail infants shown in Figure 3.

Table 2
Plasma Growth Hormone (ng/ml) at 2 and 24 hr After Separation

Time Since Separation	Bonnet		Pigtail	
	Control	Separated	Control	Separated
2 Hr	0.4 ± 0.6[1]	2.5 ± 1.4[1]	4.7 ± 3.0[1]	9.0 ± 3.9[1]
24 Hr	4.4 ± 5.4[2]	9.0 ± 16.0[2]	11.1[3]	13.2[3]

Note.
[1] $n = 4$ (±*SEM*). [2] $n = 2$ (±*SEM*). [3] $n = 1$.

Hormonal Relationships to Behavior

Plasma cortisol levels, but not growth hormone, were related to behaviors observed during the first week of separation for the separated but not the matched control groups. When species, sex, and age at separation were entered as the first step in the multiple regression for the separated subjects, none of these variables accounted for significant variance in either plasma free or total cortisol. However, for plasma growth hormone, both species, $p = .006$, and sex, $p = .045$, contributed significantly in the first step with pigtail monkeys and males of both species showing higher plasma growth hormone levels. After removing the variance attributable to these variables, several behaviors continued to make significant contributions to the variance in plasma free cortisol, but for the first week of separation only. These behaviors included frequency of vocalization, $p = .007$, duration of time in huddled slouched postures, $p = .035$, duration of time playing with others, $p = .003$, and duration of time in proximity to others, $p = .014$. Distress behaviors (vocalization and slouch) were positively related to plasma cortisol and social behaviors (proximity and play with others) were negatively related to plasma cortisol levels. None of the other behaviors contributed significantly to the variance in plasma free cortisol during the first week of separation. Similar relationships were also noted for total cortisol, with the exception that locomotion on the first day of separation, $p = .026$, also contributed significantly to the variance in total cortisol. During the second week of separation, none of the behaviors contributed significantly to variance in any of the hormone levels with one exception. Duration of slouched huddled postures contributed significantly to plasma growth hormone levels, $p = .048$, during the second week of separation. Table 3 indicates the results of the multiple regression analysis for free cortisol for the first week of separation. Similar results were obtained for total cortisol.

In matched control subjects, the multiple regression analysis indicated that neither species, sex, nor age accounted for significant variance in mean plasma total cortisol, free cortisol, and growth hormone during the first and second weeks of separation. When behaviors (vocalization, huddled postures, locomotion, play other, and proxim-

Table 3

Multiple Regression Analysis for Plasma Free Cortisol During the First Week of Separation for Separated Subjects Only

Independent Variables	Standardized β	p
STEP ONE		
Species	−.646	.132
Sex	.324	.309
Age at separation	.651	.110
STEP TWO		
Vocalization	.573	.007
Slouch	.508	.035
Play	−.830	.003
Proximity	−.558	.014
Locomotion first day	−.024	.876
Locomotion rest of week	−.029	.865

Note.
$F(3,9) = 24.47$, $p = .013$, $r = 0.993$, Adjusted $r^2 = 0.946$.

ity other) were entered in the second step of the regression, none of these behaviors accounted for any significant variance in hormone levels.

Discussion

The present study supports previous observations of the impact of a 2-week maternal separation of socially housed bonnet and pigtail macaque infants on behavior (Laudenslager et al., 1990; Reite et al., 1981; Reite et al., 1989), and further adds observations of plasma total and free cortisol and GH. Removal of the mother from the social group was followed by a rise in vocalizations that abated by the end of the first week of separation and frequently was reduced by the end of the first day after the mother was removed. Several other behaviors indicative of disturbance or distress increased in response to separation, including slouched huddled postures and reduced time spent resting (e.g., asleep or quiet). Ingestive behaviors and proximity to others increased in the absence of the mother for both species. The large majority of these behaviors had returned to baseline levels by the beginning of the second week of separation.

Previously observed species differences were less common in the present study. This may be attributable in part to the older average age of the separated pigtail infants. Thus, allomaternal care and social interactions in the bonnets and the older age of the separated pigtail infants may have abrogated the impact of maternal separation in both species. However, these influences were not sufficient to attenuate the effects of maternal separation on plasma cortisol levels in both species and the rise in plasma growth hormone in the pigtail infants. Regardless of the lack of differences between species in their behavioral response to separation, activation of the HPA as evidenced by plasma free and total cortisol levels were related to simultaneous behaviors of the separated subjects.

A multiple regression analysis, conducted separately for separated and matched control subjects, indicated that following the removal of variance associated with species, sex, and age at separation, several behaviors accounted for significant variance in plasma free and total cortisol for separated subjects and only during the first week of separation. Generally, distress behaviors were positively related to plasma cortisol and social behaviors were negatively related to plasma cortisol as was expected for this model of social separation. That is, social interactions should mitigate the negative consequences of separation and distress behaviors should be associated with indication of HPA activation.

As significant species differences in the glucocorticoid response to stressors have been noted among macaque species (Clarke, Mason, & Moberg, 1988), it was unexpected when we failed to observe species differences in the glucocorticoid response to maternal separation in bonnet and pigtail macaques, which may be related to the age differences as discussed. There was a species difference in the GH response following maternal separation such that a slowly developing increase in GH was noted in the pigtail infants only, which only approached statistical significance. This pattern of GH changes was in contrast to previous observations (Champoux et al., 1989), which noted a rapid increase in plasma GH associated with a brief separation in 30-day-old rhesus monkeys. In the subjects that were sampled shortly following separation at 2 and 24 hr, plasma GH was elevated slightly above matched controls but the small number precluded statistical analysis. As a matched control group was available for comparison, the impact of venipuncture and handling (Herndon, Turner, Perachio, Blank, & Collins,

1984) is controlled for in the present study. The variability of plasma GH in the pigtail monkeys may reflect the episodic nature of GH release (Quabbe, Gregor, Bumke-Vogt, Witt, & Giannella-Neto, 1983), although this was not apparent as increased variability in the bonnet monkey. In fact, variability in the bonnet monkeys was quite small as indicated in Figure 3. Continuous sampling protocols are not possible in the mother–infant separation paradigm and we are thus unable to control for episodic release in free-ranging subjects through the use of continuous sampling techniques.

In human newborns, reduced GH has been noted 90 min following repeated heel sticks (Stubbe & Wolfe, 1970). Based on these observations, Champoux et al., (1989) predicted that prolonged separation experiences in young macaques might be accompanied by reduced GH release. In contrast, we noted a slow increase in plasma GH in the pigtail but not bonnet infants experiencing maternal separation. This increase in plasma GH was not associated with behaviors that were associated with plasma cortisol. A similar dissociation of behavior and GH has been noted in humans. Examination stress, although associated with increased self-reports of stress, was not associated with increases in plasma GH measured over a 24-hr period (Malarkey, Hall, Pearl, Kiecolt-Glaser, & Glaser 1991). We did note a significant partial correlation between plasma GH and the presence of postural huddle in a small subset of separated subjects during the second week of separation. To the extent that postural huddle represents a more dramatic behavioral response to social separation, the observation that a portion of the variance in GH was associated with time spent in these postures suggests that GH may change only in the face of a more profound response to a social stressor.

To summarize, a 2-week maternal separation was associated with an increase in both free and total cortisol in both bonnet and pigtail infants. In the pigtails, plasma GH increased slowly during the separation. Cortisol but not growth hormone was positively related to distress behaviors and negatively related to behaviors reflecting social interactions. Growth hormone was related to a single distress behavior occurring during the second week of separation. Finally, plasma free and total cortisol were not correlated with plasma growth hormone. It appears that processes controlling the magnitude of the behavioral response to a stressor (e.g., maternal separation in this study), the HPA response, and GH response may not completely overlap.

Notes

References

Altmann, J. (1974). Observational study of behavior: Sampling methods. *Behaviour, 49*, 227–267.

Boccia, M. L., Reite, M. L., & Laudenslager, M. L. (1991). Early social environment may alter the development of attachment and social support: Two case reports. *Infant Behavior and Development, 14*, 253–260.

Champoux, M., Coe, C. L., Schanberg, S. M., Kuhn, C. M., & Suomi, S. J. (1989). Hormonal effects of early rearing conditions in the infant rhesus monkey. *American Journal of Primatology, 19*, 111–117.

Clarke, A. S. (1991). ACTH and glucocorticoid responses under two conditions of stress in macaques. *American Journal of Primatology, 25*, 115–124.

Clarke, A. S., Mason, W. A., & Moberg, G. P. (1988). Differential behavioral and adrenocortical response to stress among three macaque species. *American Journal of Primatology, 14*, 37–52.

Coe, C. L. (1993). Psychosocial factors and immunity in nonhuman primates—A review. *Psychosomatic Medicine, 55*, 298–308.

Coe, C. L., Glass, J. C., Wiener, S. G., & Levine, S. (1983). Behavioral, but not physiological, adaptation to repeated separation in mother and infant primates. *Psychoneuroendocrinology, 8*, 401–409.

Gala, R. R. (1991). Prolactin and growth hormone in regulation of the immune system. *Proceedings of the Society of Experimental Biology and Medicine, 198*, 513–527.

Gunnar, M. R. (1992). Reactivity of the hypothalamic–pituitary–adrenal system to stressors in normal infants and children. *Pediatrics, 90*, 491–497.

Herndon, A. G., Turner, J. J., Perachio, A. A., Blank, M. S., & Collins, D. C. (1984). Endocrine changes induced by venipuncture in rhesus monkeys. *Physiology & Behavior, 32*, 673–676.

Kalin, N. H., Shelton, S. E., & Turner, J. G. (1991). Effects of alprazolam on fear-related behavioral, hormonal, and catecholamine response in infant rhesus monkeys. *Life Sciences, 49*, 2031–2044.

Keith, L. D., Winslow, J. R., & Reynolds, R. W. (1978). A general procedure for estimation of corticosterone response in individual rats. *Steroids, 31*, 523–531.

Kelley, K. W., & Dantzer, R. (1991). Growth hormone and prolactin as natural antagonists of glucocorticoids in immunoregulation. In N. Plotnikoff, A. Murgo, R. Faith, & J. Wybran (Eds.), *Stress and immunity* (pp. 433–452). Ann Arbor: CRC Press.

Kirschbaum, C., & Hellhammer, D. H. (1989). Salivary cortisol in psychobiological research: An overview. *Neuropsychobiology, 22*, 150–169.

Laudenslager, M. L., & Fleshner, M. R. 1994. Stress and immunity: Of mice, monkeys, models, and mechanisms. In R. Glaser & J. Kiecolt-Glaser (Eds.), *Human stress* (pp. 161–181). New York: Academic Press.

Laudenslager, M. L., Held, P. E., Boccia, M. L., Reite, M. L., & Cohen, J. J. (1990). Behavioral and immunological consequences of brief mother–infant separation: A species comparison. *Developmental Psychobiology, 23*, 247–264.

Levine, S., Franklin, D., & Gonzalez, C. A. (1984). Influence of social variables on the biobehavioral response to separation in rhesus monkey infants. *Child Development, 55*, 1386–1393.

Levine, S., & Wiener, S. G. (1988). Psychoendocrine aspects of mother–infant relationships in nonhuman primates. *Psychoneuroendocrinology, 13*, 143–154.

MacMahon, W., Thompson, J., Bowers, W., & Sgoutas, D. (1983). A simplified ultrafiltration method for determination of serum-free cortisol. *Clinica Chimica Acta, 131*, 171–184.

Malarkey, W. B., Hall, J. C., Pearl, D. K., Kiecolt-Glaser, J. K., & Glaser, R. (1991). The influence of academic stress and season on 24-hr concentrations of growth hormone and prolactin. *Journal of Clinical Endocrinology and Metabolism, 73*, 1089–1092.

Mendel, C. M. (1989). The free hormone hypothesis: A physiologically based mathematical model. *Endocrine Reviews, 10*, 232–274.

Mineka, S., & Suomi, S. J. (1978). Social separation in monkeys. *Psychological Bulletin, 85*, 1376–1400.

Noldus, L. P. J. J. (1991). The Observer: A software system for collection and analysis of observational data. *Behavior Research Methods, 23*, 415–429.

Quabbe, H., Gregor, M., Bumke-Vogt, C., Witt, J., & Giannella-Neto, D. (1983). Endocrine rhythms in a nonhuman primate, the rhesus monkey. *Advances in Biological Psychiatry, 11*, 48–63.

Reite, M. L., Kaemingk, K., & Boccia, M. L. (1989). Maternal separation in bonnet monkey infants: Altered attachment and social support. *Child Development, 60*, 473–480.

Reite, M., Short, R., Seiler, C., & Pauley, J. D. (1981). Attachment, loss, and depression. *Journal of Child Psychology and Psychiatry, 22*, 141–169.

Richardson, R. P., Rhyne, C. D., Kumar, A., Antonacci, A. C., & Calvano, S. E. (1988). Rapid determination of free plasma corticosterone concentrations from small sample volumes. *FASEB Journal, 2*, 1322 (Abstract).

Rodbard, D., & Lewald, J. E. (1970). Computer analysis of radioligand assay and radioimmunoassay data. *Acta Endocrinologica, 64* (Suppl.), 70–103.

Stubbe, P., & Wolf, H. (1970). The effect of stress on growth hormone, glucose, and glycerol in newborn infants. *Hormone and Metabolic Research Journal, 3*, 175–179.

Wheeler, M. D., & Styne, D. M. (1988). The nonhuman primate as a model of growth hormone physiology in the human being. *Endocrine Reviews, 9*, 213–246.