



## Physiological and behavioural responses to weaning conflict in free-ranging primate infants



Tara M. Mandalaywala<sup>a, b, \*</sup>, James P. Higham<sup>c</sup>, Michael Heistermann<sup>d</sup>, Karen J. Parker<sup>e</sup>, Dario Maestriperi<sup>a, b</sup>

<sup>a</sup> Institute for Mind and Biology, The University of Chicago, Chicago, IL, U.S.A.

<sup>b</sup> Department of Comparative Human Development, The University of Chicago, Chicago, IL, U.S.A.

<sup>c</sup> Department of Anthropology, New York University, New York, NY, U.S.A.

<sup>d</sup> Endocrinology Laboratory, German Primate Centre, Goettingen, Germany

<sup>e</sup> Department of Psychiatry and Behavioural Sciences, Stanford University School of Medicine, Stanford, CA, U.S.A.

### ARTICLE INFO

#### Article history:

Received 26 March 2014

Initial acceptance 28 April 2014

Final acceptance 18 August 2014

Available online 13 October 2014

MS. number: A14-00253R2

#### Keywords:

energetics  
infant  
primate  
rhesus macaque  
stress  
weaning

Weaning, characterized by maternal reduction of resources, is both psychologically and energetically stressful to mammalian offspring. Despite the importance of physiology in this process, previous studies have reported only indirect measures of weaning stress from infants, because of the difficulties of collecting physiological measures from free-ranging mammalian infants. Here we present some of the first data on the relationship between weaning and energetic and psychological stress in infant mammals. We collected data on 47 free-ranging rhesus macaque infants on Cayo Santiago, Puerto Rico, showing that faecal glucocorticoid metabolite (fGCM) concentrations were directly related to the frequency of maternal rejection, with fGCM concentrations increasing as rates of rejection increased. Infants with higher fGCM concentrations also engaged in higher rates of mother following, and mother following was associated with increased time on the nipple, suggesting that infants that experienced greater weaning-related stress increased their efforts to maintain proximity and contact with their mothers. Infants experiencing more frequent rejection uttered more distress vocalizations when being rejected; however, there was no relationship between rates of distress vocalizations and fGCM concentrations, suggesting a disassociation between behavioural and physiological stress responses to weaning. Elevated glucocorticoid concentrations during weaning may function to mobilize energy reserves and prepare the infant for continued maternal rejection and shortage of energetic resources.

© 2014 The Association for the Study of Animal Behaviour. Published by Elsevier Ltd. All rights reserved.

According to parent–offspring conflict theory (Trivers, 1974), offspring should demand a disproportionate amount of parental resources for themselves rather than share them equally with their siblings. In contrast, parents are equally related to all their offspring and are therefore selected to invest their resources equally among offspring. As a result, there should be conflict between parent and offspring over the amount of parental investment as well as the duration of the investment period, with individual offspring being selected to demand more investment from their parents than their parents are selected to give (Trivers, 1974).

In nonhuman primates, as in many other mammals, maternal investment is mainly expressed through the production and transfer of milk and through energetically costly infant carrying (Maestriperi, 2002). In most species of anthropoid primate,

mothers carry their infants continuously and provide unrestricted access to the nipple during the first few days of lactation. As infants grow older, maternal carrying and nursing are gradually reduced as the survival benefits to the infant decrease and the costs to the mother increase (baboons: Altmann & Samuels, 1992; macaques: Hinde, 1977; Hinde & Atkinson, 1970). However, across mammalian species there are clear benefits to the offspring in extending maternal investment such as increased body weight (e.g. piglets: Bøe, 1991; Jarvis et al., 2008; lambs: Watson & Gill, 1991; primates: Lee, Majluf, & Gordon, 1991; ungulates: Lee et al., 1991), decreased parasite load and increased immune function (e.g. lambs: Watson & Gill, 1991) and decreased infant mortality (e.g. vervet monkeys: Lee, 1984).

In primates, mother–infant behavioural conflict over time spent in contact and infant access to the nipples is observable throughout the first year of an infant's life (e.g. Hinde & Spencer-Booth, 1967, 1971; Maestriperi, 1994a), and weaning conflict is particularly intense when mothers resume cycling and mating (e.g. Berman,

\* Correspondence and present address: T. M. Mandalaywala, Department of Psychology, 6 Washington Place, New York University, New York, NY 10003, U.S.A.  
E-mail address: [tmandalaywala@nyu.edu](mailto:tmandalaywala@nyu.edu) (T. M. Mandalaywala).

Rasmussen, & Suomi, 1993). Mothers reject their infants' attempts at making bodily contact or gaining access to their nipples, and infants respond with distress calls and temper tantrums and by increasing their attempts to maintain contact or proximity. Although weaning conflict is readily observed in many species of primates and other mammals (e.g. deer: Gauthier & Barrette, 1985; sheep: Berger, 1979; macaques: Maestriperieri, 1994a; apes: Codner & Nadler, 1984), the direct consequences and physiological response to this conflict are rarely measured in infants.

The lack of data on the direct effects of weaning are unfortunate as maternal rejection is likely to induce both energetic and psychological stress in the infant. In reaction to a social or ecological stressor, mammals mount a multifaceted stress response across different physiological systems (Romero, Dickens, & Cyr, 2009), including an endocrine response via activation of the hypothalamic–pituitary–adrenal (HPA) axis and the sympathetic nervous system (SNS). Activation of the HPA axis and SNS causes the release of glucocorticoid hormones and catecholamines, which in turn promote responses necessary to respond to or escape from the stressor and reduce nonessential functions (McEwen, 2001; Sapolsky, Romero, & Munck, 2000). From a monkey infant's perspective, weaning conflict and denial of access to the food resources of the nipple are likely to be both an energetic and a psychosocial stressor. Therefore, examining changes in infant glucocorticoid hormone (GC) concentrations in response to maternal rejection is important to understand both the energetic and the psychological aspects of infant responses to weaning stress.

Glucocorticoids are essentially metabolic hormones. The release of GCs leads to a stimulatory and preparatory metabolic response by decreasing appetite and mobilizing energy stored in tissues, enabling an individual to cope with temporary food restriction (Sapolsky et al., 2000). In addition, GCs act in a preparatory manner by minimizing energy expenditure and increasing glycogen storage in the liver, readying an individual to cope with a continued lack of food (Sapolsky et al., 2000). Release of GCs in response to the deprivation of expected food (e.g. when an infant attempts to nurse but is prevented) can be characterized as an adaptive response that enables the body to deal with decreased resources and prepares it for the possibility that resources will be limited in the future. Although previous studies have examined the effect of prolonged mother–infant separation on GCs (e.g. piglets: Colson, Orgeur, Foury, & Mormède, 2006; Poletto, Steibel, Siegford, & Zanella, 2006; rhesus macaques: Gunnar, Gonzalez, Goodlin, & Levine, 1981; squirrel monkey: Levine, Wiener, & Coe, 1993), infant GC response to naturally occurring maternal rejection behaviour as it occurs in real-time has yet to be quantified in any mammalian species.

In addition to shedding light on the functional, adaptive value of GCs on metabolism during the weaning period, studying and quantifying infant physiological stress in response to naturally occurring maternal rejection is important for understanding the psychological consequences of weaning. In nonhuman primates, infants sometimes display behavioural resistance to maternal rejection, such as screams or tantrums (e.g. baboons: Altmann, 1980; DeVore, 1963; langurs: Jay, 1963), and there are often strong individual differences in infant reaction to maternal rejection and separation (e.g. rhesus macaques: Hinde & Spencer-Booth, 1970). For example, some infants may show 'protest' or 'distress' in response to maternal separation or rejection and vocalize frequently (e.g. rhesus macaques: Berman et al., 1993; baboons; Altmann, 1980; DeVore, 1963; great apes: Codner & Nadler, 1984), while others may react with depression and not vocalize at all (e.g. rhesus macaques: Berman et al., 1993). Studies on captive squirrel monkeys in which infants were experimentally separated from their mothers reported that while infant vocalizations and behavioural agitation habituated

over time or multiple separations, cortisol levels remained elevated, suggesting that behavioural and physiological indicators of stress sometimes operate on different timescales and thus do not always correlate (e.g. Hennessy, 1986; Levine et al., 1993). To understand the multiple ways in which infants are affected by a reduction in maternal resources, it is necessary to measure changes in both infant GC concentrations as well as infant behaviour across the period of peak mother–infant conflict.

In this study we investigated the relationship between naturally occurring maternal rejection behaviour and GCs in free-ranging infant rhesus macaques on the island of Cayo Santiago, Puerto Rico. We observed mother–infant pairs when infants were 5–12 months of age, encapsulating the period of peak weaning conflict (Hinde & Spencer-Booth, 1967; Maestriperieri, 1994a), and measured faecal glucocorticoid metabolite (fGCM) concentrations as a proxy for circulating levels of GCs (e.g. Heistermann, Palme, & Gaswindt, 2006). fGCM concentration provides an integrated measure of adrenocortical activity (i.e. stress), allowing for analysis of a basal HPA axis activity (over a period of days), as opposed to acute HPA reactivity to stress assessed by measuring hormone concentrations in blood or saliva (over a period of minutes). Thus, our approach allowed us to examine weekly changes in GC concentration in response to weekly variation in behaviours and interactions.

We tested the hypothesis that maternal rejection is energetically and psychologically stressful, predicting that maternal rejection would be (1) accompanied by a GC response in infants, such that high rates of maternal rejection behaviours would be temporally associated with elevated concentrations of fGCMs and (2) associated with behavioural indicators of anxiety. To our knowledge, this is the first study to use noninvasive endocrine assessment to quantify real-time changes in GCs and infant behaviour in relation to maternal rejection during the weaning period in mammalian infants.

## METHODS

This study was conducted on Cayo Santiago, a 15.2 ha island located 1 km off the east coast of Puerto Rico (Rawlins & Kessler, 1986). The Cayo Santiago colony contains approximately 1000 free-ranging rhesus macaques; macaques on Cayo Santiago are provisioned with monkey chow and rainwater, but also forage naturally on vegetation. Behavioural and physiological data were collected in Groups R and S, which contained approximately 250 and 115 individuals, respectively, at the time of data collection. Rhesus macaques are seasonal breeders (Gordon, 1981), with the majority of births on Cayo Santiago occurring between August and October (per Caribbean Primate Research Centre census records). Data were collected for the present study from March through August 2012 on infants born in the previous (2011) birth season. March–August encompasses the period of peak weaning conflict, as determined by infant age during this period (Maestriperieri, 1994a; Hinde & Spencer-Booth, 1967).

### *Behavioural Data Collection*

Infants were observed between 0700 and 1430 hours, 5 days a week, using continuous focal animal sampling (Altmann, 1974). Behavioural data were collected from 47 infants when the infants were approximately 5–12 months of age (27–56 weeks). Infants were observed for one 30 min period each week using a hand-held event recorder, the Psion Workabout ([www.pSIONteklogix.com](http://www.pSIONteklogix.com)) loaded with 'Behaviour' software (Syscan International Inc., Montreal, Quebec). The order of focal animal sampling was randomized and behavioural data were converted into mean hourly frequencies (event behaviours) or mean hourly durations (state behaviours) for

analysis. Before data collection began, all observers ( $N = 3$ ) were trained in behaviour observation and Psion use by T.M.M. Observers only began behavioural data collection once interobserver reliability reached Cohen's  $k = 0.90$ , as determined by tests of agreement on individual occurrences of behaviours as defined in the ethogram (e.g. Caro, Roper, Young, & Dank, 1979; see Table 1).

As we were primarily interested in the relationship between maternal rejection and fGCM concentration, we only analysed behavioural data collected in weeks in which a weekly fGCM concentration could be determined (e.g. behavioural data from Subject A during week 30 was matched with fGCM concentration from Subject A during week 30). After matching fGCM and behavioural data, our data set contained 109.5 h of observational data (mean  $\pm$  SE behavioural observation frequency across all infants:  $2.3 \pm 0.2$  h, range 0.5–3 h, total  $N = 219$  observations). Female dominance ranks are known on Cayo Santiago as they are maternally inherited, but we additionally collected data on the outcome of dyadic agonistic interactions between females to determine sex-specific dominance hierarchies and to control for rank effects in our statistical analyses. These dyadic interaction data were placed into a winner–loser matrix and MatMan was used to generate dominance hierarchies (cf. Higham & Maestripieri, 2010). Following 10 000 iterations, significant linear hierarchies were produced (linearity test using Landau's linearity index corrected for unknown relationships, Group R:  $P = 0.03$ ; Group S:  $P = 0.03$ ), which were combined with matriline data to categorize each adult female and her infant as high, middle or low ranking by dividing subjects in each social group equally into the top, middle or bottom third of the hierarchy, respectively.

We collected observational data on interactions between the mother and infant, including the frequency with which the infant experienced maternal rejection and was kept from gaining access to the nipple. Maternal rejection behaviours include forcibly removing infants from the body and walking away from them, preventing infants from making contact by keeping them at arm's distance, and discouraging infants from attempts to make bodily contact by hitting or biting them. In some cases, mothers may allow infants to make bodily contact and be carried, but they restrict access to the nipple by blocking infants with a hand or arm (e.g. Maestripieri, 1994a). We also recorded the amount of time that the infant spent on the nipple, the frequency with which the infant followed the mother ('mother following') and the frequency of distress vocalizations (i.e. screams: Maestripieri, 1994b) and self-directed behaviours, which can be interpreted as behavioural indicators of anxiety (Maestripieri, 1993a, 1993b; Maestripieri, Schino, Aureli, & Troisi, 1992) (see Table 1 for description of

behaviours and Table 2 for information on the distribution of each variable). As GC concentration can change as a consequence of activity level (reviewed in Mastorakos, Pavlatou, Diamanti-Kandarakis, & Chrousos, 2005), time spent in locomotor activity was taken into account; movement time was the summed total of time spent in exploratory behaviour (nondirected movement), travel (directed movement) and solitary and social play (Table 1).

#### Physiological Data Collection and Analysis

Faecal samples were collected from infants between 0700 and 1430 hours. Evaluation of faecal GC values collected from the same individual on the same day showed no difference between morning and afternoon values in this population (e.g. Rahkovskaya & Heistermann, n.d.). However, time of sample collection was recorded and incorporated into analyses. We collected 219 faecal samples across the 7-month study period. On average, a faecal sample was collected from each individual every 3 weeks (mean  $\pm$  SE faecal sample collection frequency:  $3.0 \pm 0.22$  weeks, range 1–12 samples/individual) to enable accurate estimation of changes in fGCM concentration across the entire weaning period.

Faecal samples (uncontaminated with urine) were collected only after defecation was observed directly and the subject could be identified. Samples were then homogenized by mixing, nonfaecal debris was removed and a small bolus of approximately 0.5–2 g wet weight was placed in a 20 ml tube. Tubes were then placed into a cooler containing ice packs (Hodges & Heistermann, 2011). Upon return to the mainland (at either 1130 or 1430 hours), tubes were frozen at  $-20^{\circ}\text{C}$  until they were shipped on dry ice to the German Primate Centre for analysis. All faecal samples arrived in Germany still frozen. Faecal samples were prepared for enzyme-immuno assay (EIA) by being lyophilized and pulverized, followed by extraction of an aliquot (50–70 mg) of the faecal powder with 3 ml of 80% methanol by vortexing for 15 min (e.g. Heistermann, Finke, & Hodges, 1995). All faecal samples were analysed for  $11\beta$ -hydroxyetiocholanolone ( $3\alpha,11\beta$ -dihydroxy-CM), a major metabolite of cortisol in primate faeces (e.g. Heistermann et al., 2006) using an enzyme-immuno assay described in detail elsewhere (Heistermann, Ademmer, & Kaumanns, 2004). The assay has been biologically validated for use in rhesus macaques by demonstrating the well-known GC response to anaesthetization (Hoffman et al., 2011). Interassay variation, measured by repeated measurement of high- and low-value quality controls on each microtitre plate was 10.4% (high) and 13.8% (low), while intra-assay variation was 6.6% (high) and 7.9% (low). All assay results are standardized for differences in faecal weight and are expressed as fGCM concentration (ng) per dry faecal weight (g). To achieve a normal distribution, fGCM concentrations were log transformed for all analyses.

**Table 1**

Ethogram of maternal and other social behaviours of rhesus macaques analysed in the present study

Behaviour	Variable
<b>Maternal interaction</b>	
Maternal rejection	Frequency of aggressive or nonaggressive movement to prevent infant access to the nipple
Mother following	Frequency of the infant following the mother
Suckling	Time spent on the mother's nipple
<b>Anxiety</b>	
Self-directed behaviour	Self-scratching, self-grooming, shaking or yawning
Distress vocalization	Scream or gecker given by the infant
<b>Movement</b>	
	Time spent in movement resulting from directed (travel) or nondirected (exploration) locomotor behaviour, as well as solitary and social play

#### Data Analysis

Linear mixed models (LMMs) were used to examine the relationships between behavioural and physiological variables. To determine whether nonbehavioural factors affected fGCM

**Table 2**

Mean, SE and range for behavioural variables examined in free-ranging rhesus macaques

	Mean	SE	Range
Maternal rejections/h	2.20	0.34	0–37.73
Mother follows/h	4.59	0.36	0–30.61
Time on nipple (min)/30 min	4.38	0.53	0–29.97
Distress vocalizations/h	2.60	0.45	0–49.48
SDBs/h	16.47	0.94	0–99.83

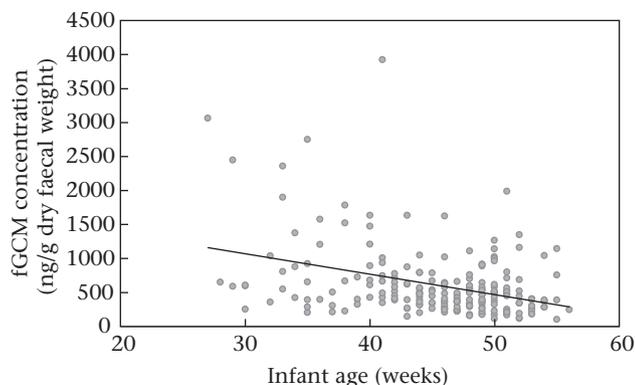
SDB: self-directed behaviour.

concentration, the first model tested whether fGCM concentration (dependent variable) varied with respect to time of faecal sample collection, infant sex, group membership, maternal rank, body size or infant age (in weeks) (all fixed effects). Only infant age was found to affect fGCM concentration ( $F_{1,201.5} = 46.8$ ,  $P < 0.001$ ; all other  $P$ s  $> 0.1$ ; see Results, Fig. 1); thus, infant age was incorporated as a random effect in all subsequent analyses.

The second set of models tested for relationships between independent behavioural variables, both mother–infant interactions (i.e. maternal rejection, mother following, time spent on the nipple) as well as behavioural indicators of anxiety (i.e. self-directed behaviours, distress vocalizations). Because we found several significant relationships between behavioural variables (see Results, Table 3), we incorporated each variable separately as a fixed effect in the third set of models.

To determine the relationship between mother–infant interactions, behavioural indicators of anxiety and fGCM concentration, the third set of models tested whether fGCM concentration (dependent variable) varied with respect to maternal interaction variables or behavioural indicators of anxiety (independent variables) (Table 3). As the second set of LMMs revealed relationships between several independent variables, each variable was incorporated into its own model as a fixed effect (e.g. rate of maternal rejection was incorporated as a fixed effect in a separate model from rate of distress vocalizations, etc.). To determine whether variation in fGCM concentration was better explained by variation in mother–infant interactions or behavioural indicators of anxiety than by activity level, the movement variable was incorporated into the third set of models as a fixed effect.

We conducted an additional analysis for any dependent variables found to have an overall relationship to fGCM concentration to attempt to address the causal question of whether changes in behaviour led to changes in fGCM concentrations, or vice versa. To do this, we divided the data in two smaller subsets. There is a 2–3 day excretion lag before hormone concentrations in blood are reflected in faeces; thus, samples collected 2–3 days following behavioural observation ('post' sample subset) are most likely to be reflective of the behaviours we observed, whereas faecal samples collected before or around the time of observation (i.e. the day before, the day of and the day after behavioural observation, 'pre' sample subset) could not be indicative of behaviours observed given the excretion lag. Using the same parameters applied in the third set of models, we then undertook our analyses separately for the pre and post subsets to attempt to assess the more likely causal pathway of relationships between behaviour and fGCM concentrations.



**Figure 1.** Relationship between infant age and faecal glucocorticoid metabolite (fGCM) concentration in free-ranging rhesus macaques across all observations.

**Table 3**  
Results of LMMs conducted for model set 2 and model set 3

	Model set 2 <sup>a</sup>			Model set 3 <sup>b</sup>	
	Mother follow	Time on nipple	Distress vocalization	SDB	fGCM concentration
Maternal rejection	NS	<b><math>P &lt; 0.001</math></b>	<b><math>P &lt; 0.001</math></b>	NS	<b><math>P = 0.023</math></b>
Mother follow		<b><math>P = 0.036</math></b>	NS	NS	$P = 0.055$
Time on nipple			<b><math>P = 0.002</math></b>	NS	NS
Distress vocalization				NS	NS
SDB					NS

SDB: self-directed behaviour; fGCM: faecal glucocorticoid metabolite.

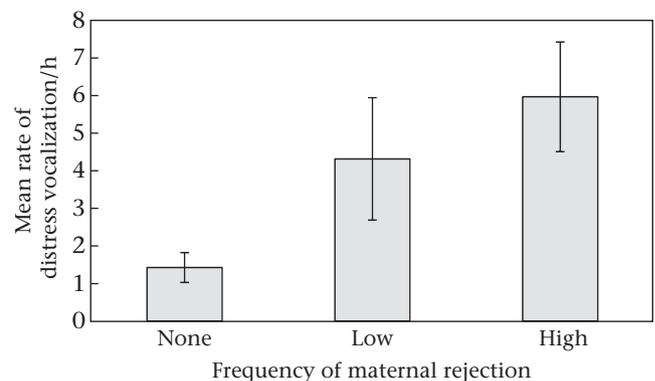
<sup>a</sup> Models tested for relationships between independent behavioural variables (i.e. mother–infant interactions and behavioural indicators of infant anxiety).

<sup>b</sup> Models tested whether fGCM concentration (dependent variable) varied with respect to maternal interaction variables or behavioural indicators of anxiety (independent variables).

Each observation was a single data point for each type of variable, meaning that analyses were simultaneously within and between subjects, depending on the number of observations and faecal samples for each individual. To control for repeated observations of the same individual and to avoid pseudoreplication, infant ID was included as a random factor in all LMMs. Infant age was incorporated as a random factor in the second and third set of models to control for possible behavioural changes across development, or age effects on fGCM concentration, respectively. All tests were two tailed with  $P < 0.05$  considered significant, and all analyses were performed in SPSS 22.0 (IBM, Armonk, NY, U.S.A.).

## RESULTS

Infant age was negatively associated with fGCM concentration ( $F_{1,201.5} = 46.8$ ,  $P < 0.001$ ; Fig. 1); therefore, age was controlled for in all subsequent analyses. Infants gave more distress vocalizations when experiencing maternal rejection ( $F_{1,188.4} = 17.74$ ,  $P < 0.001$ ; Fig. 2), suggesting that they found rejection to be stressful. There was a positive relationship between time spent on the nipple and rates of distress vocalizations ( $F_{1,213.4} = 9.71$ ,  $P = 0.002$ ; Table 3, model set 2). There was no relationship between rates of self-directed behaviours and vocalizations, or the three maternal interaction variables (all  $P$ s  $> 0.1$ ; Table 3, model set 2). Increased rates of mother following were associated with increased time on the nipple ( $F_{1,214.6} = 4.46$ ,  $P = 0.036$ ; Table 3, model set 2). There was a positive relationship between rates of maternal rejection and time on the nipple ( $F_{1,195.0} = 28.6$ ,  $P < 0.001$ ; Table 3, model set 2).



**Figure 2.** Mean  $\pm$  SE rate of distress vocalizations by free-ranging rhesus macaque infants across observations in which weaning was not observed (none,  $1.42 \pm 0.40$ ,  $N = 150$ ), was observed at a frequency below the median (low,  $4.31 \pm 1.63$ ,  $N = 34$ ) and was observed at a frequency above the median (high,  $5.97 \pm 1.45$ ,  $N = 35$ ),  $P < 0.001$  (LMM).

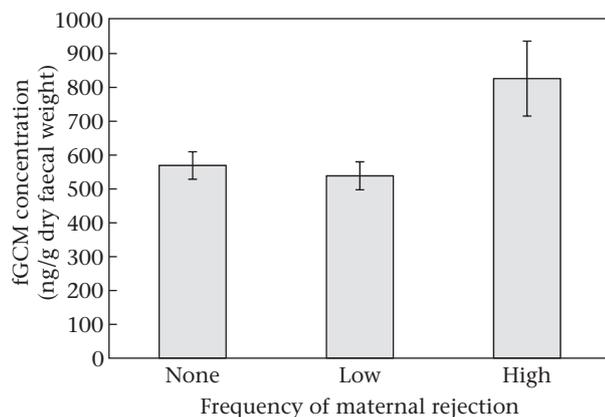
We found a significant positive relationship between the frequency of maternal rejection and fGCM concentration ( $F_{1,206,0} = 5.23$ ,  $P = 0.023$ ; Fig. 3, Table 3, model set 3), which was not affected by activity level (movement:  $P > 0.1$ ) and while controlling for infant age. In addition, there was a nonsignificant tendency for the frequency of mother following to increase with fGCM concentration ( $F_{1,196,0} = 3.73$ ,  $P = 0.055$ ; Table 3, model set 3). We found no relationship between fGCM concentration and the amount of time spent on the nipple, or between fGCM concentration and the frequency of distress vocalizations or self-directed behaviours (all  $P$ s  $> 0.1$ ; Table 3, model set 3).

Looking at our data as two smaller subsets, we found a nonsignificant relationship between maternal rejection rate and fGCM concentration in faecal samples collected 2–4 days following behavioural observation ('post' samples:  $F_{1,32,0} = 3.11$ ,  $P = 0.087$ ), but no relationship between maternal rejection rate and fGCM concentration from 'pre' samples collected prior to observation ( $F_{1,110,0} = 2.62$ ,  $P = 0.109$ ). Similarly, we found a significant relationship between mother following and fGCM concentration for the 'post' samples ( $F_{1,32,0} = 8.76$ ,  $P = 0.006$ ), but not for the 'pre' samples ( $F_{1,115,3} = 2.04$ ,  $P = 0.156$ ).

## DISCUSSION

Our study provides the first evidence for an infant physiological stress response to weaning in a free-ranging population of mammals. Our results show that when infants experience maternal rejection, they have higher fGCM concentrations, suggesting that maternal rejection may be an energetic and/or a psychosocial stressor for the offspring, one to which the body reacts by activating GC release to mobilize energy stores. Analysis of the 'pre' versus 'post' subsets of the faecal sample data revealed that the relationship between fGCM concentration and both maternal rejection rate and mother follow rate was stronger in faecal samples collected 2–4 days after observation than it was in samples collected prior to observation. This suggests that it was not changes in glucocorticoid concentrations that led to variation in behaviour, but rather variation in behaviour that led to changes in glucocorticoid concentrations.

Rates of infant distress vocalizations and maternal rejection were positively correlated, but there was no significant relationship between distress vocalizations and fGCM concentration, suggesting that infants do experience distress when being rejected, but that changes in GC concentration in relation to maternal rejection are



**Figure 3.** Mean  $\pm$  SE faecal glucocorticoid metabolite (fGCM) concentration of free-ranging rhesus macaque infants across observations in which weaning was not observed (none,  $568.8 \pm 40.4$ ,  $N = 150$ ), was observed at a frequency below the median (low,  $538.3 \pm 41.5$ ,  $N = 34$ ) and was observed at a frequency above the median (high,  $825.2 \pm 110.6$ ,  $N = 35$ ).  $P = 0.023$  (LMM).

more likely to be indicative of energetic stress rather than psychological stress. Although it is not possible to infer causality from our correlational results, overall our results suggest that infants show physiological and behavioural changes concurrently with maternal rejection, which potentially act in concert to reduce the energetically and psychologically stressful effects of reduced access to resources during the weaning period.

Time spent on the nipple and rates of rejection were positively correlated, suggesting either that mothers rejected infants that spent more time on the nipple more frequently, or that the more infants made nipple contact the more they were rejected. It is difficult to determine whether more time spent on the nipple is associated with increased milk intake (see Hinde, Power, & Oftedal, 2009; Hinde, 2009, for evidence of variation in milk content and flow among rhesus macaque mothers). Although it is currently impossible to measure the quality and quantity of milk that each infant receives accurately in a free-ranging primate population, collection of urine samples from infants to quantify body condition and energy stores via urinary C-peptide analysis (e.g. Girard-Buttaz et al., 2011) is a feasible option that should be incorporated into future studies of parent–offspring conflict.

fGCM concentrations and rates of mother following tended towards being positively correlated, which may suggest that infants that experience more weaning stress increase their efforts at maintaining proximity to their mothers. However, an alternative explanation is that infant fGCM concentrations rose as a response to increased mother following, which might be potentially stressful. Although faecal samples were collected as frequently as possible, sampling was inevitably patchy because of the difficulty associated with sampling young infants. Future research on weaning stress in primates would benefit from increased frequency of behavioural and physiological sampling as this could clarify the precise relationship between these variables.

The finding that infants gave more distress vocalizations when experiencing rejection suggests that infants find rejection to be a distressing event, even though we did not find any relationship between rates of self-directed behaviour or distress vocalizations and fGCM concentrations. Although it is possible that infants that gave more distress vocalizations were rejected more frequently, this seems very unlikely as there is ample research across primates to suggest that rejection is often perceived as a distressing event, with infants reacting to rejection with distress vocalizations (reviewed in Maestripieri, 2002). In addition, it is unlikely that infants that vocalized more frequently were attempting to elicit more resources as there was no relationship between distress vocalizations and mother following, or between mother following and maternal rejection.

There are several potential explanations for the dissociation between behavioural and physiological indicators of stress (reviewed in Higham, Maclarnon, Heistermann, Ross, & Semple, 2009). First, fGCM concentrations are an integrated measure of HPA axis activity, reflecting the past 2–3 days of activity, whereas distress vocalizations are an acute reaction to an unpleasant experience (i.e. maternal rejection). Thus, distress vocalizations are possibly reflective of anxiety and acute activation of the HPA axis or SNS, in which case an integrated, long-term measure of stress reactivity is on the wrong timescale to pick up short-term increases in anxiety in response to maternal rejection. As such, a noninvasive measure of acute stress response (e.g. salivary cortisol) might better pick up quick changes in HPA axis reactivity in response to a distressing event and correlate with behavioural indicators of acute stress more accurately. Previous research in captive rhesus macaques has demonstrated changes in salivary cortisol in response to a psychosocial stressor (Lutz, Marinus, Chase, Meyer, & Novak, 2003). Distress vocalizations also may be stimulated by activation

of the SNS and catecholamine release, in which case assessment of SNS activity, and not HPA axis activity, would be most appropriate (e.g. measurement of salivary alpha-amylase, sAA). Recent advances in noninvasive endocrine assessment have made analysis of salivary cortisol and sAA possible in free-ranging rhesus macaques (Higham, Vitale, Rivera, Ayala, & Maestripieri, 2010). Finally, it is possible that distress vocalizations are used as a coping strategy either to reduce anxiety (e.g. Levine & Wiener, 1988) or in an attempt to elicit additional resources from the mother (e.g. Semple, Gerald, & Suggs, 2009), especially as we found a relationship between rates of distress vocalizations and time spent in contact with the nipple. Additional research is necessary to clarify the exact relationship between physiological or behavioural indicators of stress and arousal on a more immediate timescale than fGCM analyses permit.

Examining the infant's short-term physiological responses to maternal rejection can be important for understanding the potential costs of mother–infant conflict and, therefore, have important implications for its evolutionary significance. As GC release results in a cascade of metabolic changes that improve one's ability to cope with temporary food shortage (reviewed in Sapolsky et al., 2000), increased GC concentrations in response to maternal rejection and a reduction in resources would appear to be a highly adaptive response. In addition to helping an infant cope with potentially reduced resources, stressor-induced releases of GCs and catecholamines also have immediate, short-term cognitive consequences, such as enhancing memory and learning (reviewed in McEwen & Sapolsky, 1995). However, the long-term physiological and cognitive consequences of natural, temporary stressors, such as weaning, are less well studied. Although chronic elevation of GCs has been shown to result in dendritic atrophy and cognitive impairments (McEwen & Sapolsky, 1995), it is possible that moderate exposure to stressors, and thus moderate amounts of HPA axis and SNS activation, actually exert a beneficial effect on an individual, shaping how individuals interact with and learn from their environment (Parker & Maestripieri, 2011).

Previous studies have shown that exposure to different rates of maternal rejection early in life affects the infant's anxiety and behavioural reactivity to the environment later in life in primates (Maestripieri et al., 2006; Fairbanks, 2003). For example, in Japanese macaques, maternal rejection leads to increased and earlier independence in infants, and maternal protectiveness delays it (Bardi & Huffman, 2002). Studies have also shown that exposure to different rates of maternal rejection early in life can result in long-term changes in the activity of stress-sensitive physiological systems such as the brain serotonergic system and the HPA axis (Maestripieri et al., 2006; Sanchez et al., 2010), which play an important role in emotion regulation and stress reactivity. Thus, studying and understanding the short-term effects of maternal behaviour on infant physiological systems can be crucial for understanding how early experience can result in long-term behavioural, emotional and neuroendocrine alterations later in life.

## Acknowledgments

We thank Julie Cascio, Sean Coyne, Constance Dubuc, Jesus Madrid, Greg Ruber and Paola Rullan for assistance in collecting behavioural and physiological data, and the staff of the Caribbean Primate Research Center (CPRC) for logistical assistance and maintenance of the census records. We are extremely grateful to Andrea Heistermann for analysing the faecal samples, to Luis Jimenez and Rory McDaniel for technical assistance with data management and to John Addicott for helping us to set up our Access database. We thank Jeffrey French and two anonymous referees for their constructive and insightful feedback. This

research was supported by National Institutes of Health (NIH) grant R01-HD067175 to D.M. and K.J.P. This project was supported by grant number 8 P40 OD012217-25 from the National Centre for Research Resources (NCRR) and the Office of Research Infrastructure Programs (ORIP) of NIH to the CPRC of the University of Puerto Rico. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of NCRR or NIH.

## References

- Altmann, J. (1974). Observational study of behavior: sampling methods. *Behaviour*, 49, 227–266.
- Altmann, J. (1980). *Baboon mothers and infants*. Cambridge, MA: Harvard University Press.
- Altmann, J., & Samuels, A. (1992). Costs of maternal care: infant carrying in baboons. *Behavioral Ecology and Sociobiology*, 29, 391–398.
- Bardi, M., & Huffman, M. A. (2002). Effects of maternal style on infant behavior in Japanese macaques (*Macaca fuscata*). *Developmental Psychobiology*, 41, 364–372.
- Berger, J. (1979). Weaning conflict in desert and mountain bighorn sheep (*Ovis canadensis*): an ecological interpretation. *Zeitschrift für Tierpsychologie*, 200, 188–200.
- Berman, C. M., Rasmussen, K. L. R., & Suomi, S. J. (1993). Reproductive consequences of maternal care patterns during estrus among free-ranging rhesus monkeys. *Behavioral Ecology and Sociobiology*, 32, 391–399.
- Bøe, K. (1991). The process of weaning in pigs: when the sow decides. *Applied Animal Behaviour Science*, 30, 47–59.
- Caro, T. M., Roper, R., Young, M., & Dank, G. (1979). Inter-observer reliability. *Behaviour*, 69, 303–315.
- Codner, M. A., & Nadler, R. D. (1984). Mother–infant separation and reunion in the great apes. *Primates*, 25, 204–217.
- Colson, V., Orgeur, P., Foury, A., & Mormède, P. (2006). Consequences of weaning piglets at 21 and 28 days on growth, behaviour and hormonal responses. *Applied Animal Behaviour Science*, 98, 70–88.
- DeVore, I. (1963). Mother–infant relations in free-ranging baboons. In H. L. Rieingold (Ed.), *Maternal behavior in mammals* (pp. 305–335). New York, NY: Wiley.
- Fairbanks, L. A. (2003). Attachment. In D. Maestripieri (Ed.), *Primate psychology* (pp. 144–170). Cambridge, MA: Harvard University Press.
- Gauthier, D., & Barrette, C. (1985). Suckling and weaning in captive white-tailed and fallow deer. *Behaviour*, 94, 128–149.
- Girard-Buttoz, C., Higham, J. P., Heistermann, M., Wedegärtner, S., Maestripieri, D., & Engelhardt, A. (2011). Urinary C-peptide measurement as a marker of nutritional status in macaques. *PLoS One*, 6, e18042.
- Gordon, T. (1981). Reproductive behavior in the rhesus monkey: social and endocrine variables. *American Zoologist*, 21, 185–195.
- Gunnar, M., Gonzalez, C., Goodlin, B., & Levine, S. (1981). Behavioral and pituitary-adrenal responses during a prolonged separation period in infant rhesus macaques. *Psychoneuroendocrinology*, 6, 65–75.
- Heistermann, M., Ademmer, C., & Kaumanns, W. (2004). Ovarian cycle and effect of social changes on adrenal and ovarian function in *Pygathrix nemaeus*. *International Journal of Primatology*, 25, 689–708.
- Heistermann, M., Finke, M., & Hodges, J. K. (1995). Assessment of female reproductive status in captive-housed Hanuman langurs (*Presbytis entellus*) by measurement of urinary and fecal steroid excretion patterns. *American Journal of Primatology*, 37, 275–284.
- Heistermann, M., Palme, R., & Ganswindt, A. (2006). Comparison of different enzymeimmunoassays for assessment of adrenocortical activity in primates based on fecal analysis. *American Journal of Primatology*, 273, 257–273.
- Hennessy, M. B. (1986). Multiple, brief maternal separations in the squirrel monkey: changes in hormonal and behavioral responsiveness. *Physiology & Behavior*, 36, 245–250.
- Higham, J. P., Maclarnon, A. M., Heistermann, M., Ross, C., & Semple, S. (2009). Rates of self-directed behaviour and faecal glucocorticoid levels are not correlated in female wild olive baboons (*Papio hamadryas anubis*). *Stress*, 12, 526–532.
- Higham, J. P., & Maestripieri, D. (2010). Revolutionary coalitions in male rhesus macaques. *Behaviour*, 147, 1889–1908.
- Higham, J. P., Vitale, A. B., Rivera, A. M., Ayala, J. E., & Maestripieri, D. (2010). Measuring salivary analytes from free-ranging monkeys. *Physiology & Behavior*, 101, 601–607.
- Hinde, K. (2009). Richer milk for sons but more milk for daughters: sex biased investment during lactation varies with maternal life history in rhesus macaques. *American Journal of Human Biology*, 21, 512–519.
- Hinde, K., Power, M. L., & Oftedal, O. T. (2009). Rhesus macaque milk: magnitude, sources, and consequences of individual variation over lactation. *American Journal of Physical Anthropology*, 138, 148–157.
- Hinde, R. A. (1977). Mother–infant separation and the nature of inter-individual relationships: experiments with rhesus monkeys. *Proceedings of the Royal Society B: Biological Sciences*, 196, 29–50.
- Hinde, R. A., & Atkinson, S. (1970). Assessing the roles of social partners in maintaining mutual proximity, as exemplified by mother–infant relations in rhesus monkeys. *Animal Behaviour*, 18, 169–176.

- Hinde, R. A., & Spencer-Booth, Y. (1967). The behaviour of socially living rhesus monkeys in their first two and a half years. *Animal Behaviour*, *15*, 169–196.
- Hinde, R. A., & Spencer-Booth, Y. (1970). Individual differences in the responses of rhesus monkeys to a period of separation from their mothers. *Journal of Child Psychology and Psychiatry*, *11*, 157–176.
- Hinde, R. A., & Spencer-Booth, Y. (1971). Effects of brief separation from mother on rhesus monkeys. *Science*, *173*, 111–118.
- Hodges, J. K., & Heistermann, M. (2011). Field endocrinology: monitoring hormonal changes in free-ranging primates. In J. M. Setchell, & D. J. Curtis (Eds.), *Field and laboratory methods in primatology: A practical guide* (pp. 353–370). Cambridge, UK: Cambridge University Press.
- Hoffman, C. L., Higham, J. P., Heistermann, M., Coe, C. L., Prendergast, B. J., & Maestripieri, D. (2011). Immune function and HPA axis activity in free-ranging rhesus macaques. *Physiology & Behavior*, *104*, 507–514.
- Jarvis, S., Moinard, C., Robson, S. K., Sumner, B. E. H., Douglas, A. J., Seckl, J. R., et al. (2008). Effects of weaning age on the behavioural and neuroendocrine development of piglets. *Applied Animal Behaviour Science*, *110*, 166–181.
- Jay, P. C. (1963). Mother–infant relations in langurs. In H. L. Rheingold (Ed.), *Maternal behavior in mammals* (pp. 282–304). New York, NY: Wiley.
- Lee, P. (1984). Ecological constraints on the social development of vervet monkeys. *Behaviour*, *91*, 245–262.
- Lee, P., Majluf, P., & Gordon, I. (1991). Growth, weaning and maternal investment from a comparative perspective. *Journal of Zoology*, *225*, 99–114.
- Levine, S., Wiener, S., & Coe, C. (1993). Temporal and social factors influencing behavioral and hormonal responses to separation in mother and infant squirrel monkeys. *Psychoneuroendocrinology*, *18*, 297–306.
- Levine, S., & Wiener, S. G. (1988). Psychoendocrine aspects of mother–infant relationships in nonhuman primates. *Psychoneuroendocrinology*, *13*, 143–154.
- Lutz, C., Marinus, L., Chase, W., Meyer, J., & Novak, M. (2003). Self-injurious behavior in male rhesus macaques does not reflect externally directed aggression. *Physiology & Behavior*, *78*, 33–39.
- Maestripieri, D. (1993a). Maternal anxiety in rhesus macaques (*Macaca mulatta*) II. Emotional bases of individual differences in mothering style. *Ethology*, *95*, 19–31.
- Maestripieri, D. (1993b). Maternal anxiety in rhesus macaques (*Macaca mulatta*): I. Measurement of anxiety and identification of anxiety-eliciting situations. *Ethology*, *95*, 19–31.
- Maestripieri, D. (1994a). Mother–infant relationships in three species of macaques (*Macaca mulatta*, *M. nemestrina*, *M. arctoides*). I. Development of the mother–infant relationship in the. *Behaviour*, *131*, 75–96.
- Maestripieri, D. (1994b). Mother–infant relationships in three species of macaques (*Macaca mulatta*, *M. nemestrina*, *M. arctoides*). II. The social environment. *Behaviour*, *131*, 97–113.
- Maestripieri, D. (2002). Parent–offspring conflict in primates. *International Journal of Primatology*, *23*, 923–951.
- Maestripieri, D., Higley, J. D., Lindell, S. G., Newman, T. K., McCormack, K. M., & Sanchez, M. M. (2006). Early maternal rejection affects the development of monoaminergic systems and adult abusive parenting in rhesus macaques (*Macaca mulatta*). *Behavioral Neuroscience*, *120*, 1017–1024.
- Maestripieri, D., Schino, G., Aureli, F., & Troisi, A. (1992). A modest proposal: displacement activities as an indicator of emotions in primates. *Animal Behaviour*, *44*, 967–979.
- Mastorakos, G., Pavlatou, M., Diamanti-Kandarakis, E., & Chrousos, G. P. (2005). Exercise and the stress system. *Hormones*, *4*, 73–89.
- McEwen, B. S. (2001). From molecules to mind. Stress, individual differences, and the social environment. *Annals of the New York Academy of Sciences*, *935*, 42–49.
- McEwen, B. S., & Sapolsky, R. M. (1995). Stress and cognitive function. *Current Opinion in Neurobiology*, *5*, 205–216.
- Parker, K. J., & Maestripieri, D. (2011). Identifying key features of early stressful experiences that produce stress vulnerability and resilience in primates. *Neuroscience and Biobehavioral Reviews*, *35*, 1466–1483.
- Poletto, R., Steibel, J. P., Siegford, J. M., & Zanella, A. J. (2006). Effects of early weaning and social isolation on the expression of glucocorticoid and mineralocorticoid receptor and 11 $\beta$ -hydroxysteroid dehydrogenase 1 and 2 mRNAs in the frontal cortex and hippocampus of piglets. *Brain Research*, *1067*, 36–42.
- Rahkovskaya, M., & Heistermann, M. (n.d.). [Faecal glucocorticoid metabolite (fGCM) concentrations of rhesus macaques on Cayo Santiago, Puerto Rico]. Unpublished raw data.
- Rawlins, R. G., & Kessler, M. J. (1986). *The Cayo Santiago macaques: History, behavior, and biology*. Albany, NY: State University of New York Press.
- Romero, L. M., Dickens, M. J., & Cyr, N. E. (2009). The reactive scope model: a new model integrating homeostasis, allostasis, and stress. *Hormones and Behavior*, *55*, 375–389.
- Sanchez, M. M., McCormack, K., Grand, A. P., Fulks, R., Graff, A., & Maestripieri, D. (2010). Effects of sex and early maternal abuse on adrenocorticotropin hormone and cortisol responses to the corticotropin-releasing hormone challenge during the first 3 years of life in group-living rhesus monkeys. *Development and Psychopathology*, *22*, 45–53.
- Sapolsky, R. M., Romero, L. M., & Munck, A. U. (2000). How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. *Endocrine Reviews*, *21*, 55–89.
- Semple, S., Gerald, M. S., & Suggs, D. N. (2009). Bystanders affect the outcome of mother–infant interactions in rhesus macaques. *Proceedings of the Royal Society B: Biological Sciences*, *276*, 2257–2262.
- Trivers, R. (1974). Parent–offspring conflict. *American Zoologist*, *14*, 249–264.
- Watson, D. L., & Gill, H. S. (1991). Effect of weaning on antibody responses and nematode parasitism in Merino lambs. *Research in Veterinary Science*, *51*, 128–132.