Hormones and Behavior

The Official Journal of the Society for Behavioral Neuroendocrinology

Available online at
ScienceDirect
www.sciencedirect.com

Focused grooming networks and stress alleviation in wild female baboons

Roman M. Wittig [a,*], Catherine Crockford [b], Julia Lehmann [c], Patricia L. Whitten [d],
Robert M. Seyfarth [b], Dorothy L. Cheney [a]

[a] *Department of Biology, University of Pennsylvania, Philadelphia, PA 19104, USA*
[b] *Department of Psychology, University of Pennsylvania, Philadelphia, PA 19104, USA*
[c] *School of Biological Sciences, University of Liverpool, Liverpool, L69 7ZB, UK*
[d] *Department of Anthropology, Emory University, Atlanta, GA 30322, USA*

Abstract

We examine the relationship between glucocorticoid (GC) levels and grooming behavior in wild female baboons during a period of instability in the alpha male rank position. All females' GC levels rose significantly at the onset of the unstable period, though levels in females who were at lower risk of infanticide began to decrease sooner in the following weeks. Three factors suggest that females relied on a focused grooming network as a coping mechanism to alleviate stress. First, all females' grooming networks became less diverse in the weeks following the initial upheaval. Second, females whose grooming had already focused on a few predictable partners showed a less dramatic rise in GC levels than females whose grooming network had been more diverse. Third, females who contracted their grooming network the most experienced a greater decrease in GC levels in the following week. We conclude that close bonds with a few preferred partners allow female baboons to alleviate the stress associated with social instability.
© 2008 Elsevier Inc. All rights reserved.

Keywords: Female baboons; Coping mechanism; Social stressors; Fecal glucocorticoids; Grooming; Affiliation; Social instability

Introduction

Allostasis is the process by which organisms adjust to threats – whether real or imagined – to survival and reproduction (McEwen and Wingfield, 2003). Over the short term, an increase in allostatic load is adaptive, because it is usually accompanied by an increase in glucocorticoid (GC) concentrations, which trigger behaviors that help the organism cope with environmental and social challenges. When successful, these behaviors subsequently also function to lower GC levels, allowing the organism to avoid the deleterious effects of chronic stress (Sapolsky et al., 2000; Goymann and Wingfield, 2004). Grooming appears to be one of the primary coping strategies adopted by female monkeys to reduce allostatic load (Boccia et al., 1989; Aureli et al., 1999; Gust et al., 1993; Shutt et al., 2007). Little is known, however, about how and why grooming might function to alleviate stress. For example, do females' grooming patterns change during periods of social instability? If so, do these changes cause GC levels to decline?

The grooming interactions of female baboons (*Papio hamadryas* ssp.), like those of females in other monkey species, are highly differentiated. Although a female may groom with all other females in her group during the course of a year, most of her grooming is concentrated among a few, predictable partners who are usually, but not always, close female kin (Silk et al., 2006a,b; Silk, 2007). These focused grooming interactions appear to be adaptive, because socially integrated females experience higher infant survival than less integrated ones (Silk et al., 2003b).

The importance of a stable social network is also reflected in females' GC levels. In a previous study of female baboons (*Papio hamadryas ursinus*) living in the Okavango Delta of Botswana, Engh et al. (2006a) found that females' GC levels rose significantly when they lost a close relative and grooming partner to predation. These 'bereaved' females also temporarily expanded their grooming network in an apparent effort to

* Corresponding author.
E-mail address: rmw12@st-andrews.ac.uk (R.M. Wittig).

identify new partners. In contrast, during periods of social and demographic stability, when their grooming networks are intact and undamaged, female baboons who restrict the majority of their grooming interactions to a few, consistent partners have lower GC levels than females whose grooming networks are more diffuse (Crockford et al., 2008). A focused grooming network may function to lower GC levels in part because it provides females with a dependable and controllable number of social partners. Social support is well known to alleviate psychological stress in both humans and other animals (reviewed by e.g. Carter, 1998; Panskepp, 1998; Thorsteinsson and James, 1999; DeVries et al., 2003; Rosal et al., 2004).

The results of the study by Crockford et al. (2008) suggest a number of predictions about females' behavioral responses during periods of social instability. First, as long as their grooming networks are not damaged by the death of a close partner, females who have a focused grooming network should experience a smaller rise in GC levels than females whose grooming networks were more diffuse. Second, all females should attempt to alleviate stress by further restricting their grooming interactions to their preferred partners, and this behavior should cause their GC levels to decrease (Crockford et al., 2008). Here we test these predictions by examining changes in females' grooming interactions and GC levels following two psychological stressors: male immigration and upheaval in the male dominance hierarchy. In the Okavango Delta, male immigrants who achieve alpha status are known to commit infanticide, and both immigration and instability in the alpha male rank position have been shown to elevate GC levels in lactating females (Beehner et al., 2005; Engh et al., 2006b).

Methods

Subjects and study site

Data were gathered between May and November 2005, during a period of instability in the male dominance hierarchy and following the period of social and demographic stability documented in Crockford et al. (2008). Data collection was part of a long-term study of wild chacma baboons in the Moremi Game Reserve in the Okavango Delta of Botswana (Cheney and Seyfarth, 2007). The group has been observed since 1978 and all animals are fully habituated to human observers on foot. At the time of this study, the group contained approximately 70 individuals, including nine adult males and 24 adult females (six years and older). The subjects of this study were 22 reproductive adult females.

As in other species of Old World monkeys, female baboons form stable, linear dominance hierarchies based on the direction of approach–retreat interactions and aggression. Females assume ranks similar to their mothers', so that matrilineal relatives typically occupy adjacent ranks (Silk et al., 1999, 2006a,b; Cheney et al., 2004). Females remain in their natal groups throughout their lives and maintain close bonds with close matrilineal kin, whereas males usually emigrate to neighbouring groups at sexual maturity.

Events during the time of male rank instability

After over eight months of no challenges to the alpha position (Crockford et al., 2008), adult male FT re-immigrated into the group on 31 May 2005 and began to challenge the alpha male SO. On 4 June, SO disappeared, leaving the alpha position vacant. There ensued several months of intense competition, initially involving FT and a natal adult male NA. On 4 August 2005 another male HA immigrated into the group and also began competing for the alpha position.

HA finally attained the alpha position on 5 October. NA and FT continued to fight for the beta position until NA defeated FT on 15 October. No further changes in the top of the male dominance hierarchy occurred in the ensuing two months.

External stressors are known to affect females differently in different reproductive states depending upon the relative risk the stressor imposes (French et al., 2004; Engh et al., 2006b; Beehner et al., 2006; Power and Schulkin, 2006). In the Okavango, dominant immigrant males are often infanticidal, Cheney et al., 2004) and therefore pose a significant threat to lactating females. This threat is reflected in increased glucocorticoid levels of lactating females after male immigration (Engh et al., 2006b). In this paper, therefore, we classify lactating females as being at *higher risk* (*HR*) of infanticide. Due in part to sample size constraints, we also classify females in late pregnancy (i.e. in the third trimester, or last two months of gestation) as being at *higher risk*. Conversely, cycling and females in early pregnancy (i.e. in the first two trimesters of gestation) are classified as being at *lower risk* (*LR*) of infanticide. We divide pregnant females into these two categories for two reasons.

First, and most important, there were no differences in the increase in GC levels between cycling and early pregnant females in the week after male immigration (cycling: GC increase$\pm$SD$=101.4$ ng/g±51.3, $N=5$; early pregnancy: GC increase$\pm$SD$=91.3$ ng/g±46.7, $N=5$; t-test: $t=0.325$, DF$=8$, $P=0.753$), or between lactating females and late pregnant females (lactating: GC increase$\pm$SD$=166.8$ ng/g±87.9, $N=5$; late pregnancy: GC increase$\pm$SD$=274.0$ ng/g±175.8, $N=6$; t-test: $t=1.233$, DF$=9$, $P=0.249$). There was, however, a nearly significant difference in GC level increase between females in early and late pregnancy (t-test: $t=-2.240$, DF$=9$, $P=0.052$).

Second, although two studies of East African baboons have reported an increase in abortion rates in some early pregnant females following male immigration (Pereira, 1983; Alberts et al., 1992), no cases of abortion following male immigration have been observed in our study group. Instead, infant mortality is due to infanticide that occurs largely during the first few months after the immigrant male starts to challenge the alpha position. Because the likelihood of infanticide varies depending on the number of resident males in the group at the time, their relative competitive abilities, and the timing of the immigrant's rise to the alpha position (Palombit et al., 2000), it is not possible to demarcate the precise period of infanticidal risk. We could document 18 cases of confirmed or strongly suspected infanticide (Cheney et al., 2004) involving females who were pregnant when a dominant male immigrated into the group. Fifteen females were in the last trimester of pregnancy when the male immigrated, and three were in the first two trimesters. It therefore appears that the greatest risk of infanticide falls not only on lactating females but also on pregnant females during their last months of gestation.

Because this paper focuses on the relationship between grooming and GC levels following a stress-inducing event, we restrict our analysis mainly to one six-week period: the week before, the week during, and the four weeks after the initial male rank upheaval on 31 May. During this time period there were no other events known to influence females' GC levels, such as predation and infanticide. After 4 July there were numerous other destabilizing events that would have confounded our analysis (3 cases of probable predation, 3 probable infanticides, 1 additional male immigration, 2 re-immigrations, regular intergroup hostility, and temporary associations with other baboon groups). We discuss these events elsewhere (Cheney et al. in preparation).

Behavioral sampling

We conducted 10-min focal animal sampling (Altmann, 1974) on each adult female at least once per week. During each focal sample we recorded all of the subject's social and vocal interactions (including aggression, submission, grooming, proximity etc.; see Crockford et al., 2008), as well as all approaches to within 2 m of the subject. We also used ad libitum sampling (Altmann, 1974) to note all grooming interactions and the grooming partners involved.

Hormone collection and analysis

We extracted hormones from a total of 532 fecal samples during the study period for the entire unstable period. 136 samples were obtained from 21 females from the week before the immigration event and from 22 females after the initial immigration event (one female had just started her first cycle ever and was added to the fecal sample list the week of the immigration event). Each

individual was sampled once a week, and no female was sampled twice before a sample had been obtained from all other subjects. All samples were collected between 6:20am and 12:30pm (mean±SD of sample collection time=9:55am± 1:06 h), and GC levels did not vary with the hour of collection: GC±SD=304.3 ng/ g [range=279.8–326.2]±175.3 [range=151.5–189.3]). Thus, confounding effects due to diurnal changes in GC levels (van Cauter, 1990; Gust et al., 2000; Touma and Palme, 2005) were unlikely.

Hormones were extracted from feces in the field using methods described by Beehner and Whitten (2004; see also Beehner et al., 2005) and analyzed in the laboratory of Dr. Patricia Whitten (Emory University). All samples were assayed for GC metabolites using a commercially available radioimmunoassay (RIA) kit (for a detailed description see Crockford et al., 2008). This method has been validated for use with baboons (Beehner and Whitten, 2004) and has been used in several preceding studies of the same population (Beehner et al., 2005; Bergman et al., 2005; Engh et al 2006a,b; Crockford et al., 2008). The intra-assay coefficients of variation (CV) averaged 4.4% over 512 sample duplicates ranging from 0.003–49 pg/ml. The inter-assay CVs for low and high serum controls from 17 assays were 15.1% and 8.6%, respectively. This research was reviewed and approved by the Animal Care and Use Committee at the University of Pennsylvania (Protocol #19001).

Demographic and behavioral variables

We examined four individual and five behavioral attributes that might affect GC levels.

1. *Reproductive state.* We classified four reproductive states: cycling (females in any stage of the estrous cycle); two states of pregnancy (assigned post hoc from the birth of an infant and starting at the final detumescence of the oestrus cycle): early pregnancy (first four months, or 1st and 2nd trimester), and late pregnancy (last two months, or last trimester); and lactating (the period following birth until the resumption of cycling). Females develop a red perenium during pregnancy, facilitating the detection of any fetal loss.
2. *Age (or: year of birth).* Aging in captive rhesus macaques, rats and humans seems to blunt the stress response (Gust et al., 2000). It may also influence GC levels in wild populations. The birth dates of all females were known (Cheney et al., 2004).
3. *Rank.* Each female's dominance rank was calculated monthly based on the direction of approach–retreat interactions (Silk et al., 1999). All females' ranks remained stable during the study period.
4. *Number of close female relatives* was calculated as the number of females aged 3 years and older who were related to the subject through the maternal line by more than $r \geq 0.25$ (i.e. mothers, daughters, matrilineal sisters, grandmothers, grand-daughters). Matrilineal relatedness was known for all females (Cheney et al., 2004).
5. Two rates of aggression were calculated:
a.) Rate of aggression received was calculated as the number of aggressive events individuals received from other group members per observation time.
b). Rate of aggression given was calculated as the number of aggressive events individuals directed to other group members per observation time.
6. Three measures of grooming among females were calculated:
a) *Shannon-Wiener Diversity Index (SWDI)* measured the diversity of a female's grooming partners. The *SWDI* was calculated using the formula from Wilson and Bossert (1971): $SWDI = -\sum_{i=1}^{s} p_i \log p_i$, with p_i being the proportion of grooming events with female i, s the number of female grooming partners, and log the natural logarithm. This index is a measure not of grooming network size but of grooming partner skew. For example, an individual who grooms with a large number of other females but restricts most of her grooming to one to two preferred partners, will have a low SWDI. Conversely, an individual who grooms with a large number of other females but distributes her grooming evenly among all her partners, will have a high SWDI. Previous research has found that females with a low SWDI during periods of social stability have lower GC levels than those with a high SWDI (Crockford et al., 2008).
b) *Hinde index for grooming partners (HIGP)* measured the proportion of partners whom a subject groomed relative to those who groomed the subject (derived from Hinde and Akinson, 1970): $HIGP = \frac{\#P_A}{\#(P_A + P_P)}$ with P_A being partners that the subject groomed and P_P being partners that the subject was
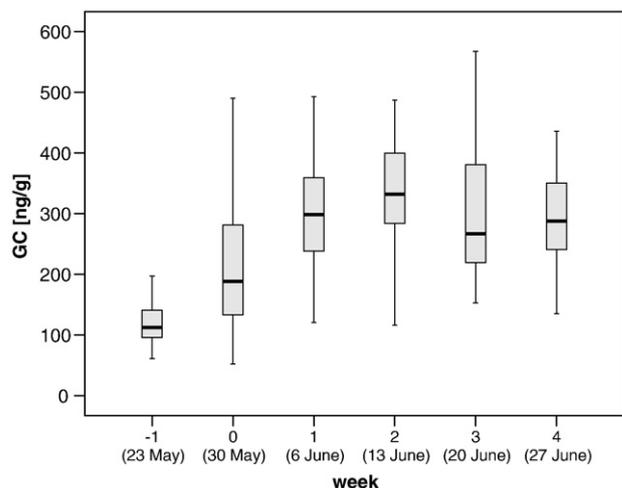

Fig. 1. Weekly glucocorticoid (GC) levels of all adult females between 23 May and 3 July 2007. FT re-immigrated on 31 May and SO disappeared on 4 June (both week 0), starting the male rank instability. All females have been lumped together. Thick lines show averages, boxes represent 50% and whiskers show SD.

groomed by). This index is a measure for skew in active versus passive grooming. A high *HIGP* indicates that the subject grooms more females than she is groomed by; a low index indicates that she is groomed by more females than she grooms.
c) *Percent of grooming events with close kin* measured the proportion of events during which subjects engaged in grooming with close kin as opposed to other females. This measure assesses bias toward grooming with close kin.

Statistical Analysis

We designated the week in which FT immigrated and SO disappeared as week 0, and the ensuing weeks as week 1, 2, and so on. The last week of stability preceding week 0 was set as week −1 and served as a baseline for comparison.

We focused the majority of our analyses on the six-week period surrounding the initial challenge to the male dominance hierarchy (Fig. 1), from one week prior to the upheaval (week −1) through the four weeks afterwards (week 4). No other confounding events (e.g. predation, infanticide, further immigration) were observed during this time. We extracted weekly values for each subject's GC level (in most cases there was one sample per individual; on occasions when there was more than one sample, the mean value was used), as well as the four individual attributes, two aggression rates and three grooming variables described above.

In some analyses, we compared absolute values of variables within females between two weeks (e.g. between week −1 and week 1, when the question was about changes due to the start of the rank upheaval). In other analyses, we compared the change within one variable between two consecutive weeks with the change within another variable between two different consecutive weeks. These comparisons allow us to hypothesize a causal relation between a change in one variable and a subsequent change in another. For example, to examine whether the change in *SWDI* during week 2 had an effect on GC levels in week 3, we compared the difference of females' *SWDI* between week 1 and week 2 with the difference of females' GC level between week 2 and week 3.

Although every female in the group engaged in regular grooming interactions with other females, there were some weeks when an individual was not observed in any grooming interactions during our behavioral samples. When this occurred, the female was not included in the *SWDI* analyses involving that week, due to mathematical constrains of the index. There was no pattern to the females who were not observed in grooming bouts from one week to the next. For example, five (all *lower risk*) females were not included in the analysis that compared changes in *SWDI* indices between week−1 and week 1. One female was observed grooming in week −1 but not in week 1, two were not observed grooming in either week, and two were observed grooming in week 1 but not in week −1.

Females who were not observed in grooming bouts in some weeks did not have higher or lower GC levels than other females, nor did they differ from other females in their change in GC levels following the male immigration event.

We used multiple regression analysis to assess the effects of females' individual attributes on changes in GC levels from one week to another. A stepwise backwards method was employed to reduce the number of independent variables until the probability of F for each independent variable was >0.1. We conducted two separate series of multiple regression analyses, depending on the females' risk for infanticide.

Whenever possible we conducted parametric statistics (paired *t*-tests when differences between the samples were normally distributed; Pearson correlations when both variables were normally distributed). Otherwise, Kendall Tau correlations were used.

Sample sizes were not constant across weeks, either because the reproductive states of females had changed or because not all females were observed in grooming interactions in every week. Values outside the 95% confidence interval were treated as outliers. They are presented in the figures but were excluded from the analyses. Tests were performed in SPSS 15.0 (SPSS Inc., Chicago, IL, USA) and conducted as two-tailed with an $\alpha = 0.05$.

Results

Females' physiological responses to male rank instability

After eight months of stability in the alpha male position, FT re-immigrated into the group and began to challenge the alpha male SO. Females' GC levels rose dramatically during this initial week of instability (week 0) and continued to rise in the following week 1 (Fig. 1). We compared the 22 females' GC levels in the week before instability began with those in the week after. All females showed increased GC levels in week 1 compared to week −1 (Fig. 1; paired *t*-test: DF=20, $t = -5.91$, $P < 0.001$). This increase was independent of the relative risk of infanticide. Both *lower risk* (LR) and *higher risk* (HR) females experienced a significant increase in GC levels (LR: paired *t*-test: DF=9, $t = -4.912$, $P = 0.001$; HR: paired *t*-test: DF=10, $t = -4.546$, $P = 0.001$). However, *higher risk* females tended to show a greater increase than *lower risk* females (independent *t*-test: GC increase$_{LR} \pm$SD=116 ng/g$\pm$74.6, GC increase$_{HR} \pm$SD=208 ng/g$\pm$ 151.4, N_{LR}=10, N_{HR}=11, $t = -1.78$, $P = 0.095$).

Over the next three weeks, females' mean GC levels did not change significantly (Fig. 1; paired *t*-tests: week 1 to 2: DF=21, $t = -0.024$, $P = 0.981$; week 2 to 3: DF=21, $t = 0.603$, $P = 0.553$; week 3 to 4: DF=21, $t = -0.240$, $P = 0.813$). Indeed, most females' GC levels remained elevated above baseline for the next 15 weeks.

Three weeks after HA attained the alpha position in October, and two weeks after the resolution of the beta rank position, females' GC levels dropped significantly (GC$_{week20} \pm$SD=385 ng/g$\pm$190.4; GC$_{week21} \pm$SD=146 ng/g$\pm$53.8; paired *t*-test: DF=18, $t = 4.963$, $P < 0.001$), to levels similar to those during the previous stable period (GC$_{week-1} \pm$SD=127 ng/g$\pm$46.7; GC$_{week21} \pm$SD=150 ng/ g$\pm$52.9; paired *t*-test: DF=17, $t = -1.387$, $P = 0.183$).

Behavioral responses to male rank instability

We first conducted within-individual comparisons to examine females' behavioral responses to the destabilizing immigration event. We found that the grooming diversity indices of both *higher risk* (HR) and *lower risk* (LR) females decreased
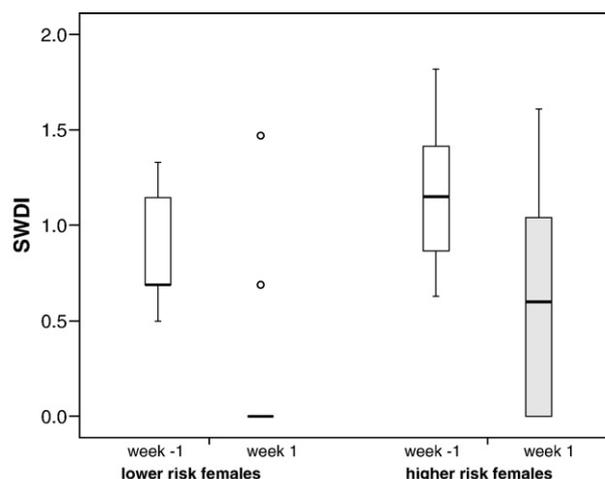

Fig. 2. Grooming diversity (SWDI) of *lower risk* and *higher risk* females during week −1 (white bars: before immigration event) and week 1 (grey bars: after immigration event). Legend as in Fig. 1.

significantly following the start of male rank instability, from week −1 to week 1 (Fig. 2; LR: paired *t*-test: DF=6, $t = 7.349$, $P < 0.001$; HR: paired *t*-test: DF=9, $t = 3.158$, $P = 0.012$;). The decrease in grooming diversity appeared to occur because most females were concentrating their grooming interactions on their close female kin. The percentage of grooming events with close kin increased in week 1 compared to week −1 (paired *t*-test: grooming kin$_{week-1} \pm$SD=29%$\pm$28, grooming kin$_{week1} \pm$SD=65%$\pm$47, DF=13, $t = -2.719$, $P = 0.018$).

Females' grooming diversity indices remained lower than they had been before the onset of instability through week 4 (paired *t*-test, LR: SWDI$_{week-1} \pm$SD=0.89$\pm$0.32, SWDI$_{week4} \pm$ SD=0.28$\pm$0.35, DF=6, $t = 4.205$, $P = 0.006$; HR: SWDI$_{week-1} \pm$ SD=1.20$\pm$0.40, SWDI$_{week4} \pm$SD=0.74$\pm$0.70, DF=9, $t = 1.905$, $P = 0.089$).

The decrease in females' grooming diversity did not occur as a result of a decrease in grooming rates. There was no change in females' grooming rates during the week following the immigration event (paired *t*-test: grooming rate$_{week-1} \pm$SD=139s/ h$^{-1} \pm$156.7, grooming rate$_{week1} \pm$SD=128s/h$^{-1} \pm$292.8, DF=21, $t = 0.143$, $P = 0.888$). Similarly, females showed no change in the Hinde Index of grooming given to grooming received (paired *t*-test: meanHIPG$_{week-1} \pm$SD=0.51$\pm$0.33, meanHIPG$_{week1} \pm$ SD=0.43$\pm$0.40, DF=16, $t = 0.652$, $P = 0.524$).

As predicted (Crockford et al., 2008), we found that cycling and pregnant females with a low grooming diversity index (SWDI) during the three months preceding the male rank upheaval showed smaller increases in GC levels in the week following the event than those who had a higher SWDI (Fig. 3; Pearson: $r = 0.608$, $N = 12$, $P = 0.036$). In contrast, the GC levels of lactating females appeared to be unaffected by their previous SWDI (Pearson lactating: $r = -0.504$, $N = 5$, $P = 0.386$). This result was not surprising; during the previous stable period, lactating females' grooming diversity indices had already been generally higher and less predictable than females' in other reproductive states, and their GC levels had also been correspondingly higher (Crockford et al., 2008).
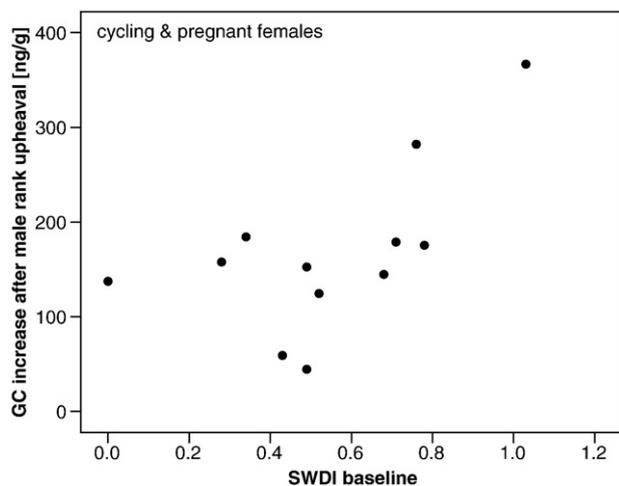
Fig. 3. Correlation between cycling and pregnant females' grooming diversity (*SWDI*) before the male rank upheaval and their increase in glucocorticoid (GC) levels in the following week (increase $= GC_{week1} - GC_{week-1}$). One outlier was removed.

Although there was a general increase in male–male aggression after the immigration of male FT (male aggression given to males$_{week-1} = 0.75$ events h^{-1}; male aggression given to males$_{week1} = 3$ events h^{-1}; $N=8$ males), females received aggression from males at a lower, rather than a higher, rate in the week after the immigration event (male aggression received by females$_{week-1} \pm SD = 0.40$ events h$^{-1} \pm 0.59$; male aggression received by females$_{week1} \pm SD = 0.11$ events h$^{-1} \pm 0.17$; *t*-test: $t=2.422$, DF$=21$, $P=0.025$). Furthermore, there was no change in rates of female–female aggression (female–female aggression$_{week-1} \pm SD = 0.19$ events h$^{-1} \pm 0.07$; female–female aggression$_{week1} \pm SD = 0.08$ events h$^{-1} \pm 0.08$; *t*-test: $t=0.981$, DF$=21$, $P=0.338$).

Table 1
Multiple regression analyses of changes in GC levels between consecutive weeks in *lower risk* females during the weeks following the onset of instability in the male dominance hierarchy

GC level change	F	N	R^2	P	predictor	β	T	P
Week −1 and 1	3.785	10*	0.321	0.088				
					(constant)		2.283	0.044
					# close kin	0.567	1.945	0.088
Week 1 and 2	5.920	10	0.425	0.041				
					(constant)		−1.675	0.132
					rank	0.652	2.433	0.041
Week 2 and 3	–	9	0	NS				
Week 3 and 4	5.581	9	0.444	0.051				
					(constant)		−1.569	0.161
					# close kin	0.666	2,362	0.051

Dependent variable is the difference in GC levels between week X and Y (calculated as difference$=$GC level of week Y $-$ GC level of week X); individual attributes entered in the model were: # close kin, year of birth and rank; only the final models of the *stepwise backward* method are presented in the table; * although there were 11 *lower risk* females in the group, one female was not sampled before the immigration event.
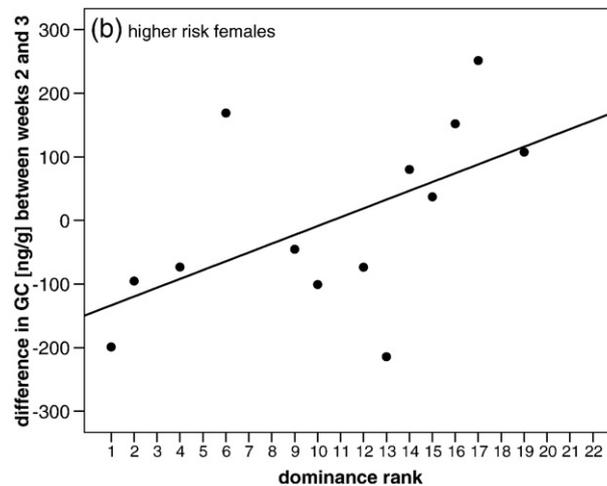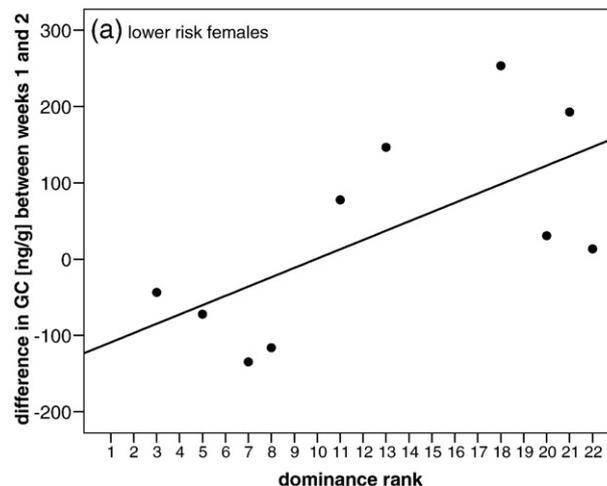

Fig. 4. Regression between females' dominance rank and the change in their glucocorticoid (GC) levels: (a) *lower risk* females' change in GC levels between weeks 1 and 2 (change index $= GC_{week2} - GC_{week1}$) and (b) *higher risk* females' change in GC levels between week 2 and 3 (change index $= GC_{week3} - GC_{week2}$).

The relation between individual attributes and GC levels

We used multiple regression to investigate whether changes in females' GC levels over consecutive weeks were due to individual differences in dominance rank, age, or the number of close female kin. The results of the four regression analyses for *lower risk* females are summarized in Table 1. The only significant model describes changes in week 2. The model shows that 42.5% of the variation in changes in females' GC levels was explained by dominance rank. The GC levels of higher-ranking females started to decrease in week 2 (Fig. 4), while low-ranking females' GC levels continued to increase.

The results of the four regression analyses for *higher risk* females (Table 2) show a similar effect, but a week later than for *lower risk* females. The model comparing weeks 2 and 3 shows that 53.7% of the variation in changes in *higher risk* females' GC levels was explained by the females' rank and age. As with the *lower risk* females, the GC levels of higher-ranking *higher risk* females started to drop in week 3 (Fig. 4), while those of lower-ranking females were still increasing.

Table 2
Multiple regression analyses of changes in GC levels between consecutive weeks in *higher risk* females during the weeks following the onset of instability in the male dominance hierarchy

GC level change	F	N	R^2	P	predictor	β	T	P
Week −1 and 1	–	11	0	NS				
Week 1 and 2	–	12	0	NS				
Week 2 and 3	5.796	13	0.537	0.021				
					(constant)		−2.379	0.039
					year of birth	0.474	2.191	0.053
					rank	0.510	2.357	0.040
Week 3 and 4	–	13	0	NS				

Legend as in Table 1.

As expected for a female philopatric monkey species, females' rank was independent of age (Kendall Tau: $r=-0.072$, $N=22$, $P=0.649$). Higher-ranking females tended to have more close female kin (Kendall Tau: $r=-0.280$, $N=22$, $P=0.094$), but whether or not a female had any close female kin at all was not correlated with rank (Kendall Tau: $r=-0.228$, $N=22$, $P=0.210$). Perhaps as a result, we could detect no consistent relation between decreases in females' GC levels and the number of close kin.

The relation between changes in grooming and changes in glucocorticoid levels

As described in Table 1, *lower risk* females' GC levels began to drop in week 2. Interestingly, there was a significant positive correlation between the decrease in *lower risk* females' *SWDI* in week 1 and the decrease in their GC levels in week 2. Females who decreased their *SWDI* more in week 1 had a greater decrease in GC levels in week 2 (Pearson: $r=0.940$, $N=5$; $P=0.017$), suggesting a causal relation between grooming diversity and reduced GC levels. This decrease in grooming diversity appeared to occur because females were concentrating their grooming on close kin. Though small sample size precluded a definitive test, females who increased the percentage of grooming with close female kin in week 1 had a greater decrease in GC levels in week 2 (Pearson: $r=-0.848$, $N=4$, $P=0.152$).

Higher risk females' GC levels started to decrease during week 3 (Table 2). In contrast to *lower risk* females, there was no correlation between the change in *higher risk* females' *SWDI* in week 2 and in the change in their GC levels in week 3 (Pearson: $r=0.007$, $N=10$, $P=0.984$). However, *higher risk* females who increased their percentage of grooming with kin in week 2 showed a greater decrease in GC levels in week 3 (Pearson: $r=-0.740$, $N=8$, $P=0.036$).

There was no evidence that the drop in GC levels was due to changes in rates of aggression. Females received aggression at similar rates throughout the four weeks following the male rank upheaval (paired *t*-tests week 1 to 2: DF=21, $t=-0.099$, $P=0.922$; week 2 to 3: DF=21, $t=-1.613$, $P=0.122$; week 3 to 4: DF=21, $t=-0.057$, $P=0.955$).

Discussion

All females' GC levels increased in the week after the initial upheaval in the male dominance hierarchy, irrespective of their reproductive state. Females' GC levels remained elevated until the male dominance hierarchy stabilised 21 weeks later. Despite females' generally elevated GC levels during this period of instability, however, there was some decrease in GC levels in the four weeks immediately following the initial destabilizing events. The GC levels of females who were not vulnerable to infanticide (*lower risk* females) started to decrease in the second week after the initial upheaval, whereas those of females vulnerable to infanticide (*higher risk* females) started to decrease in the third week.

These results differ somewhat from those obtained in previous research on this same group of baboons, which suggested that male immigration and rank instability caused an increase primarily in lactating females' GC levels (Beehner et al., 2005; Engh et al., 2006b). The different results obtained in this study probably occurred because fecal samples were gathered at almost double the rate of that in earlier studies, enabling us to examine GC levels on a weekly rather than a monthly basis. Because *lower risk* females' GC levels dropped sooner than *higher risk* females', monthly GC averages may not detect short-term rises in *lower risk* females' GC levels.

Strikingly, all females reduced their grooming diversity in the weeks following the onset of the unstable period, to focus more strongly on a few preferred partners. For most females, these preferred partners were close kin. Two sorts of data suggest that this more focused pattern of grooming acted as a coping strategy to alleviate stress. First, for both *low*er and *higher risk* females the change in grooming diversity preceded and was correlated with a decrease in GC levels one week later. Second, cycling and pregnant females with a low *SWDI* during the previous stable period showed a lower stress response at the onset of the unstable period.

The decrease in grooming diversity was more pronounced for *lower risk* than for *higher risk* females. This was probably due to the fact that many *higher risk* females were lactating. Lactating females with young infants are extremely attractive to other females, who attempt to groom and interact with them at high rates (Silk et al., 2003a; Crockford et al., 2008). As a result of these unsolicited grooming initiations, lactating females may have less control over their grooming interactions and may be less successful at focusing their grooming interactions on a few preferred partners. Their GC levels are also correspondingly higher, even during times of social stability.

There was no consistent evidence that females' GC levels were affected by the number of close kin. Female baboons tend to concentrate their grooming among a few close relatives (Silk et al., 1999, 2006a,b). However, when kin are not available females form stable grooming partnerships with non-relatives, which may mitigate any detrimental effects on stress or GC levels associated with the lack of close kin.

Females' GC responses may have been influenced in part by dominance rank, since high-ranking females' GC levels began to decrease at least one week earlier than low-ranking females'.

It seems possible that high-ranking females exert greater control over the timing and nature of their social interactions, allowing them to experience less stress during periods of instability. However, during the previous period of stability in the same social group, there was no relation between female dominance rank and GC levels, a finding supported by other studies of female primates (Abbott et al., 2003; Sapolsky, 2005). Whether there is any fitness advantage to being able to reduce GC levels a week earlier than low-ranking females remains to be determined.

Working with the same group of baboons, Engh et al. (2006b) found that females whose grooming network had been damaged by the death of a close female relative expanded their grooming network in subsequent months. In contrast, control females whose grooming networks remained intact either contracted their grooming network or showed no change in grooming diversity. The females in this study behaved in a similar way as the control females in the Engh et al. study. The period of male rank instability occurred at a time when there had been no female mortality for several months, and all females' grooming networks were undamaged. At the onset of instability, females appeared to turn to their closest partners for support, and their grooming networks became correspondingly less diverse.

These results complement those obtained in previous research on stress alleviation. It has long been known that humans and other animals rely on social affiliation when under stress (Sapolsky et al., 1997; Carter, 1998; Panskepp, 1998). The pituitary hormone oxytocin, which can be released by physical contact and affiliation, plays an important role in affiliation-mediated stress reduction, and this effect is particularly pronounced in females (reviewed by Taylor et al., 2000). In humans, women show a greater desire than men to seek social contact during times of stress, and they are also more likely to turn to smaller support groups (Taylor et al., 2000). In both sexes, the number of 'core' individuals on whom people rely for support during times of crisis (3–5 individuals) tends to be significantly smaller than their circle of mutual friends (12–20) or regular acquaintances (30–50) (Dunbar and Spoor, 1995; Hill and Dunbar, 2003; Zhou et al., 2005). In the elderly, strong social networks enhance survival (Giles et al., 2005), and when humans perceive future social opportunities to be limited or at risk – either as they age or when they become ill – they contract their social networks and become more selective in their social relationships (Carstensen 1992, 1995).

Several previous studies have suggested that grooming functions in part to decrease stress and anxiety (e.g. Boccia et al., 1989; Aureli et al., 1999; Shutt et al., 2007). We could detect no relation between changes in GC levels and grooming given or received. Our data suggest more specifically that a stable grooming network focused on a few predictable partners is more important than grooming direction in reducing stress. During periods of social stability, female baboons with more concentrated grooming networks have lower GC levels than those with more diverse ones (Crockford et al., 2008). During periods of instability, the grooming networks of all females – regardless of their immediate risk – became less diverse, and females whose grooming networks were previously more focused experience

smaller increases in GC levels. Finally, when female baboons' grooming networks are damaged due to the death of a close relative and grooming partner, they appear to attempt to compensate for this loss by temporarily expanding their grooming network to identify new grooming partners. Because socially integrated females have a higher reproductive success (Silk et al., 2003b; Silk, 2007), the establishment and maintenance of close bonds with preferred partners is likely to be highly adaptive.

Acknowledgments

References

Abbott, D.H., Keverne, E.B., Bercovitch, F.B., Shively, C.A., Mendoza, S.P., Saltzman, W., Snowdon, C.T., Ziegler, T.E., Banjevic, M., Garland, T.J., Sapolsky, R.M., 2003. Are subordinates always stressed? A comparative analysis of rank differences in cortisol levels among primates. Horm. Behav. 43, 67–82.

Alberts, S.C., Sapolsky, R.M., Altmann, J., 1992. Behavioral, endocrine and immunological correlates of immigration by an aggressive male into a natural primate group. Horm. Behav. 26, 167–178.

Altmann, J., 1974. Observational study of behavior: sampling methods. Behaviour 49, 227–267.

Aureli, F., Preston, S.D., de Waal, F.B.M., 1999. Heart rate responses to social interactions in free-moving rhesus macaques (*Macaca mulatta*): a pilot study. J. Comp. Psych. 113, 59–65.

Beehner, J.C., Whitten, P.L., 2004. Modifications of a field method for fecal steroid analysis in baboons. Physiol. Behav. 82, 269–277.

Beehner, J.C., Bergman, T.J., Cheney, D.L., Seyfarth, R.M., Whitten, P.L., 2005. The effect of new alpha males on female stress in free-ranging baboons. Anim. Behav. 69, 1211–1221.

Beehner, J.C., Nguyen, N., Wango, E.O., Alberts, S.C., Altmann, J., 2006. The endocrinology of pregnancy and fetal loss in wild baboons. Horm. Behav. 49, 688–699.

Bergman, T.J., Beehner, J.C., Cheney, D.L., Seyfarth, R.M., Whitten, P.L., 2005. Correlates of stress in free-ranging male chacma baboons. Anim. Behav. 70, 703–713.

Boccia, M.L., Reite, M., Laudenslager, M., 1989. On the physiology of grooming in a pigtail macaque. Physiol. Behav. 45, 667–670.

Carstensen, L.L., 1992. Social and emotional patterns in adulthood: support for socioemotional selectivity theory. Psychol. Aging 7, 331–338.

Carstensen, L.L., 1995. Evidence for a life-span theory of socioemotional selectivity. Curr. Dir. Psychol. Sci. 4, 151–156.

Carter, C.S., 1998. Neuroendocrine perspectives on social attachment and love. Psychoneuroendocrinology 23, 779–818.

Cheney, D.L., Seyfarth, R.M., 2007. Baboon Metaphysics: Evolution of a Social Mind. University of Chicago Press, Chicago.

Cheney, D.L., Seyfarth, R.M., Fischer, J., Beehner, J., Bergman, T., Johnson, S.E., Kitchen, D.M., Palombit, R.A., Rendall, D., Silk, J.B., 2004. Factors affecting reproduction and mortality among baboons in the Okavango Delta, Botswana. Int. J. Primatol. 25, 401–428.

Crockford, C., Wittig, R.M., Whitten, P.L., Seyfarth, R.M., Cheney, D.L., 2008. Social stressors and coping mechanisms in wild female baboons (*Papio hamadryas ursinus*). Horm. Behav. 53, 254–265.

DeVries, A.C., Glasper, E.R., Detillion, C.E., 2003. Social modulation of stress responses. Physiol. Behav. 79, 399–407.

Dunbar, R.I.M., Spoor, M., 1995. Social networks, support cliques, and kinship. Hum. Nat. 6, 273–290.

Engh, A.E., Beehner, J.C., Bergman, T.J., Whitten, P.L., Hoffmeier, R.R., Seyfarth, R.M., Cheney, D.L., 2006a. Behavioural and hormonal responses to predation in female chacma baboons (*Papio hamadryas ursinus*). Proc. Roy. Soc. Lond., B. 273, 707–712.

Engh, A.E., Beehner, J.C., Bergman, T.J., Whitten, P.L., Hoffmeier, R.R., Seyfarth, R.M., Cheney, D.L., 2006b. Female hierarchy instability, male immigration, and infanticide increase glucocorticoid levels in female chacma baboons. Anim. Behav. 71, 1227–1237.

French, J.A., Koban, T., Rukstalis, M., Ramirez, S.M., Bardi, M., Brent, L., 2004. Excretion of urinary steroids in pre- and postpartum female baboons. Gen. Comp. Endocrinol. 137, 69–77.

Giles, L.C., Glonek, G.F.V., Luszcz, M.A., Andrews, G.R., 2005. Effects of social networks on 10 year survival in very old Australians: the Australian longitudinal study of aging. J. Epid. Comm. Health 59, 574–579.

Goymann, W., Wingfield, J.C., 2004. Allostatic load, social status, and stress hormones — the costs of social status matter. Anim. Behav. 67, 591–602.

Gust, D.A., Gordon, T.P., Hambright, M.K., Wilson, M.E., 1993. Relationship between social factors and pituitary-adrenocortical activity in female rhesus monkeys (*Macaca mulatta*). Horm. Behav. 27, 318–331.

Gust, D.A., Wilson, M.E., Stocker, T., Conrad, S., Plotsky, P.M., Gordon, T.P., 2000. Activity of the hypothalamic–pituitary–adrenal axis is altered by aging and exposure to social stress in female rhesus monkeys. J. Clin. Endocrinol. Metab. 85, 2556–2563.

Hill, R.A., Dunbar, R.I.M., 2003. Social network size in humans. Hum. Nat. 14, 53–72.

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. Anim. Behav. 18, 169–176.

McEwen, B.S., Wingfield, J.C., 2003. The concept of allostasis in biology and biomedicine. Horm. Behav. 43, 2–15.

Palombit, R., Cheney, D., Seyfarth, R., Rendall, D., Silk, J., Johnson, S., Fischer, J., 2000. Male infanticide and defense of infants in chacma baboons. In: van Schaik, C., Janson, C. (Eds.), Infanticide by Males and Its Implications. Cambridge University Press, Cambridge, pp. 123–152.

Panksepp, J., 1998. Affective Neuroscience: The Foundations of Human and Animal Emotions. Oxford University Press, New York.

Pereira, M.E., 1983. Abortion following the immigration of an adult male baboon (*Papio cynocephalus*). Am. J. Primatol. 4, 93–98.

Power, M.L., Schulkin, J., 2006. Functions of corticotropin-releasing hormone in anthropoid primates: From brain to placenta. Am. J. Hum. Biol. 18, 431–447.

Rosal, M.C., King, J., Ma, Y., Reed, G.W., 2004. Stress, social support, and cortisol: inverse associations? Behav. Med. 30, 11–21.

Sapolsky, R.M., 2005. The influence of social hierarchy on primate health. Science 308, 648–652.

Sapolsky, R.M., Alberts, S.C., Altmann, J., 1997. Hypercortisolism associated with social subordinance or social isolation among wild baboons. Arch. Gen. Psychiatry. 54, 1137–1142.

Sapolsky, R.M., Romero, L.M., Munck, A.U., 2000. How do glucocorticosteroids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. Endocr. Rev. 21, 55–89.

Shutt, K., MacLarnon, A., Heistermann, M., Semple, S., 2007. Grooming in Barbary macaques: better to give than to receive. Biol. Lett. 3, 231–233.

Silk, J.B., 2007. Social components of fitness in primate groups. Science 317, 1347–1351.

Silk, J.B., Seyfarth, R.M., Cheney, D.L., 1999. The structure of social relationships among female baboons in the Moremi Reserve, Botswana. Behaviour 136, 679–703.

Silk, J.B., Rendall, D., Cheney, D.L., Seyfarth, R.M., 2003a. Natal attraction in adult female baboons (*Papio cynocephalus ursinus*) in the Moremi Reserve, Botswana. Ethology 109, 627–644.

Silk, J.B., Alberts, S.C., Altmann, J., 2003b. Social bonds of female baboons enhance infant survival. Science 302, 1231–1234.

Silk, J.B., Altmann, J., Alberts, S.C., 2006a. Social relationships among adult female baboons (*Papio cynocephalus*): I Variation in the strength of social bonds. Behav. Ecol. Sociobiol. 61, 183–195.

Silk, J.B., Alberts, S.C., Altmann, J., 2006b. Social relationships among adult female baboons (*Papio cynocephalus*): II. Variation in the quality and stability of social bonds. Behav. Ecol. Sociobiol. 61, 197–204.

Taylor, S.E., Cousino Klein, L., Lewis, B.P., Gruenewald, T.L., Gurung, R.A., Updegraff, J.A., 2000. Biobehavioral responses to stress in females: tend-and-befriend, not fight-or-flight. Psychol. Rev. 107, 411–429.

Thorsteinsson, E.B., James, J.E., 1999. A meta-analysis of the effects of experimental manipulations of social support during laboratory stress. Psychol. Health 14, 869–886.

Touma, C., Palme, R., 2005. Measuring fecal glucocorticoid metabolites in mammals and birds: The importance of validation. Ann. N.Y. Acad. Sci. 1046, 54–74.

Van Cauter, E., 1990. Diurnal and ultradian rhythms in human endocrine function — a minireview. Horm. Res. 34, 45–51.

Wilson, E.O., Bossert, W.H., 1971. A Primer of Population Biology. Sinauer, Sunderland, MA.

Zhou, W.X., Sornette, D., Hill, R.A., Dunbar, R.I.M., 2005. Discrete hierarchical organization of social group size. Proc. Roy. Soc. Lond., B. 272, 439–444.