

RESEARCH ARTICLE

The Relationship Between Socio-Sexual Behavior and Salivary Cortisol in Bonobos: Tests of the Tension Regulation Hypothesis

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Bonobos are known for their pacifistic behavior and their large repertoire of behaviors that are thought to serve conflict resolution. One is an unusual form of ventro-ventral mounting that facilitates genital contacts (GC). Various hypotheses have been proposed to explain its function. In this study we tested predictions of the tension regulation hypothesis using salivary cortisol as a marker for social stress. The results indicate a temporal relationship between GC and cortisol levels. Compared with baseline data and matched samples of unrestricted food access, rates of GC increased when access to food sources was restricted. Cortisol levels were highest when access to food was constrained. However, because the behavioral and hormonal responses occurred when viewing the stimulus at a distance and preceded the physical presence of the stimulus, we conclude that the anticipation of a competitive situation was sufficient to induce social stress. Contrary to our prediction, targets of aggression did not have higher rates of GC nor did they solicit GC more often than others. Furthermore, higher GC rates did not correlate with a more pronounced decrease in cortisol levels. Not all results obtained in this study supported the predictions concerning the regulatory function of GC on social tension and further research is needed to explore this question. However, the results indicate that the anticipation of competition may be sufficient to induce a costly physiological response, and that high levels of resource competition may have lasting effects on physical stress and stress management. *Am. J. Primatol.* 70:1–10, 2008. © 2008 Wiley-Liss, Inc.

Key words: bonobo; *Pan paniscus*; socio-sexual behavior; genital contact; tension regulation; stress; salivary cortisol

INTRODUCTION

Studies on primates and other social mammals have shown that nonaggressive conflict resolution is a common and efficient alternative to aggressive behaviors [Aureli & de Waal, 2000; Silk, 1998]. Among primates, bonobos are known for low rates of aggressive behavior, and a large repertoire of behaviors that are thought to serve consolation, reconciliation and conflict resolution [de Waal, 1987]. One of these behaviors is an unusual form of ventro-ventral mounting. These mounts facilitate genital contact (GC), which can be amplified by lateral movement. Although orangutans and chimpanzees may also engage in GC [Anestis, 2004; van Schaik et al., 2003], bonobos are the only nonhuman primates that perform GC habitually and in both captive and natural environments [Kuroda, 1980; de Waal, 1987]. The use of GC is biased toward mature females but males and immatures of both sexes also perform this behavior [Hohmann & Fruth, 2000]. It has been proposed that GC serves to “dissolve inter-individual tension” [Kuroda, 1980, p 190]. Field

observations at Lomako revealed that rates of GC increased in small food patches, suggesting that the behavior is associated with increasing levels of resource competition. Applying an ethological concept of reconciliation, de Waal [1987] tested for two different functions of GC in a study on captive bonobos, arousal transformation and tension regulation, and found strong support for tension regulation.

In behavioral studies, the term tension is widely used but not always well defined. Tension is often used as a synonym for stress, which is defined as a condition typically characterized by symptoms of

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mental and physical tension or strain. If this tension is caused by external stressors, then it should lead to a stress response, which after a cascade of events leads to cortisol secretion by the adrenal cortex [Sapolsky, 2002]. Although the stress response is an adaptive reaction to external challenges, its chronic activation can lead to pathologies. Therefore, animals have developed coping mechanisms that allow them to alleviate the effect of stressors by lowering cortisol levels [Sapolsky et al., 2000]. One primary coping strategy for primates seems to be grooming [Aureli et al., 1999; Boccia et al., 1989; Gust et al., 1993] and evidence suggests that the presence of the grooming partner alone can reduce stress levels [Gust et al., 1994, 1996]. Another mechanism to reduce social tension and stress is the performance of socio-sexual behavior [Vasey, 1995; Wickler, 1967] and the exchange of GC among bonobos has been interpreted as a tension regulation mechanism [de Waal, 1987].

In this study, we specify the tension regulation hypothesis by predicting that increased social tension that arises during situations such as competition over clumped food resources leads to an elevated stress response and that the exchange of GC serves as a means to reduce the stress. The study combines data from behavioral observations and measurements of salivary cortisol levels. The data are used to compare variation of cortisol levels across situations that differ in levels of resource competition and to test the relationship between cortisol levels and performance of GC. If GC serves as a tension reduction mechanism during situations of feeding competition, we expect support for the following predictions: (1) Rates of GC and cortisol levels increase at times when resource competition is high; (2) performance of GC is followed by a decrease in cortisol levels; (3) individuals who are more likely to be the target of aggression are more often involved in GC and solicit GC more frequently than others and (4) individuals who are more likely to be the target of aggression have a stronger or longer lasting cortisol response.

Evidence suggests that kinship affects the mechanisms used to resolve social conflicts [Aureli et al., 1997] as well as cortisol levels [Ziegler & Sousa, 2002]. Because the study group contained two adult mother–daughter pairs, we made notes on kinship for all dyads engaging in GC. Accordingly, we also predict that: (5) dyads of unrelated females perform GC more frequently than related individuals.

The diet of bonobos is dominated by fruit and most food items occur in discrete patches. In spite of that, it is thought that competition is low enough to allow females to aggregate [Wrangham, 1986]. Resident females entertain differentiated and friendly relations with other females, which reflect in food sharing and agonistic aid [Vervaecke et al.,

2000]. However, social tension may arise when access to food sources is ambiguous [Parish, 1994]. The threat of aggression can activate the flight and fight response, and thereby lead to an elevated secretion of stress hormones such as corticosteroids [Sapolsky, 2002]. The temporal association between competitive situations and performance of GC found in some studies [Hohmann & Fruth, 2000; de Waal, 1987] suggests that this behavior may be a strategy to cope with elevated stress levels. Although previous studies vary in terms of the functional relationship between GC and social tension [e.g. Wrangham, 1993], they converge in so far as all associate the performance of the behavior with potentially tense situations. The work reported here takes a similar perspective but adds physiological parameters that have not been used in the other behavioral studies on bonobos.

METHODS

Behavioral Observations

Data were collected for 9 months from bonobos at the zoo in Frankfurt am Main, Germany, between January and October 2004. During most of this period the group consisted of one adult male, five adult females and two immatures (Table I). In July, one female died after giving birth. Bonobos had access to two indoor and two outdoor enclosures and remained in social groups at any time of the day and at night. Food was offered five times a day and consisted mainly of a melange of fresh fruit and vegetables. In addition to installations for climbing, bonobos had access to a large variety of objects such as wood wool, card boxes and others. Caretakers conducted regular training programs that stimulated subjects to perform certain tasks, including oral manipulation of dental cotton pads (see below). The methods used in this study to collect observational

TABLE I. Composition of the Bonobo Group at Frankfurt Zoo at the Time of this Study

Subject identity		Kinship ties	Rank
Salonga ^a	Adult female	Daughter of Margret	First
Natalie	Adult female	Mother of Ukela	Second
Ukela	Adult female	Daughter of Natalie	Third
Kamiti	Adult female	No kin relations	Fourth
Margret	Adult female	Mother of Salonga	?
Ludwig	Adult male	No kin relations	Fifth
Heri	Juvenile male	Son of Natalie	?
Haiba	Juvenile female	Daughter of Ukela	?

^aThis female died close to the end of the study. After her disappearance, Natalie became the top-ranking female but the rank order remained stable. Owing to lack of interactions, the rank of adult female Margret could not be assessed.

data and saliva samples are in compliance with animal care regulations.

Behavioral records came from 5-min focal follows of the five adult females, the adult male and the two immatures (total number of focal scores: 1,266; for details see Table II). Codes for observed behaviors were entered into data sheets or recorded on audio tapes. During the 9 months, data were collected every week for 3 days, each time under a different condition (Table II): stimulus ($N = 28$ trials), matched sample ($N = 27$) and baseline ($N = 21$). During the stimulus condition, bonobos were offered food in an opaque plastic box ($25 \times 40 \times 20$ cm). After filling it with food, the box was installed in one indoor enclosure. Bonobos could access the content of the box through holes and with the help of long wooden probes that were provided along with the food. Installation of the food box was preceded by 40 min of prestimulus observations (PRE-phase) and followed by 40 min of poststimulus observations (POST-phase). Each individual was observed for one 5-min focal interval during each phase, the PRE- and the POST-phase, and the order of observing individuals was randomized. Matched samples were identical to the stimulus condition except that food items were not clumped in a single patch but spread across the enclosure that had the following measures: 5.60 m length, 5.10 m width, 3.25 m height. The critical difference between these two conditions was that food was clumped (stimulus) or dispersed (matched sample). In both conditions bonobos were able to watch food preparation and preparation of the food box by caretakers. In contrast to stimulus and matched sample, baseline observations included active periods in the morning and early afternoon without food provisioning. The minimum time separating successive stimuli was 7 days. Stimuli and corresponding matched samples were separated by 1 or 2 days. To prevent habituation, the order between stimuli and matched samples alternated in a random order.

To identify changes in social interactions, we scored behaviors that were considered to be indicative of social tension: self-scratching, GC, displays and contact aggression. *Scratching* (SCR) is self-directed and refers to relatively stereotypic movements of hands and arms, which facilitate

contact between fingertips of one hand with other body parts [Aureli & de Waal, 1997]. It differs from other forms of grooming by its high rate of repetition, the mode of limb movement and the lack of visual monitoring. GC refers to ventro-ventral mounting during which participants move their genitals with lateral movements against each other. There are asymmetries in terms of initiation of the contact as well as spatial position and movement. In order to assess the differences in the motivation, we scored the identity of the bonobo who solicited a GC defining solicitation by ventral presenting. *Displays* (DIS) are undirected or directed motor performances without physical aggression such as bipedal running, which are interpreted as a sign of excitement and arousal. The term *aggression* (AGR) refers to actions leading to physical contact such as pulling, slapping, scratching or biting that an actor (aggressor) directs against another group member (target). Rank differences between individuals were based on approach-retreat interactions. Rank order remained stable throughout the study period. One female died close to the end of the study. As a result, numerical ranks of all individuals ranking below this female moved one step up. One adult female could not be ranked because of the lack of interactions with others.

Hormonal Analysis

Physiological assessments of short-term changes in stress levels are usually based on measurements of glucocorticoid concentrations in either blood or saliva. In primates and other vertebrates, invasive sampling is not only technically difficult and ethically undesirable but might be stressful in itself and, therefore, mask the pattern under investigation. Saliva, however, has been collected noninvasively from nonhuman primates [Lutz et al., 2000] and studies in humans, dogs and other animals have demonstrated a predictable relationship between stress events and cortisol excretion in saliva [Pollard, 1995]. In humans, peak concentrations in saliva are reached 20–30 min after the onset of the stress condition [Kirschbaum & Hellhammer, 1989].

We collected 613 saliva samples from five adult females, one adult male and two immatures under stimulus condition and on matched sample days

TABLE II. Protocol of Data Collection

	Condition		
	Stimulus ($N = 28$)	Matched sample (27)	Baseline (21)
Duration/session (min)	80	80	360
Daytime	08:00–12:00 a.m.	08:00–12:00	08:00–16:00
Food distribution	Clumped	Dispersed	No food
#Focal scores	404	398	464
Saliva samples	Yes	Yes	No

using Salivette[®] containers (Sarstedt). To cover the period of peak concentration of cortisol in saliva, we handed out the cotton pads to each group member 15 min before (PRE-phase) and 15 min after food provisioning (POST-phase I). The pads were returned by the animals after chewing them for several minutes. A third sample was collected 60 min after food provisioning (POST-phase II). Bonobos were trained to chew dental cotton pads and return the pads to the keeper on command. Used cotton pads were immediately put into vials and stored in a freezer at -20°C until they were transported to the lab on dry ice.

In spite of these efforts, sampling was sometimes incomplete because individuals occasionally refused to take cotton pads or did not chew long enough to obtain sufficient amounts of saliva. Therefore sample sizes varied between individuals. Average sample sizes for the stimulus condition were 14.1 (range 4–21) for the PRE-phase, 17 (5–25) for the POST-phase I and 17 (6–25) for the POST-phase II. For the matched sample condition, average sample sizes were 15.1 (range 5–21) for the PRE-phase, 12.3 (2–17) for the POST-phase I and 15.8 (6–23) for the POST-phase II.

We measured changes in cortisol levels as the mere difference between the PRE- and POST-values (i.e. $\Delta C = C\text{-PRE} - C\text{-POST}$) as well as relative changes (i.e. $\Delta C = (C\text{-PRE} - C\text{-POST})/C\text{-PRE}$).

To extract the saliva from the cotton pads, we centrifuged the tubes at 3,000 U/min for 10 min. Samples of 100 μl of saliva were vortexed together with 5 ml of diethyl ether for 10 min and then frozen for 2 hr at -20°C . The supernatant was decanted and evaporated under constant air flow at 35°C . The steroids were then reconstituted in 500 μl assay buffer and stored at -20°C until hormone analysis.

The extracts were analyzed for immunoreactive cortisol by an enzyme immunoassay (EIA) described in Palme and Möstl [1997]. The EIA used an antibody raised in a rabbit against cortisol-3-CMO:B-SA. With cortisol as a standard (100%), the antiserum showed the following cross-reactivity: corticosterone 6.4%, allodihydrocortisol 4.6%, allotetrahydrocortisol 0.8%, tetrahydrocortisol 0.1 and $<0.01\%$ for all other metabolites tested. The sensitivity of the assay determined at 90% binding was 0.63 pg/well. Intra- and interassay coefficients of variation, calculated from replicate determinations of high- and low-value quality controls measured within and between assays, gave values of 5.2 and 7.8 and 6.4 and 21.2%, respectively.

Statistical Analyses

As the most data tested were clearly nonnormally distributed (e.g. highly skewed and with large numbers of tied observations), we used nonparametric tests throughout, which we chose according to

the rationales and assumptions as described in Siegel and Castellan [1988]. Tests were conducted on data either comprising one value per individual or repeated observations per individual. To avoid pseudo-replication [Hurlbert, 1984] owing to multiple observations of the same individuals and confounding by other factors than the one investigated, we usually applied these tests separately for each individual and each level of all the factors not being investigated. For instance, to compare cortisol values between the PRE- and POST-phases we conducted tests separately for each combination subject and stimulus condition/matched sample.

A potential problem in such analyses of data comprising repeated observations of the same subject is nonindependence of data. We addressed this by correlating (using Spearman's ρ) rates of behaviors with the sequence of experimental days, for each combination of behavior, subject, condition (baseline, experimental day, matched sample) and PRE-/POST-experimental, but only for those such combinations in which the subject at least once showed the behavior (resulting in a total of 62 correlations). Combining the P -values using Fisher's omnibus test (see below) revealed a clearly significant result ($\chi^2 = 166.3$, $df = 124$, $P = 0.007$) suggesting that the frequency of the behaviors changed over the course of the study period. However, the average correlation was 0.02 (one-sample t -test: $t_{61} = 0.56$, $P = 0.58$; see below), and also after splitting the file by behavior, condition and PRE-/POST-experimental none of the subsets of correlations differed much from zero (largest absolute average $r_s = 0.18$, all $P > 0.1$, only combinations with $N \geq 5$ considered). Hence, we concluded that nonindependence of data was not a major issue.

To account for multiple testing we used Fisher's omnibus test. This procedure combines a number of P -values into a single χ^2 -distributed variable with its degrees of freedom equaling twice the number of P -values [Haccou & Meelis, 1994]. To combine correlation coefficients we used one-sample t -tests of the null hypothesis stating that the average correlation coefficient equals zero. We calculated Spearman correlations and Mantel tests using programs written by one of the authors (R. M.), and binomial tests using the function "binomdist" provided by Excel (version 2002, SP3). Fisher's omnibus test was calculated by hand. For all other tests we used SPSS 15.0.0 for Windows. Whenever required, we used exact nonparametric tests [Mundry & Fischer, 1998; Siegel & Castellan, 1988] or corresponding approximate permutation procedures in the case of having larger samples but many tied observations ("Monte-Carlo" significance in SPSS). We generally used at least 1,000 permutations but in the case of a P -value being close to the critical threshold we used 10,000 permutations. With the exception of Fisher's omnibus test, all indicated

P-values are two-tailed. We used Mann–Whitney *U*-tests as post hoc tests after a significant Kruskal–Wallis *H*-test and Wilcoxon tests as post hoc tests applied following a significant Friedman test. We used these instead of the standard procedures to keep the power at an acceptable level and to prevent from type II errors [Cohen & Cohen, 1983].

RESULTS

Behavioral Scores

Comparison of individual rates of scratching, GC, display and aggression revealed significant differences between baseline, stimulus condition and matched samples (Fisher's omnibus test combining four Friedman tests: $\chi^2 = 17.8$, $df = 8$, $P = 0.02$; Table III). Considering results of the individual Friedman tests revealed only GC to be displayed at different rates at baseline, stimulus condition and matched samples (AGR: $\chi^2 = 2.33$, $df = 2$, $P = 0.34$; DIS: $\chi^2 = 4.0$, $df = 2$, $P = 0.17$; rate of GC: $\chi^2 = 9.48$, $df = 2$, $P = 0.005$; SCR: $\chi^2 = 2.33$, $df = 2$, $P = 0.43$). Post hoc tests showed that the rate of GC was significantly higher under stimulus condition compared with baseline days as well as matched samples (Wilcoxon test: both $T^+ = 21$, both $N = 6$, both $P = 0.031$) but did not differ between baseline and matched samples ($T^+ = 8$, $N = 5$ (one tie), $P = 1$; Fig. 1).

There was no obvious relation between frequencies of GC and scratching (correlations, calculated separately for each combination of sample/stimulus condition, PRE/POST and subject, combined using Fisher's omnibus test: average $r_s = 0.01$, $\chi^2 = 34.5$, $df = 28$, $P = 0.184$).

The rate of aggressive behaviors was 4.1/100 hr of observation (averaged across all subjects and conditions) and was highest under stimulus condition and lowest during baseline observations (Table III). Aggression came most often from adult females (94%) and the adult male was most often (70%) the target of aggression. Displays occurred twice as often as aggressive behaviors (not tested),

TABLE III. Frequencies of Behaviors (per Hour) During Baseline, Matched Samples and Under Stimulus Condition

	Baseline	Matched sample	Stimulus
Display	0.04	0	3.7
Scratching	0.27	1.0	1.1
Genital contact	0.13	0.6	2.2
Aggression	0.04	0.1	0.24
Target of aggression			
Adult female	10%		
Adult male	70%		
Immature	19%		

and more often under stimulus condition than in matched samples ($T^+ = 36$, $N = 8$, $P = 0.008$).

Contrary to our predictions, we found that subjects who were more frequently the target of aggression solicited GC less frequently (Spearman correlation of rates: $r_s = -0.84$, $N = 6$, $P = 0.044$) and also tended to be less frequently invited by others to join in GC ($r_s = -0.79$, $N = 6$, $P = 0.056$). Higher-ranking individuals tended to direct aggression against others more frequently ($r_s = -0.9$, $N = 5$, $P = 0.083$), but there was no obvious relation between the rank and the solicitation rate ($r_s = 0.8$, $N = 5$, $P = 0.13$) or the rank and the rate with which individuals performed GC ($r_s = 0.36$, $N = 5$, $P = 0.63$).

GC and Kinship

Overall, relatives performed GC less frequently than unrelated females and, under the stimulus condition, GC involved only unrelated dyads. However, there was no significant relation between kinship of females and the frequency with which they had GC (Mantel test: $r_s = -0.53$, $N_{\text{subjects}} = 5$, exact $P = 0.13$; Table IV).

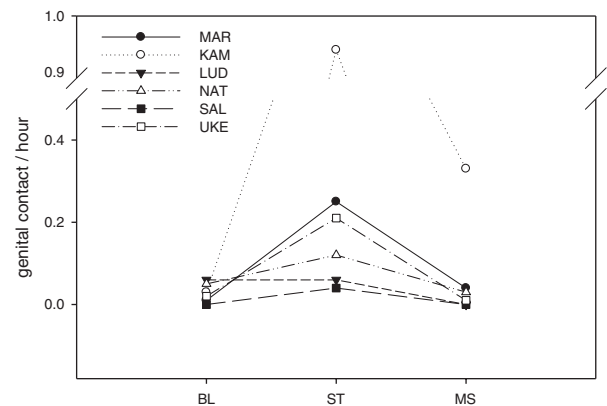


Fig. 1. Rates of genital contacts of mature (adult and adolescent) bonobos during baseline (BL), stimulus condition (ST) and matched sample days (MS). The dotted line refers to rates of genital contacts of Kamiti, the only female group member without a close kin, and has been adapted to fit into the graph.

TABLE IV. Matrix Shows the Distribution of Genital Contacts Among Female Dyads

	MAR	SAL	NAT	UKE	KAM
MAR	–	0	3	5	14
SAL		–	1	0	5
NAT			–	0	16
UKE				–	17
KAM					–

Two dyads (MAR–SAL and NAT–UKE) represent mother–adult daughter pairs.

Salivary Cortisol Levels

Cortisol levels are known to decline during the day [Desir et al., 1980; Weitzman et al., 1971] and therefore shifts in cortisol values over time may reflect the differences in sampling time. As expected, there was a weak though significant decrease in cortisol values over the course of the day (average $r_S = -0.20$; one-sample t -test: $t_{14} = -2.95$, $P = 0.01$).

During periods of social instability, both male and female cortisol levels can change significantly in primate groups [Beehner et al., 2005; Engh et al., 2006a,b; Sapolsky, 1983, 1993]. Although no major rank reversal had been observed, we wanted to exclude the possibility that minor instabilities undetected by the observer had caused temporal variations in cortisol levels. However, we found no indications for changes in cortisol levels within subjects (correlations of cortisol values with day, calculated separately for each individual, stimulus condition and matched samples as well as prior and after the stimulus, combined using Fisher's omnibus test: $\chi^2 = 54.2$, $df = 56$, $P = 0.54$) and also the average correlation coefficient did not indicate any systematic change (average $r_S = -0.015$; one-sample t -test: $t_{27} = 0.259$, $P = 0.797$).

Measurements of salivary cortisol values of adult bonobos ranged between 0.11 and 3.66 ng/ml (min and max across all subjects; Fig. 2 and Table V). Average cortisol values differed between the adult subjects (Kruskal-Wallis H -tests, conducted separately for each combination of PRE/POST and stimulus condition/matched sample, combined using Fisher's omnibus test: $\chi^2 = 74.56$, $df = 8$, $P < 0.001$), with the adult male generally exhibiting higher cortisol values than the adult females (pairwise Mann-Whitney U -tests: 15 out of 20 comparisons with $P < 0.05$; comparisons between females: 7 (4 subjects) or 11 (1 subject) out of 20 significant). The average cortisol value of one of the two immatures (Haiba) was the lowest of all subjects, whereas that of the other immature (Heri) was within the range of the adult females (Fig. 2 and Table V).

A comparison of cortisol values from samples collected under stimulus condition and matched

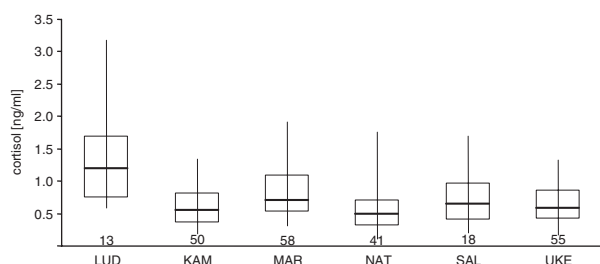


Fig. 2. Means, SD and range of individual cortisol values of the adult male (LUD) and the five adult females (KAM, MAR, NAT, SAL and UKE) during MS. Figures under each bar refer to the number of saliva samples used here. MS, matched sample.

samples showed that, overall, cortisol values were significantly higher under stimulus condition (Fisher's omnibus test, combining Wilcoxon tests conducted separately for each subject: $\chi^2 = 18.68$, $df = 10$, $P = 0.0446$). Overall, cortisol values declined between the first (PRE) and second (POST) part of the observation session (Fisher's omnibus test, combining Wilcoxon tests conducted separately for each combination of subject and stimulus condition/matched sample: $\chi^2 = 36.94$, $df = 20$, $P = 0.0119$). In the stimulus condition bonobos had higher cortisol values during the PRE-phase compared with the POST-phase (Fisher's omnibus test, combining Wilcoxon tests conducted separately for each subject: $\chi^2 = 22.27$, $df = 10$, $P = 0.0138$; Fig. 3). However, this effect was owing to the differences in the samples of

TABLE V. Average Values and Standard Deviations of Salivary Cortisol of the Bonobos Involved in This Study and Comparative Data From an Orangutan [Elder & Menzel, 2001] and From Humans [Kirschbaum et al., 1992]

Bonobo		Mean cortisol (ng/ml)
Salonga	Adult female	0.66 ± 0.4
Natalie	Adult female	0.66 ± 0.3
Ukela	Adult female	0.71 ± 0.3
Kamiti	Adult female	0.57 ± 0.2
Margret	Adult female	0.81 ± 0.3
Ludwig	Adult male	1.67 ± 0.9
Heri	Juvenile male	0.61 ± 0.3
Haiba	Juvenile female	0.53 ± 0.2
Orangutan	Adult female	1.11
Human	Female	1.75
	Male	3.52

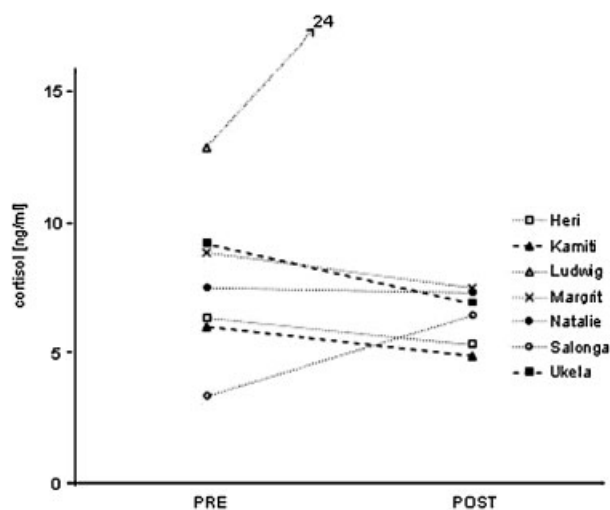


Fig. 3. Salivary C-values collected under stimulus condition before (PRE) and after (POST) presentation of the food box (stimulus condition). Note that the figure includes only the lower values of the adult male (LUD) and that the peak value is given only numerically.

most females, whereas the male and the alpha female had higher cortisol values during the POST-phase (Fig. 3). In the corresponding values from matched sample days, cortisol values did not differ between the PRE- and POST-phases (Fisher's omnibus test: $\chi^2 = 14.67$, $df = 10$, $P = 0.115$).

GC and Cortisol Levels

There was no obvious relationship between daily rate of GC and cortisol levels (correlations, calculated separately for each combination of individual and stimulus and matched samples condition, combined using Fisher's omnibus test: $\chi^2 = 11.76$, $df = 20$, $P = 0.924$; average $r_S = -0.04$, $t_9 = -0.773$, $P = 0.459$; only subjects with sample size ≥ 5 included). Correlation of the same values but this time across subjects and separately for each day did not change the result ($\chi^2 = 14.37$, $df = 28$, $P = 0.984$; average $r_S = -0.18$, $t_{13} = -1.5$, $P = 0.157$; only days with sample size ≥ 5 included).

There was no significant relationship between the frequency of GC and change in cortisol levels (correlation between the change in cortisol level from PRE- to POST-phase and the rate of GC during PRE-phase, calculated separately for combinations of subject and stimulus/matched samples condition, combined using Fisher's omnibus tests) neither with regard to relative changes in cortisol level ($\chi^2 = 15.0$, $df = 22$, $P = 0.86$) nor with regard to absolute changes ($\chi^2 = 16.0$, $df = 22$, $P = 0.82$). However, there was a tendency for relative cortisol levels to decrease more when rates of GC were higher (average $r_S = -0.11$, $t_{10} = -0.208$, $P = 0.094$) but no such trend for absolute changes in cortisol levels (average $r_S = -0.09$, $t_{10} = 1.29$, $P = 0.23$). Comparing cortisol values in samples collected before and after the stimulus was set, we found that neither absolute nor relative changes in cortisol levels differed between days at which subjects actively, passively or not at all participated in GC (Kruskal-Wallis H -tests, conducted separately for each subject and experimental days and matched samples, combined using Fisher's omnibus test: $\chi^2 = 29.36$, $df = 44$, $P = 0.96$).

DISCUSSION

The aim of this study was to test the tension regulation hypothesis based on predictions concerning the relationship between GC and salivary cortisol titers. The central prediction was that GC serves to reduce social tension and that this is reflected in reduced levels of the stress hormone, cortisol. The aim of the experiment was to induce social stress by offering food in a dense patch. However, measurements of salivary cortisol demonstrate that anticipation of food instead of its presentation served as a strong stimulus. Although not intended, this finding highlights the psychological effect of competition. Regarding the predictions listed in the introduction,

it appears that support for the tension regulation hypothesis is inconsistent. Under stimulus conditions, rates of GC and salivary cortisol concentrations were higher than those during matched sampling and baseline sampling (support for P-1). However, the second prediction (P-2) was only partially supported: except for the alpha female, the samples from females showed a decline of cortisol between the first and the second half of a sampling period under stimulus condition, whereas rates of GC remained elevated throughout the sampling period. In contrast, cortisol levels in the samples from the alpha female, the adult male and the immatures increased or remained elevated throughout the stimulus condition. Contrary to the third prediction (P-3), targets of aggression did not have higher rates of GC nor did they solicit GC more often than others. The finding that cortisol levels in samples of the adult male and the two immatures—the preferred targets of aggression—remained elevated for a longer time than those in samples from females supports P-4. We also found that kinship had the predicted effect on the exchange of GC, supporting P-5.

The study design limits interpretations of the results. Only a small number of individuals participated in this study. Sampling of saliva was not always successful and, therefore, the comparison of cortisol values from sampling under stimulus conditions and matched samples was based on a reduced sample set. Moreover, published records on the performance of GC by males are not available and therefore we cannot make firm conclusions about the relationship between stress, GC and social status of male bonobos. Because of the spatial constraints and the female-biased group composition, males of captive groups may be exposed to more stress than males in natural environments. Given that the subjects of this study consumed food during the period of sampling, we cannot exclude the possibility that food consumption had an effect on saliva characteristics and thereby the concentration of salivary cortisol. However, food consumption does not necessarily alter salivary cortisol levels [Gröschl et al., 2001] and some of the results such as the consistent differences in cortisol values between experiment and matched sample days cannot be attributed to the consumption of food.

In spite of these limitations, the results contribute to our understanding of the function of GC in the context of resource competition. When food sources were dispersed, both rates of GC and cortisol levels did not differ from baseline levels. When access to food sources was constraint, both parameters changed in the predicted direction. An interesting result is that the rise of cortisol and performance of GC preceded the actual stimulus. Watching the preparation of the food box appeared to be a sufficient stimulus to induce social stress, whereas

watching the conventional mode of food preparation did not. The visibility of the food box and thereby the anticipation of a competitive situation, but not food, was sufficient to induce cortisol excretion. The behavioral and hormonal responses suggest that bonobos associated the food box with the competition for access to food before food provisioning took place. Behavioral observations of captive bonobos suggested that socio-sexual behavior may actually prevent conflicts [e.g. de Waal, 1987] and experimental work by Hare et al. [2007] revealed that bonobos were better than chimpanzees in cooperating when food sources can be monopolized. On the one hand, this illustrates that potential problems and confounding factors may be encountered when conducting behavioral experiments with nonhuman primates. On the other hand, it demonstrates the ability of primates to associate current activities with a prospective context. The result from bonobos resembles data from humans showing that anticipation of stress can be sufficient to trigger an increase of salivary cortisol [Kirschbaum et al., 1999].

The five females were able to take food from the food box, whereas the adult male was excluded from access to it. The two juveniles had also low success rates in food fishing and mainly received food from their mothers. The restricted accessibility to the food box seems to provide a plausible explanation for high cortisol values of the male during food provisioning. However, the increase of cortisol between pre- and poststimulus in samples of the alpha female (Salonga) requires explanation. High-ranking individuals tend to be more often in control of preferred food patches and are therefore more likely to be the target of pestering and food solicitation behaviors by subordinates [Fruth & Hohmann, 2000]. By inference, individuals who control access to food sources may be exposed to higher levels of stress than participants. The alpha female was the only female with increasing cortisol excretion and it is tempting to speculate that the hormonal response may reflect pressure exerted by those who compete for access to the food box. Again, owing to the sample size, we are not able to distinguish between effects related to sex and the hormonal responses of the adult male and the alpha female remain ambiguous.

Inter-individual variation of stress hormone levels indicates that subjects reacted differently to social tension. In response to the preparation of the food box, cortisol levels increased in all individuals but the adult male and the two immatures had higher peaks and a longer lasting response than the adult females. This difference in hormonal response can be explained in various ways. In the bonobo group at Frankfurt zoo, the adult females were dominant over the adult male and the two immature individuals, and it is likely that the prospect of having better access to the food patch had an impact on cortisol excretion. However, it has been shown

that the relationship between hormonal stress response and dominance status varies across species [Goyman et al., 2003]. Additional data are required before the observed differences in hormonal stress response between the adult female bonobos and the adult male can be explained. Data from human studies indicate consistent sex differences in the response to stress with males showing a higher increase in salivary cortisol than females [Kirschbaum et al., 1992]. Future studies using samples from a larger set of males living in multimale multifemale groups should help to distinguish between the role of dominance status and identity of sex. Additional data are also required to explore the relationship between GC and stress hormones in the context of resource competition. The lack of consistency in the relationship between behavioral and hormonal responses suggests that conflict regulation is driven by a complex mechanism that is likely to be modulated by a variety of variables. Individual rates of GC might be influenced as well by other hormones such as testosterone [Jurke et al., 2000], whereas individual cortisol levels of females may be affected by changes in female sex steroids during their menstrual cycle [e.g. Kirschbaum et al., 1999].

Our data on the relationship between GC and cortisol do not match precisely with the predicted temporal relationship between hormones and behavior. However, the coincidence of adrenal and behavioral responses to an external stimulus suggests a link between GC and stress. The fact that GC remained elevated when cortisol titers had already decreased does not mean that GC did not down-regulate hormone levels.

Evidence suggests that chimpanzees and bonobos differ in their behavioral response to social tension [Hare et al., 2007; de Waal, 1992]. Given the close phylogenetic link, the two sister species offer an interesting model for further studies of the functional relationship between socio-sexual behavior and physiology. Making use of noninvasive sampling techniques offers a new avenue to explore the regulatory function of behaviors in *Pan* and other nonhuman primates.

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