Testosterone and cortisol are negatively associated with ritualized bonding behavior in male macaques

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Abstract

Neuroendocrine research on the formation of social bonds has primarily focused on the role of nonapeptides, however, steroid hormones often act additionally to either inhibit or facilitate bonding. Testosterone is proposed to mediate a trade-off between male reproductive competition and nurturing; therefore, low levels are predicted during periods of nurturing infant care and social bonding. In species where social bonding and support regulates hypothalamic-pituitary-adrenal axis activity, we also expect glucocorticoid levels to be low during bonding periods. Here we investigated how urinary testosterone and cortisol were related to male-infant-male interactions – a ritualized male bonding behavior – as well as infant care in male Barbary macaques. We collected >3000 hours of behavioral observation data during full-day focal animal follows from 14 adult males and analyzed testosterone and cortisol levels from 650 urine samples. As predicted both testosterone and cortisol levels were negatively correlated with rates of male-infant-male interactions. Infant care was positively correlated to testosterone and cortisol levels. The observed negative relationship between testosterone male-infant-male interactions may be beneficial to lower competitive tendencies between adult males and to not inhibit bond formation. Concurrently, lowered cortisol could reflect increased bonding and perceived social support as male-infant-male interactions predict future coalition formation in this species. Alternatively, lowered cortisol levels may be reflective of buffered tensions between males. The positive relationship of testosterone and cortisol with infant care suggests it may be done under competitive rather than nurturing contexts in this species. Collectively our results suggest that low steroid levels may be beneficial to same-sex bonding. Measuring steroid hormones in relation to bonding and nurturing can help us interpret behaviors within the ecological contexts that they occur.

Keywords: Steroid/peptide theory, testosterone, cortisol, social bond, male-infant-male interaction, Barbary macaque

1 Introduction

Steroid and nonapeptide hormones are involved in regulating animal behavior with the potential for cross-communication between different regulatory axes, allowing for fine-tuning of behavioral responses to different social contexts (Neumann, 2002; Neumann and Landgraf, 2012; van Anders et al., 2011; Viau, 2002). In particular, the steroid/peptide theory of social bonds (S/P theory) proposes that testosterone (regulated by the hypothalamic-pituitary-gonadal axis; HPG axis) and oxytocin (regulated by the oxytocinergic system) interact to promote different behavioral goals (van Anders et al., 2011). The oxytocinergic system likely evolved to promote mother-offspring bonds in mammals, yet the system was co-opted to also promote bonds between adults in both sexual and non-sexual contexts (Ross and Young, 2009; Ziegler and Crockford, 2017). The HPG axis regulates reproduction, with testosterone – the major androgen produced in males – promoting sexual contact and competition (Wingfield et al., 1990). Thus, while high oxytocin levels promote bonding and intimacy, variation in testosterone levels in conjunction with high oxytocin should be sensitive to different types of intimacy. For example, the S/P theory predicts that high oxytocin and low testosterone levels promote nurturing intimacy, whereas high oxytocin and high testosterone levels promote sexual intimacy (van Anders et al., 2011).

The S/P theory (van Anders et al., 2011) builds on the challenge hypothesis (Wingfield et al., 1990), which proposes that high testosterone levels promote male reproductive competition and is traded-off against paternal care. Across several vertebrate species males exhibit testosterone levels much higher than required for sperm production when competition for mates is high, such as during the mating season or territorial defense, and low testosterone levels during periods of paternal care (Desjardins et al., 2006; Gettler et al., 2011; Hirschenhauser and Oliveira, 2006; Lynn, 2008; Muller, 2017; Oliveira et al., 2002; Wingfield et al., 1990). High testosterone potentially inhibits paternal care as experimentally increasing testosterone levels can reduce rates of offspring provisioning and time spent incubating nests in various bird species (Lynn, 2008), including when such increases are within the natural range secreted by an individual (Goymann and Flores, 2017). However, testosterone levels are positively related to paternal care when it is

competitive in nature, such as when defending offspring from conspecifics (Lynn, 2008; Moore et al., 2019; Muller, 2017). In some species, particularly fish, paternal care primarily involves defending nests and is a behavior positively selected for by females (Stiver and Alonzo, 2009). In such cases there is no trade-off between paternal care and mating effort as high testosterone levels promote both (Moore et al., 2019; Stiver and Alonzo, 2009). The S/P theory resolves this inconsistency in how testosterone relates to paternal care by proposing that high testosterone levels inhibit nurturing behaviors and intimacy in general rather than paternal care per se (van Anders, 2013; van Anders et al., 2011).

Beyond competition and nurture, testosterone is also relevant to bonding between same-sex adults. Social bonds between same-sex adults are usually characterized by regular affiliative contact and mutual social support (Massen et al., 2010; Ostner and Schülke, 2018) and share many qualities that are similar to nurturing infant care. Therefore, testosterone may not only be detrimental to nurturing paternal care, but also detrimental to the formation of adult bonds. Same-sex adults may see each other as reproductive rivals and as such, high testosterone levels may interfere with the bonding process by cognitively priming individuals to be competitive rather than nurturing (Eisenegger et al., 2011). Consequently, the S/P theory predicts that the ideal physiological conditions to induce the formation of social bonds are high levels of oxytocin coupled with low levels of testosterone (van Anders et al., 2011). There is provisional support for this prediction in same-sex adults as high oxytocin and lowered testosterone has been linked to meat-sharing in male chimpanzees (*Pan troglodytes*: Sobolewski et al., 2012; Wittig et al., 2014), a behavior that may be used as a tool to form social bonds (Mitani and Watts, 2001; Wittig et al., 2014). Similarly, both low basal levels of testosterone, as well as decreases in levels from baseline, were associated with increased feelings of closeness following a friendship formation task in same-sex adult human dyads (Ketay et al., 2017). Further studies are needed to see if these results are replicated in other species that form strong same-sex bonds.

The HPG axis and the oxytocinergic system are also involved in cross-communication with the hypothalamic-pituitary-adrenal (HPA) axis (Neumann, 2002; Salvador, 2012; Viau, 2002), including in contexts relevant to social bonds. The HPA axis regulates the release of

100 glucocorticoid (GC) steroids and is responsive to actual or perceived stressors (Sapolsky,
101 2002). The presence of or interaction with closely bonded partners during a potentially
102 stressful event often mitigates HPA axis activity, a phenomenon known as social buffering
103 that may be moderated by oxytocin (Hennessy et al., 2009; Hostinar et al., 2014; Smith and
104 Wang, 2014). Being well socially integrated or being able to count on others for social
105 support may additionally help to regulate HPA axis activity even in the immediate absence
106 of stressors (Brent et al., 2011; Rosal et al., 2004; Stocker et al., 2016; Wittig et al., 2016). As
107 with testosterone, cortisol levels also decline in humans after increasing feelings of
108 personal closeness with a task (Ketay et al., 2019, 2017), and individuals with low cortisol
109 had *partners* that desired to be closer to them (Ketay et al., 2017). Thus, while not explicitly
110 part of the S/P theory, mutually lowered GCs and testosterone may have synergistic
111 facilitative effects in the context of same-sex bonding.

112 The aim of this study was to test predictions of the S/P theory (van Anders et al., 2011)
113 regarding nurturing and bonding in male Barbary macaques (*Macaca sylvanus*) in relation
114 to testosterone levels. Given the potential cross-talk between the HPG axis and the
115 oxytocinergic system with the HPA axis, we also explored the relationship between GCs
116 with nurturing and bonding. The male Barbary macaque is a good model for investigating
117 same-sex relationships because males of this species form strong, equitable and stable
118 social bonds with other males (Young et al., 2014b) that physiologically buffer strongly
119 bonded males, leading to a reduced GC (i.e. cortisol) response during stressful situations
120 (Young et al., 2014a). Furthermore, there are two types of interactions relevant to the S/P
121 theory that male Barbary macaques regularly engage in. First, triadic male-infant-male
122 interactions are one proposed mechanism for bonding between males of this species (Deag,
123 1980; Paul et al., 1996). Male-infant-male interactions are ritualistic and involve two males
124 sitting in body contact while holding an infant in-between them, sharing affiliative facial
125 signals and often inspecting the infant's genitals (Deag, 1980; Deag and Crook, 1971). Male-
126 infant-male interactions occur primarily during the non-mating season and predict
127 coalition formation in the following mating season (Berghänel et al., 2011). Since male
128 Barbary macaques prefer to form coalitions with closely bonded partners (Young et al.,
129 2014b) this supports the notion that male-infant-male interactions indeed serve to form

130 social bonds (Kalbitz et al., 2017; Paul et al., 1996). Another proposed function for male-
131 infant-male interactions is that infants may be used as an 'agonistic buffer', to allow two
132 males to approach each other in a non-threatening context (Deag and Crook, 1971; Paul et
133 al., 1996). Grooming between adult males is much rarer than male-infant-male interactions
134 (Paul et al., 1996) and mostly occurs with adult females, and is thus expected to play a
135 comparatively minor role in adult male-male bonding. As per the S/P theory we would
136 predict high oxytocin and low testosterone levels to be associated with male-infant-male
137 interactions. In a previous study, we found mixed results for a positive relationship
138 between male-infant-male interactions and oxytocin levels (Rincon, Deschner, et al., 2019).
139 To complement this study, we now test the other half of the S/P theory and predicted that
140 urinary testosterone levels would be negatively correlated with rates of male-infant-male
141 interactions. Given the potential bonding and/or agonistic buffering function of male-
142 infant-male interactions, we additionally predicted that this behavior would be negatively
143 correlated with urinary cortisol levels.

144 Infant care is the second interaction relevant to the S/P theory that male Barbary macaques
145 take part in. Independent of male-infant-male interactions, male care involves huddling,
146 carrying, and grooming infants (Whitten, 1987). In this (Kubenova et al., 2019b) and other
147 primate species, males bias their care based on the likelihood of paternity, either genetic
148 paternity or past mating success (Buchan et al., 2003; Kerhoas et al., 2016; Langos et al.,
149 2013; Moscovice et al., 2010; Ostner et al., 2013). As the behaviors displayed by males
150 towards infants can be classified as nurturing in nature, we predicted that urinary
151 testosterone levels would be negatively correlated with infant care. A previous study in this
152 species found that male infant care was positively correlated with fecal GC levels (Henkel et
153 al., 2010). Here we tried to replicate this finding and predicted a positive correlation
154 between infant care and urinary cortisol, the primary GC excreted by the HPA axis in
155 Barbary macaques. We link urinary hormone levels with rates of behavior exhibited during
156 6 to 13 hours preceding urination from full-day follows of a single individual. This
157 approach matches hormones to behavior rates over a much shorter period than traditional
158 methods that average behavior rates over days, weeks or longer.

2 Materials and methods

2.1 Study site and animals

Study subjects belonged to one group of Barbary macaques (group C) living in semi-free ranging conditions within 14.5 ha of enclosed forest at Affenberg Salem, Germany (de Turckheim and Merz, 1984). Monkeys were provisioned once a day with fruits, vegetables, grains and had *ad libitum* access to water and monkey chow. Data were collected from 31 March to 26 October 2016 during one non-mating season. The study group consisted of 14 adult males (7 to 25 years old), 20 adult females (5 to 27 years old), 2 large subadult males (6 years old), 8 immature males (1 to 4 years old), 10 immature females (1 to 4 years old) and 1 newborn infant male (<1 years old). The oldest adult male died during the study period on 20 August 2016. All group members could be individually recognized by observers though a combination of unique characteristics including body size, coat color and condition, facial spots and scars, gait and identification tattoos on the inner thigh.

2.2 Behavioral data collection and dominance hierarchy

Behavioral data were collected from 14 adult males using continuous focal animal sampling (Martin and Bateson, 2007) during individual full-day focal animal follows (total = 3276 hours, mean ± standard deviation = 234 ± 19 hours per individual). The occurrence of all social interactions, including the identity of social partner(s) were recorded.

To construct a dominance hierarchy, we used agonistic interactions recorded during continuous focal animal sampling and *ad libitum* sampling. An agonistic interaction was defined by the occurrence of at least one aggressive (open-mouth threat, stare, lunge, charge, chase, physical aggression) and/or submissive (make room, give ground, flee) behavior. Aggressive and submissive behaviors occurring in quick succession of each other were considered as part of the same conflict. We calculated one dominance hierarchy for the entire study period using normalized David's score (de Vries et al., 2006) in R (version 3.5.3; R Core Team, 2018) using the function DS from the package EloRating (version 0.46.8; Neumann and Kulik, 2019). A higher David's score indicates higher dominance rank. The dominance hierarchy was stable throughout the whole study period.

2.3 Urine sample collection

Urine samples were collected from focal males whenever possible by catching it with a plastic bag or otherwise from leaves, plant litter or ground, using a disposable plastic pipette to transfer the sample to 2 ml cryotubes. When pipetting was not possible we absorbed urine using a salivette (Salivette Cortisol, Sarstedt, Nümbrecht, Germany) (Danish et al., 2015). Samples were only collected if they were not contaminated with fecal matter or urine from other individuals. Both samples in cryotubes and salivettes were stored in a thermos container filled with ice while in the field. At the end of the day, samples collected by salivette were centrifuged at 1500 rpm for 5 minutes to extract the urine, which was afterwards transferred to 2 ml cryotubes. All samples were then stored in a freezer at -20°C. At the end of the field season samples were transported on dry ice to the University of Göttingen where they were stored again at -20°C until analysis.

Based on radiometabolism studies assessing the time for hormone excretion in macaque urine, we considered behaviors that occurred up to six hours prior to sample collection to affect urinary testosterone levels (Möhle et al., 2002) and behaviors that occurred within the same day (up to 13.5 hours) prior to sample collection to affect urinary cortisol levels (Bahr et al., 2000). We excluded samples from which we had < 2 hours of observation time prior to sample collection (N = 60), leaving a total of 650 urine samples for analysis. Table 1 shows descriptive information for each study subject.

Table 1: Descriptive information per study subject for the study period

Male ID	Observation days	Observation hours	Dominance rank (norm David's score)	Male-infant-male interactions (per obs hr.)	Infant care (% obs time)	Age (years)	Urine samples (N)
W3	29	237	14.7	0.85	3.46	12	24
U2	29	242	13.1	0.47	1.17	13	42
M3	30	234	12.6	0.06	2.17	21	20
T3	31	242	11.1	0.59	2.42	14	56
M4	30	247	10.9	0.10	0.09	21	49
W7	28	223	10.3	0.33	0.66	12	44
B5	30	237	7.9	1.93	6.70	7	66
B6	29	247	7.3	0.66	2.14	7	61
B7	30	240	7.2	1.75	3.25	7	49
Z5	28	238	5.2	0.09	0.15	9	49
Z6	28	238	4.4	0.37	1.19	9	73
A6	28	237	3.9	0.19	0.06	8	59
I1	14	171	2.2	0.01	0.03	25	13
B1	28	244	2.0	0.28	0.19	7	45

2.4 Hormone analysis

The measurement of cortisol and testosterone from the urine of male Barbary macaques has been previously validated (Rincon, Ostner, et al., 2019). For the accurate quantification of testosterone, but not cortisol, urine samples needed to be enzymatically hydrolyzed prior to hormone measurement (Rincon, Ostner, et al., 2019). Hydrolysis was carried out by mixing 20 - 100 µl urine of each sample with 40 µl β-Glucuronidase (K12 strain

214 *Escherichia coli*, Prod. No. BGALS-RO, Sigma-Aldrich) and 900 µl phosphate buffer
215 (composed of 6.0 g NaH_2PO_4 x H_2O + 14.5 g Na_2HPO_4 x 2 H_2O dissolved in 500 ml water; pH
216 6.9) and incubating in a shaking water bath at 37°C overnight. Hydrolyzed samples were
217 then purified and extracted using solid phase extraction cartridges (Chromabond HR-X,
218 30mg, 1ml, Macherey-Nagel, Dueren, Germany) placed onto a 12-port vacuum manifold.
219 Prior to sample loading, cartridges were conditioned first with 1 ml of MeOH, followed by 1
220 ml H_2O and 1 ml phosphate buffer. Columns were not allowed to run dry at this step.
221 Hydrolyzed urine samples were then pipetted into pre-labelled cartridges and the solution
222 was let to sink in. Cartridges were subsequently rinsed three times with 1 ml H_2O followed
223 by two times with 1 ml MeOH/H_2O (40/60) solution, after which cartridges were dried by
224 applying a vacuum. Steroids were finally eluted with 1 ml absolute MeOH followed by 1 ml
225 ethyl acetate, and eluates were then evaporated to dryness at 40°C under pressurized air.
226 Then, 1 ml hydrolysis phosphate buffer was added to the dried sample eluates and
227 vortexed for 5 min. Afterwards 150 µl 10% K_2CO_3 buffer and 5 ml tert. butyl methyl ether
228 (TBME) was added to each tube for extraction of the liberated steroids. Tubes were sealed
229 with caps and parafilm, vortexed for 10 min on a multi-tube vortexer, then centrifuged for
230 5 min at 2000 rpm and subsequently store at -20°C until the aqueous layer had frozen out.
231 The ether phase was then poured into new collection tubes, the ether was evaporated to
232 around 1 ml, and briefly vortexed to concentrate steroids in the bottom of the tube, and
233 finally evaporated to dryness. Dried samples were reconstituted in 1 ml absolute MeOH by
234 vortexing for 10 min. Extracts were then transferred into 2 ml eppendorf safe-lock tubes
235 and stored at -20°C until analysis for testosterone concentrations. The efficiency of the
236 combined hydrolysis and extraction procedure was assessed using internal controls of
237 testosterone glucuronide run together with each set of samples as described earlier
238 (Rincon, Ostner, et al., 2019). The combined hydrolysis/extraction efficiency was 62.1 ±
239 4.2% mean ± SD (range: 50-70%; N = 36).

240 We measured immunoreactive urinary testosterone from samples following extraction and
241 hydrolysis (described above), and immunoreactive urinary cortisol from unprocessed
242 diluted urine by using enzyme immunoassays as described in detail in Rincon, Ostner, et al.
243 (2019). For the testosterone assay, sensitivity at 90% binding was 3.0 pg; intra-assay CVs

244 of high and low value quality controls were <10%, while figures for inter-assay CVs were
245 13.2% (high) and 19.5% (low). For the cortisol assay, sensitivity at 90% binding was 0.6
246 pg; intra-assay CVs of high and low value quality controls were <10%, while figures for
247 inter-assay CVs were 9.2% (high) and 10.1% (low). To account for differences in the
248 volume and concentration across urine samples, urinary testosterone and cortisol levels
249 were corrected for creatinine and are expressed as ng/mg Cr (Bahr et al., 2000).

250 *2.5 Statistical analyses*

251 To test whether male-infant-male interactions influenced urinary testosterone and cortisol
252 levels, we fitted two Bayesian multilevel linear regression models with a Gaussian response
253 distribution and identity link function. Urinary testosterone (model 1) and urinary cortisol
254 (model 2) were our response variables. Both hormone values had a strong positive skew so
255 we did a natural log transformation to achieve a more symmetrical distribution and used
256 these values in analysis. Our test predictors included a count of male-infant-male
257 interactions and male infant care, defined as the time males spent in body contact with
258 infants including huddling, carrying and grooming in both models. We included duration of
259 grooming, count of male-male aggression, dominance rank (normalized David's score), and
260 time of sample collection (daytime) as control predictors in both models. Counts of male-
261 infant-male interactions and aggression, and duration of infant care were corrected for the
262 observation time of the urine sample window. Each male had one dominance rank score for
263 the entire study period (Table 1). All predictors were z-transformed to a mean of 0 and
264 standard deviation of 1 to improve model convergence and interpretation of results
265 (Schielzeth, 2010).

266 We fitted models in R (version 3.5.2; R Core Team, 2018) using the function brm from the
267 package brms (version 2.9.0; Bürkner, 2017). The package brms calls on the computational
268 framework Stan (https://mc-stan.org) to fit Bayesian models (Bürkner, 2017). We included
269 male identity as a random effect in all models to control for multiple observations (urine
270 samples) per subject. We also included random slopes and correlation parameters between
271 random intercepts and random slopes for all predictors because they had at least three
272 unique values in over half of subjects (Barr et al., 2013; Schielzeth and Forstmeier, 2009).

However, we had to exclude correlations between random intercepts and random slopes in the testosterone model (model 1) to ensure there were no divergent transitions after warm-up. We ran all models with 5000 iterations over four MCMC chains, which included 1000 "warm up" iterations for each chain, resulting in a total of 16000 posterior samples (Bürkner, 2017). In all models, there were no divergent transitions during warm up, all Rhat values were equal to 1.00 and visual inspection of a plot of the chains showed that they were able to converge. We used weakly informative priors to improve convergence, guard against overfitting and regularize parameter estimates (Lemoine, 2019; McElreath, 2016). As a prior for the intercept and beta coefficients we used a normal distribution with mean 0 and standard deviation 10; for the standard deviation of group level (random) effects and sigma we used a Half-Cauchy distribution with location 0 and scale parameter 1; for the correlation between random slopes we used LKJ Cholesky prior with eta 2.

We report model estimates as the mean of the posterior distribution with 95% credible intervals (CI). To decide whether predictor variables were substantially affecting the response (hormone levels), we calculated the proportion (Pr) of posterior samples that fell on the same side of 0 as the mean. The Pr ranges from 0.5 to 1.0, with Pr = 1.00 indicating that, given the model, the effect of a predictor was entirely positive or negative, whereas Pr = 0.5 indicates that the effect was centered around 0. We considered Pr > 0.95 as substantial evidence that a predictor was associated with an outcome.

3 Results

We recorded a total of 1832 triadic male-infant-male interactions during 3276 hours of observation in one non-mating season. All males of the study group were observed to partake in male-infant-male interactions at least once (Table 1). The majority (66%) of male-infant-male interactions involved the one newborn male, 25% involved yearlings and 9% involved juveniles. Rates of male-infant-male interactions were negatively correlated with both testosterone (Table 2; Fig. 1 *a*, *b*) and cortisol (Table 3; Fig. 2, *a*, *b*) levels.

As with triadic interactions, all males of the group were observed caring for infants (≤ 1 years old), including time spent huddling, carrying, or grooming (Table 1). Male infant care

was positively correlated with both testosterone (Table 2; Fig. 1, *a*, *c*) and cortisol (Table 3; Fig. 2, *a*, *c*) levels.

Table 2: Urinary testosterone levels in relation to male-infant-male interactions and male infant care (model 1). Male identity was included as a random effect, N subjects = 14, N urine samples = 650. CI = 95% credible intervals, Pr = proportion of the posterior samples that fall on the same side of 0 as the mean.

	Estimate	SD	CI lower	CI upper	Pr
Intercept	3.04	0.09	2.85	3.22	1.00
Test predictors					
Male-infant-male	-0.18	0.06	-0.32	-0.07	>0.99
Infant care	0.10	0.05	-0.00	0.20	0.97
Control predictors					
Grooming	0.03	0.04	-0.04	0.11	0.82
Aggression	-0.02	0.04	-0.10	0.06	0.68
Dominance rank	-0.17	0.08	-0.33	-0.01	0.98
Daytime	-0.03	0.03	-0.09	0.04	0.79

Table 3: Urinary cortisol levels in relation to male-infant-male interactions and male infant care (model 2). Male identity was included as a random effect, N subjects = 14, N urine samples = 650. CI = 95% credible intervals, Pr = proportion of the posterior samples that fall on the same side of 0 as the mean.

	Estimate	SD	CI lower	CI upper	Pr
Intercept	5.17	0.12	4.94	5.42	1.00
Test predictors					
Male-infant-male	-0.32	0.15	-0.67	-0.08	0.99
Infant care	0.08	0.05	-0.02	0.20	0.95
Control predictors					
Grooming	0.00	0.06	-0.12	0.12	0.53
Aggression	-0.03	0.03	-0.10	0.03	0.83
Dominance rank	-0.18	0.10	-0.38	0.01	0.97
Daytime	-0.23	0.04	-0.31	-0.15	1.00

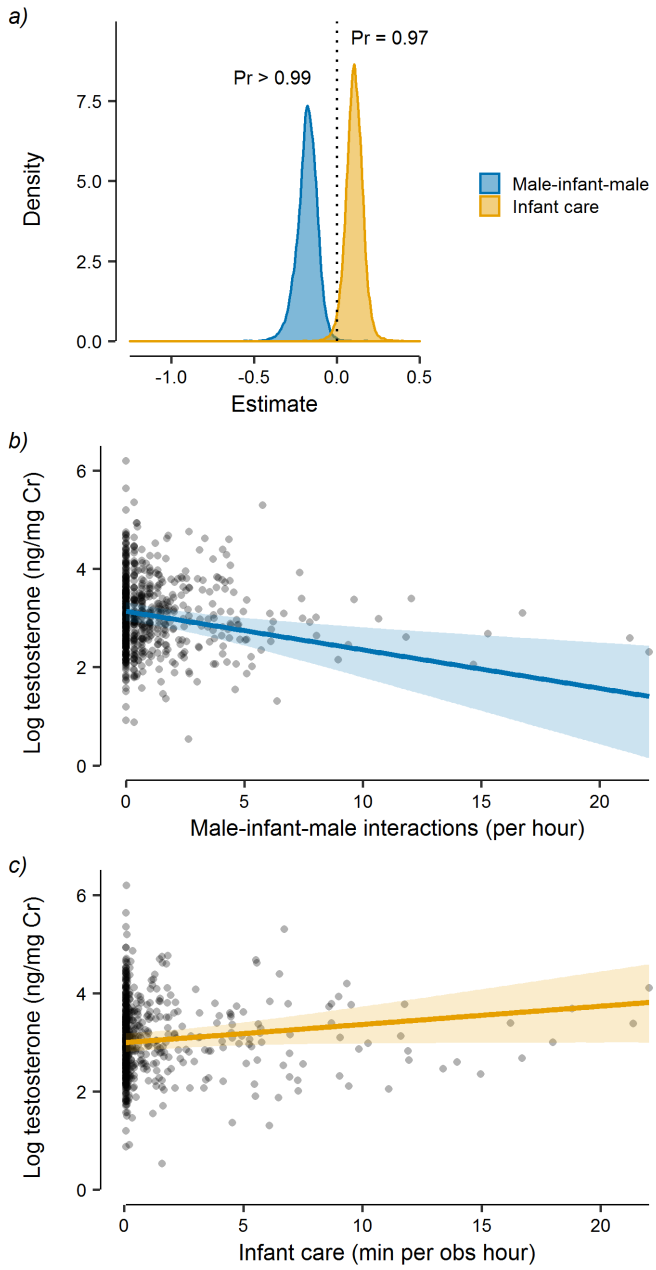

Fig. 1: Urinary testosterone levels in relation to male-infant-male interactions and infant care (model 1). a) Posterior probability distribution of the slope estimate. Pr = proportion of the posterior samples that fall on the same side of 0 as the mean. b) and c) Model fitted values (solid line) and 95% credible intervals (shaded area) when all other predictors are at their mean. Cr = creatinine. Circles indicate raw data points (urine samples). Note that the fitted model line is based on model results with additional controls that are not reflected in the raw data points.

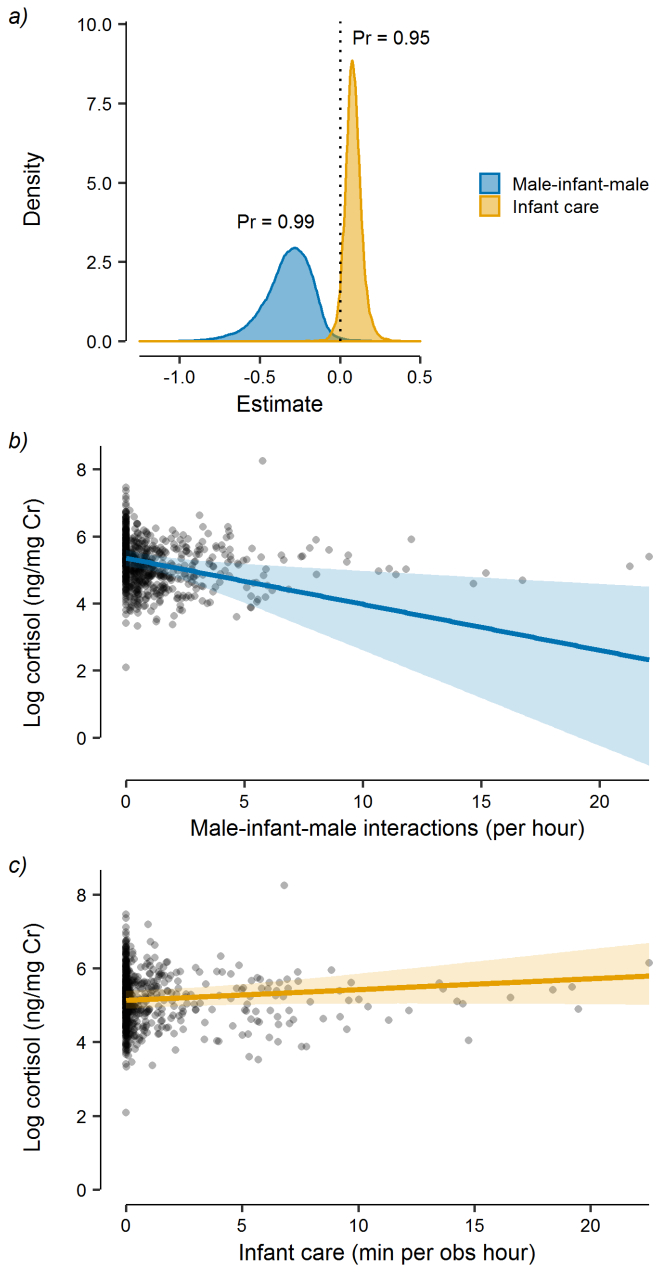

Fig. 2: Urinary cortisol levels in relation to male-infant-male interactions and infant care (model 2). a) Posterior probability distribution of the slope estimate. Pr = proportion of the posterior samples that fall on the same side of 0 as the mean. b) and c) Model fitted values (solid line) and 95% credible intervals (shaded area) when all other predictors are at their mean. Cr = creatinine. Circles indicate raw data points (urine samples). Note that the fitted model line is based on model results with additional controls that are not reflected in the raw data points.

4 Discussion

In this study, we investigated how testosterone and cortisol levels related to social bonding and nurturing in male Barbary macaques, testing predictions of the steroid/peptide theory of social bonds (S/P theory: van Anders et al., 2011). As predicted, urinary testosterone and cortisol levels were negatively correlated with triadic male-infant-male interactions, a ritualized behavior hypothesized to promote and/or maintain social bonds in male macaques (Berghänel et al., 2011; Kalbitz et al., 2017; Paul et al., 1996). We also investigated whether male care for infants was negatively associated with testosterone (van Anders et al., 2011) and positively associated with cortisol levels (Henkel et al., 2010). Our predictions were partially supported as both testosterone and cortisol levels were positively correlated with male infant care, suggesting that infant care may be done under competitive, rather that nurturing contexts.

Our finding that urinary testosterone levels were negatively correlated with male-infant-male interactions in Barbary macaques supports predictions of the S/P theory that low testosterone levels are conducive to nurturing relationships and bonding (van Anders, 2013; van Anders et al., 2011). These results are also in line with previous studies in humans and chimpanzees, which found that behaviors promoting bond formation between same-sex adults are associated with lowered testosterone levels (Ketay et al., 2017; Sobolewski et al., 2012). Low testosterone during bond formation in same-sex dyads may reduce competitive tendency and avoid expression of behaviors that are incompatible with social bonding. For example, testosterone has an inhibitory role in trust (Boksem et al., 2013; Bos et al., 2010), facial mimicry (Hermans et al., 2006) and empathy (Ronay and Carney, 2013; van Honk et al., 2011). Human, chimpanzee and macaque males readily form close social bonds and long-term cooperative relationships (Apicella and Silk, 2019; Kalbitz et al., 2016; Watts, 2002; Young et al., 2014b). As these cooperative relationships are likely adaptive (Aktipis et al., 2018; Schülke et al., 2010; Watts, 2002; Young et al., 2013a), males may have been under selective pressure to down-regulate testosterone during periods of affiliation to facilitate bond formation. It would be interesting to test whether lowered testosterone after affiliation between same-sex dyads is only found in species where long-term cooperative relationships increase fitness. Beyond the formation of same-sex social

359 bonds, high testosterone levels may also be detrimental to the maintenance of these bonds
360 in the long term. For example, territory holder male manakins (*Pipra filicauda*), a lekking
361 bird species, must perform cooperative display with floater males to attract females (Ryder
362 et al., 2009). Territory holders whose testosterone levels are too high fail to attract and
363 maintain stable display partnerships with floater males (Ryder et al., 2019), reducing their
364 ability to compete for females.

365 We additionally found that urinary cortisol levels were negatively correlated with rates of
366 male-infant-male interactions in male Barbary macaques. One explanation for this finding
367 is that male-infant-male interactions decrease tensions between adult males, as per the
368 agonistic buffering hypothesis (Deag and Crook, 1971; Paul et al., 1996), and consequently
369 reduce cortisol levels. Another non-mutually exclusive explanation relates to the
370 potentially bond-formation properties of male-infant-male interactions, resulting in
371 integration into the male social network (Henkel et al., 2010) and increased social support
372 (Berghänel et al., 2011). Social support is a powerful regulator of the HPA axis and thus of
373 GC levels (Hennessy et al., 2009; Kikusui et al., 2006). Merely the presence of closely
374 bonded conspecifics during a stressor is enough to buffer HPA axis activity in a variety of
375 species (Hennessy et al., 2009; Kikusui et al., 2006). Indeed male Barbary macaques with
376 strong social bonds show attenuated GC responses to social and environmental stressors
377 (Young et al., 2014a). Across primates, subordinate individuals exhibit higher GC levels
378 than dominants only in societies in which they are harassed by dominants but lack access
379 to social support (Abbott et al., 2003). Having reliable social support may also help to
380 downregulate HPA activity even in the immediate absence of stressors (Brent et al., 2011;
381 Fürtbauer et al., 2014; Rosal et al., 2004; Wittig et al., 2016), possibly due to increased
382 psychological wellbeing (Lakey and Orehek, 2011; Massen et al., 2010; Thoits, 2011). Thus
383 on days when the study males engaged in male-infant-male interactions most frequently,
384 they may have perceived themselves as having strengthened their social bonds and ability
385 to call on support if needed (Berghänel et al., 2011), consequently lowering cortisol.

386 The process of bonding itself is likely driven by hormones other than testosterone and
387 cortisol. For instance, the S/P theory predicts that high oxytocin levels are needed to
388 promote social bonding (van Anders et al., 2011). In a previous study on the same group of

Barbary macaques we did not find an increase in oxytocin levels following male-infant-male interactions in general but found that oxytocin levels were only higher after male-infant-male interactions with non-bond partners (Rincon, Deschner, et al., 2019). If it is the combination of low testosterone and high oxytocin that is responsible for the bonding effects, then male-infant-male interactions may function to form bonds selectively between non-bonded partners while physiologically not affecting existing bonds between bond partners. In this interpretation, male-infant-male interactions are a tool for bond formation rather than bond maintenance, although, low testosterone levels after interactions with bond partners may still be beneficial to avoid damaging existing relationships. In our study subjects, 45% of male-infant-male interactions occurred with non-bond partners [Rincon et al. unpublished data] indicating that there is substantial variation in partner identity and thus the potential to bond with previously non-bonded partners is high. Finally, the low levels of testosterone associated with male-infant-male interactions occur independently of infant care, suggesting that it is not merely the presence of an infant that lowers testosterone or cortisol levels but that the male-infant-male interaction itself is what is salient.

Low cortisol in conjunction with low testosterone may act synergistically to reduce tension and facilitate bonding. During a dyadic friendship formation task in humans, participants desired to be closer to their partners if they (their partner) had low cortisol levels (Ketay et al., 2017), suggesting that affiliative interactions in a relaxed state are beneficial for both partners. While low testosterone and low cortisol may be beneficial to bond formation between same-sex adults, this pattern may not generalize to other types of dyads or contexts. Our findings are in contrast to a previous study in humans where testosterone levels were negatively related to friendship *formation* and positively related to friendship *maintenance* within a social network, whereas the opposite was true for cortisol levels, suggesting that testosterone and cortisol have opposite effects with regard to friendship formation and maintenance (Kornienko et al., 2016). One difference between our study and Kornienko et al. (2016) is that the social network comprised a mixed sex group. Steroid dynamics with relation to opposite-sex bonding may differ from that for same-sex bonding due to opposite-sex dyads having greater potential for a sexual relationship and same-sex

419 dyads having greater potential to be reproductive competitors. For example, high
420 testosterone levels promote men to affiliate with women (van der Meij et al., 2012) and
421 facilitate the initial stage of sexual or romantic relationships (Roney and Gettler, 2015).
422 Although high testosterone levels may be detrimental to satisfaction in long-term romantic
423 relationships (Edelstein et al., 2014; Roney and Gettler, 2015), it is unclear if this is also the
424 case for long-term opposite-sex friendships.

425 Contrary to our predictions, male infant care was positively correlated with testosterone
426 levels. The same was true for cortisol levels, corroborating a previous finding in this
427 species (Henkel et al., 2010). These results suggest that, from the male's perspective, infant
428 care is neither nurturing nor relaxing and may instead be done under a competitive
429 context. Infant caretakers have a protective role in the majority of Old World nonhuman
430 primates (Muller, 2017), and male care most likely evolved as an adaptation against
431 infanticide, even if it is no longer the main function of the behavior (Paul et al., 2000). The
432 threat of infanticide has been shown to elicit positive testosterone and GC responses in
433 primates (Cheney et al., 2015; Muller, 2017; Ostner et al., 2008). Infanticide is rare in
434 Barbary macaques and most likely reflects effective counter measures employed by
435 females to induce paternity confusion such as promiscuity (Small, 1990), reproductive
436 synchrony (Young et al., 2013a) and post-conception copulations (Young et al., 2013b).
437 Nevertheless, males do prefer to interact with infants based on their past mating success
438 with the mother (Kubenova et al., 2019b), which may be indicative of paternal investment
439 and serve as motivation to defend or support an infant against conspecifics. Infant care
440 during the non-mating season also predicts future copulations with the mother in the
441 following mating season in Barbary macaques (Kubenova et al., 2019b; Ménard et al.,
442 2001). Thus, male infant care in Barbary macaques could also be considered as mating
443 effort. Whether infant care is done as a protective role or as part of mating effort, a positive
444 relationship with testosterone and cortisol could be beneficial in both cases.

445 Another motivation for males to care for infants may be to engage in affiliative contact with
446 other males (Henkel et al., 2010; Paul et al., 1996) with the ultimate purpose of
447 strengthening male bonds, forging alliances and increasing/maintaining status. For
448 instance, infant-holders are more likely to initiate male-infant-male interactions with males

who are higher ranking or adjacent in rank to themselves (Kubenova et al., 2019a; Paul et al., 1996) – males that would constitute the best coalitionary allies. Therefore, infants are attractive commodities and could create competition among males to gain access to them due to there being only a limited number of infants relative to adult males. Once an infant is in a male's 'possession' they may need to fend off interest from other group members and scout their social environment for potential affiliation opportunities with preferred males. In this scenario, caring for infants could be rather stressful and/or requiring a state of alertness. Moderate increases in testosterone and cortisol during infant care may not only help retain the infant in possession but also promote the use of infants as tools to affiliate with other males. For instance, in humans, testosterone promotes males to behave in such a way to increase/maintain social status even if such behaviors are prosocial in nature (Boksem et al., 2013; Eisenegger et al., 2011; Terburg and van Honk, 2013). Similarly, moderate increases in GC levels serve to promote seeking of affiliative contact in group-living species with complex social systems (Raulo and Dantzer, 2018). Therefore, elevated testosterone and cortisol levels during infant care are not necessarily a 'cost' to enter the male network as previously suggested (Henkel et al., 2010), but a beneficial endocrine profile that allows males to better navigate their social environment.

Collectively our results provide support for the S/P theory which stipulates that high testosterone levels reflect mating effort and low levels are ideal for nurturing relationships including in same-sex adult bonding. Social behavior and dynamics is a complex process. Theoretical frameworks that integrate multiple endocrine systems, such as the S/P theory (van Anders et al., 2011) and others (e.g. Soares et al., 2010), are useful in painting a more complete picture of the social context and ultimate function of particular social interactions.

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