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

Alan V. Rincon[a,b], Michael Heistermann[c], Oliver Schülke[a,b,d], Julia Ostner[a,b,d]

[a]*Department of Behavioral Ecology, Johann-Friedrich-Blumenbach Institute for Zoology and Anthropology, University of Goettingen, Goettingen, Germany*

[b]*Leibniz ScienceCampus Primate Cognition, Goettingen, Germany*

[c]*Endocrinology Laboratory, German Primate Center, Leibniz Institute for Primate Research, Goettingen, Germany*

[d]*Research Group Social Evolution in Primates, German Primate Center, Leibniz Institute for Primate Research, Goettingen, Germany*

Corresponding author: Alan V. Rincon, Department of Behavioral Ecology, University of Goettingen, Kellnerweg 6, 37077 Goettingen, Germany

Email: avrincon1@gmail.com

14   **Abstract**

15   Neuroendocrine research on the formation of social bonds has primarily focused on the role of

16   nonapeptides. However, steroid hormones often act simultaneously to either inhibit or facilitate

17   bonding. Testosterone is proposed to mediate a trade-off between male mating effort and nurturing

18   behavior; therefore, low levels are predicted during periods of nurturing infant care and social

19   bonding. In species where social bonding and support regulates hypothalamic-pituitary-adrenal

20   axis activity, we also expect glucocorticoid levels to be low during bonding periods. We investigated

21   how immunoreactive urinary testosterone (iuT) and cortisol (iuC) were related to triadic male-

22   infant-male interactions – a ritualized male bonding behavior – as well as infant care in male

23   Barbary macaques (*Macaca sylvanus*). We collected >3000 hours of behavioral observation data

24   during full-day focal animal follows from 14 adult males and quantified iuT and iuC from 650 urine

25   samples. As predicted, both iuT and iuC were negatively correlated with rates of triadic interactions

26   within-subjects in the hours preceding urination. We found no relationship between iuT and iuC

27   with triadic interactions between-subjects. Infant care was weakly positively correlated to iuT and

28   iuC within-subjects, but not between-subjects. The observed negative relationship between iuT and

29   triadic interactions may be beneficial to lower competitive tendencies between adult males and to

30   not inhibit bond formation. Lowered iuC could reflect increased bonding and perceived social

31   support as triadic interactions predict future coalition formation in this species. Additionally,

32   lowered iuC may be reflective of buffered tensions between males. The positive relationship of iuT

33   and iuC with infant care suggests that the handling of infants in may be less nurturing but rather

34   protective or competitive in this species. Measuring steroid hormones in relation to bonding and

35   nurturing can help us interpret behaviors within the ecological contexts that they occur.

36   Keywords: Testosterone, cortisol, steroid/peptide theory, social bond, triadic male-infant-male

37   interaction, Barbary macaque

1   Introduction

Steroid hormones and behavior are intricately linked and often affect each other in a reciprocal manner (Goymann, 2009). Stable differences in baseline hormone levels could result in stable differences in behavioral profiles across individuals. Additionally, hormone levels can change dynamically within individuals to help them respond adaptively to a changing social environment. Distinguishing whether hormone-behavior interactions operate at the within- or between-subjects level (or both) can help us understand behavioral variation among individuals and behavioral flexibility within individuals (Hau and Goymann, 2015).

The challenge hypothesis (Goymann et al., 2019; Wingfield et al., 1990), proposes that high testosterone levels (regulated by the hypothalamic-pituitary-gonadal axis; HPG) promotes male mating effort and inhibits paternal care. Across vertebrates, males exhibit elevated testosterone levels when competing for mates, such as during the mating season or territorial defense, and low testosterone levels during periods of paternal care (Grebe et al., 2019; Hirschenhauser and Oliveira, 2006; Lynn, 2008; Muller, 2017; Wingfield et al., 1990). Testosterone potentially inhibits paternal care as experimentally increasing testosterone levels reduces paternal behavior in various bird species (Lynn, 2008), including increases within an individual's natural range (Goymann and Flores, 2017). However, testosterone levels are positively related to paternal care when care is competitive in nature and concerns offspring defense from conspecifics (Moore et al., 2019; Muller, 2017). The steroid peptide theory of social bonds (S/P theory) builds on the challenge hypothesis to resolve 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 et al., 2011).

Beyond mating effort and nurturing care, testosterone is relevant to bonding between same-sex adults. Same-sex adult bonds are usually characterized by affiliative contact and mutual social

62   support (Massen et al., 2010; Ostner and Schülke, 2018) and share many qualities with nurturing

63   infant care. Consequently, the S/P theory proposes that low levels of testosterone are ideal for bond

64   formation to not inhibit the bonding process (van Anders et al., 2011). Consistent with this

65   prediction, low testosterone has been linked to meat-sharing in male chimpanzees (*Pan troglodytes*:

66   Sobolewski et al., 2012), a behavior implicated in bond formation (Wittig et al., 2014). Similarly, low

67   basal levels of testosterone, and decreased levels from baseline, were associated with feelings of

68   closeness following a friendship formation task in same-sex adult human dyads (Ketay et al., 2017).

69   Glucocorticoid steroids (primarily corticosterone in birds and rodents and cortisol in other

70   mammals) are also involved in regulating animal social behavior, including in contexts relevant to

71   nurture and social bonds. Glucocorticoid release is regulated by the hypothalamic-pituitary-adrenal

72   (HPA) axis and is responsive to actual or perceived stressors (Sapolsky, 2002). The presence of or

73   interaction with bonded partners during a potentially stressful event often attenuates HPA axis

74   activity, a phenomenon known as social buffering (Cohen and Wills, 1985; Hostinar et al., 2014).

75   Being well socially integrated or being able to count on others for social support may help to

76   regulate HPA axis activity even in the immediate absence of stressors (Rosal et al., 2004; Wittig et

77   al., 2016). As with testosterone, cortisol levels also decline in humans after increasing feelings of

78   personal closeness, and individuals with low baseline cortisol had *partners* that desired to be closer

79   to them (Ketay et al., 2017). Thus, mutually lowered cortisol and testosterone may have synergistic

80   facilitative effects in the context of same-sex bonding.

81   Similarly to testosterone, a male's glucocorticoid response to infant care may be dependent on

82   social context. Within nurturing contexts, infant care appears to be negatively related to

83   glucocorticoid levels. For example, cortisol levels decline following father-offspring interactions

84   under non-stressful conditions in humans (Gettler et al., 2011b; Storey et al., 2011). Similarly, male

85   marmosets (*Callithrix kuhlii*) that frequently carried infants had significantly lower glucocorticoid

86   levels than males that carried infants less frequently (Nunes et al., 2001). However, glucocorticoid

87   levels may be positively related to paternal care under more stressful contexts. For example,

88   cortisol levels increase in response to infant cries in men (Fleming et al., 2002). In chacma baboons

89   (*Papio ursinus*), males whose infants were at immediate risk of infanticide showed significant

90   increases in glucocorticoid levels compared to a stable period, and had overall higher levels than

91   males whose infants were not at risk (Cheney et al., 2015).

92   The aim of this study was to investigate how urinary testosterone and cortisol relate to same-sex

93   bonding and nurturing behaviors in male Barbary macaques (*Macaca sylvanus*). Male Barbary

94   macaques form stable social bonds with other males (Young et al., 2014b) that physiologically

95   buffer bonded males during stressful situations (Young et al., 2014a). Two types of interactions are

96   relevant to our aims: triadic male-infant-male interactions (hereafter triadic interactions) and

97   infant care. Triadic interactions are ritualistic and involve two adult males sitting in body contact

98   with an infant or young juvenile in-between them, exchanging affiliative facial signals and often

99   lifting up the immature or inspecting its genitals (Deag and Crook, 1971; Paul et al., 1996). These

100   interactions are of short duration, occur primarily during the non-mating season, predict coalition

101   formation in the following mating season, and have been implicated in bond maintenance

102   (Berghänel et al., 2011; Kalbitz et al., 2017). Triadic interactions may also function in 'agonistic

103   buffering', where the presence of the infant allows two males to approach each other in a non-

104   threatening context (Deag and Crook, 1971; Paul et al., 1996). Given the potential bonding and/or

105   agonistic buffering function of triadic interactions, we predicted a negative correlation with both

106   urinary testosterone and cortisol levels.

107   In addition to performing triadic interactions, male Barbary macaques also care for infants and

108   yearlings on a dyadic level (in the absence of other males) by huddling, carrying, and grooming with

109   them, sometimes for prolonged periods of time (Whitten, 1987). The exact function of male infant

110   care in this species remains ambiguous. Males generally prefer to care for infants based on their

111   probability of paternity inferred from their previous mating success with the mother (Kubenova et

112   al., 2019), although their preferred infant is not always their genetic offspring (Ménard et al., 2001).

113   One possible explanation is that males care for infants in a nurturing context. Another possibility is

114   that infant care may be considered part of mating effort to increase a male's mating success with

115   the mother on the following mating season (Ménard et al., 2001). A previous study on this species

116   suggested that handling infants might be stressful as it was associated with elevated fecal

117   glucocorticoid levels (Henkel et al., 2010). Depending on the function of infant care, we may expect

118   different relationships between this behavior with testosterone and cortisol. If infant care is

119   nurturing in nature, we predict a negative relationship with testosterone (van Anders et al., 2011;

120   Wingfield et al., 1990) and cortisol (Nunes et al., 2001; Storey et al., 2011). If the function of infant

121   care is protective, or if infants are used as tools for mating effort, we expect a positive relationship

122   between this behavior and testosterone (Muller, 2017; van Anders et al., 2011) and cortisol

123   (Cheney et al., 2015).

124   Finally, as steroids can influence behavior within- or between-subjects we explicitly differentiate

125   between both types of effects in our analyses. We predicted that the directionality of results would

126   be the same whether effects are within- or between-subjects and that both types of effects would be

127   found.

128   **2   Materials and methods**

129   *2.1   Study site and animals*

130   Study subjects belonged to one group of Barbary macaques (group C) living in semi-free ranging

131   conditions within 14.5 ha of enclosed forest at Affenberg Salem, Germany (de Turckheim and Merz,

132   1984). Monkeys were provisioned once a day with fruits, vegetables, grains and had *ad libitum*

133   access to water and monkey chow. Data were collected from 31 March to 26 October 2016 during

134   one non-mating season. The study group consisted of 14 adult males (7 to 25 years old), 20 adult

135   females (5 to 27 years old), 2 large subadult males (6 years old) and 19 immatures (up to 4 years

136   old, 10 females, 11 males, including 2 male and 1 female yearlings, and one newborn male). The

137   oldest adult male died during the study period on 20 August 2016. All group members could be

138   individually recognized by observers though a combination of unique characteristics including

139   body size, coat color and condition, facial spots and scars, gait and identification tattoos on the

140   inner thigh.

141   *2.2   Behavioral data collection and dominance hierarchy*

142   Behavioral data were collected from 14 adult males using continuous focal animal sampling (Martin

143   and Bateson, 2007). Focal males were followed continuously for an entire observation day (8-13.5

144   hours per day; total = 3289 hours, mean ± standard deviation = 235 ± 19 hours per individual;

145   Table 1). The occurrence of all social interactions, including the identity of social partner(s) were

146   recorded. We defined infant care as the time males spent in body contact with infants including

147   huddling, carrying, and grooming, with 'infants' defined as < 1.5 years old (i.e. newborns and

148   yearlings, N = 4). As infant care can occur for prolonged periods of time, we recorded the duration

149   of this behavior. We defined triadic interactions as those that occurred between two adult males

150   and any immature individual (0-4 years old, N = 19; see introduction for a description). Although

151   triadic interactions can develop from a dyadic male-infant situation in some cases, they also

152   develop when a male without an infant is approached by a male carrying an infant or by two males

153   spontaneously converging on the same infant without prior male-infant interactions. In addition,

154   the group of immatures involved in either of the two immature-related behaviors differed, making

155   the two behaviors rather independent. As triadic interactions are often short in duration, we

156   recorded the frequency of this behavior.

157   To construct a dominance hierarchy, we used agonistic interactions recorded during continuous

158   focal animal sampling and *ad libitum* sampling. An agonistic interaction was defined by the

159   occurrence of at least one aggressive (open-mouth threat, stare, lunge, charge, chase, physical

160   aggression) and/or submissive (make room, give ground, flee) behavior. Aggressive and submissive

161   behaviors occurring in quick succession (<20 seconds) of each other were considered as part of the

162   same conflict. We calculated one dominance hierarchy for the entire study period using normalized

163   David's score (de Vries et al., 2006) in R (version 3.6.2; R Core Team, 2019) using the function DS

164   from the package EloRating [version 0.46.8], generating a continuous measure of individual

165   winning success. A higher David's score indicates higher dominance rank. The dominance hierarchy

166   was stable throughout the whole study period.

167   *2.3   Urine sample collection*

168   Urine samples were collected from focal males whenever possible without disturbing the animal by

169   catching it with a plastic bag or otherwise from leaves, plant litter or ground, using a disposable

170   plastic pipette to transfer the sample to 2 ml cryotubes. When pipetting was not possible we

171   absorbed urine using a salivette (Salivette Cortisol, Sarstedt, Nümbrecht, Germany; Büttler et al.,

172   2018). Samples were only collected if they were not contaminated with fecal matter or urine from

173   other individuals. Both samples in cryotubes and salivettes were stored in a thermos container

174   filled with ice while in the field. At the end of the day, samples collected by salivette were

175   centrifuged at 1500 rpm for five minutes to extract the urine, which was afterwards transferred to

176   2 ml cryotubes. All samples were then stored in a freezer at -20°C. At the end of the field season

177   samples were transported on dry ice to the University of Göttingen where they were stored again at

178   -20°C until analysis.

179   We determined the steroid excretion window in macaque urine based on previous

180   radiometabolism studies (Bahr et al., 2000; Möhle et al., 2002). For cortisol, we considered

181   behaviors that occurred within the same day (up to 13.5 hours) prior to sample collection to affect

182   urinary hormone levels (Bahr et al., 2000). For testosterone, levels were at their highest in the urine

183   up to six hours after injection, although they still remained somewhat elevated for up to one day

184   (around 13.5 hours; Möhle et al., 2002). Therefore, we considered behaviors that occurred up to six

185   hours prior to sample collection to affect urinary testosterone levels and used this time window in

186   the main analysis of the study. Nevertheless, to determine the robustness of results we conducted

187   additional analyses using a 13.5 hour clearance window and present these results in the

188   supplementary material (Table S1a and Table S1b). The results were similar regardless of which

189   excretion window was used. We excluded samples from which we had < 2 hours of observation

190   time prior to sample collection for both testosterone and cortisol (N = 60), leaving a total of 650

191   urine samples for cortisol analysis. Out of these 650 urine samples, 78 (12%) were collected with

192   salivettes [Salivette Cortisol, Sarstedt, Nümbrecht, Germany]. Samples collected by salivette were

193   excluded from testosterone analyses but not cortisol analyses as they were validated for the former

194   and not the latter (Büttler et al., 2018), leaving a total of 572 samples. To determine the robustness

195   of results, we present cortisol analysis with salivette samples excluded in the supplementary

196   material (Table S2a and Table S2b). Results including or excluding samples collected via sallivete

197   were similar. Table 1 shows descriptive information and sample sizes for each study subject.

198   Table 1: Descriptive information per study subject for the study period. Behavior rates shown here

199   were calculated based on all observation days for descriptive purposes. Behavior rates used in

200   analyses were calculated individually for each urine sample and hormone excretion window.

Male ID	Observation days	Observation hours	Dominance rank (norm David's score)	Triadic interactions (per obs. hr.)	Infant care (% obs. time)	Age (years)	Testosterone samples (N)	Cortisol samples (N)
W3	19	238	14.6	0.81	3.32	12	13	24
U2	20	243	13.1	0.45	1.16	13	42	42
M3	21	235	12.6	0.06	2.16	21	19	20
T3	21	242	11.1	0.51	2.42	14	42	56
M4	21	247	10.9	0.04	0.07	21	43	49
W7	19	224	10.3	0.31	0.64	12	40	44
B5	20	238	7.9	1.80	6.65	7	55	66
B6	21	247	7.3	0.65	2.14	7	57	61
B7	21	241	7.2	1.58	3.19	7	44	49
Z5	20	240	5.2	0.08	0.15	9	48	49
Z6	20	239	4.4	0.36	1.19	9	68	73
A6	21	238	3.9	0.17	0.06	8	54	59
I1	14	172	2.2	0.01	0.03	25	12	13
B1	20	246	2.0	0.26	0.19	7	35	45

201

202   *2.4   Hormone analysis*

203   The measurement of immunoreactive cortisol (iuC) and testosterone (iuT) from the urine of male

204   Barbary macaques has been previously validated (Rincon et al., 2019). Furthermore, we found a

205   negative correlation between cortisol and time of day, thus further biologically validating our

206   cortisol measurement (Table 3). For the accurate quantification of testosterone, but not cortisol,

207   urine samples needed to be enzymatically hydrolyzed prior to hormone measurement (Rincon et

208   al., 2019). Hydrolysis was carried out by mixing 20 - 100 µl urine of each sample with 40 µl β-

209   Glucuronidase (K12 strain *Escherichia coli*, Prod. No. BGALS-RO, Sigma-Aldrich) and 900 µl

210   phosphate buffer (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

211   water; pH 6.9) and incubating in a shaking water bath at 37°C overnight. Hydrolyzed samples were

212   then purified and extracted using solid phase extraction cartridges (Chromabond HR-X, 30mg, 1ml,

213   Macherey-Nagel, Dueren, Germany) placed onto a 12-port vacuum manifold. Prior to sample

214   loading, cartridges were conditioned first with 1 ml of MeOH, followed by 1 ml H_2O and 1 ml

215   phosphate buffer. Columns were not allowed to run dry at this step. Hydrolyzed urine samples were

216   then pipetted into pre-labelled cartridges and the solution was let to sink in. Cartridges were

217   subsequently rinsed three times with 1 ml H_2O followed by two times with 1 ml MeOH/H_2O

218   (40/60) solution, after which cartridges were dried by applying a vacuum. Steroids were finally

219   eluted with 1 ml absolute MeOH followed by 1 ml ethyl acetate, and eluates were then evaporated

220   to dryness at 40°C under pressurized air. Then, 1 ml hydrolysis phosphate buffer was added to the

221   dried sample eluates and vortexed for 5 min. Afterwards 150 µl 10% K_2CO_3 buffer and 5 ml tert.

222   butyl methyl ether (TBME) was added to each tube for extraction of the liberated steroids. Tubes

223   were sealed with caps and parafilm, vortexed for 10 min on a multi-tube vortexer, then centrifuged

224   for 5 min at 2000 rpm and subsequently stored at -20°C until the aqueous layer had frozen out. The

225   ether phase was then poured into new collection tubes, the ether was evaporated to around 1 ml,

226   and briefly vortexed to concentrate steroids in the bottom of the tube, and finally evaporated to

227   dryness. Dried samples were reconstituted in 1 ml absolute MeOH by vortexing for 10 min. Extracts

228   were then transferred into 2 ml eppendorf safe-lock tubes and stored at -20°C until analysis for

229   testosterone concentrations. The efficiency of the combined hydrolysis and extraction procedure

230   was assessed using internal controls of testosterone glucuronide run together with each set of

231   samples as previously described (Rincon et al., 2019). The combined hydrolysis/extraction

232   efficiency was 62.1 ± 4.2% mean ± SD (range: 50-70%; N = 36).

233   We measured iuT from samples following extraction and hydrolysis (described above), and iuC

234   from unprocessed diluted urine by using enzyme immunoassays as described in detail in Rincon et

235   al. (2019). For the testosterone assay (Palme and Möstl, 1994), sensitivity at 90% binding was 0.3

236   pg; intra-assay CVs of high and low value quality controls were <10%, while figures for inter-assay

237   CVs were 13.2% (high) and 19.5% (low). For the cortisol assay (Palme and Möstl, 1997), sensitivity

238   at 90% binding was 0.6 pg; intra-assay CVs of high and low value quality controls were <10%, while

239   figures for inter-assay CVs were 9.2% (high) and 10.1% (low). To account for differences in the

240   volume and concentration across urine samples, iuT and iuC levels were corrected for creatinine

241   and are expressed as ng/mg Cr (Bahr et al., 2000).

242   *2.5   Statistical analyses*

243   To test whether triadic interactions were associated with urinary testosterone and cortisol levels,

244   we fitted Bayesian multilevel linear regression models with a Gaussian response distribution and

245   identity link function. Immunoreactive urinary testosterone (iuT; model 1) and cortisol (iuC; model

246   2) were our response variables. Distributions of both hormone values had a strong positive skew so

247   we used a natural log transformation to achieve more symmetrical distributions and used these

248   values in analysis. Our test predictors were count of triadic interactions and duration of male infant

249   care. We included duration of grooming between two adults (male-female or male-male), count of

250   male-male aggression, a continuous measure of dominance rank (normalized David's score), and

251   time of sample collection (daytime) as control predictors in both models. Counts of triadic

252   interactions and male-male aggression, and duration of infant care and grooming were calculated

253   for the hormone excretion window of each urine sample and corrected for the observation time of

254   the hormone excretion window. Each male had one dominance rank score for the entire study

255   period (Table 1). All predictors were z-transformed to a mean of 0 and standard deviation of 1 to

256   improve model convergence and interpretation of results (Schielzeth, 2010).

257   To decouple whether triadic interactions and infant care influenced hormone levels within- or

258   between-subjects, we computed within-subjects centering as described in van de Pol and Wright

259   (2009). We first calculated the mean rates of triadic interactions for each male. Then we calculated

260   relative rates of triadic interactions by subtracting a male's mean rate of interactions from the rate

261   at a given observation. Therefore, relative rates of triadic interactions are centered around zero,

262   with negative and positive values denoting deviations from the mean. The mean rates of triadic

263   interactions were included in the models as an expression of between-subjects variation and the

264   relative rates of triadic interactions at a given observation were included as an expression of

265   within-subjects variation. This procedure was repeated analogously for infant care. Mean rates of

266   triadic interactions were strongly correlated with the mean duration of infant care ($r = 0.90$ in the

267   testosterone excretion window, $r = 0.88$ in the cortisol excretion window), making it difficult to

268   distinguish independent effects for the between-subjects effect of each behavior. Therefore, we

269   decided not to include both predictors within the same model to avoid issues of collinearity and

270   instead fit two versions of the same model: one model included the mean duration of infant care

271   and one the other included mean rates of triadic interactions. Triadic interactions and infant care

272   within-subjects were not strongly correlated and therefore could be included in the same model.

273   Here we report models including mean duration of infant care (and excluding mean rates of triadic

274   interactions) and include results of the complementary model (that includes mean rates of triadic

275   interactions but excludes mean duration of infant care) in the supplementary material (Table S3,

276   Table S4). Regardless of which behavior was included, model estimates for all predictors remained

277   remarkably similar and thus did not change the interpretation of results.

278   We fitted models in R (version 3.6.2; R Core Team, 2019) using the function brm from the package

279   brms (version 2.11.1; Bürkner, 2017). The package brms calls on the computational framework

280   Stan (https://mc-stan.org) to fit Bayesian models (Bürkner, 2017). We included male identity as a

281   random effect in all models to control for multiple observations (urine samples) per subject. We

282   also included random slopes and correlation parameters between random intercepts and random

283   slopes for all predictors that varied within-subjects (triadic interactions within-subjects, infant care

284   within-subjects, grooming, aggression, daytime) (Barr et al., 2013). We ran all models with 4000

285   iterations over four MCMC chains, which included 1000 "warm up" iterations for each chain,

286   resulting in a total of 12000 posterior samples (Bürkner, 2017). In all models, there were no

287   divergent transitions during warm up, all Rhat values were equal to 1.00, and visual inspection of a

288   plot of the chains indicated that the models were able to converge. We used weakly informative

289   priors to improve convergence, guard against overfitting, and regularize parameter estimates

290   (McElreath, 2016). As a prior for the intercept and beta coefficients we used a normal distribution

291   with a mean of 0 and a standard deviation of 1; for the standard deviation of group level (random)

292   effects and sigma we used a Half-Cauchy distribution with location 0 and scale parameter 1; for the

293   correlation between random slopes we used LKJ Cholesky prior with eta 2.

294   We report model estimates as the mean of the posterior distribution with 95% credible intervals

295   (CI). To aid in the interpretation of whether predictor variables substantially affected the response

296   (hormone levels), we calculated the proportion (Pr) of posterior samples that fell on the same side

297   of 0 as the mean. The Pr ranges from 0.5 to 1.0, with Pr = 1.00 indicating that, given the model, the

298   effect of a predictor was entirely positive or negative, whereas Pr = 0.5 indicates that the effect was

299   centered around 0.

300   The data and code to reproduce the analyses in this paper are available at

301   https://osf.io/4dwgu/?view_only=732edeb15854463983a00ecf5abdb18d.

302   **3   Results**

303   We recorded a total of 1701 triadic interactions during 3289 hours of observation in one non-

304   mating season. All males of the study group were observed to partake in triadic interactions at least

305   once (Table 1). The majority (64%) of triadic interactions involved the one newborn male, 27%

306   involved yearlings (two males and one female) and 9% involved older juveniles up to four years of

307   age. Rates of triadic interactions were negatively correlated with iuT levels within-subjects (Table

308   2; Fig. 1 *a*, *b*), but not between-subjects (Estimate = 0.10, 95% CI: -0.07, 0.26, Pr = 0.88; Table S3).

309   Likewise, rates of triadic interactions were negatively correlated with iuC levels within-subjects

310   (Table 3; Fig. 2, *a*, *b*), but not between-subjects (Estimate = -0.14, 95% CI: -0.40, 0.10, Pr = 0.88;

311   Table S4).

312   All adult males of the group were observed caring for the newborn infant and/or yearlings (≤ 1.5

313   years old), including time spent huddling, carrying, or grooming (Table 1). Male infant care tended

314   to be positively correlated with iuT levels within-subjects (Table 2; Fig. 1, *a*, *c*), but was not related

315   to iuT levels between-subjects (Table 2). Male infant care tended to be positively correlated with

316   iuC levels within-subjects (Table 3; Fig. 2, *a*, *c*), but was not related to iuC levels between-subjects

317   (Table 3).

318   Table 2: Immunorective urinary testosterone levels (iuT) in relation to the frequency of triadic
319   interactions and duration of male infant care (model 1). Male identity was included as a random
320   effect, N subjects = 14, N urine samples = 572. CI = 95% credible intervals, Pr = proportion of the
321   posterior samples that fall on the same side of 0 as the mean. The complementary model with
322   results for triadic interactions between-subjects is provided in the supplementary material (Table
323   S3) and estimates are also provided in the text.

	Estimate	SD	CI lower	CI upper	Pr
Intercept	3.06	0.09	2.88	3.22	1.00
Test predictors					
Triadic interactions (within subjects)	-0.18	0.07	-0.33	-0.06	>0.99
Infant care (within subjects)	0.07	0.05	-0.04	0.17	0.92
Infant care (between subjects)	0.08	0.09	-0.10	0.26	0.82
Control predictors					
Aggression	-0.03	0.04	-0.10	0.04	0.78
Dominance rank	-0.16	0.08	-0.32	0.00	0.97
Grooming	0.07	0.04	-0.01	0.14	0.96
Daytime	-0.01	0.04	-0.10	0.08	0.63

324

325   Table 3: Immunoreactive urinary cortisol levels (iuC) in relation to the frequency of triadic

326   interactions and duration of male infant care (model 2). Male identity was included as a random

327   effect, N subjects = 14, N urine samples = 650. CI = 95% credible intervals, Pr = proportion of the

328   posterior samples that fall on the same side of 0 as the mean. The complementary model with

329   results for triadic interactions between-subjects is provided in the supplementary material (Table

330   S4) and estimates are also provided in the text.

	Estimate	SD	CI lower	CI upper	Pr
Intercept	5.15	0.14	4.86	5.39	1.00
Test predictors					
Triadic interactions (within subjects)	-0.24	0.11	-0.47	-0.04	0.99
Infant care (within subjects)	0.07	0.05	-0.03	0.17	0.93
Infant care (between subjects)	-0.14	0.13	-0.41	0.12	0.88
Control predictors					
Aggression	-0.03	0.04	-0.11	0.03	0.85
Dominance rank	-0.17	0.11	-0.38	0.04	0.95
Grooming	0.00	0.06	-0.12	0.11	0.51
Daytime	-0.23	0.04	-0.31	-0.15	1.00

331

332
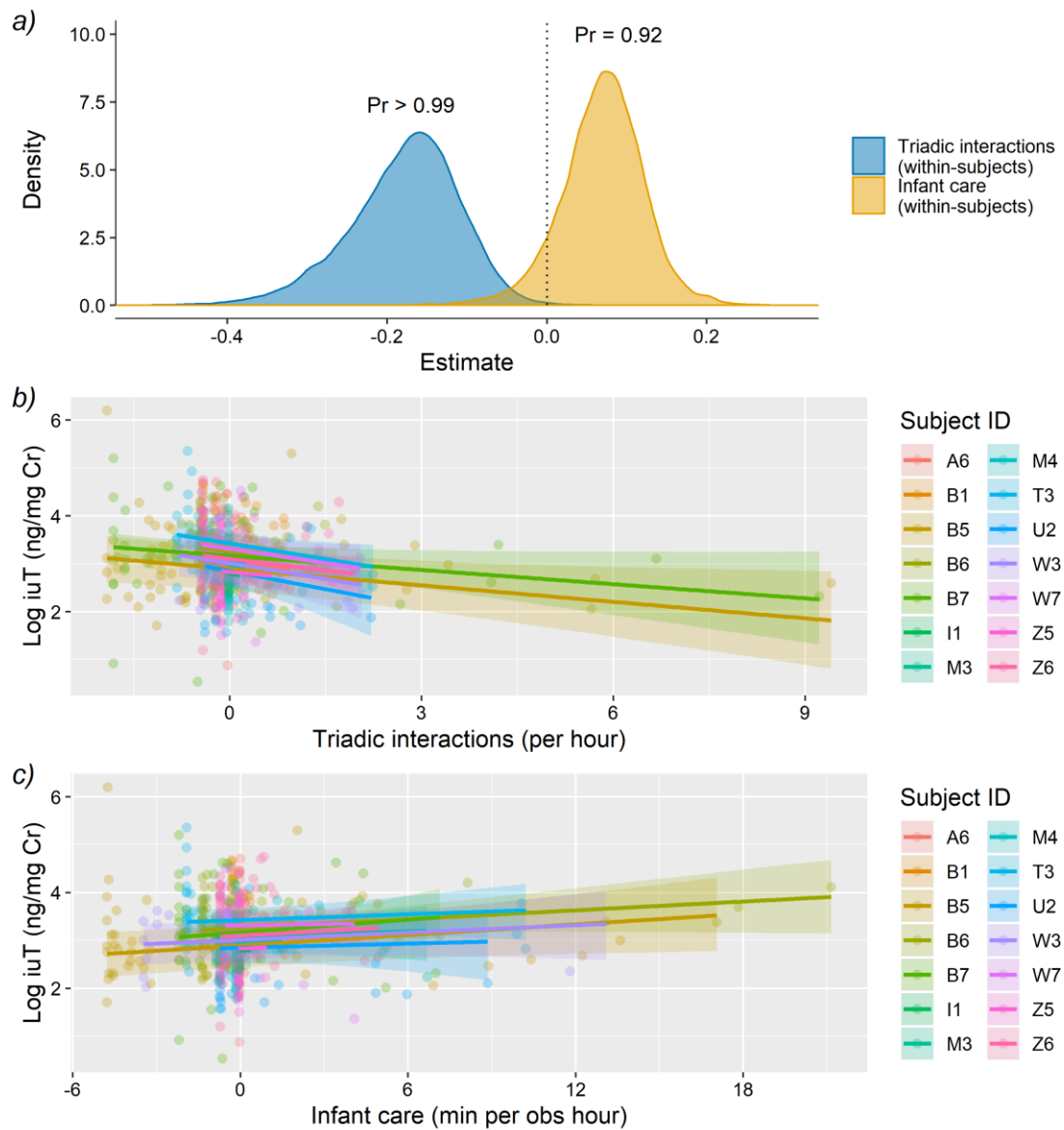
Fig. 1: Immunoreactive urinary testosterone levels (iuT) in relation the frequency of triadic interactions and duration of male infant care within-subjects (model 1). a) Posterior probability distribution of the slope estimate. Pr = proportion of the posterior samples that fall on the same side of 0 as the mean. b) and c) Model fitted values (solid lines) and 95% credible intervals (shaded areas) per focal male, when all other predictors are at their mean. Note that the x-axis has been centered within-subjects, thus positive and negative values indicate deviations from the mean. Circles indicate raw data points (urine samples; N = 572). Note that multiple behaviors can occur within the excretion window per urine sample and each behavior can influence hormone levels in opposing directions. The individual effects of each behavior are controlled for in the full statistical model. Cr = creatinine.

343
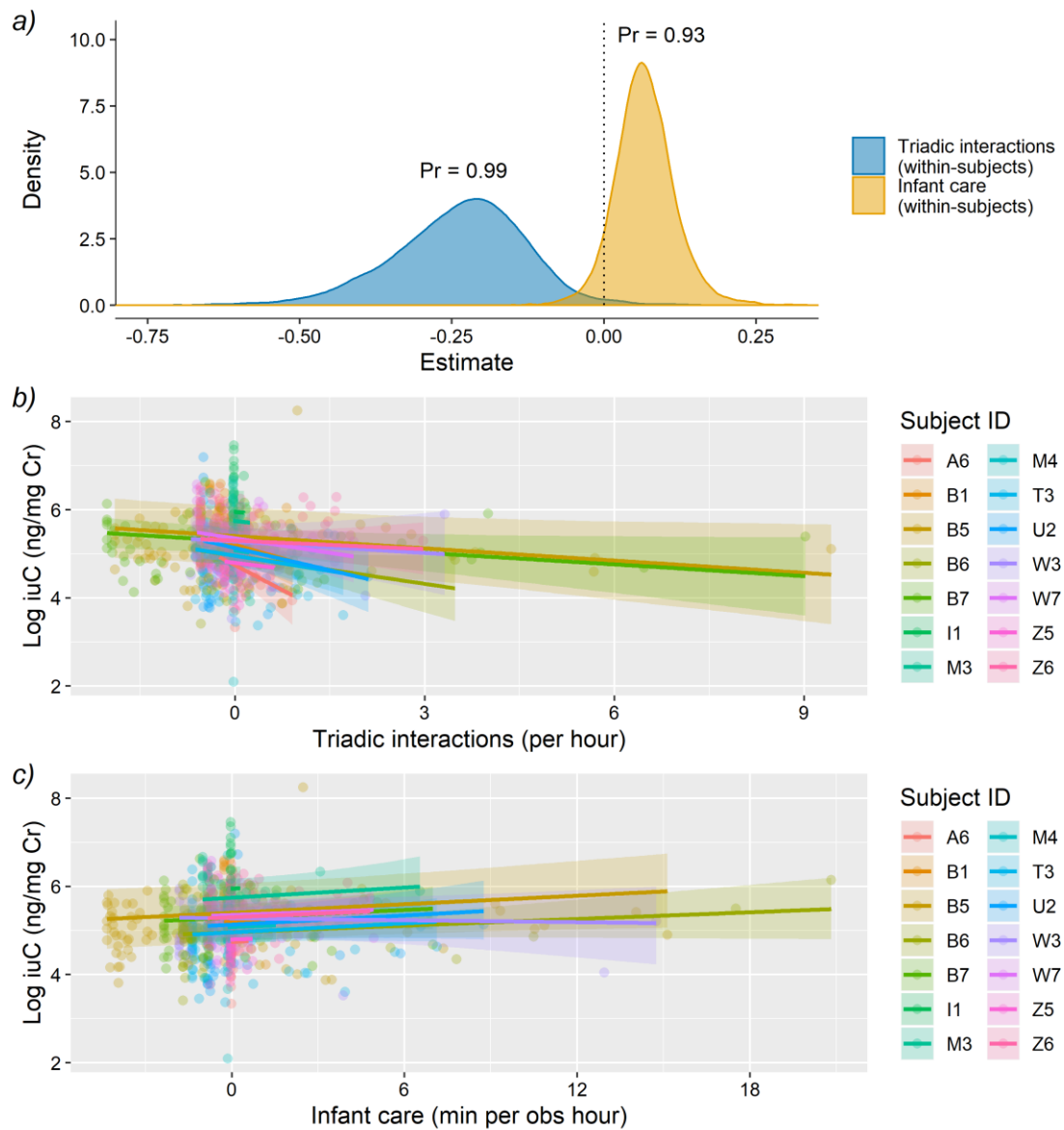
Fig. 2: Immunoreactive urinary cortisol levels (iuC) in relation to the frequency of triadic interactions and duration of male infant care within-subjects (model 2). a) Posterior probability distribution of the slope estimate. Pr = proportion of the posterior samples that fall on the same side of 0 as the mean. b) and c) Model fitted values (solid lines) and 95% credible intervals (shaded areas) per focal male, when all other predictors are at their mean. Note that the x-axis has been centered within-subjects, thus positive and negative values indicate deviations from the mean. Circles indicate raw data points (urine samples; N = 650). Note that multiple behaviors can occur within the excretion window per urine sample and each behavior can influence hormone levels in opposing directions. The individual effects of each behavior are controlled for in the full statistical model. Cr = creatinine.

354   **4   Discussion**

355   In this study, we investigated how testosterone and cortisol levels were related to social bonding

356   and nurturing in male Barbary macaques. We first tested for a relationship between testosterone

357   and triadic male-infant-male interactions, a ritualized behavior hypothesized to promote and/or

358   maintain social bonds in male macaques (Berghänel et al., 2011; Kalbitz et al., 2017; Paul et al.,

359   1996). We found that iuT was negatively correlated to frequency of triadic interactions within-

360   subjects but was not correlated to mean rates of interactions between-subjects. In other words, the

361   more frequently a male engaged in this behavior, the lower his iuT levels. This finding supports the

362   S/P theory, which proposes that low testosterone levels are preferred during bonding behaviors, so

363   as to not inhibit the bonding process (van Anders et al., 2011). These results are also in line with a

364   previous study in humans that found decreases in testosterone levels from baseline following a

365   friendship formation task between same-sex adults (Ketay et al., 2017). Similarly, chimpanzee

366   males that participated in meat-sharing after a hunt - a bond promoting behavior - had lower

367   testosterone than males that did not hunt or share, although it is unclear whether this resulted from

368   differences in baseline levels or reactivity (Sobolewski et al., 2012). Same-sex adults may see each

369   other as reproductive rivals and high testosterone levels may consequently interfere with the

370   bonding process by cognitively priming individuals to be competitive rather than nurturing

371   (Boksem et al., 2013; Eisenegger et al., 2011). Human, chimpanzee, and macaque males readily form

372   close social bonds and long-term cooperative relationships with a few co-resident males that

373   compete with the other co-residents (Aktipis et al., 2018; Schülke et al., 2010; Watts, 2002; Young et

374   al., 2013). Thus, males may have been under selective pressure to downregulate testosterone

375   during periods of affiliation to facilitate bond formation. It would be interesting to test whether

376   lowered testosterone after affiliation between same-sex dyads is primarily found in species where

377   long-term cooperative relationships increase fitness or if low testosterone is also found after

378   opportunistic short-term affiliation. Beyond the formation of same-sex social bonds, high

379   testosterone levels may also be detrimental to the maintenance of these bonds in the long term. For

380   example, territory holder male manakins (*Pipra filicauda*, a lekking bird species), must perform

381   cooperative displays with floater males to attract females; however, territory holders with high

382   testosterone levels fail to maintain stable display partnerships with floater males, thus reducing

383   their ability to compete for females (Ryder et al., 2020).

384   We additionally found that iuC levels were negatively correlated within-subjects with rates of

385   triadic interactions in male Barbary macaques. Triadic interactions decrease tensions between

386   adult males, as per the agonistic buffering hypothesis (Deag and Crook, 1971; Paul et al., 1996), and

387   thus may consequently reduce cortisol and testosterone levels. Another non-mutually exclusive

388   explanation relates to the potential bond-formation properties of triadic interactions, resulting in

389   integration into the male social network (Henkel et al., 2010) and increased social support

390   (Berghänel et al., 2011). Social support is a powerful regulator of the HPA axis and thus of

391   glucocorticoid levels (Hostinar et al., 2014). Merely the presence of closely bonded conspecifics

392   during the occurrence of a stressor is enough to buffer HPA axis activity in a variety of species

393   (Hostinar et al., 2014). Indeed, male Barbary macaques with strong social bonds show attenuated

394   glucocorticoid responses to social and environmental stressors (Young et al., 2014a). Having

395   reliable social support may also help to downregulate HPA activity even in the immediate absence

396   of stressors (Rosal et al., 2004; Wittig et al., 2016). Thus, study males of this study may have

397   perceived themselves as having strengthened their social bonds and ability to call on support if

398   needed on days when they frequently engaged in triadic interactions (Berghänel et al., 2011),

399   consequently lowering cortisol.

400   While lowered testosterone levels may be necessary to prevent the inhibition of bonding, the

401   process of bonding itself is likely driven by other hormones. For instance, the S/P theory predicts

402   that high oxytocin levels are needed to promote social bonding (van Anders et al., 2011). In a

403 previous study on the same group of Barbary macaques we did not find an increase in oxytocin

404 levels following triadic interactions in general but found that oxytocin levels were only higher after

405 triadic interactions with non-bond partners (Rincon et al., 2020). If the combination of low

406 testosterone and high oxytocin is responsible for bonding, then triadic interactions may function to

407 form bonds selectively between non-bonded partners while physiologically not affecting existing

408 bonds between strongly bonded partners. In this interpretation, triadic interactions are a tool for

409 bond formation rather than bond maintenance, although, low testosterone levels after interactions

410 with bond partners may still be beneficial to avoid damaging existing relationships (Ryder et al.,

411 2020), and perhaps to not inhibit friendly interactions altogether. In our study subjects, 45% of

412 triadic interactions occurred with non-bond partners (Rincon et al., unpublished data) indicating

413 that there is substantial variation in partner identity and thus the potential to bond with previously

414 non-bonded partners is high. Finally, the low levels of testosterone associated with triadic

415 interactions occur independently of infant care, suggesting that it is not merely the presence of an

416 immature individual that lowers testosterone or cortisol levels, but that the triadic interaction itself

417 is what is salient.

418 Low cortisol in conjunction with low testosterone may act synergistically to reduce tension and

419 facilitate bonding. During a dyadic friendship formation task in humans, participants desired to be

420 closer to their partners if their partners had low cortisol levels (Ketay et al., 2017), suggesting that

421 affiliative interactions in a relaxed state are beneficial for both partners. While low testosterone and

422 low cortisol may be beneficial to bond formation between same-sex adults, this pattern may not

423 generalize to other types of dyads or contexts. For example, our findings are in contrast to a

424 previous study in humans where testosterone levels were negatively related to friendship

425 formation and positively related to friendship maintenance within a social network, whereas the

426 opposite was true for cortisol levels (Kornienko et al., 2016). One difference between our study and

427 Kornienko et al. (2016) is that the social network comprised a mixed-sex group in their study.

428 Steroid dynamics in relation to opposite-sex bonding may differ from that for same-sex bonding as

429 opposite-sex dyads having greater potential for a sexual relationship and same-sex dyads having

430 greater potential to be reproductive competitors. Indeed, high testosterone levels most likely

431 promotes males to affiliate with females and facilitates the initial stage of sexual or romantic

432 relationships (Goymann et al., 2019; Roney and Gettler, 2015; van Anders et al., 2011). Although

433 high testosterone levels may be detrimental to satisfaction in long-term romantic relationships

434 (Roney and Gettler, 2015), it is unclear if this is also the case for long-term opposite-sex platonic

435 friendships.

436 We found that male infant care was weakly positively correlated with testosterone levels within-

437 subjects but not between-subjects. Cortisol exhibited the same pattern, which is in line with a

438 previous finding in this species (Henkel et al., 2010). These results suggest that, from the male's

439 perspective, infant care is neither nurturing nor relaxing and may instead be performed under a

440 competitive context. Across many mammals, infants are at risk of infanticide in species where the

441 period of lactation is long relative to gestation (van Schaik, 2000), as is the case in primates. Infant

442 caretakers perform a protective role in the majority of Old World nonhuman primates and the

443 threat of infanticide has been shown to elicit elevated testosterone and glucocorticoid responses

444 (Cheney et al., 2015; Muller, 2017). Beyond defense against infanticide, fathers support or tolerance

445 of immatures can have additional benefits. For instance, male baboons (*Papio cynocephalus*) often

446 support their offspring in agonistic conflicts against other group members, which may help in

447 gaining dominance rank and reducing stress or injury (Buchan et al., 2003). Father-offspring

448 associations can also lead to improved feeding opportunities and ultimately faster maturation for

449 juveniles (Huchard et al., 2013). Male Barbary macaques prefer to interact with infants based on

450 their past mating success with the mother (Kubenova et al., 2019), which may be indicative of

451 paternal investment. Thus, modest increases in testosterone and cortisol when males care for

452 infants could serve to make them more alert to their social environment and more effective at

453   providing protective support. Additionally, a positive relationship between testosterone and infant

454   care is in line with the suggestion that infants can be used as tools for mating effort in this species

455   (Ménard et al., 2001). It has also previously been suggested that parental effort may enhance status

456   in young human fathers thus resulting in elevated testosterone (Mazur, 2014). It is not clear if

457   infant care enhances status in Barbary macaque males, but it does seem likely that these behaviors

458   are perceived positively by the infant's mother (Ménard et al., 2001).

459   Differences in baseline or mean hormone levels among individuals could potentially result in

460   different behavioral profiles where individuals perform certain behaviors at a higher or lower rate

461   on average (Hau and Goymann, 2015). In our Barbary macaques males, however, we found that

462   mean differences in iuT and iuC levels did not result in mean differences in rates of triadic

463   interactions or duration of infant care. Instead, variation in steroid levels within-subjects were

464   linked to these two behaviors. It is unclear why we found within- but not between-subjects effects

465   given that both paternal care and friendship formation tasks influence baseline steroid levels as

466   well as reactivity, at least in humans (e.g. Gettler et al., 2011a; Ketay et al., 2017). Differences in

467   steroid receptor densities among male Barbary macaques could account for differences in

468   sensitivity to hormone levels and thus result in a lack of between-subjects variation in behavior.

469   Another possibility is that triadic interactions and infant care are expressed only sporadically and

470   unpredictably throughout the day. Thus, selection may have acted on an individual's ability to

471   flexibly up- or downregulate hormone levels in response to the social environment.

477   the manuscript. This research was funded by the Deutsche Forschungsgemeinschaft (DFG, German

478   Research Foundation) - Project number 254142454 / GRK 2070.

479   **References**

480   Aktipis, A., Cronk, L., Alcock, J., Ayers, J.D., Baciu, C., Balliet, D., Boddy, A.M., Curry, O.S., Krems, J.A.,

481   Muñoz, A., Sullivan, D., Sznycer, D., Wilkinson, G.S., Winfrey, P., 2018. Understanding cooperation

482   through fitness interdependence. Nat. Hum. Behav. 2, 429–431. https://doi.org/10.1038/s41562-

483   018-0378-4

484   Bahr, N.I., Palme, R., Möhle, U., Hodges, J.K., Heistermann, M., 2000. Comparative aspects of the

485   metabolism and excretion of cortisol in three individual nonhuman primates. Gen. Comp.

486   Endocrinol. 117, 427–438. https://doi.org/10.1006/gcen.1999.7431

487   Barr, D.J., Levy, R., Scheepers, C., Tily, H.J., 2013. Random effects structure for confirmatory

488   hypothesis testing: Keep it maximal. J. Mem. Lang. 68, 255–278.

489   https://doi.org/10.1016/j.jml.2012.11.001

490   Berghänel, A., Ostner, J., Schröder, U., Schülke, O., 2011. Social bonds predict future cooperation in

491   male Barbary macaques, *Macaca sylvanus*. Anim. Behav. 81, 1109–1116.

492   https://doi.org/10.1016/j.anbehav.2011.02.009

493   Boksem, M.A.S., Mehta, P.H., Van den Bergh, B., van Son, V., Trautmann, S.T., Roelofs, K., Smidts, A.,

494   Sanfey, A.G., 2013. Testosterone inhibits trust but promotes reciprocity. Psychol. Sci. 24, 2306–

495   2314. https://doi.org/10.1177/0956797613495063

496   Buchan, J.C., Alberts, S.C., Silk, J.B., Altmann, J., 2003. True paternal care in a multi-male primate

497   society. Nature 425, 179–181. https://doi.org/10.1038/nature01866

498   Bürkner, P.-C., 2017. Brms: An R package for Bayesian multilevel models using Stan. J. Stat. Softw.

499   80, 1–28. https://doi.org/10.18637/jss.v080.i01

500   Büttler, R.M., Bagci, E., Brand, H.S., Heijer, M. den, Blankenstein, M.A., Heijboer, A.C., 2018.

501   Testosterone, androstenedione, cortisol and cortisone levels in human unstimulated, stimulated

502   and parotid saliva. Steroids 138, 26–34. https://doi.org/10.1016/j.steroids.2018.05.013

503   Cheney, D.L., Crockford, C., Engh, A.L., Wittig, R.M., Seyfarth, R.M., 2015. The costs of parental and

504   mating effort for male baboons. Behav. Ecol. Sociobiol. 69, 303–312.

505   https://doi.org/10.1007/s00265-014-1843-3

506   Cohen, S., Wills, T.A., 1985. Stress, social support, and the buffering hypothesis. Psychol. Bull. 98,

507   310–357. https://doi.org/10.1037/0033-2909.98.2.310

508   Deag, J.M., Crook, J.H., 1971. Social behaviour and "agonistic buffering" in the wild Barbary macaque

509   *Macaca sylvana* L. Folia Primatol. 15, 183–200. https://doi.org/10.1159/000155378

510   de Turckheim, G., Merz, E., 1984. Breeding Barbary macaques in outdoor open enclosures, in: Fa, J.E.

511   (Ed.), The Barbary Macaque: A Case Study in Conservation. Springer US, Boston, MA, pp. 241–261.

512   https://doi.org/10.1007/978-1-4613-2785-1_10

513   de Vries, H., Stevens, J.M.G., Vervaecke, H., 2006. Measuring and testing the steepness of dominance

514   hierarchies. Anim. Behav. 71, 585–592. https://doi.org/10.1016/j.anbehav.2005.05.015

515   Eisenegger, C., Haushofer, J., Fehr, E., 2011. The role of testosterone in social interaction. Trends

516   Cogn. Sci. 15, 263–271. https://doi.org/10.1016/j.tics.2011.04.008

517   Fleming, A.S., Corter, C., Stallings, J., Steiner, M., 2002. Testosterone and prolactin are associated

518   with emotional responses to infant cries in new fathers. Horm. Behav. 42, 399–413.

519   https://doi.org/10.1006/hbeh.2002.1840

520   Gettler, L.T., McDade, T.W., Feranil, A.B., Kuzawa, C.W., 2011a. Longitudinal evidence that

521   fatherhood decreases testosterone in human males. Proc. Natl. Acad. Sci. 108, 16194.

522   https://doi.org/10.1073/pnas.1105403108

523   Gettler, L.T., Mcdade, T.W., Kuzawa, C.W., 2011b. Cortisol and testosterone in Filipino young adult

524   men: Evidence for co-regulation of both hormones by fatherhood and relationship status. Am. J.

525   Hum. Biol. 23, 609–620. https://doi.org/10.1002/ajhb.21187

526   Goymann, W., 2009. Social modulation of androgens in male birds. Gen. Comp. Endocrinol. 163,

527   149–157. https://doi.org/10.1016/j.ygcen.2008.11.027

528   Goymann, W., Flores, D.P., 2017. Acute peaks of testosterone suppress paternal care: Evidence from

529   individual hormonal reaction norms. Proc. R. Soc. B 284, 20170632.

530   https://doi.org/10.1098/rspb.2017.0632

531   Goymann, W., Moore, I.T., Oliveira, R.F., 2019. Challenge hypothesis 2.0: A fresh look at an

532   established idea. BioScience 69, 432–442. https://doi.org/10.1093/biosci/biz041

533   Grebe, N.M., Sarafin, R.E., Strenth, C.R., Zilioli, S., 2019. Pair-bonding, fatherhood, and the role of

534   testosterone: A meta-analytic review. Neurosci. Biobehav. Rev. 98, 221–233.

535   https://doi.org/10.1016/j.neubiorev.2019.01.010

536   Hau, M., Goymann, W., 2015. Endocrine mechanisms, behavioral phenotypes and plasticity: Known

537   relationships and open questions. Front. Zool. 12, S7. https://doi.org/10.1186/1742-9994-12-S1-

538   S7

539   Henkel, S., Heistermann, M., Fischer, J., 2010. Infants as costly social tools in male Barbary macaque

540   networks. Anim. Behav. 79, 1199–1204. https://doi.org/10.1016/j.anbehav.2010.02.005

541   Hirschenhauser, K., Oliveira, R.F., 2006. Social modulation of androgens in male vertebrates: Meta-

542   analyses of the challenge hypothesis. Anim. Behav. 71, 265–277.

543   https://doi.org/10.1016/j.anbehav.2005.04.014

544   Hostinar, C.E., Sullivan, R.M., Gunnar, M.R., 2014. Psychobiological mechanisms underlying the

545   social buffering of the HPA axis: A review of animal models and human studies across development.

546   Psychol. Bull. 140. https://doi.org/10.1037/a0032671

547   Huchard, E., Charpentier, M.J., Marshall, H., King, A.J., Knapp, L.A., Cowlishaw, G., 2013. Paternal

548   effects on access to resources in a promiscuous primate society. Behav. Ecol. 24, 229–236.

549   https://doi.org/10.1093/beheco/ars158

550   Kalbitz, J., Schülke, O., Ostner, J., 2017. Triadic male-infant-male interaction serves in bond

551   maintenance in male Assamese macaques. PLoS ONE 12, e0183981.

552   https://doi.org/10.1371/journal.pone.0183981

553   Ketay, S., Welker, K.M., Slatcher, R.B., 2017. The roles of testosterone and cortisol in friendship

554   formation. Psychoneuroendocrinology 76, 88–96. https://doi.org/10.1016/j.psyneuen.2016.11.022

555   Kornienko, O., Schaefer, D.R., Weren, S., Hill, G.W., Granger, D.A., 2016. Cortisol and testosterone

556   associations with social network dynamics. Horm. Behav. 80, 92–102.

557   https://doi.org/10.1016/j.yhbeh.2016.01.013

558   Kubenova, B., Ostner, J., Schülke, O., Majolo, B., Smilauer, P., Waterman, J., Tkaczynski, P., Konecna,

559   M., 2019. The male and female perspective in the link between male infant care and mating

560   behaviour in Barbary macaques. Ethology 0, 1–11. https://doi.org/10.1111/eth.12948

561   Lynn, S.E., 2008. Behavioral insensitivity to testosterone: Why and how does testosterone alter

562   paternal and aggressive behavior in some avian species but not others? Gen. Comp. Endocrinol. 157,

563   233–240. https://doi.org/10.1016/j.ygcen.2008.05.009

564   Martin, P., Bateson, P., 2007. Measuring behaviour: An introductory guide, 3rd ed. Cambridge

565   University Press, Cambridge.

566   Massen, J., Sterck, E., Vos, H. de, 2010. Close social associations in animals and humans: Functions

567   and mechanisms of friendship. Behaviour 147, 1379–1412.

568   https://doi.org/10.1163/000579510X528224

569   Mazur, A., 2014. Testosterone of young husbands rises with children in the home. Andrology 2,

570   125–129. https://doi.org/10.1111/j.2047-2927.2013.00164.x

571   McElreath, R., 2016. Statistical rethinking: A Bayesian course with examples in R and Stan. Chapman

572   and Hall/CRC. https://doi.org/10.1201/9781315372495

573   Ménard, N., von Segesser, F., Scheffrahn, W., Pastorini, J., Vallet, D., Gaci, B., Martin, R.D., Gautier-

574   Hion, A., 2001. Is male-infant caretaking related to paternity and/or mating activities in wild

575   Barbary macaques (*Macaca sylvanus*)? C. R. Acad. Sci. III, Sci. Vie 324, 601–610.

576   https://doi.org/10.1016/S0764-4469(01)01339-7

577   Moore, I.T., Hernandez, J., Goymann, W., 2019. Who rises to the challenge? Testing the challenge

578   hypothesis in fish, amphibians, reptiles, and mammals. Horm. Behav.

579   https://doi.org/10.1016/j.yhbeh.2019.06.001

580   Möhle, U., Heistermann, M., Palme, R., Hodges, J.K., 2002. Characterization of urinary and fecal

581   metabolites of testosterone and their measurement for assessing gonadal endocrine function in

582   male nonhuman primates. Gen. Comp. Endocrinol. 129, 135–145. https://doi.org/10.1016/S0016-

583   6480(02)00525-7

584   Muller, M.N., 2017. Testosterone and reproductive effort in male primates. Horm. Behav. 91, 36–51.

585   https://doi.org/10.1016/j.yhbeh.2016.09.001

586   Nunes, S., Fite, J.E., Patera, K.J., French, J.A., 2001. Interactions among paternal behavior, steroid

587   hormones, and parental experience in male marmosets (*Callithrix kuhlii*). Horm. Behav. 39, 70–82.

588   https://doi.org/10.1006/hbeh.2000.1631

589   Ostner, J., Schülke, O., 2018. Linking sociality to fitness in primates: A call for mechanisms, in:

590   Naguib, M., Barrett, L., Healy, S.D., Podos, J., Simmons, L.W., Zuk, M. (Eds.), Advances in the Study of

591   Behavior. Academic Press, pp. 127–175. https://doi.org/10.1016/bs.asb.2017.12.001

592   Palme, R., Möstl, E., 1997. Measurement of cortisol metabolites in faeces of sheep as a parameter of

593   cortisol concentration in blood. Int. J. Mamm. Biol. 62, 192–197.

594   Palme, R., Möstl, E., 1994. Biotin-streptavidin enzyme immunoassay for the determination of

595   oestrogens and androgens in boar faeces, in: Görög, S. (Ed.), Advances in Steroid Analysis '93.

596   Akademiai Kiado, Budapest, pp. 111–117.

597   Paul, A., Kuester, J., Arnemann, J., 1996. The sociobiology of male-infant interactions in Barbary

598   macaques, *Macaca sylvanus*. Anim. Behav. 51, 155–170. https://doi.org/10.1006/anbe.1996.0013

599   R Core Team, 2019. R: A language and environment for statistical computing. R Foundation for

600   Statistical Computing, Vienna, Austria. https://www.R-project.org/

601   Rincon, A.V., Deschner, T., Schülke, O., Ostner, J., 2020. Oxytocin increases after affiliative

602   interactions in male Barbary macaques. Horm. Behav. 119, 104661.

603   https://doi.org/10.1016/j.yhbeh.2019.104661

604   Rincon, A.V., Ostner, J., Heistermann, M., Deschner, T., 2019. Measuring urinary cortisol and

605   testosterone levels in male Barbary macaques: A comparison of EIA and LC–MS. Gen. Comp.

606   Endocrinol. 281, 117–125. https://doi.org/10.1016/j.ygcen.2019.05.017

607   Roney, J.R., Gettler, L.T., 2015. The role of testosterone in human romantic relationships. Curr. Opin.

608   Psychol., Relationship science 1, 81–86. https://doi.org/10.1016/j.copsyc.2014.11.003

609   Rosal, M.C., King, J., Ma, Y., Reed, G.W., 2004. Stress, social support, and cortisol: Inverse

610   associations? Behav. Med. 30, 11–22. https://doi.org/10.3200/BMED.30.1.11-22

611   Ryder, T.B., Dakin, R., Vernasco, B.J., Evans, B.S., Horton, B.M., Moore, I.T., 2020. Testosterone

612   modulates status-specific patterns of cooperation in a social network. Am. Nat. 195, 82–94.

613   https://doi.org/10.1086/706236

614   Sapolsky, R.M., 2002. Endocrinology of the stress-response., in: Becker, J.B., Breedlove, S.M., Crews,

615   D., McCarthy, M.M. (Eds.), Behavioral Endocrinology. MIT Press, Cambridge, MA, pp. 409–450.

616   Schielzeth, H., 2010. Simple means to improve the interpretability of regression coefficients.

617   Methods Ecol. Evol. 1, 103–113. https://doi.org/10.1111/j.2041-210X.2010.00012.x

618   Schülke, O., Bhagavatula, J., Vigilant, L., Ostner, J., 2010. Social bonds enhance reproductive success

619   in male macaques. Curr. Biol. 20, 2207–2210. https://doi.org/10.1016/j.cub.2010.10.058

620   Sobolewski, M.E., Brown, J.L., Mitani, J.C., 2012. Territoriality, tolerance and testosterone in wild

621   chimpanzees. Anim. Behav. 84, 1469–1474. https://doi.org/10.1016/j.anbehav.2012.09.018

622   Storey, A.E., Noseworthy, D.E., Delahunty, K.M., Halfyard, S.J., McKay, D.W., 2011. The effects of

623   social context on the hormonal and behavioral responsiveness of human fathers. Horm. Behav. 60,

624   353–361. https://doi.org/10.1016/j.yhbeh.2011.07.001

625   van Anders, S.M., Goldey, K.L., Kuo, P.X., 2011. The steroid/peptide theory of social bonds:

626   Integrating testosterone and peptide responses for classifying social behavioral contexts.

627   Psychoneuroendocrinology 36, 1265–1275. https://doi.org/10.1016/j.psyneuen.2011.06.001

628   van de Pol, M., Wright, J., 2009. A simple method for distinguishing within- versus between-subject

629   effects using mixed models. Anim. Behav. 77, 753–758.

630   https://doi.org/10.1016/j.anbehav.2008.11.006

631   van Schaik, C.P., 2000. Vulnerability to infanticide by males: Patterns among mammals, in: van

632   Schaik, C.P., Janson, C.H. (Eds.), Infanticide by Males and Its Implications. Cambridge University

633   Press, Cambridge, pp. 61–72. https://doi.org/10.1017/CBO9780511542312.005

634   Watts, D.P., 2002. Reciprocity and interchange in the social relationships of wild male chimpanzees.

635   Behaviour 139, 343–370.

636   Whitten, P.L., 1987. Infants and adult males, in: Smuts, B.B. (Ed.), Primate Societies. University of

637   Chicago Press, Chicago, pp. 343–357.

638   Wingfield, J.C., Hegner, R.E., Dufty, A.M., Ball, G.F., 1990. The "challenge hypothesis": Theoretical

639   implications for patterns of testosterone secretion, mating systems, and breeding strategies. Am.

640   Nat. 136, 829–846. https://doi.org/10.1086/285134

641   Wittig, R.M., Crockford, C., Deschner, T., Langergraber, K.E., Ziegler, T.E., Zuberbühler, K., 2014. Food

642   sharing is linked to urinary oxytocin levels and bonding in related and unrelated wild chimpanzees.

643   Proc. R. Soc. B 281, 20133096. https://doi.org/10.1098/rspb.2013.3096

644   Wittig, R.M., Crockford, C., Weltring, A., Langergraber, K.E., Deschner, T., Zuberbühler, K., 2016.

645   Social support reduces stress hormone levels in wild chimpanzees across stressful events and

646   everyday affiliations. Nat. Commun. 7, 13361. https://doi.org/10.1038/ncomms13361

647   Young, C., Hähndel, S., Majolo, B., Schülke, O., Ostner, J., 2013. Male coalitions and female behaviour

648   affect male mating success independent of dominance rank and female receptive synchrony in wild

649   Barbary macaques. Behav. Ecol. Sociobiol. 67, 1665–1677. https://doi.org/10.1007/s00265-013-

650   1577-7

651   Young, C., Majolo, B., Heistermann, M., Schülke, O., Ostner, J., 2014a. Responses to social and

652   environmental stress are attenuated by strong male bonds in wild macaques. Proc. Natl. Acad. Sci.

653   111, 18195–18200. https://doi.org/10.1073/pnas.1411450111

654   Young, C., Majolo, B., Schülke, O., Ostner, J., 2014b. Male social bonds and rank predict supporter

655   selection in cooperative aggression in wild Barbary macaques. Anim. Behav. 95, 23–32.

656   https://doi.org/10.1016/j.anbehav.2014.06.007