

1 **Relationships between affiliative social behavior and hair cortisol concentrations in semi-**  
2 **free ranging rhesus monkeys**

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23 **Abstract**

24           Sociality is a fundamental aspect of human behavior and health. One benefit of affiliative  
25 social relationships is reduced short-term levels of glucocorticoids (GCs), which are indicative of  
26 physiological stress. Less is known, however, about chronic GC production in relation to  
27 affiliative social behavior. To address this issue, we studied a semi-free ranging troop of rhesus  
28 macaques (*Macaca mulatta*) and collected hair samples to measure hair cortisol concentrations  
29 (HCCs), as a measure of chronic GC production, during routine biannual exams. We collected  
30 social behavior (both aggressive and affiliative) and hair samples for 32 adult female rhesus  
31 macaques over one year (Experiment 1). Our results indicated that adult females who initiated  
32 higher levels of social affiliation had significantly lower levels of HCCs. Neither the initiation nor  
33 the receipt of aggression were significantly related to HCCs in this study. In a second experiment  
34 we studied 28 mother-infant dyads for the first 90 days postpartum to examine mother-infant  
35 facial interactions (i.e. mutual gazing). We analyzed HCCs during weaning approximately one  
36 year later, which is a major transitional period. We found that infants that engaged in higher  
37 levels of mutual gazing in the first 90 days postpartum had significantly lower levels of HCCs  
38 during weaning. Finally, we studied 17 infant rhesus macaques (13 males) to examine whether  
39 social behavior (such as play) in the first five months of life correlated with infant HCCs over  
40 those months (Experiment 3). We found that infant males that engaged in more social play had  
41 significantly lower levels of HCCs. By relying on an animal model, our study shows that  
42 affiliative social traits are associated with lower long-term GC production. Future research should  
43 address the complex interactions between social behavior, chronic GC production, and mental  
44 and physical health.

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46 **Keywords:** *Macaca mulatta*; HPA axis; play; grooming; face-to-face interactions

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## 49 **1. Introduction**

50 Group living mammals have developed a complex system of social interactions that have  
51 evolved to create stable social bonds over time, which have reproductive and lifespan-extension  
52 benefits (Kappeler et al., 2015; Silk et al., 2010). For example, individuals with strong social  
53 bonds live longer (Silk et al., 2010; Yee et al., 2008), possibly because social support has been  
54 linked to lower disease risks (Seeman, 1996) and faster recovery (Kulik and Mahler, 1989). The  
55 health benefits of affiliative social relationships are also likely related to reduced levels of  
56 circulating glucocorticoids (GCs), hormones that participate in the physiological stress response,  
57 that result from affiliative social interactions (Heinrichs et al., 2003; Shutt et al., 2007). Indeed,  
58 chronic high levels of circulating GCs can result in cardiovascular impairments, reproductive  
59 inhibition, immunosuppression (Sapolsky, 2005), and neurobiological changes such as dendritic  
60 atrophy, which is a risk factor for depression (Qiao et al., 2016). Moreover, loneliness, or  
61 perceived social isolation, is a risk factor for mental and physical health issues (Hawkey and  
62 Cacioppo, 2013).

63 Studies examining sociality and endocrine measures of stress have relied on short-term  
64 samples of GCs, which are typically collected from urine, feces, saliva, and blood and are subject  
65 to a number of confounds including circadian rhythm, temporal restrictions, and environmental  
66 variability (Davenport et al., 2006; Meyer and Novak, 2012). Additionally, these samples relay  
67 little information about chronic hypothalamic-pituitary-adrenocortical (HPA) axis activity, unless  
68 numerous sequential samples are taken over time, which can pose various challenges (Davenport  
69 et al., 2006).

70 A recent tool to measure chronic HPA axis activity is the collection of hair to quantify  
71 hair cortisol concentrations (HCCs; Davenport et al., 2006; Meyer and Novak, 2012). Hair  
72 reflects chronic retrospective HPA axis activity over a period of weeks to months without the  
73 need for multiple samplings. HCCs have been increasingly relied upon in both animal and human  
74 biobehavioral research, and studies have revealed relationships between HCCs and mental

75 disorders such as depression (Qin et al., 2015) and generalized anxiety disorder (Staufenbiel et  
76 al., 2013), as well as physical disorders such as cardiovascular disease (Manenschijn et al., 2013)  
77 and myocardial infarction (Pereg et al., 2011). In addition, HCCs may be a biomarker of major  
78 life stressors (Karlén et al., 2011) such as childhood abuse (Schreier et al., 2015) and chronic  
79 stress in dementia caregivers (Stalder et al., 2014). However, inconsistent results between self-  
80 reported stress and HCCs have been found in humans (associations: Gow et al., 2011; Kalra et al.,  
81 2007; Qi et al., 2014; no associations: O'Brien et al., 2013; Olstad et al., 2016; Stalder et al.,  
82 2010; 2017; Wells et al., 2014), with researchers hypothesizing that subjective measures do not  
83 capture experiences of stress in the same way as physiological measures (Olstad et al., 2016).  
84 Indeed, studies utilizing more objective measures have established relationships with HCCs  
85 (Geng et al., 2016; Stalder et al., 2017). One objective measure may be overt social behavior,  
86 such as measured frequencies of aggression and affiliation. In this regard, several recent studies  
87 have begun examining the relationships between aggression and HCCs in mammals (Feng et al.,  
88 2016; Salas et al., 2016; Tennenhouse et al., 2016; Yamanashi et al., 2016). However, even  
89 though affiliation plays a fundamental role in social organization in humans and non-human  
90 primates, little research has examined the relationship between affiliation and chronic HPA axis  
91 activation.

92         One particular type of affiliative social behavior that has not been examined with respect  
93 to HCCs is the influence of maternal interactions. Previous research in animal models has  
94 suggested that mothers act as a buffering mechanism for stressors in infants, and infants who are  
95 deprived maternal care may lack the ability to regulate the HPA system (Dettmer et al., 2012;  
96 2016a; Feng et al., 2011; Hennessy et al., 2009). However, less is known about how natural  
97 variations in maternal care, rather than the lack of maternal care *per se*, as well as other social  
98 behaviors such as play, relate to long-term HPA axis activity. Addressing this gap will elucidate  
99 the impacts that variable maternal care has on downstream infant HPA axis regulation and social  
100 development.

101 To address these literature gaps, we studied social behavior and HCCs in a troop of  
102 rhesus macaques (*Macaca mulatta*) living in a naturalistic, semi-free ranging environment.  
103 Rhesus macaques are an ideal animal model for these processes in humans because they have  
104 natural variations in the tendency of affiliation (Capitanio et al., 2014) and maternal behavior  
105 (McCormack et al., 2006), and they form strict dominance hierarchies characterized by frequent  
106 aggression. Aggression may be seen as a proxy for bullying in humans, which has also been  
107 related to GCs (Hansen et al., 2006.) In Experiment 1, we studied 32 adult female rhesus  
108 macaques over one year. We predicted that 1) high rates of received aggression would be  
109 associated with higher levels of HCCs, and 2) high rates of affiliation would conversely be  
110 associated with lower levels of HCCs. In Experiment 2, we studied 28 mother-infant dyads for  
111 the first 90 days postpartum to determine the relationship between early mother-infant face-to-  
112 face interactions (i.e. mutual gazing: Dettmer et al., 2016b; 2016c; Ferrari et al., 2009) and later  
113 offspring HCCs. Hair samples were obtained at routine semiannual health exams. We analyzed  
114 the hair samples taken at weaning age, which typically occurs after the birth of their next sibling,  
115 at an average of 12 months of age (Fooden, 2000). Because the transition from nursing to a  
116 weanling is a period of marked maternal-infant conflict (Trivers, 1974), we sought to explore  
117 whether variations in early maternal face-to-face interactions would relate to infant HPA axis  
118 regulation during this critical time of development. We therefore predicted that 3) higher  
119 frequencies of mutual gazing after birth would negatively correspond with HCCs during the time  
120 of weaning. In Experiment 3, we studied 17 infant rhesus macaques throughout the first five  
121 months of life to report on the relationship between infant social behavior and HCCs. We  
122 predicted that 4) infant social behavior, particularly social play (the most frequent peer-to-peer  
123 behavior at this age), would negatively relate to HCCs.

124

## 125 **2. Methods**

### 126 *2.1 Subjects and housing*

127 In Experiment 1, subjects were 32 adult female rhesus macaques (age range: 3 to 18  
128 years; mean  $\pm$  SD:  $8.19 \pm 3.34$  years) observed from August 2014 to August 2015. The females  
129 represented all major lineages (i.e. matriline; N=3) in the troop (matriline 3: N=19; matriline 4:  
130 N=11, matriline 1: N=2). In Experiment 2, subjects were 28 mother-infant dyads (16 female  
131 infants; 12 males) studied in the first 90 days postpartum in 2013 and 2014. In Experiment 3,  
132 subjects were 17 infant rhesus macaques (13 males, 4 females) born between March and May  
133 2015 and studied from birth through August 2015. All subjects were born and reared at the  
134 Laboratory of Comparative Ethology (LCE) field station at the NIH Animal Center in Poolesville,  
135 Maryland. The field station was a 5-acre (2.0ha) open-air enclosure with natural vegetation, a  
136 pond (0.9ha) with a centralized island (0.07ha), and climbing structures and enrichment. Three  
137 corncrib shelters (4.88 x 4.88x 5.79 m) and three indoor climate controlled runs (2.74 x 5.79 x  
138 4.27m) provided protection from inclement weather. Commercial lab diet (Purina Monkey Chow  
139 #5038, St. Louis, MO), natural vegetation, and water were available *ad libitum* and supplemented  
140 with fresh fruits and seeds/nuts twice a day. All procedures described below adhered to the NIH  
141 Guide for the Care and Use of Laboratory Animals and were approved by the NICHD Animal  
142 Care and Use Committee (ACUC).

143

## 144 2.2 Data collection

### 145 2.2.1 Experiment 1: Aggression

146 A total of 4,898 dominance interactions were recorded from August 2014 to August 2015  
147 through both focal and *ad libitum* sampling (Altmann, 1974). Interactions included both  
148 aggressive (threat, chase, attack) and submissive interactions (fear grimace, displacement; see  
149 Wooddell et al., 2016). Dominance ranks were established via Elo-rating (Neumann et al., 2011;  
150 Wooddell et al., 2016).

151

### 152 2. 2. 2. Experiment 1 and 3: Adult and infant focal behavioral data

153 Adult behavioral data were collected via modified frequency sheets (Novak et al., 1998;  
154 Wooddell et al., 2016) by two primary observers (LJW and AMD; inter-rater reliability  $\geq 85\%$ )  
155 from August 11, 2014 through August 10, 2015 using a 5-minute continuous focal animal  
156 sampling method (Altmann, 1974). Each 5-minute session was divided into 20, 15-second  
157 intervals. Any behavior that occurred within the 15-seconds was recorded in chronological order.  
158 The maximum frequency a behavior could occur therefore was 20 intervals per session. Each  
159 female (N=32) was coded for 1-2 sessions per week in both morning (900 to 1200) and afternoon  
160 sessions (1200 to 1700). A total of 1,833 adult observations (mean  $\pm$  SEM:  $57.28 \pm 3.55$  sessions  
161 per female) were collected during the study period, totaling 153 hours. Each infant born in 2015  
162 received three weekly sessions following this coding scheme starting at approximately one month  
163 of age. A total of 446 infant focal observations (mean  $\pm$  SEM:  $26.23 \pm 0.24$  sessions per infant)  
164 were collected during the study period, totaling approximately 37 hours. Total focal data  
165 collection time (for both adults and infants) thus was approximately 190 hours.

166 Behaviors collected included nonsocial behaviors (locomotion, foraging, etc.) and social  
167 behaviors. For the purposes of this study, we only analyzed social behaviors including affiliative  
168 behaviors (contact, groom, grooming present, lipsmack, mount, play), aggressive behaviors  
169 (threat, chase, attack), and submissive behaviors (fear grimace, displacement).

170

### 171 *2.2.3 Experiment 2: Maternal face-to-face interactions*

172 Focal observations were conducted 3-5 times per week for the first 90 days postpartum  
173 for 28 mother-infant dyads in 2013 and 2014 by two primary observers (AMM and KJB: inter-  
174 rater reliability  $\geq 85\%$ ). The frequency of mutual gazing, which was defined as eye-to-eye contact  
175 between mother and infant lasting at least 3 seconds, in each 15-minute session (see Dettmer et  
176 al., 2016b, 2016c, for a more detailed description) was recorded. A total of 915 15-minute  
177 sessions were recorded (mean  $\pm$  SEM:  $32.68 \pm 1.23$  sessions per infant). Total data collection  
178 time for mother-infant interactions was thus 229 hours.

179

180 *2.2.4 Hair cortisol concentrations (HCCs)*

181           Routine biannual health exams were conducted every six months in February and August  
182 of every year. Beginning in 2012, during these health exams, hair samples were taken by shaving  
183 the back of the animals' necks for every individual in the population (infants, juveniles, and  
184 adults). Because these samples were taken every six months (always from the same location on  
185 the back of the neck following a shave-reshave procedure), the HCCs values reflected chronic  
186 retrospective activity over the past six months (since the last time it was shaved), during which  
187 cortisol was incorporated into the growing hair shaft.

188           In Experiment 1, hair samples were collected by shaving the back of the animals' necks  
189 during routine biannual health exams in February 2015 (reflecting activity from August 2014 to  
190 February 2015) and August 2015 (reflecting activity from February 2015 to August 2015) as part  
191 of a longitudinal study, reflecting the time period in which behavioral data were collected  
192 (August 2014 to August 2015). Samples were stored in a foil pouch at -80°C until shipment to  
193 the Hormone Assay Core Laboratory at the University of Massachusetts Amherst. Following  
194 Meyer et al. (2014), samples were weighed, washed twice with isopropanol and dried for 5-7 days  
195 under a fume hood. For all hair assays, we used the entire length of the shaved hair, as the  
196 founding study validating the hair cortisol assay in rhesus macaques found no significant  
197 difference in proximal versus distal hair segments when hair samples were divided in half  
198 (Davenport et al., 2006). After washing and drying, samples were then ground to a fine powder  
199 with a ball mill grinder (MM200; Retsch, Newtown, PA) and incubated in methanol for 24 hours  
200 to extract cortisol from the samples. Aliquots of the methanol extract were dried down and  
201 reconstituted with assay buffer, then analyzed via enzyme immunoassay (EIA) using a salivary  
202 cortisol kit (#1-3002; Salimetrics, Carlsbad, PA). Resulting values ( $\mu\text{g}/\text{dL}$ ) were converted to  
203  $\text{pg}/\text{mg}$  for analysis. Inter- and intra-assay coefficients of variation were <10% based on aliquots  
204 of the same extracted pooled hair sample analyzed repeatedly across assays.



205 In Experiment 1, two adult subjects had no hair sample taken at either time-point in  
206 February or August. Thus the total sample size for Experiment 1 was n=30.

207 In Experiment 2, hair samples were collected from infants born in 2013 and 2014 in  
208 August 2014 and August 2015 at the routine biannual health exams, depending on the cohort  
209 (infants born in 2013 had their weaning age hair sample taken in 2014, and infants born in 2014  
210 had their weaning age hair sample taken in 2015). It is important to note that these subjects had  
211 hair samples taken every six months (every February and August) from birth onwards, so the hair  
212 samples reflected chronic retrospective activity over the past six months. However for the  
213 purposes of this study, we were interested in HCCs during weaning, as this is an important  
214 behavioral and developmental milestone, and we therefore included the samples from the  
215 weaning age. It is also important to note that the behavioral data were collected in the first few  
216 months of life, whereas the hair samples were taken during weaning approximately 1 year later.  
217 The selection of this approach was dictated by our desire to examine long-term relationships  
218 between early maternal affiliation and later HCCs in the offspring, rather than assess the possible  
219 relationship between neonatal behavior and contemporaneous cortisol levels. Four subjects were  
220 not available for hair sampling. Thus, the total sample size for Experiment 2 was n=24.

221 In Experiment 3, hair samples were taken in August 2015 at the health exams. The  
222 infants were born from March to May, thus the HCCs reflected activity over the first five months  
223 of life (during which the behavioral data were collected). Five of the infants born in 2015 in  
224 Experiment 3 were not available for hair sampling. The total sample size for Experiment 3 was  
225 n=12.

226

### 227 *2.3 Statistical analyses*

228 As instances of aggression were collected from all observed occurrences (focal + *ad*  
229 *libitum*) during other unrelated longitudinal projects, aggression for the present study was  
230 calculated as the proportion of aggressive interactions initiated and received by the individual

231 divided by the total number of observed aggressive interactions. Therefore out of 108 attacks, an  
232 individual that initiated 5 attacks would have a proportion of 5/108, meaning that this individual  
233 initiated a proportion of 0.046 (4.6%) of the attacks. This was done for each aggressive behavior  
234 (threat, chase, attack) and a composite aggression score.

235 All initiated and received affiliative social behaviors (contact, groom, grooming present,  
236 lipsmack, mount, play) per session were combined to create a total initiated and received sociality  
237 score. Average initiated and received sociality scores were then calculated to represent the  
238 average frequency an individual engaged in initiated or received social behaviors per 5-minute  
239 session. The maximum frequency was 20 intervals. Therefore an average frequency of 5 indicated  
240 that the individual initiated or received a social behavior in 25% of the intervals (5/20).

241 Average rates of mutual gazing per session between mother and infant were calculated  
242 for the first 90 days postpartum. The data did not follow a normal distribution, so the average  
243 frequency of mutual gazing was log transformed.

244 HCCs were log transformed prior to analysis to ensure normality. In Experiment 1, the  
245 two hair samples were averaged to represent the entire accumulation of HCCs that occurred over  
246 the course of a year for adult females.

247 In Experiment 1, linear regression tested whether social behaviors (affiliative and  
248 agonistic) predicted a significant proportion of the total variance in average HCCs over one year  
249 in adult female rhesus macaques. In Experiment 2, linear regression tested whether mutual gazing  
250 during the first 90 days postpartum (as well as age and sex) predicted a significant proportion of  
251 the total variance in HCCs at approximately the age of weaning (circa 1 to 1.5 years). In  
252 Experiment 3, Spearman correlations (due to the small sample size) were used to test the  
253 associations between infant social behavior and HCCs over the first five months of life. All alpha  
254 values were set at  $P < 0.05$ . SPSS 22 was used for all analyses.

255

256 **3. Results**

257 *3.1 Experiment 1: General results for adult female rhesus macaque HCCs*

258 Average adult female HCCs ranged from 28.99 to 68.92 pg/mg (mean  $\pm$  SEM: 44.01  $\pm$   
 259 1.58). There were no significant effects of age ( $F(1,28)=0.04$ ,  $P=0.84$ ,  $R^2=0.002$ ,  $\beta=-0.04$ ) or  
 260 dominance rank ( $F(1,28)=0.64$ ,  $P=0.43$ ,  $R^2=0.02$ ,  $\beta=0.15$ ) on HCCs.

261

262 *3.1.1 Experiment 1: Aggression and HCCs in adult female rhesus macaques*

263 Submissive interactions were the most common dominance interaction (43% of all  
 264 interactions), followed by chases (27.56%) and threats (27.07%). Interactions that involved  
 265 physical contact (attacks) were relatively infrequent, occurring in only 2.6% of all observed  
 266 interactions.

267 Contrary to *prediction 1*, neither the amount of initiated nor received aggression was  
 268 significantly related to adult female HCCs (initiated:  $F(1,28)=0.25$ ,  $P=0.62$ ,  $R^2=0.09$ ,  $\beta=0.09$ ;  
 269 received:  $F(1,28)=0.002$ ,  $P=0.97$ ,  $R^2=0.008$ ,  $\beta=0.008$ ), even when segregated based on the  
 270 intensity of aggression (threats, chases, attacks, in that order of intensity; all  $P>0.05$ ).

271

272 *3.1.1 Experiment 1: Sociality and HCCs in adult female rhesus macaques*

273 Social contact was the most commonly initiated social behavior (65.48%), followed by  
 274 grooming (32.18%). Adult females initiated grooming presents, (1.08%), play (0.57%), lipsmacks  
 275 (0.38%), and mounts (0.3%) relatively infrequently. The average frequency of initiated social  
 276 affiliation ranged from 4.28 intervals per 5-minute session (maximum intervals=20) to 11.96  
 277 intervals (mean  $\pm$  SEM: 7.99  $\pm$  0.34).

278 In support of *prediction 2*, linear regression revealed that the average frequency of  
 279 initiated social affiliation (contact, groom, grooming present, lipsmack, mount, play) was  
 280 negatively associated with adult female average HCCs over the course of a year ( $F(1,28)=15.26$ ,  
 281  $P<0.001$ ,  $R^2=0.35$ ,  $\beta=-0.59$ , see Figure 1a). In addition, when examining the two primary  
 282 initiated social behaviors (contact and grooming) independently, both behaviors yielded

283 significant results with average HCCs (contact:  $F(1,28)=6.08$ ,  $P=0.02$ ,  $R^2=0.18$ ,  $\beta=-0.42$ ;  
 284 grooming:  $F(1,28)=4.86$ ,  $P=0.036$ ,  $R^2=0.15$ ,  $\beta=-0.39$ ). Surprisingly, received social affiliation did  
 285 not significantly relate to average HCCs ( $F(1,28)=1.83$ ,  $P=0.16$ ,  $R^2=0.06$ ,  $\beta=-0.25$ , Figure 1b),  
 286 even when independently examining contact and grooming (received contact:  $F(1,28)=1.94$ ,  
 287  $P=0.17$ ,  $R^2=0.07$ ,  $\beta=-0.26$ ; received grooming:  $F(1,28)=0.14$ ,  $P=0.71$ ,  $R^2=0.07$ ,  $\beta=-0.07$ ).

288

289 **Figure 1: Relationship between affiliative social behaviors initiated (a) and received (b) and**  
 290 **HCCs in adult female rhesus macaques**

291 The average frequency (maximum frequency of 20 intervals) of initiated affiliative social  
 292 behavior per 5-minute session negatively corresponded to HCCs (pg/mg) in adult female rhesus  
 293 macaques over a one year time period. No significant relationship was found for received social  
 294 affiliation.

295

296 *3.2 Experiment 2: Early maternal face-to-face interactions and HCCs*

297 Average frequencies of mutual gazing ranged from 0 to 4 bouts per 15-minute session  
 298 (mean  $\pm$  SEM:  $0.41 \pm 0.15$ ). Linear regression revealed sex as a main predictor of mutual gazing,  
 299 with male infants engaging in higher rates of mutual gazing than females ( $F(1,23)=5.69$ ,  $P=0.03$ ,  
 300  $R^2=0.19$ ,  $\beta=-0.45$ ). We therefore examined sex effects in subsequent analyses. Because an age  
 301 related decline has also been observed in HCCs in young primates (Fourie and Bernstein, 2011),  
 302 we also examined age effects.

303 Weaning HCCs ranged from 27.47 pg/mg to 66.40 (mean  $\pm$  SEM:  $42.77 \pm 1.79$ ).

304 Supporting *prediction 3*, linear regression revealed that the average frequency of mutual gazing  
 305 90 days postpartum negatively predicted HCCs during the weaning transition (mean  $\pm$ SEM age at  
 306 hair sampling:  $476.57 \pm 6.85$  days;  $F(1,19)=8.15$ ,  $P=0.01$ ,  $R^2=0.30$ ,  $\beta=-0.55$ , see Fig. 2). No age  
 307 ( $F(1,22)=2.39$ ,  $P=0.14$ ,  $R^2=0.10$ ,  $\beta=-0.31$ ) or sex effects ( $F(1,22)=0.005$ ,  $P=0.94$ ,  $R^2=0.00$ ,  $\beta=-$

308 0.02) on HCCs were revealed. Mother's dominance rank also did not predict any significant  
 309 variation in HCCs during weaning ( $F(1,22)=0.34$ ,  $P=0.57$ ,  $R^2=0.02$ ,  $\beta=0.12$ ).

310

311 **Figure 2: Mutual gazing during the first 90 days of life and HCCs at weaning age**

312 Infants who engaged in higher frequencies of mutual gazing with their mothers per 15-minute  
 313 session in the first 90 days postpartum also displayed lower levels of hair cortisol (pg/mg) during  
 314 their transition to weaning approximately one year later.

315

316 *3.3 Experiment 3: Infant social behavior and HCCs*

317 Social contact was the most common social behavior (84.76% of social interactions)  
 318 initiated by infants, followed by play (14.63%). Grooming (0.43%) and mounting (0.18%) were  
 319 initiated relatively infrequently. Lipsmacking and grooming presents occurred negligibly. It is  
 320 important to note that these interactions included all social partners (other infants, juveniles, and  
 321 adults). Infant HCCs ranged from 40.45 pg/mg to 169.42 (mean  $\pm$  SEM:  $103.14 \pm 11.34$ ).

322 The average frequency of initiated and received social affiliation was not significantly  
 323 related to infant HCCs (initiated:  $r_s=-0.30$ ,  $P=0.34$ ,  $N=12$ ; received:  $r_s=-0.37$ ,  $P=0.24$ ,  $N=12$ ),  
 324 partially failing to support *prediction 4* for infants. However, *prediction 4* was partially supported  
 325 by a significant negative relationship between initiated play and infant HCCs ( $r_s=-0.58$ ,  $P=0.048$ ,  
 326  $N=12$ ). In contrast, no significant relationship was observed between infant HCCs and received  
 327 social play ( $r_s=-0.33$ ,  $P=0.30$ ,  $N=12$ ). However, there was a significant difference in the  
 328 frequency of initiated play between males and females (Mann Whitney U test,  $U=44$ ,  $P=0.037$ ),  
 329 with males exhibiting significantly more play (mean  $\pm$  SEM: males:  $1.55 \pm 0.24$ ; females:  $0.55 \pm$   
 330  $0.12$ ). When examining sex differences, the correlation between initiated play and HCCs was  
 331 only significant for males ( $r_s=-0.70$ ,  $P=0.036$ ,  $N=9$ , see Fig. 3), as there was not a large enough  
 332 sample size of females for analyses ( $N=3$ ). Mother's dominance rank ( $r_s=-0.11$ ,  $P=0.73$ ,  $N=12$ )  
 333 and age ( $r_s=-0.43$ ,  $P=0.16$ ,  $N=12$ ) did not significantly correlate with infant HCCs.

334

**335 Figure 3: Male infant social play and HCCs**

336 Male infants who initiated higher frequencies of social play per 5-minute session (maximum  
337 frequency of 20 intervals) displayed significantly lower hair cortisol (pg/mg) during the first five  
338 months of life. No significant relationship was found for received social play or other types of  
339 social affiliation.

340

**341 4. Discussion**

342 We sought to determine whether social behavior was associated with chronic HPA axis  
343 activity, reflected in HCCs, in semi-free ranging adult and infant rhesus macaques. For adults, we  
344 predicted that more agonistic social behavior (e.g. aggression) would be associated with higher  
345 levels of HCCs, and high rates of social affiliation (e.g. grooming) would be associated with  
346 lower levels of HCCs. In addition, we predicted that maternal face-to-face interactions would  
347 promote HPA axis regulation during a transitional period in life (e.g. weaning) and that affiliative  
348 social behavior would contribute to lower levels of HCCs in infants. Our results demonstrate that  
349 affiliative social interactions were associated with lower long-term HPA axis activity across  
350 development, although causation cannot yet be determined with the existing data.

351 Surprisingly, we found no significant association between levels of aggression and  
352 chronic HPA axis activity. At first this seems counterintuitive, as a number of studies have  
353 reported that frequent exposure to agonistic social behavior is associated with higher HCCs (Feng  
354 et al., 2016; Salas et al., 2016; Tennenhouse et al., 2016; Yamanashi et al., 2016). One possible  
355 explanation is that individuals who receive high rates of aggression, which is common in the  
356 despotic society of rhesus macaques, may often be able to predict aggression before it begins, and  
357 predictability has been associated with lower cortisol responses (Galhardo et al., 2011). This  
358 predictability may help offset the immediate stressors of agonistic behavior. This may also  
359 partially be supported by the finding that the most common type of dominance interaction was

360 submission (moving away from or displaying a grimace to a dominant animal) rather than  
361 aggression, signaling that there may be some degree of predictability in dominance interactions  
362 (avoiding a dominant animal before aggression begins). In addition, individuals may reconcile  
363 following aggressive interactions (de Waal a Yoshihara, 1983), which may help mitigate the HPA  
364 axis response. Our results suggest that aggression alone may not be sufficient enough to explain  
365 significant proportions of the variance in chronic HPA axis activity, as individuals may be able to  
366 cope via other mechanisms. Thus further research is warranted, especially across a number of  
367 species, including humans, with various dominance styles ranging from tolerant to highly  
368 despotic (Thierry, 2007).

369 As predicted, we found that adult females who initiated more social affiliation also had  
370 significantly lower levels of HCCs over the course of a year, similar to studies using short-term  
371 measures of HPA axis activity (Shutt et al., 2007). These findings suggest either that females who  
372 were more social had subsequently lower levels of HCCs, or that females who had lower levels of  
373 HCCs (due to some trait-like aspect) were more likely to seek out social interactions, although  
374 our study cannot determine causation. Future experimental studies will be able to establish causal  
375 links between sociality and HPA axis activity. Perhaps the more interesting question is why there  
376 is no relationship between received social affiliation and HCCs. Similar to our results, Shutt et al.  
377 (2007) also found a significant relationship with only initiated social grooming and fecal GCs,  
378 suggesting that the initiation of affiliative social behaviors may yield physiologically different  
379 results than receiving such behaviors. The social and biological benefits (coalitionary support,  
380 HPA axis activity, general health) and consequences (vigilance, foraging) of initiated and  
381 received social behaviors warrant further study. Future research should address the complex  
382 interactions between sociality, the HPA axis, and beneficial physiological outcomes. Moreover,  
383 Novak and Meyer (2017, unpublished data) found that in a large sample of 117 rhesus macaques,  
384 HCCs remained highly stable over time. Similarly, our results suggest that HCCs may reflect  
385 some trait-like aspect of the animal's behavior (such as social affiliation). However, this is not to

386 say that social affiliation alone may relate to HCCs, as hair samples reflect a long period of time  
387 spanning cumulative months and events. Indeed, age (Fourie and Bernstein, 2011; Dettmer et al.,  
388 2014) genetics (Fourie and Bernstein, 2011), population density (Dettmer et al., 2014), early life  
389 adversity (Dettmer et al., 2012; 2016a; Feng et al., 2011) and dominance rank (Dettmer et al.,  
390 2016a; Feng et al., 2016) have all been shown to influence HCCs in monkeys (primarily rhesus  
391 macaques). Future research should address the significant predictors of monkey HCCs, much like  
392 a recent meta-analysis done in humans (Stalder et al., 2017). This will then allow us to investigate  
393 the role social affiliation has in HCCs, whether it be a main or a modulating effect.

394 We also provide evidence that early mother-infant face-to-face interactions (i.e. mutual  
395 gazing) in infancy negatively corresponded to HCCs during a highly transitional and stressful  
396 period in primate life: weaning. We previously found that early mother-infant face-to-face  
397 interactions were associated with more infant sociality later in development (Dettmer et al.,  
398 2016c). The present results indicate that early mother-infant affiliative bonds may also have  
399 downstream effects on offspring HPA axis activity, thus suggesting a potential mechanism for the  
400 Dettmer et al. (2016c) results. This promotion of social behavior may help regulate the HPA axis,  
401 especially during a major transitional period. It is therefore possible that the mediation of stress  
402 by maternal affiliation has long lasting influences, extending past the early months of life. Our  
403 results suggest that infant primates that engage in maternal-infant interactions more frequently  
404 may be better able to cope with the physiological and psychological stressor of weaning, possibly  
405 via enhanced social interactions. We do however acknowledge that the frequencies of mutual  
406 gazing were rare (ranging from 0 to 4 bouts per 15 minute session; mean  $\pm$  SEM:  $0.40 \pm 0.15$ )  
407 and thus urge other researchers to replicate these findings. In addition, future studies should  
408 examine social behavior of the infants (both maternal and peer interactions) during the weaning  
409 period, as it is possible that the effects on chronic HPA axis activity of these early mother-infant  
410 interactions may extend to other social behaviors (i.e. grooming, play, etc.) during the transition  
411 to weaning. Moreover, future studies are necessary before we can be confident that more frequent



412 face-to-face interactions between mothers and infants benefit the offspring in other socially and  
413 physiologically stressful times (e.g., emigration, transition to puberty, etc.).

414 Finally, we demonstrate that social play also negatively correlated with infant HCCs, but  
415 only for males. While we did not find any significant relationships with social affiliation overall,  
416 these results support the idea that social play may be an especially important social behavior for  
417 male infant macaques (Kulik et al., 2015), which is fundamentally different than other forms of  
418 social affiliation (contact, grooming, etc.). This was additionally found in a recent study in  
419 nursery-reared rhesus macaques in which lip-smacking imitative ability in the first week of life  
420 predicted higher levels of social play (but not social grooming) in males at one year of age  
421 (Kaburu et al., 2016). As play occurs most frequently in young mammals, these results suggest  
422 there may be some inherent benefit of play during development, especially for males. Indeed,  
423 research with animal models has suggested that play is modulated by the brain's reward system  
424 (Manduca et al., 2016) and promotes brain and sensorimotor development (Pellis & Pellis, 2007).  
425 Furthermore, animals that are deprived of social play have impaired social interactions as adults  
426 (Hol et al., 1999) and altered dendritic morphology, which increases vulnerability to social stress  
427 (Burlison et al., 2016). Our results suggest that social play may additionally be related to the  
428 development of long-term HPA axis activity. However, as the results were correlational, it is  
429 difficult to interpret whether play behavior may have been an important coping mechanism  
430 thereby regulating HPA axis activity, or that males who were better able to cope with  
431 physiological stress were more able to allocate more time to social play. In addition, why some  
432 individuals engage in more social play than others may not only be a product of sex, but also  
433 previous maternal experience. In line with this, previous research has suggested that infant  
434 macaques that engage in higher frequencies of maternal mutual gazing during infancy have  
435 enhanced social interactions later (Dettmer et al., 2016c). We hypothesize that early maternal  
436 interactions may promote social behavior in infancy, and this enhanced sociality may help  
437 regulate the HPA axis. Further experimental studies will be able to identify the causal links

438 between maternal behavior and play, which may provide valuable information on the downstream  
439 effects maternal behaviors have on the offspring, even into adulthood.

440         Several limitations of the present study warrant mention. One limitation is that adult  
441 behavioral data were only analyzed for females, and thus it is unclear whether these findings  
442 extend to males. Given that rhesus macaque society is female philopatric and males emigrate  
443 around the time of puberty, females develop strong social bonds and engage in more frequent  
444 social affiliation than males (Drickamer, 1976). While we did collect extensive behavioral  
445 observations on our adult males, the small sample size (N=4) made any analyses difficult to  
446 interpret. Therefore, an interesting opportunity exists for populations that have a larger number of  
447 males to study variations in social affiliation and chronic HPA axis activity. Another important  
448 limitation is that our findings do not address social behavior and HCCs during the juvenile period,  
449 which contains important behavioral, physiological, and developmental milestones for primates  
450 (Pereira & Fairbanks, 2002). Unfortunately during the time of this study, focal data on juveniles  
451 were not recorded due to limited researcher availability. Therefore, future studies should focus on  
452 the relationships between social behavior and chronic HPA axis activity in juveniles, which may  
453 have important implications for adult outcomes. For example, in mother-peer-reared but not  
454 nursery-reared rhesus macaques, the change in HPA axis activity during the juvenile period  
455 negatively predicted adult rank, suggesting that an ability to regulate the HPA system may  
456 promote later social competence and rank (Dettmer et al., 2016a). However the Dettmer et al.  
457 (2016a) study did not record affiliative social interactions, which may have also contributed to  
458 HPA axis regulation and subsequent adult social rank. Indeed, nursery-reared rhesus macaque  
459 infants have impaired social interactions (Andrews and Rosenblum 1994) and thus HPA axis  
460 activity might not only be a product of rearing environment but also of impaired social  
461 interactions. Future work should address social behavior and HPA axis activity in juvenility in  
462 relation to adult outcomes.

463 Finally, although our longitudinal shave-reshave procedure was designed to assess  
464 adrenocortical activity over the six month period between shavings, it appeared that some  
465 monkeys had complete hair regrowth within the sampling area at the time of sample collection.  
466 For those subjects, therefore, the period of cortisol deposition may have been somewhat less than  
467 the full six months. Our methodology therefore does not allow us to determine the exact time  
468 course of hair cortisol accumulation in each individual monkey, and therefore parts of the  
469 behavioral measurements may not have been precisely contemporaneous with HCC  
470 accumulation. In the future, precise hair growth measurements are needed to account for  
471 individual variability in growth rates and to determine the specific time period of hormone  
472 incorporation for each subject. Examining the factors that promote varying degrees of hair growth  
473 in monkeys would also be invaluable in determining the biological and behavioral significance of  
474 hair cortisol. Finally, a longitudinal assessment of serially collected salivary cortisol in  
475 conjunction with HCCs would provide a robust validation into the temporal summation of  
476 cortisol into hair in monkeys, much like what has been observed in humans (Short et al., 2016).

477

## 478 **5. Conclusion**

479 Given that sociality relates to long-term HPA axis activity, and that long-term HPA axis  
480 activity can negatively affect health (Sapolsky, 2005), our results suggest that a potential  
481 mechanism for the link between sociality and health may be via mediation by the HPA axis.  
482 Because sociality is a fundamental aspect of both human behavior and human health, our study,  
483 conducted with a rhesus macaque animal model, warrants further investigations into complex  
484 interactions of sociality, HPA axis activity, and health outcomes. Further research should  
485 investigate immune function, reproduction, neurobiology, cardiovascular functions, and mental  
486 health in regards to social behavior and HCCs. This will help elucidate the downstream effects  
487 sociality has on health outcomes, possibly via the HPA axis.

488

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495

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497

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