



## OPEN Associations between fecal glucocorticoid levels and social bonds vary with relatedness in juvenile rhesus macaques

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Strong social bonds in gregarious adult animals have been associated with lower levels of glucocorticoids. However, similar research is lacking for juvenile primates. We examined relationships between social bonds and mean concentrations of fecal glucocorticoid metabolites (fGCMs) in 44 free-ranging juvenile rhesus macaques (*Macaca mulatta*) on Cayo Santiago, Puerto Rico. We measured frequencies of affiliative behavior (grooming, play, approaches and proximity) with other same-sex, same-aged juveniles (peers) and the total number of affiliative peer relationships. We found a positive relationship between fGCMs and grooming frequencies. Females that spent more time in proximity to peers also had higher fGCMs. In contrast, among juveniles with more closely related peers, those with more affiliative peers or more frequent play bouts had lower fGCMs. However, strong peer bonds in most juveniles did not appear to be associated with reduced glucocorticoid levels. fGCMs were higher for females than males, but were unassociated with physical activity, aggression, or peer seeking tendencies. We propose that the establishment and navigation of some peer bonds at this life stage may involve increased metabolic demand.

For social animals, including humans, strong affiliative and supportive relationships (i.e., social bonds) have been shown to attenuate the effects of stressors<sup>1–6</sup>. Based on findings of inverse relationships between social bonds and glucocorticoids (GCs) (hormones secreted by the adrenal gland in response to many types of stressors), social buffering posits that social bonds buffer the potential negative effects of various social stressors that individuals might encounter and/or block individuals' exposure to stressors<sup>7,8</sup>. Most work exploring relationships between social bonds and hypothalamic-pituitary-adrenal (HPA) axis activity has focused on adults. Consequently, we know less about the extent to which they are related in juveniles, a period in life marked by the development of social skills and the expansion of social networks<sup>9</sup>.

The primary function of GCs is to maintain energy homeostasis<sup>10</sup>. GCs are often elevated through activation of the HPA axis during exposure to energetic stressors, including vigorous physical activity<sup>11</sup>, emotional arousal (e.g., aggression<sup>12</sup>), but also during situations of psychosocial stress<sup>13</sup>. Social buffering is possible for both acute and chronic stressors via direct and indirect support from conspecifics<sup>8,14</sup>. For example, social bonds may buffer chronic psychosocial stressors by increasing predictability within social interactions and stabilizing the social environment<sup>8</sup>. Although long-term responses to chronic stressors are associated with deleterious effects<sup>15</sup> and traditionally considered maladaptive<sup>16</sup>, chronic stress does not always result in higher GC levels nor lower individual fitness<sup>17</sup>. The stress response has been shown to be highly flexible, context-dependent, and may reflect different selection pressures (reviewed in<sup>14</sup>).

There is evidence that both the quantity and strength of social bonds appear to buffer increases in GCs and to have both short and long term fitness benefits for adult primates<sup>4,18</sup>. Engh and colleagues<sup>19</sup> highlighted the importance of quantities of social bonds in adult female baboons (*Papio hamadryas ursinus*); females that lost a close relative to predation initially experienced a rise in GC levels. They subsequently expanded their grooming networks to include a larger number of partners, after which their GC levels rapidly returned to baseline. Also, adult female baboons that maintained strong social bonds lived longer<sup>20</sup> and had higher offspring survival rates<sup>21</sup>. For them, the strength of relationships appeared to be more important than the quantity. To date, the question of whether the quantity or strength of social bonds serve distinct roles for juveniles is unclear.

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The period before sexual maturity is crucial for nonhuman juvenile primates to hone social skills and expand their social networks while navigating competition over access to resources and social partners from older, more experienced group members<sup>9</sup>. Enduring social bonds, often formed during infancy or the juvenile period<sup>22</sup>, have been hypothesized to contribute to the development of social skills and access to resources during this developmental stage and beyond<sup>9</sup>. The availability of social support has been shown to buffer artificially imposed acute stressors in captive juveniles. For instance, juveniles placed in a novel setting exhibited fewer behavioral and physiological indicators of stress when placed with their most preferred peer versus non-preferred peers or alone<sup>23,24</sup>. However, less is known about the extent to which free-ranging immatures vary in basal levels of GCs and are able to use social relationships to buffer or avoid everyday stressors. Also unclear is the relative importance of quantities vs. strength of social bonds. Nor do we know how social buffering abilities seen in adults develop during ontogeny.

Despite the lack of research on juvenile social bonds and HPA axis activity, there is suggestive evidence that social interactions may also pose stressors during this developmental period that may be reflected in high GC levels. Captive juvenile chimpanzees (*Pan troglodytes*) that spent more time engaged in affiliative interactions with peers had higher rather than lower GC levels<sup>25</sup>. Moreover, Thompson and colleagues<sup>26</sup> found that wild juvenile blue monkeys (*Cercopithecus mitis*) that groomed more also had higher basal GCs. Both these studies suggest that social interaction may involve increased metabolic demand and/or that juveniles that have high GC levels may seek out social interaction. In humans, there is evidence that peers are not as effective social buffers as parents; GC levels in adolescents exposed to a social stressor increased more when the teens were with a close friend versus a parent<sup>27</sup>. Finally, meta-analytic research in toddlers suggested that elevated GC levels are positively related to time spent with peers in daycare compared to time spent with parents at home<sup>28</sup>. However, whether this is due to exposure to peers or separation from parents is unclear.

We examined the relationship between social bonds and basal GC levels among juvenile rhesus macaques (*Macaca mulatta*). Rhesus macaques form large multi-male, multi-female social groups in which the permanent core consists of a number of adult females and their immature offspring<sup>29</sup>. Female rhesus macaques remain in their natal group for life and continue to associate closely with their maternal kin, whereas males typically disperse during adolescence<sup>30</sup>. Around one year of age, when rhesus are fully independent nutritionally (usually between 6 months – 1 year), a stable linear dominance hierarchy can be constructed from agonistic interactions between (same-aged group members) that correlates with the hierarchy among their mothers<sup>31</sup>. Yearlings and juveniles (2 and 3 year olds) are then subject to within group competition for food and other resources including access to social partners.

Juveniles actively establish and maintain affiliative social relationships with similarly aged group members and their relationships with other group members are largely independent from those of the mother, having differentiated from those of the mother with the same individuals in strength and quality<sup>22,32,33</sup>. Although they spend more time grooming with siblings than with other juveniles, they also affiliate with distantly related and unrelated juveniles more than with distant kin and non-kin of other ages<sup>34,35</sup>. In addition, both male and female juvenile rhesus macaques interact more often with same-sex than opposite sex peers in both affiliative and agonistic interactions<sup>36</sup>. However, males and females differ in their patterns of interaction with other juveniles; males participate more frequently in rough-and-tumble play<sup>37</sup>, whereas females spend more time socially grooming<sup>36</sup>. For this reason, we focused on kin and non-kin relationships with same-aged and same-sex juveniles, hereafter referred to as peers, since these relationships are likely to represent particularly substantial influences at this stage.

Here, we address associations between same-sex peer social bonds and HPA-axis activity, assessed by individual mean concentrations of fecal glucocorticoid metabolites (fGCMs) (**Aim 1**). We measured the strength of social bonds as the frequency of affiliative behavior (grooming, play, proximity, and nonaggressive approaches) with other same-sex juveniles and by the total number of affiliative peers. We then (**Aim 1A**) examined the relationship between social bond strength and fGCM concentrations. Negative relationships between fGCM concentrations and these social bond measures would be consistent with the idea that social bonds buffer the potential negative effects of social stressors (i.e., social buffering). Alternatively, positive relationships between fGCM concentrations and social bond measures would be consistent with the idea that social interaction with peers may pose challenges that may be reflected in high GC levels. (**Aim 1B**) In addition, since male and female juveniles interact primarily with same-sex peers and differ in their patterns of interaction with them<sup>36</sup>, we investigate possible sex differences in both fGCM concentrations and in associations between fGCM concentrations and social bond measures.

In **Aim 2**, we attempt to examine alternative explanations for any observed relationships between fGCM concentrations and social behavior. We ask whether a relationship between fGCMs and social bonds could be driven in part by variations in physical activity<sup>11</sup> or emotional arousal, e.g., during aggressive displays<sup>12</sup> by testing whether physical activity (**Aim 2A**) and aggression directed at other group members (**Aim 2B**) were related to fGCM concentrations and social bond measures. We also ask whether any observed relationship between fGCMs and social bonds could be due to a tendency for juveniles that have relatively strong bonds to avoid receiving aggression from other group members<sup>7</sup>, by testing whether rates of aggression received by juveniles from other group members (**Aim 2C**) were related to fGCM concentrations and social bond measures. Finally, we ask whether relationships between fGCMs and social bonds may be due in part to tendencies for juveniles with high fGCM concentrations to seek more interaction with peers<sup>38</sup>. To do this we use the Hinde index<sup>39</sup> to ask whether juveniles that display relatively high fGCM concentrations (**Aim 2D**) are more likely to initiate and maintain bouts of proximity, play and grooming than those with low fGCM concentrations. Taken together, these aims address unanswered questions about the types of social bonds among juveniles that are associated with reductions versus increases in metabolic demand and generate useful information relevant to the ontogeny of adult stress buffering systems.

## Methods

### Study site and subjects

The study was conducted from May to October 2016, on Cayo Santiago, a 15-ha island off the coast of Puerto Rico that has been home to a free-ranging, predator free population of rhesus macaques brought from India in 1938. At the time of study, the population contained six naturally composed, species-typically structured social groups. Individuals were well-habituated, easily observed, and easily identified. Maternal genealogical relationships of all individuals were known<sup>40</sup>. Cayo Santiago provides an environment which minimizes variation in nonsocial factors (e.g. predation, climate, diet)<sup>41</sup> that may influence individual GC levels, providing researchers more opportunity than wild populations to focus on the consequences of social stressors and on social mechanisms for coping with them. The macaques were provisioned with commercial monkey chow once per day from feeders located in three 0.25-acre corrals; this consistent provisioning likely lessened diet-related variation of GCs related to caloric intake and specific nutrients<sup>42</sup> compared with those seen in some wild populations. Water was provided *ad libitum* in drinking stations around the island. Amounts of provisioned food and water were ample, however accessing food and water was competitive. Although the Cayo Santiago population is not wild, many rhesus macaques living in native habitats have been generously provisioned most likely for thousands of years by humans who also have discouraged predators in their ranges<sup>43</sup>.

The study group (KK) consisted of two matriline and 192 members at the start of the study, ranking fourth in size and dominance status among the six groups living on the island. Although it was somewhat larger than the average group size of 32 for wild non-provisioned rhesus and 77 for provisioned rhesus<sup>44</sup>, this likely enhanced our ability to detect variation in GC levels and their relationships to social bonds. Larger groups display higher levels of within group competition on Cayo than smaller groups, and there is variation among individuals within groups for access to food<sup>45</sup>.

Focal subjects included all juveniles born within the group during two consecutive birth seasons, i.e., 2013 and 2014, ( $n=44$ : males  $n=25$ , females  $n=19$ ). Observations began prior to sexual reproduction and male dispersal when juveniles were close to completing their second ( $1.8 \pm 0.09$  y, mean  $\pm$  SD) and third years of life ( $2.7 \pm 0.07$  y, mean  $\pm$  SD). These ages represent a pre-reproductive phase, between infancy and sub-adulthood in rhesus macaques. Females on Cayo Santiago usually first give birth at 4 y of age<sup>46</sup>. Males typically leave their natal group during the mating season between 3 and 5 years (median = 4.5 y)<sup>30,47</sup>. Although a few females may first give birth at 3 years<sup>48</sup>, none in our subject sample did so (the youngest gave birth at 4.9 y of age). No male subject left the group; the youngest transferred at 4.7 y of age. This study was conducted in compliance with the ARRIVE guidelines<sup>49</sup>. In addition, all research methods were approved and performed in accordance with the regulations provided by the Institutional Animal Care and Use Committees (IACUC) of the University of Puerto Rico and the Caribbean Primate Research Center (CPRC) (protocol #A090115), and the University at Buffalo (protocol #ANT05065N).

### Behavioral data collection

KB and three assistants collected behavioral data in 10 min focal-animal samples on all subjects<sup>50</sup> with tablets (Samsung Galaxy T355) using Prim8 software<sup>51</sup>. At any one time, one observer recorded data on a juvenile subject, one on a juvenile's mother, while a third assistant collected fecal samples. We recorded durational state behaviors (grooming, social play, solitary play, traveling, resting) and events, including affiliative behaviors (non-aggressive approaches and leaves, grooming presents), and agonistic behaviors (contact aggression, non-contact aggression, displacements, avoidances, open mouth threats, fear grimaces, displays) (see Table S1 for behavioral definitions). For all interactions, initiators and receivers were identified. We also conducted instantaneous scan sampling at 5-minute intervals in the beginning, middle, and end of each focal session identifying all individuals within proximity (0–2 m) to the focal. We divided data collection days into hourly time blocks so that individual juveniles were observed once per day and had an approximately equal number of focal animal samples conducted for each observational hour over the course of the study. Each juvenile was observed for the same amount of time (11 h/juvenile, 484 h total for the study). We used behavioral data that were collected after inter-rater reliability among observers for identification and behavior reached Cohen's kappa of 0.85.

### Dominance rank

We constructed linear rank orders for juvenile subjects from the outcomes of aggressive and submissive dominance interactions derived from focal follows and *ad-libitum* observations<sup>50</sup>. We calculated dominance ranks for male and female juveniles based on same-sex interactions only using winner-loser outcomes with the *EloRating* package<sup>52</sup> in R (R version 4.3.2<sup>53</sup>).

### Sample collection and hormone metabolite analysis

Fecal collection occurred opportunistically between 0730 h and 1330 h, for all juvenile subjects and their mothers. While it has been hypothesized that juvenile responses to acute stressors are related to those of their mothers in humans<sup>54</sup>, juvenile mean fGCM concentrations in the present study apparently were independent of those of their mothers (see Supplemental Table S2). Thus, it was not necessary to control for maternal concentrations in the analyses. Fecal samples provide an estimate of all glucocorticoid secretions during the previous 24–48 h and as a result provide a more accurate assessment of long-term glucocorticoid concentrations than saliva or blood<sup>55</sup>. We collected fecal samples over the entire study, overlapping with our behavioral data collection. We were interested in basal GC levels that occur over a period of time rather than responses to specific events<sup>56</sup>. The conventional way to measure basal levels is by averaging several sample levels taken periodically over an extended period of time. We collected samples only if they were free from urine and water contamination and taken directly after defecation. Fecal boluses were homogenized with 0.5–2.0 g of fecal matter placed in polypropylene tubes. Samples were kept on ice for up to 7 h until transferred to CPRC's field station where they

were stored in the freezer (-20 C) until the end of the study. We attempted to collect one sample per subject each day, for a total collection of 328 samples for juveniles ( $7.45 \pm 3$  per juvenile,  $M \pm SD$ , range: 3–13) and 270 samples for the juvenile's mothers ( $9.43 \pm 2.5$  per adult female,  $M \pm SD$ , range: 3–15). Only two juveniles and one mother were represented by as few as three samples.

Fecal extracts were analyzed using an enzyme-immuno-assay (EIA) for concentrations of immunoreactive 11 $\beta$ -hydroxyetiocholanolone, a major cortisol metabolite in mammals, including primates (e.g.,<sup>57</sup>) (see Supplemental Material). This EIA was previously validated and applied successfully for assessing adrenocortical activity and glucocorticoid output from feces of macaques (e.g.,<sup>57,58</sup>).

### Social bonds

To measure affiliative peer relationships, i.e., social bonds, we used behavioral data obtained from focal-animal sampling and scan sampling. From these data, for each individual, we calculated the frequency of dyadic (1) grooming interactions ( $7.68 \pm 7.94$  per female,  $M \pm SD$ ,  $5.8 \pm 4.05$  per male,  $M \pm SD$ ), (2) play bouts ( $1.81 \pm 1.29$  per female,  $M \pm SD$ ,  $7.73 \pm 1.73$  per male,  $M \pm SD$ ), (3) nonaggressive approaches ( $14.63 \pm 1.61$  per female,  $M \pm SD$ ,  $19.52 \pm 2.57$  per male,  $M \pm SD$ ), and (4) the number of scans each dyad was seen in proximity (within 2 m of another peer without grooming or playing) ( $54.89 \pm 20.91$  per female,  $M \pm SD$ ,  $62.92 \pm 19.73$  per male,  $M \pm SD$ ) with all its same-sex, same-aged peers. These affiliative measures are commonly used to evaluate social bonds in rhesus macaques and other primates (e.g.,<sup>59</sup>). We then calculated the total number of affiliative peers per juvenile. For this measure, we were interested in the presence of an affiliative relationship irrespective of its strength. To do this, we totaled the number of peers a juvenile was observed giving or receiving grooming, engaged in a playing bout, seen within proximity (within 2 m of another peer), and/or engaged in nonaggressive approaches.

### Statistical analysis

#### Mixed models

To evaluate relationships with mean fGCM concentrations (the response variable) and social bond variables (grooming, play, approaches, proximity, and total number of affiliative peers) (**Aim 1**), we generated a series of linear mixed models (LMMs), using the *nlme* package<sup>60</sup>.

To reduce the risk of overfitting our dataset, our analysis comprised five LMMs with a single predictor of interest (social bond variable) and our control variables (dominance rank, sex, and maternal relatedness). Since each juvenile was observed for the same amount of time, it was not necessary to offset frequencies of behavior by observation time. By including sex as a fixed effect in each of the models, we were able to assess the relationship between fGCM concentrations and individual sex, while controlling for the social bond variables, rank and maternal relatedness. We did not include the juvenile's age as a fixed effect because fGCM concentrations were not significantly different between juveniles born in the 2013 and 2014 cohorts, and juvenile age overall was not a significant predictor of fGCM concentrations (Figure S1). Our study group included four sets of maternally related sisters and two sets of maternally related brothers. Hence, we also controlled for maternal kinship due to the possible confounds of the heritability of stress responses<sup>61</sup>. To do so, we calculated mean degrees of maternal relatedness with same-sex, same-age related peers ( $r=0.25$  for half siblings,  $0.125$  for half uncles, aunts, nieces, nephews, and  $0.063$  for half first cousins,  $r=0$  for more distant kin and unrelated peers) for each subject and entered it as a fixed effect. Interactions between the predictor of interest (social bond variables) and our control variables were tested one at a time against the base model to see whether the reduced models improved fits. We ran log-likelihood ratio tests (LLRTs) with the function *anova* for comparisons<sup>62</sup> (see SI for further details).

Next, to rule out alternative explanations for variation in fGCM concentrations a second set of LMMs were run with mean fGCM concentrations as the response variable, while controlling for sex, rank and maternal relatedness (**Aims 2 A-D**).

- **Aim 2A.** Since vigorous physical activity has been associated with elevated GC levels<sup>11</sup>, we examined a physical activity index created by summing individual rates (s/h) of durational state behaviors recorded during focal-animal sampling: traveling, solitary play, and social play (play with group members of all ages and both sexes).
- **Aim 2B.** Aggressive arousal has also been associated with increases in GC levels<sup>12</sup>, we calculated rates at which each juvenile directed contact and noncontact aggression towards other group members (of all ages and both sexes) and engaged in aggressive displays (i.e., branch shaking).
- **Aim 2C.** We tested whether any relationship between fGCMs and social bonds we find could be due in part to a tendency for juveniles that have relatively strong bonds to receive less aggression from others, since social bonds have been shown to play a role in reducing exposure to stressors in some studies<sup>7</sup>. To do this, we calculated individual rates at which each juvenile received contact and noncontact aggression from other group members (of all ages and both sexes).
- **Aim 2D.** We used the Hinde index<sup>39</sup> to assess whether juveniles that displayed relatively high fGCM concentrations sought out peer interaction more than others and were relatively more responsible than their partners for maintaining social interactions. We computed the number of initiated approaches and leaves of proximity for each same-sex dyad, the number of initiated and terminated bouts of grooming for female-female dyads and the number of initiated and terminated bouts of play for male-male dyads. The index was calculated for each same-sex dyad as the percentage of approaches and initiations minus the percentage of leaves and terminations by focal A directed towards partner B. The Hinde's index ranges from +1 to -1. Positive values indicate focal A was primarily responsible for initiating and maintaining interaction, whereas negative values indicate partner B was primarily responsible for initiating and maintaining interaction. The index controls for differences in activity levels (i.e., baseline frequencies of initiations and terminations of interaction) between

the focal and its partners. To calculate a single value per juvenile, we calculated the index using sums of initiations and terminations for each juvenile with all its same-sex, same-aged peers.

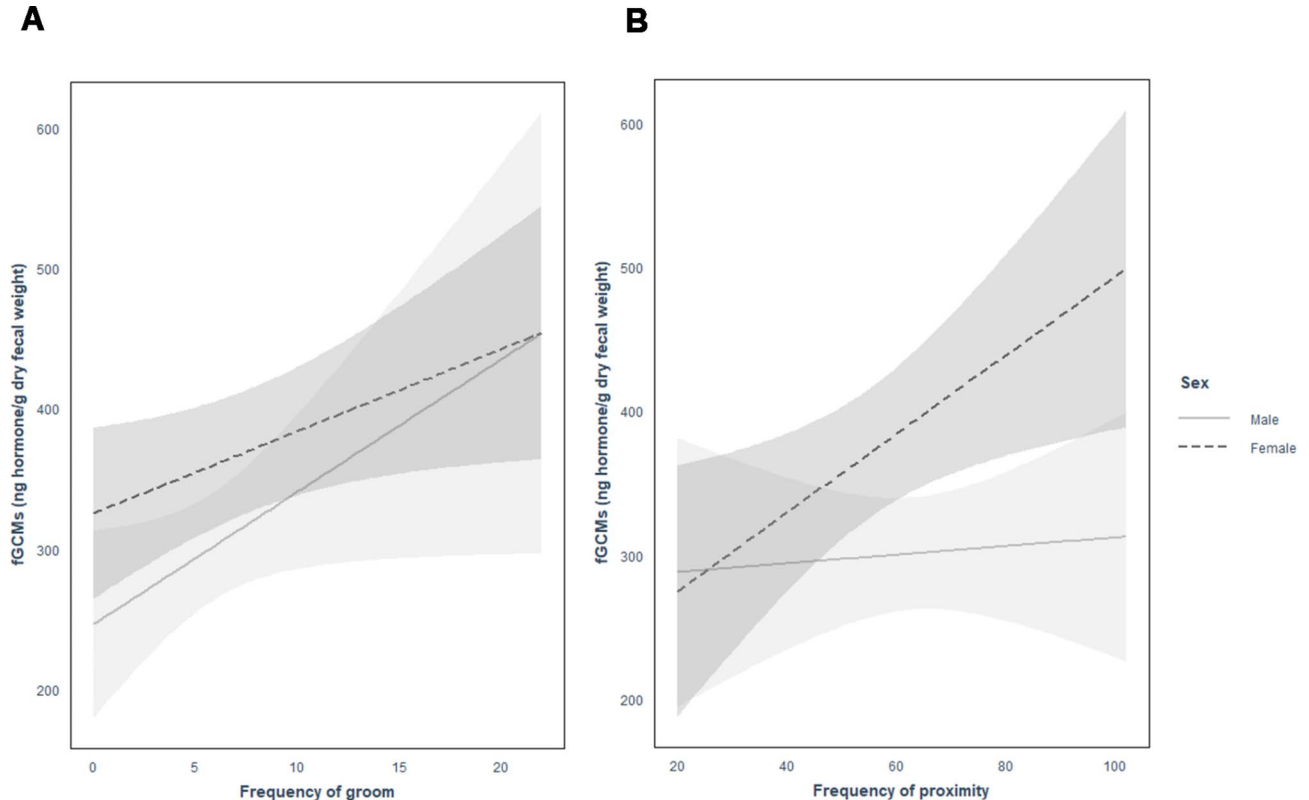
Assumptions were visually checked using the performance package. Fixed effects were log-transformed or a Yeo-Johnson power transformation was used when normality assumptions were not met<sup>63</sup>. Continuous predictors were centered and rescaled for interpretation, when necessary. There was no evidence of multicollinearity between factors (all variance inflation factors ranged between 1.05 and 2.95). The Benjamini-Hochberg false discovery rate was used to calculate the adjusted p-values for all models with fGCM concentrations as the outcome variable (alpha 0.05). Simple slopes analysis in the *interactions* package was used to interpret significant interaction effects. In addition, given our sample size, we calculated *f* and *p*-values for interactions using permutation tests with 1000 iterations. All models included ID as a random effect.

## Results

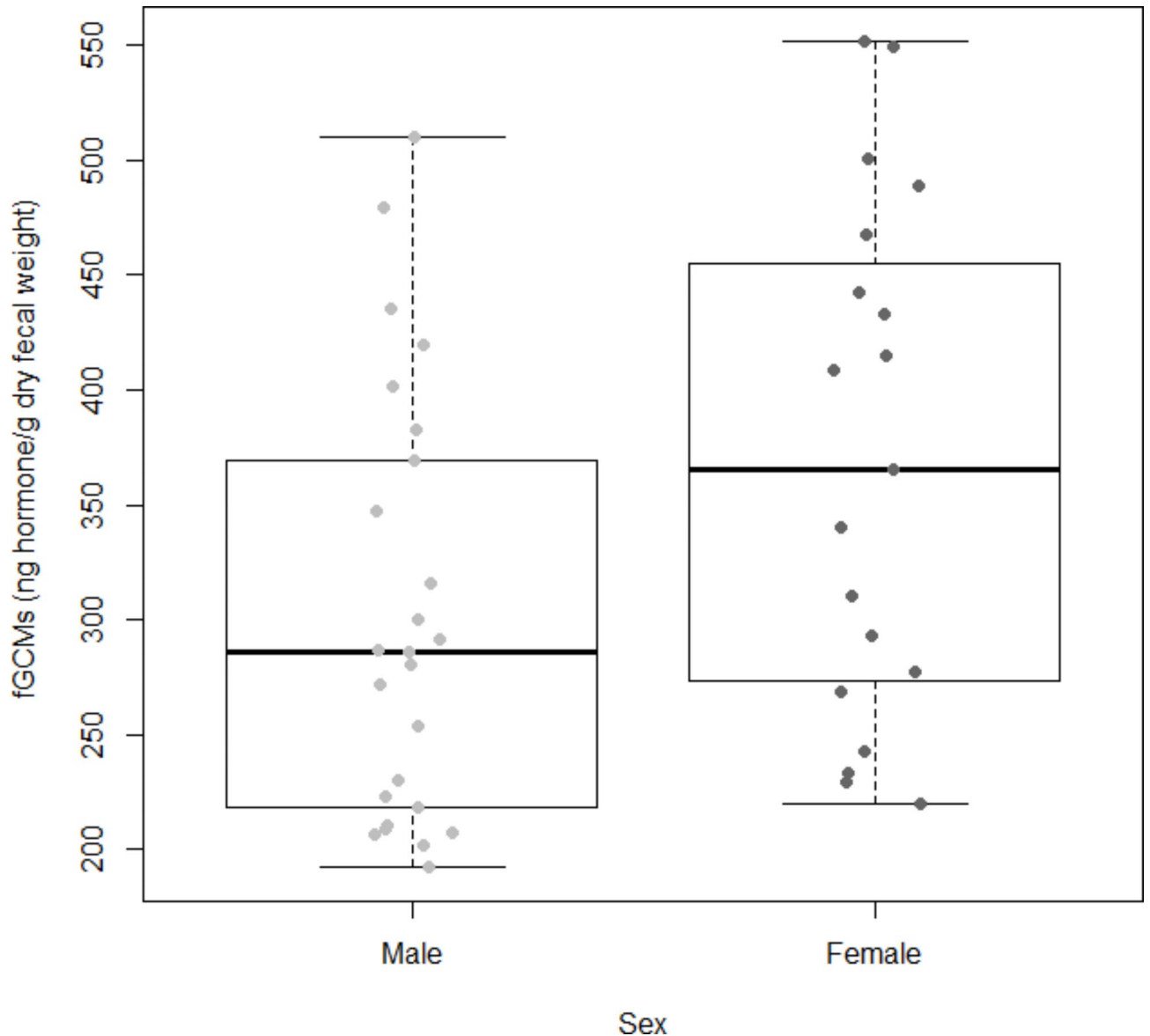
### Aim 1: Social bonds and fGCM concentrations

Mean fGCMs were positively and significantly related to dyadic grooming frequencies, while controlling for rank, sex and maternal relatedness ( $\beta = 7.74$ ,  $SE = 2.48$ ,  $t(39) = 3.11$ ,  $p = 0.02$ ) (Fig. 1a). There was a significant positive interaction between proximity and sex ( $f(39) = 4.26$ ,  $p < 0.001$ ). Females, but not males, with a higher frequency of proximity to other peers had significantly higher fGCMs (slope  $\pm SE = 2.73 \pm 1.06$ ) (Fig. 1b). While females had higher individual mean fGCM concentrations than males ( $\beta = 69.19$ ,  $SE = 30.96$ ,  $t(39) = 2.23$ ,  $p = 0.03$ ) (Fig. 2), the slope of the grooming relationship with fGCM did not differ by sex (female = 0.04 versus male = 0.02,  $p = 0.11$ ). Interactions between sex and grooming, play, approaches and number of affiliative peers were not significant, suggesting that the relationships between fGCM and these social bond measures were independent from sex (Table 1).

Approaches were not related to mean fGCM concentrations ( $\beta = 2.34$ ,  $SE = 1.27$ ,  $t(39) = 1.83$ ,  $p = 0.25$ ). There were significant interactions between maternal relatedness and both number of affiliative peers and play frequencies (Table 1). There was a negative association between fGCM concentrations and number of affiliative peers for juveniles whose affiliative peers were most closely related. Juveniles with a high number of affiliative peers and whose affiliative peers were more closely related had significantly lower fGCM concentrations (slope  $\pm SE = -17.47 \pm 5.71$ ,  $p < 0.001$ ) (Fig. 3a). Similarly, juveniles with more frequent play and more closely related affiliative peers had significantly lower fGCM concentrations (slope  $\pm SE = -16.34 \pm 6.22$ ,  $p < 0.001$ ) (Fig. 3b). There were no significant main effects or interactions involving rank (Table S3 and S4).



**Fig. 1.** Relationship between fGCM concentrations of samples collected from juvenile female ( $n = 19$ ) and juvenile male ( $n = 25$ ) rhesus macaques and (a) frequency of grooming interactions, and (b) significant interaction between frequency of proximity and sex. Shaded bands indicate 95% CIs.



**Fig. 2.** Individual mean fGCM concentrations for juvenile female ( $n=19$ ) and juvenile male ( $n=25$ ) rhesus macaques. The mean line represents the average fGCM value of each group.

## Aim 2: alternative explanations

### *Physical activity*

Individual rates of physical activity were not related to mean fGCM concentrations ( $\beta=0.06$ ,  $SE=0.25$ ,  $t(39)=0.80$ ,  $p=0.84$ ) (Table 2), grooming ( $\beta=0.06$ ,  $SE=0.01$ ,  $t(39)=0.46$ ,  $p=0.64$ ), or to proximity ( $\beta=0.04$ ,  $SE=0.03$ ,  $t(39)=1.20$ ,  $p=0.23$ ) when controlling for rank, sex and maternal relatedness. However, both the number of affiliative peers and play were positively related to physical activity (number of affiliative peers:  $\beta=0.01$ ,  $SE=0.01$ ,  $t(39)=2.78$ ,  $p<0.01$ ; play: ( $\beta=0.01$ ,  $SE=0.01$ ,  $t(39)=3.97$ ,  $p<0.001$ ), and negatively related to sex (number of affiliative peers:  $\beta=-3.81$ ,  $SE=0.77$ ,  $t(39)=-4.94$ ,  $p<0.001$ , play: ( $\beta=-4.59$ ,  $SE=0.53$ ,  $t(39)=-8.52$ ,  $p<0.001$ ); nevertheless, when we examined males alone, we still found no relationship between fGCM concentrations and physical activity when controlling for rank and maternal relatedness ( $\beta=0.09$ ,  $SE=0.29$ ,  $t(21)=0.32$ ,  $p=0.80$ ). These results suggest that the variation in physical activity cannot explain the relationships we found between fGCM concentrations and social bond variables (see SI Tables for variables that were not significant predictors).

### *Aggressive behavior*

Variation in directed aggressive behavior also failed to explain the relationships between fGCM concentrations and social bond variables. Individual rates of aggression directed toward all group members were unrelated to fGCM concentrations ( $\beta=-92.91$ ,  $SE=42.17$ ,  $t(39)=-2.20$ ,  $p=0.17$ ) (Table 2), grooming ( $\beta=-1.69$ ,  $SE=2.56$ ,  $t(39)=-0.66$ ,  $p=0.51$ ), proximity ( $\beta=-1.90$ ,  $SE=6.95$ ,  $t(39)=-0.27$ ,  $p=0.78$ ), play ( $\beta=0.03$ ,  $SE=0.68$ ,

Relationship between mean fGCM concentrations and social bond variables				
Model predictors	Estimate	SE	t	p
Fixed effects				
(Intercept)	251.97	24.61	10.23	<0.001
Groom	7.74	2.48	3.11	<b>0.02</b>
(Intercept)	182.99	66.94	2.73	<b>0.01</b>
Proximity	1.82	0.98	1.84	0.27
(Intercept)	238.01	39.79	5.98	<0.001
Approach	2.34	1.27	1.83	0.27
(Intercept)	237.89	82.77	2.87	<b>0.01</b>
Play	8.14	10.36	0.78	0.79
(Intercept)	297.17	157.25	1.88	0.06
Number of partners	0.18	7.86	0.02	0.98
Interactions			f	p
Groom*sex			0.83	0.79
Proximity*sex			4.26	<0.001
Approach*sex			0.06	0.80
Play*sex			0.02	0.80
Number of affiliative peers*sex			-0.04	0.79
Play*relatedness			-5.06	<0.001
Number of affiliative peers*relatedness			-8.13	<0.001

**Table 1.** Results of LMMs for the relationship between fGCM concentrations and social bond variables. Significance of interactions ( $f$  and  $p$  values) determined via 1000 permutations.  $P$  values are adjusted with Benjamini-Hochberg false discovery rate. Only shown is the predictor of interest (social bond variables) and interactions between the predictor of interest (social bond variables) and control variables; also see the full table in SI Appendix table S4 for other fixed predictors (control variables) and table S3 for excluded interactions based on log-likelihood ratio tests. Significant values are in bold.

$t(39)=0.05$ ,  $p=0.95$ ), or number of affiliative peers ( $\beta=1.45$ ,  $SE=0.88$ ,  $t(39)=1.65$ ,  $p=0.10$ ), or when controlling for rank, sex and maternal relatedness.

#### Aggression received

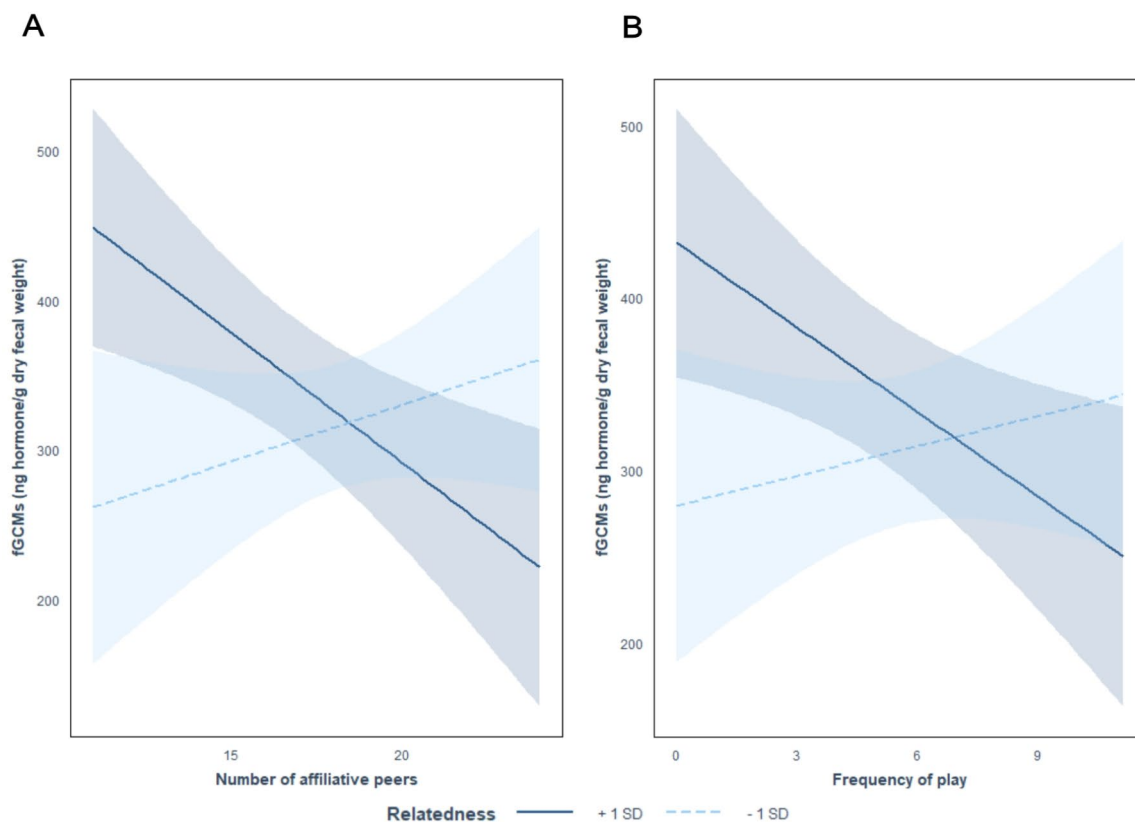
Aggression received also failed to explain the relationships between fGCM concentrations and social bond variables. Individual rates of aggression received from all group members were unrelated to fGCM concentrations ( $\beta=43.40$ ,  $SE=82.95$ ,  $t(39)=0.52$ ,  $p=0.79$ ), grooming ( $\beta=-4.35$ ,  $SE=4.75$ ,  $Z(39)=-0.91$ ,  $p=0.36$ ), proximity ( $\beta=-1.31$ ,  $SE=12.95$ ,  $t(39)=-0.10$ ,  $p=0.91$ ), play ( $\beta=1.92$ ,  $SE=1.23$ ,  $t(39)=1.55$ ,  $p=0.12$ ), or number of affiliative peers ( $\beta=1.19$ ,  $SE=1.68$ ,  $t(39)=0.70$ ,  $p=0.48$ ) when controlling for rank, sex and maternal relatedness.

#### Initiation and maintenance of social interaction

Lastly, responsibility for the initiation and maintenance of spatial proximity, play, and grooming also failed to explain the relationships between fGCM concentrations and social bond variables. Hinde indices were unrelated to fGCM concentrations ( $\beta=67.06$ ,  $SE=58.21$ ,  $t(39)=1.15$ ,  $p=0.62$ ), grooming ( $\beta=0.16$ ,  $SE=1.73$ ,  $t(39)=0.09$ ,  $p=0.92$ ), proximity ( $\beta=2.30$ ,  $SE=6.26$ ,  $t(39)=0.36$ ,  $p=0.71$ ), play ( $\beta=0.22$ ,  $SE=0.93$ ,  $t(39)=0.23$ ,  $p=0.81$ ), or number of affiliative peers ( $\beta=0.48$ ,  $SE=1.19$ ,  $t(39)=0.40$ ,  $p=0.68$ ) when controlling for rank, sex and maternal relatedness.

## Discussion

Despite a plethora of evidence that strong affiliative and supportive social relationships buffer the effects of stressors in adult humans and nonhuman primates<sup>1–4</sup>, we know little about whether juvenile nonhuman primates similarly benefit from such relationships with their peers. In particular, it has been unclear whether same-sex peer bonds mediate fGCM concentrations for juvenile primates. Our study of this question in free ranging juvenile rhesus macaques suggests that strong relationships with peers are associated with decreases in fGCM concentrations for some juveniles, but not others. Overall, our findings indicate both similarities and differences between adult and juvenile rhesus macaques on Cayo Santiago in the ways in which particular individuals may affect or be affected by same-sex social bonds<sup>64</sup>. We speculate that social bonds that involve high rates of dyadic play and high numbers of affiliative peers may buffer the effects of stressors for juveniles that have closely related peers, whereas some social bonds that involve high rates of grooming and proximity may be associated with increased metabolic demand. Below we discuss our findings and their implications.



**Fig. 3.** Predicted fGCM values for the interaction between (a) the number of affiliative peers and maternal relatedness and (b) frequency of play bouts and maternal relatedness. Solid line represents juveniles with the most related same-sex peers (> 1 SD above the mean,  $n = 10$ ) and dotted line represents juveniles with no same-sex peers (> 1 SD below the mean,  $n = 19$ ). Shaded bands indicate 95% CIs. While categories for maternal relatedness are shown, all statistical models used a continuous specification for relatedness.

### Metabolic demand—grooming and proximity

We found evidence that grooming for both sexes and proximity for females were positively related to fGCM concentrations. These findings are consistent with those that found higher GC levels in captive juvenile chimpanzees that spent more time affiliating with peers<sup>25</sup> and in wild juvenile blue monkeys that groomed more<sup>26</sup>. In addition, with important caveats, these findings are in line with studies of captive infant rhesus macaques and human children that show increased GC secretion linked to peer interactions. In rhesus macaques, nursery reared infant macaques have displayed increases in acute salivary cortisol concentrations following peer socialization arranged in the laboratory<sup>65</sup>. Furthermore, children in daycare have been shown to have elevated concentrations of cortisol when compared to being at home<sup>66</sup>. However, unlike our study, these studies of children also involved separation from parents and other family members. Hence, they cannot distinguish between responses to peers vs. parental separation. Nevertheless, social inexperience or incompetence has been hypothesized as one of the contributing factors for elevated cortisol in young children that spend a considerable amount of time with peers<sup>67</sup>. Unfortunately, no truly comparable studies are available that examined GC responses to peers per se in children, or the potential influences of numbers or relatedness of juvenile peer relationships in either human or nonhuman primates.

Given the evidence for social buffering in adults, it remains unclear precisely why higher frequencies of grooming and proximity should be associated with increased fGCM concentrations in juveniles. Since grooming represents a major means by which individuals establish and maintain affiliative and supportive relationships, individuals may compete to groom specific group members that rank higher than themselves or that have access to limited resources<sup>68</sup>. Given their social inexperience and the risks inherent in competition, (including attacks by older, larger kin or prospective peers), establishing or maintaining strong relationships with peers through grooming relationships might represent a more intense social stressor for juveniles than for adults. This might be

Relationship between mean fGCM concentrations and alternative explanations				
Model predictors	Estimate	SE	t	p
Fixed effects				
(Intercept)	283.63	72.81	3.89	<0.001
Physical activity	0.06	0.25	0.80	0.84
(Intercept)	398.07	48.46	8.21	<0.001
Aggressive behavior	-92.91	42.17	-2.20	0.17
(Intercept)	283.78	38.87	7.30	<0.001
Aggression received	43.40	82.95	0.52	0.79
(Intercept)	329.42	23.17	14.21	<0.001
Hinde index	67.06	58.21	1.15	0.62

**Table 2.** Results of LMMs for the relationship between fGCM concentrations and alternative explanations. P values are adjusted with Benjamini-Hochberg false discovery rate. Only shown is the predictor of interest (alternative explanations); also see the full table in SI Appendix table S5 for other fixed predictors (control variables). Significant values are in bold.

especially the case in species such as rhesus macaques in which intense aggression is frequently directed down the hierarchy<sup>69</sup>. Since grooming involves a high level of physical intimacy and is usually a stationary activity, it doesn't allow an individual to easily extricate itself should conflict arise. Moreover, juveniles may still be learning the fine points involved in the mechanics or individual groomees' tolerance of grooming. As a result, they may anticipate or experience more risk of conflict during grooming (and perhaps during other frequent types of interaction with peers) than do adults, resulting in elevated baseline fGCM concentrations.

Another possibility is that higher fGCM concentrations that were found for females with higher rates of proximity may represent inherent or more general differences in physiological development between the sexes. An individual's sex may influence their GC metabolism and subsequently excretion<sup>42</sup>; such sexually dimorphic effects on hormone metabolism have been found in mice (reviewed in<sup>70</sup>). Although sexual dimorphism in hormone metabolism is not fully understood among primate species, sex differences in GC levels have been observed. Hair cortisol which measures 3 to 4 months of HPA activity, were found to be consistently lower in vervet monkey males than females beginning at 3 years of age<sup>71</sup>. In humans, sex differences have also been reported; women have been found to have generally higher cortisol levels than men of all ages<sup>72</sup>. For rhesus macaque juveniles, these differences between the sexes may also reflect the long-term social structure of the group. Female juveniles stay in their natal group for life; thus establishing new relationships and expanding social networks may play a larger role during development for females than males.

It has been argued that elevated GC secretion in children in response to affiliative and supportive relationships with peers is adaptive and may play an integral role in child development. Specifically, an increased response may help children learn how to appropriately respond to stressors<sup>73</sup>. Moreover, such responses may affect social outcomes in other gregarious species (reviewed in<sup>7,14</sup>); for example, in zebra finches (*Taeniopygia guttata*), GC levels predicted a juveniles' future social network position<sup>74</sup>. Finches artificially induced with corticosterone occupied more central positions in their social networks and were more social than their untreated siblings. Further research is needed to assess whether relatively higher mean fGCM concentrations in nonhuman juvenile primates represent an adaptive response during development. Most studies of GCs in juvenile primates have examined responses to acute stressors rather than baseline fGCM concentrations. Hence, a comparative study could shed light on whether baseline fGCM concentrations are influenced by long term social pressures and motivations, as we propose here, rather than shorter term challenges.

### Social buffering—number of affiliative peers and play

In contrast to our findings that strong social bonds are related to increased metabolic demand for some juveniles, we found that juveniles with the most closely related peers, those with a higher number of affiliative peers or higher frequencies of play bouts had lower mean fGCMs. These findings were similar for males and females despite females generally displaying higher fGCM concentrations than males. Rhesus macaques develop strong affiliative and supportive bonds with close maternal kin during their first few months of life<sup>33</sup> that often endure through the juvenile phase and beyond. Hence, by the time macaques become juveniles, these relationships tend to be not only strong, but also stable and predictable in nature. At the same time, the juvenile phase of life is marked by development and refinement of social skills and by the expansion of social networks<sup>9</sup>. Given that the pursuit of new relationships is likely inherently risky<sup>9</sup>, it may be that juveniles with several closely related peers benefit from these longstanding relationships in ways that are reflected in GC levels. Not only are their longstanding relationships with related peers likely to be strong and more relaxed than those of others, but these longstanding bonds may also buffer the potential negative effects of stressors involved in the establishment and navigation of new peer relationships. Early and enduring social bonds are valuable for both philopatric females, that often maintain affiliative and supportive social bonds with other females into adulthood<sup>22</sup> and for dispersing males<sup>75,76</sup>. When male rhesus macaques migrate for the first time to new groups, they spend significantly more time with familiar, maternally related males than with unrelated males<sup>76</sup>. Rhesus macaque males on Cayo Santiago also frequently transfer into the same group as their older, maternally related brothers and spend more

time in close proximity to them than to unrelated males. This suggests that such enduring social bonds not only persist in new groups, but also facilitate the integration of younger brothers into their new group<sup>75,76</sup>.

Although no previous study of free-ranging juveniles has examined evidence for social buffering for individuals with varying numbers of related vs. unrelated peers per se, in general strong dyadic bonds have been shown to provide benefits among captive juveniles exposed to acute stressors<sup>23,24</sup>. Play behavior in particular has a role in regulating stress by mitigating tension and preventing conflict (*Callithrix jacchus*;<sup>77,78</sup>). Further, social play has been shown to affect the HPA responses of marmosets subjected to numerous social separation stressors during development. Juveniles engaged in higher social play showed decreased basal concentrations of cortisol and a greater reduction in cortisol reactivity as they aged<sup>79</sup>. However, like grooming, vigorous contact play among peers also represents an intimate, competitive activity<sup>80</sup> that carries a risk of lapsing into conflict. Play among related versus unrelated peers may help to mitigate this risk<sup>81</sup>.

### Alternative explanations

We found no evidence to support our alternative hypotheses for variation in fGCM concentrations. Males with higher frequencies of play bouts were more physically active than males with fewer play bouts, and juveniles with higher numbers of affiliative peers were more active; however, fGCM concentrations were unrelated to physical activity, suggesting that physical arousal cannot account for the relationships we found between fGCM and the social bond variables. Similarly, there was no evidence that these relationships can be explained by aggressive arousal. Frequencies of aggression directed to other group members were unrelated to the social bond variables. Nor were frequencies of aggression received related to fGCM concentrations or social bond variables, suggesting that juveniles with strong social bonds do not experience less aggression than others. Moreover, responsibility for initiating and maintaining social interaction with peers was unrelated to fGCM concentrations or social bonds variables, suggesting that juveniles with high fGCM concentrations do not seek more social contact with peers than others. Finally, given the stability of nutritional and environmental conditions on Cayo Santiago<sup>41</sup>, it is unlikely that these relationships could be explained by variation in these factors.

Given that our study group was larger than typical wild rhesus macaque groups, studies in wild groups that are likely to contain fewer related peers would also be informative. Furthermore, although our study focused on the role of peer interactions, the potential impact of social support from other group members on HPA axis activity should also be addressed in future studies. Finally, some studies have found both positive and negative relationships between parasite loads and social bonds among mammals, including nonhuman primates<sup>82,83</sup>. Although there is suggestive evidence that parasites have only minor effects on social behavior and health on Cayo Santiago<sup>84,85</sup> relationships between social bond variables, GC levels and parasites should be examined.

In summary, this study represents the first attempt to examine the relationships between adrenocortical activity as measured by mean fGCM concentrations and social bonds among free ranging juvenile nonhuman primates. Whereas many studies have shown social bond numbers and strength to moderate GC levels in adult nonhuman primates, our findings were consistent with this notion only for a minority of juveniles that had the most closely related peers. For other juveniles, mean fGCM concentrations were relatively high in those that groomed more frequently. For these juveniles, strong peer social bonds may involve an increase in metabolic demand, given that juveniles are generally less experienced socially and at a life stage during which they must hone social skills, establish relationships and expand social networks—tasks that are likely to be crucial for later reproductive success<sup>6</sup>. Further, our results highlight how little we know about the development of social buffering systems that we see in adults and suggest that ontogeny may not follow a straightforward path from immaturity to adulthood. Indeed, development of social buffering systems might begin with longstanding kin relationships. More generally, this study complements efforts to bring the study of the development of social buffering systems in humans into an evolutionary perspective and to provide information necessary to discern causal relationships and more precise parallels and differences between human and nonhuman primate social buffering and stress response systems.

### Data availability

The datasets generated during the current study are available in the Mendeley Data repository, [<https://data.mendeley.com/datasets/8p5vggxwjp/2>].

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## Author contributions

K.C.B. collected data and managed the project. M.H. and J.P.H. oversaw hormone analysis. K.C.B. and C.M.B. analyzed, interpreted data and drafted the manuscript. All authors approved of the final version of this manuscript.

## Declarations

## Competing interests

The authors declare no competing interests.

## Additional information

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