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Intermittent pair-housing, pair relationship qualities, and HPA activity in adult female rhesus macaques

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Abstract

Laboratory rhesus macaques are often housed in pairs and may be temporarily or permanently separated for research, health, or management reasons. While both long-term social separations and introductions can stimulate a stress response that impacts inflammation and immune function, the effects of short-term overnight separations and whether qualities of the pair relationship mediate these effects are unknown. In this study, we investigated the effects of overnight separations on the urinary cortisol concentration of 20 differentially paired adult female rhesus macaques (*Macaca mulatta*) at the California National Primate Research Center. These females were initially kept in either continuous (no overnight separation) or intermittent (with overnight separation) pair-housing and then switched to the alternate pair-housing condition partway through the study. Each study subject was observed for 5 weeks, during which we collected measures of affiliative, aggressive, anxious, abnormal, and activity-state behaviors in both pair-housing conditions. Additionally, up to three urine samples were collected from each subject per week and assayed for urinary free cortisol and creatinine. Lastly, the behavioral observer scored each pair on four relationship quality attributes ("Anxious," "Tense," "Well-meshed," and "Friendly") using a seven-point scale. Data were analyzed using a generalized linear model with gamma distribution and an information theoretic approach to determine the best model set. An interaction between the intermittent pairing condition and tense pair adjective rating was in the top three models of the best model set. Dominance and rates of affiliation were also important for explaining urinary cortisol variation. Our results suggest that to prevent significant changes in HPA-axis activation in rhesus macaque females, which could have unintended effects on research outcomes, pairs with "Tense" relationships and overnight separations preventing tactile contact should be avoided.

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SUPPORTING INFORMATION
Additional supporting information may be found online in the Supporting Information section at the end of the article.

Keywords

cortisol; overnight separation; pair-housing; peer interaction

1 | INTRODUCTION

It is well established that social environments, compared to solitary housing, significantly improve captive non-human primate (NHP) welfare and health (Olsson & Westlund, 2007). For example, single-housing has been associated with physiological changes, such as higher blood pressure (Coelho, Carey, & Shade, 1991) and immunosuppression (Lilly, Mehlman, & Higley, 1999), that increase the risk of acquiring pathological health conditions (e.g., cardiovascular disease or infection). Furthermore, studies in laboratory rodents have demonstrated that environments lacking complexity, such as limited cage features and insufficient outlets for expressing species adaptations, can have deleterious effects on biomedical research results (e.g., Richter et al., 2011). Consequently, regulatory pressure has increased on research facilities to socially house NHPs (Hannibal, Bliss-Moreau, Vandeleest, McCowan, & Capitanio, 2017). Although social housing is the expected and enforced norm, laboratory NHPs may experience extended periods of social separation due to colony or study protocols. For example, pair-mates may be separated to prevent a social partner from picking at and removing surgical sutures, confirm diarrhea, or menses after overnight separation, or collect overnight urine or fecal samples. The effects of these separations on the welfare, physiology, and health of laboratory NHPs are not well understood. In this paper, we investigate the effects of daily, overnight separations of paired adult female rhesus macaques (*Macaca mulatta*) on urinary cortisol, a hormonal measure that is sensitive to environmental changes and reflects physiological states that may impact research outcomes.

Among all research facilities in the United States, laboratory NHPs are primarily housed in social groups (61.51%), less often in pairs (22.84%), or singly-housed (15.65%) (Bennett, 2016). Pair-housing, the cohousing of two individuals by connected adjacent cages, has been developed and refined to maximize social contact for laboratory NHPs in a manner compatible with many research objectives (Baker, Crockett et al., 2012). Single-housing facilitates specific research objectives, but maintains individuals in separate cages. Although this allows auditory, visual, and olfactory contact with conspecifics, tactile contact is restricted to varying degrees depending on whether the separating door is solid metal, bars, grate, or mesh (Baker, Bloomsmith et al., 2014; Bennett, 2016). Single-housing, however, is prohibited by regulations, unless justified by clinical or behavioral findings that require pair separation or research needs that have been reviewed and approved by the institutional oversight office (United States Department of Agriculture, 2013). Modified forms of pair-housing are often used to accommodate research or management needs. Intermittent pair-housing involves temporary daily or weekly separations that last 12 or more hours, including overnight (Baker, 2016; Capitanio, Blozis, Snarr, Steward, & McCowan, 2017). In contrast, continuous pair-housing allows complete visual and physical access to a pair-mate, with infrequent and brief separations.

Several studies have demonstrated welfare improvements for NHPs that are pair-housed as compared to those that are singly-housed. For example, pair-housing has been associated with improved behavioral welfare indices, including reduced levels of abnormal and anxiety-related behaviors (e.g., Baker, Bloomsmith et al., 2012; Gottlieb, Maier, & Coleman, 2015), enhanced repertoires of species-specific behaviors (e.g., Baker, Bloomsmith et al., 2014), and decreased self-injurious behavior (SIB) (e.g., Rommeck, Anderson, Heagerty, Cameron, & McCowan, 2009; Weed et al., 2003). Another study found that pair-housed NHPs had better immune function than single-housed NHPs (Schapiro, Nehete, Perlman, & Sastry, 2000). While the benefits of pair-housing are now well established, pairing laboratory macaques with compatible companions is challenging and requires knowledge of and experience with species-specific social behavior (Truelove, Martin, Perlman, Wood, & Bloomsmith, 2017). Thus, research on laboratory macaque pair-housing has shifted focus to refining pairing practices to improve partner compatibility, welfare, and pairing success (e.g., Capitanio et al., 2017; Pomerantz & Baker, 2017; Truelove et al., 2017). Relatively little progress has been made, however, to improve our understanding of how frequent changes to pair-housing affect NHP physiology, despite the implications for biomedical research (reviewed in Hannibal et al., 2017).

Captive NHPs tend to have better welfare measures when they are able to express key species-specific behaviors (Lutz & Novak, 2005). Although most primate species spend a significant amount of their activity budget engaged in social behavior (Dunbar, 1991), captive pair-housed NHPs spend even more time doing so (Crockett, Bowers, Bowden, & Sackett, 1994), likely due to a limited repertoire of other activities. For both wild and captive NHPs, the longest bouts of affiliation occur when they are huddled together overnight (Anderson, 1998; Eaton, Kelley, Axthelm, Iliff-Sizemore, & Shiigi, 1994). Furthermore, NHPs actively prefer the proximity of a social partner even when there are costs associated with that choice. For example, adult rhesus macaques chose to remain in the same cage as their social companions despite tradeoffs in available space (Basile, Hampton, Chaudry, & Murray, 2007). Also, captive tufted capuchin monkeys (*Cebus apella*) often chose their companions over food, even several hours after food deprivation (Dettmer & Fragaszy, 2000). Lastly, access to social partners buffers physiological stress during stressful procedures in captivity (Hennessy, Kaiser, & Sachser, 2009; Kikusui, Winslow, & Mori, 2006; Truelove et al., 2017), such as witnessing the anesthesia of another animal in the room (Gilbert & Baker, 2011).

In contrast, separations from conspecifics can negatively impact NHP behavior and physiology. Physiological disruptions associated with permanent social group removal are “substantial” and take about 3-months to return to baseline, thus a 3-month conditioning period is recommended when previously outdoor housed NHPs are moved into indoor research settings (reviewed in Capitanio, Kyes, & Fairbanks, 2006). Temporary separations from social contact for greater than 10 hr to several days, are also known to increase negative indices of welfare in captive NHPs. For example, adolescent rhesus macaques displayed higher levels of abnormal and depressive behaviors in response to a 4-day social separation, increasing further after repeated separations (Mineka, Suomi, & DeLizio, 1981). Also, an 11-hr period of social isolation in Wied's black tufted-ear marmoset monkeys

(*Callithrix kuhli*) was associated with increased urinary cortisol concentration (Smith & French, 1997).

While the implementation of intermittent pair-housing varies among facilities, all cases involve at least some overnight separation, as previously mentioned (Baker, 2016; Capitanio et al., 2017; Roberts & Platt, 2005; Rommeck, Capitanio, Strand, & McCowan, 2011; Tardif, Coleman, Hobbs, & Lutz, 2013). Continuously paired animals still experience short daytime separations for sample collection, health checks, and husbandry procedures, but spend more than half of every day together, with the exception of serious, albeit rare, health issues. At the California National Primate Research Center (CNPRC), intermittently housed monkeys are separated from about 14:00 (just prior to the afternoon feeding) until 08:00 (after the morning feeding) the following day, providing a maximum of 6 hr of daily socialization and physical contact. These separations remove the opportunity for these individuals to receive the benefits of overnight social contact (Eaton et al., 1994; Kikusui et al., 2006). Therefore, the welfare of intermittently pair-housed NHPs needs to be characterized by incorporating indices of welfare that can capture the lasting effects of overnight separations.

Physiological indices of welfare, specifically the measurement of hypothalamic-pituitary-adrenal (HPA) axis activity, can provide insight into the impacts of overnight social separation. The main output of the HPA axis is cortisol, a glucocorticoid that can influence a variety of physiological systems, especially those involved in stress response and immune functioning (Sapolsky, Romero, & Munck, 2000). Depending on the biological source, elevated HPA axis activity can be detected several minutes (blood), hours (urine), days (feces), or months (hair) after a stressor has occurred (Novak, Hamel, Kelly, Dettmer, & Meyer, 2013). Activity of the HPA-axis is known to be highly sensitive to environmental influences (e.g., temperature, stress) (Herman et al., 2003; Vandeleest, Blozis, Mendoza, & Capitanio, 2013) including the social environment (Mendoza, Capitanio, & Mason, 2001). Social isolation and unstable social relationships can lead to elevated cortisol levels and, when chronic, can eventually lead to altered regulation of the HPA axis (Capitanio, Mendoza, Lerche, & Mason, 1998; Dettmer, Novak, Meyer, & Suomi, 2014). For example, wild male olive baboons (*Papio anubis)* that were about to lose rank had higher cortisol levels than similarly ranked males that were about to gain rank (Sapolsky, 1992). On the other hand, higher rates of positive social interactions, like grooming, have been associated with lower fecal cortisol concentrations in Barbary macaques (*Macaca slyvanus*) (Shutt, MacLarnon, Heistermann, & Semple, 2007) and with lower hair cortisol concentrations in rhesus macaques (Wooddell et al., 2017). Relative cortisol levels, thus, are only useful when informed by the context (climate, activity, rank relationships, and other social and environmental variables) and perturbations associated with changes in levels.

Research on the impact of social housing (pair- vs. single-housing) on cortisol levels has yielded mixed results. Although some previous studies found no differences in serum cortisol concentrations between single- and pair-housed macaques (e.g., Baker, Bloomsmith et al., 2012; Gust, Gordon, Brodie, & McClure, 1994; Schapiro, Bloomsmith, Kessel, & Shively, 1993), others have found higher cortisol levels in singly-housed animals (Doyle, Baker, & Cox, 2008). These studies, however, vary in a couple of potentially important ways. First, they differ in the sampling matrix used to measure cortisol levels. All of the studies failing to find a relationship between cortisol and pairing status measured serum cortisol levels, whereas the Doyle et al. (2008) study measured fecal cortisol levels. These sampling matrices reflect HPA-axis activation on a scale of minutes (serum) to days (feces) which may have impacted the measured relationships. Second, these studies varied in whether, or the degree to which, they pre-selected potential pair-mates based on criteria that tend to maximize compatibility (e.g., body weight disparity). Since positive and negative social interactions can alter HPA axis activation, the qualities of the pair relationship may be critical to the ability to detect differences in cortisol levels. Overall, the consequences of manipulating a NHPs' social environment (e.g., switching between pair-housing conditions) on their behavior and physiological functioning remain largely unknown (Hamel et al., 2017; reviewed in Hannibal et al., 2017). Pair-mate compatibility may alter the magnitude of the stress response to pair separations and reunions. Therefore, investigating the pair relationship could uncover behavioral compatibility metrics that are likely to facilitate less stressful separations and reunions. It is unlikely that there is a single metric of pair compatibility, but converging evidence from more than one behavioral or physiological metric would allow managers to use the metrics they have access to and that have predictive power.

In this study, we investigate whether changes in intermittent versus continuous pair-housing condition of adult female rhesus macaques impacts the HPA axis as measured by urinary cortisol concentrations. We further explore the impact of pair relationship quality and whether it modulates the effect of housing condition, while controlling for other aspects of the social environment, such as dominance status and affiliation rates. For NHPs adapted for a rich social life, long periods of social isolation have the potential to produce physiological variability with implications for the external validity of biomedical research conducted with such animals (Hannibal et al., 2017). If overnight separation is associated with substantial changes in HPA axis activity, then modifications of this practice should be considered for the benefit of both animal welfare and research.

2 | METHODS

This research was conducted from March to May 2015 at the California National Primate Research Center (CNPRC) in Davis, California. Animal care and research protocols for this study were approved by the Institutional Animal Care and Use Committee at the University of California Davis. This research was conducted in accordance with United States federal regulations and adhered to the American Society of Primatologist Principals for the Ethical Treatment of Animals.

2.1 | Subjects

In order to limit physiological variability of the study sample as much as possible, subject selection criteria included: (i) only females due to sex differences in physiology and the fact that most adults in the indoor colony are female; (ii) a minimum three months indoors and in their pair-housing condition, without repeated incidents of serious physical aggression and wounding; (iii) no history of conception during the past breeding season; (iv) reared in an outdoor social group; and (v) between 4 to 11 years old, (criteria based on findings and recommendations by Capitanio et al., 2006; Cavigelli & Caruso, 2015; Reeder & Kramer, 2005). Subjects were enrolled as pairs as much as possible to avoid pair separations for other colony or project needs not related to this study. Random selection and assignment of animals was not possible because the purpose of the study was to understand impact of indoor pairing practices on physiology and the pool of animals that fit our selection criteria was very small.

The study began with 24 adult female rhesus macaques. Due to our subject criteria, two females were enrolled in the study while their pair-mates were not. To maintain consistency in behavioral data collection and conduct pair-adjective ratings, these data were collected on both pair-mates for all subjects, but data from the two non-study pair-mates of subjects was not included in individual level analyses. Two study subjects, who were paired together, were dropped during the study due to intra-pair conflict and another two were dropped from analyses due to poor or insufficient urinary samples, leaving 20 subjects. Subjects were ages 4.9–10.9 years (mean = 6.7, SD = 1.8), confirmed non-pregnant by ultrasound, and were not observed to have a consistent pattern of menstrual synchronization within pairing groups (i.e., females cycled at different times throughout the study). All subjects were born and raised in outdoor large (0.2-hectare outdoor enclosures containing up to 180 NHPs) or small (43.7 m^2 outdoor enclosures containing up to 30 NHPs) social groups comprised of all age and sex classes at the CNPRC for at least the first 2.5 years of life. Subjects selected for the study had been relocated for management reasons to indoor housing at least four months prior to the study (mean = 20.7, SD = 20.0). All subjects had been housed successfully (without persistent agonism or wounding) with another female in their baseline condition (intermittent or continuous) for at least three months prior to the study (mean = 11.0, SD = 6.4). The baseline pairing condition was intermittent for 9 subjects and continuous for 11 subjects.

2.2 | Housing and pairing

Animal housing consisted of pairs of stainless steel cages (floor space 0.4 m^2, height 0.8 m). The cages of paired animals were joined by an opening (approximately 30 by 30 cm) with a sliding solid stainless-steel partition that prevented physical contact. Per management practice, intermittently paired animals were separated by the partition prior to afternoon feeding (approximately 14:00) until after morning feeding (approximately 08:00) the following day. Therefore, intermittent pairs had about 6 hr of co-housing each day. Conversely, continuously paired animals were co-housed for at least 18 hr daily and were always together overnight. All socially housed animals in the colony, regardless of housing condition, experience occasional separations for minutes, hours, or even days for sample collections, veterinary exams or treatments, and husbandry procedures. However, unless intermittently-housed, the majority of their 24-hr days are spent in social contact. For the purposes of urine sample collection and feeding regime consistency across the experimental groups, continuous pairs were separated during each feeding time (two bouts) for about an hour in the morning, and 1–3 hr in the afternoon (cumulative maximum of 4 hr per day). Afternoon feeding time coincided with urine sample collection for all pairs, to ensure correct identification and prevent cross-contamination of samples. Continuous pairs were re-paired immediately after an adequate sample was obtained or as soon as the 4-hr mark was reached.

Intermittent pairs remained separated overnight, consistent with the colony management protocol for this housing category. The short separations of continuously housed subjects for sample collection is not of long enough duration to be considered intermittent because they were only long enough to obtain samples and they were not separated overnight. Subjects were fed a standard monkey chow diet and a forage mixture of rice, split peas, and oats twice daily by animal care staff, with fresh water available ad libitum. Regular facility enrichment (e.g., mirror, chew toy, forage board, metal perch, puzzle feeders) was provided to each subject according to CNPRC standard operating procedures (SOPs) throughout the study.

2.3 | Experimental design

To compare the behavior and urinary cortisol concentration of continuously (C) versus intermittently (I) pair-housed female rhesus macaques, subjects were assigned to one of two experimental groups (i.e., CI or IC) based on their pairing condition at the beginning of the study (i.e., initial pairing condition; variable definitions are listed in Table 1). Pairs were in their initial pairing condition for two weeks (i.e., initial project phase), and then switched to their experimental condition for three weeks (i.e., experimental project phase) (Figure 1). Because it was not possible to complete data and sample collection on all subjects in one 5-week study period, subjects were studied in two cohorts, balanced by experimental group so that there were about equal numbers of CI and IC subjects in each cohort. The first cohort was studied March 23 to April 24, 2015 and the second cohort was studied April 27 to May 29, 2015. All data collection occurred on weekdays (initial project phase: 9–10 days; experimental project phase: 14–15 days).

2.4 | Behavioral data collection

Two 8-min focal observations were conducted on each pair per observation day, between 11:15 and 13:45 hr in a randomized order. Affiliative, agonistic, status, activity, self-directed, and abnormal behaviors (see Table 1 for variables comprised of these behaviors) were recorded using the HanDBase application (DDH Software, Wellington, FL) on an Android tablet. Observations were conducted solely by co-author L. C. Cassidy, who was previously trained as a CNPRC behavioral management staff member and reliable on all observation ethograms. For each pair, 18–20 observations were conducted during the initial pairing condition and 26–28 observations were conducted during the experimental pairing condition. Behaviors were recorded using one-zero sampling with 20-s sample intervals, except for self-directed behaviors which were recorded using all occurrences event sampling. For each observation day, proportions and frequencies were calculated for behaviors recorded with one-zero sampling and event sampling, respectively.

2.5 | Pair relationship adjective ratings

Four pair rating adjectives, “Anxious,” “Tense,” “Well-meshed,” and “Friendly” (see Table 1, Pair Rating variables), defined by co-author K. Chun, were used to evaluate the relationship of each pair on a 7-point scale. Adjective ratings allow observers to integrate multi-modal information about animals across time and experiences, and can be scientifically tested for reliability and validity (Meagher, 2009). Dyad ratings have been used to assess social interactions between amygdala lesioned versus control animals (Emery et al., 2001). Like personality ratings, these adjectives likely remain relatively constant across different contexts (Capitanio, 1999). It was not our aim to use adjective ratings to assess possible changes to pair relationships between the initial and experimental project phases. Rather, we incorporated them to have an overall assessment of qualities of the pair relationship, irrespective of Project Phase, to assess whether this had an impact on potential changes in physiological responses to the experiment. Pair adjective ratings for the current study were assessed based on the behavioral observer's (co-author L.C. Cassidy) direct experience with the subjects over the study period. Ratings were conducted 1–2 days after the data collection period for each cohort concluded, and again 9 days later to assess intra-observer reliability using Krippendorf's α for interval metrics (Anxious α = 0.92; Tense α = 0.88; Well-meshed α = 0.93; Friendly α = 0.84) (Hayes & Krippendorff, 2007). The mean of the two observations for each reliable pair adjective rating was used in analyses.

2.6 | Urine sample collection

Throughout the 5-week study period, urine samples were collected from each subject between the hours of 14:00 and 17:00 each weekday until up to three urine samples over 3 ml in volume (considered an adequate sample) were collected for that week. The limited and consistent collection period allowed minimal separation of the pairs and reduced variation in cortisol levels due to diurnal variation in primates (Novak et al., 2013). Urine was collected from clean stainless steel cage pans placed underneath each subject's cage. The pans were periodically checked for urine and a maximum volume of 45 ml was transferred into a 50 ml polypropylene vial. 351 samples were collected from 22 animals. On the day of collection, urine samples were stored at room temperature until 18:00. Lastly, the samples were centrifuged at 2500 RPM for 5 min to remove impurities (e.g., food particles), and the supernatant transferred to 5 ml polypropylene vials and stored at −80 °C until assay.

2.7 | Cortisol assays

Urinary free cortisol (Co) was measured using a quantitative competitive immunoassay and direct chemiluminescent technology developed and conducted by the CNPRC Primate Assay Laboratory Core. A total of 313 urine samples were assayed in duplicate for this study. Analytical sensitivity of the cortisol immunoassays was 2 ng Co/ml. Inter-assay coefficient of variation (CV) was 3.1% and intra-assay CV was 1.6%. Creatinine (Cr) was measured by a colorimetric assay to control for variations in subject body weight, urine output, and water content in each sample (Novak et al., 2013). Analytical sensitivity was 0.05 mg Cr/ml, inter-assay CV was 1.2%, and intra-assay CV was 0.5% for the creatinine assays. Urine sample concentration was normalized by dividing the cortisol concentration by the corresponding creatinine concentration. Urine samples with a creatinine concentration of 0.20 mg Cr/ml and below were excluded (n = 55) from our analysis as they could have resulted in falsely elevated normalized cortisol concentrations. Of 258 urine samples that met our analysis criteria, urinary cortisol per creatinine ranged from 32.32 ng Co/mg Cr to 1617.73 ng Co/mg Cr (mean = 362.29, SD = 283.34 ng Co/mg Cr).

2.8 | Data analysis

Data were analyzed in Stata 14.1 using a generalized linear mixed model (GLMM) for a gamma distribution (meglm command) (Hardin & Hilbe, 2007). Both subject and pair identity were considered as potential random effects. An information theoretic (IT) approach was used to evaluate models based on goodness-of-fit, sample-size-corrected Akaike Information Criterion (AICc) scores, and differences in AICc scores (ΔAICc) following methods described by Burnham and Anderson (2002) and Burnham, Anderson, and Huyvaert (2011). We included variables in the models that the literature indicates may have an impact on HPA axis activity (e.g., menses and activity) or pair compatibility (e.g., affiliation and agonistic behavior), as well as the specific variables (Current Condition and pair adjective ratings) of interest to our research questions (see Table 1 for a list of all variables). The random effects were evaluated before considering models with fixed effects and only subject ID alone was retained as a random effect. Collinear variables were not used in the same model and among collinear variables, the variable with the lowest AICc score was retained for further model comparison.

Supplementary Table S1 contains a list of all models tested and the reasons these models were rejected from consideration. We considered all models that had both a model chi-square indicating a minimally good model and an AICc score less than the random effects only model, which indicates whether a model is better than a model with no predictors. Models violating the principal of parsimony were excluded (Burnham & Anderson, 2002). Model likelihoods, Akaike weights, and evidence ratios, which measure the strength of the evidence for these models, were calculated for a candidate set of models with a ΔAICc ≤7.0 (Burnham & Anderson, 2002; Burnham et al., 2011; Grueber, Nakagawa, Laws, & Jamieson, 2011; Symonds & Moussalli, 2011). From this candidate model set, a best model set was then selected based on evidence ratios ≤ 10 and weights were then renormalized (Burnham & Anderson, 2001, 2002). The Akaike weights for the best model set were used to calculate variate weights by summing the model weights for each variate across all models in which it was included (Burnham & Anderson, 2002). Variate weights measure the relative importance of each variate for understanding the outcome, with 1 indicating it has the highest possible certainty of being important. Marginal effects (margins command) and plots (plot command) were produced from the top model for predictors of interest (Hardin & Hilbe, 2007).

3 | RESULTS

Of the models predicting urinary cortisol levels in our study animals, nine had at least some support with ΔAICc ≤ 7 (Burnham & Anderson, 2002) and were further examined as the candidate set of models (see Supplementary Table S2). From this set of candidate models, a set of best models with evidence ratios <10 were selected and the model weights renormalized (see Table 2) (Burnham & Anderson, 2001). The Akaike weight of the best model (M1) was 0.481; therefore, there was not strong enough evidence to rely on this as the single best model and information from other models in the best model set should also be considered. All models in the best model set contained main effects for Dominant (descriptives of categories: dominant = 50.0%, subordinate = 50.0%), Affiliation (descriptives of percent of observation period: mean = 36.7, min = 0.0, max = 100.0, sd = 30.5), and Current Condition (descriptives of categories: continuous = 47.9%, intermittent = 52.1%). In addition, the three models with the highest weight (M1, M2, and M3, $w = 0.929$) also contained a main effect for Tense (descriptives of score: mean = 2.7, min = 1.5, max = 5, sd = 1.4) and an interaction between Tense and Current Condition. The cumulative weight of Models 1 and 2 was 0.81 and the only differences between models 1 and 2 were the main effects of Experimental Group (seen in model 1, but not 2) (descriptives of categories: CI = 55.0%, IC = 45.0%) and Project Phase (seen in model 2, but not 1) (descriptives of categories: initial = 42.5%, 57.5%). Model 4 included Project Phase, which also occurred in model 2, as well as Total Pairing Time (descriptives of months: mean = 11.5, min = 3.6, max = 24.8, sd = 6.4) and Inactivity (descriptives of percent of observation period: mean = 38.6, min = 0.0, max = 100.0, sd = 24.3), which occurred in no other models in the best model set.

The predictors in the best models are listed by order of importance based on their corresponding variate weights (the sum of the model weights for the models containing variate j and denoted as $w_+(j)$) in Table 3. All models included the main effects of Dominant, Affiliation, and Current Condition and thus all had $w_+(j) = 1$. Tense and the interaction of Current Condition and Tense occurred in the top three models and had $w_+(j) = 0.93$. Experimental Group ($w_+(j) = 0.48$) only occurred in Model 1, Project Phase ($w_+(j) = 0.40$) only occurred in Models 2 and 4, and both Total Pairing Time and Inactivity (both had $w_+(j)$ = 0.07) only occurred in Model 4.

The results of the best model (Model 1) are presented in Table 4. Dominant animals had urinary cortisol levels that were nearly half of those in subordinate animals ($\beta = -0.497$) (see Figure 2a). An increase in affiliation by ten percentage points was associated with 0.029 times lower (about three percent lower) cortisol levels ($\beta = -0.003$) (See Figure 2b). Although significant, the main effect of Current Condition was relatively small with an increase in urinary cortisol of about 0.12 times when Tense was at the mean value (2.73) for the sample ($\beta = -0.604$, exponentiated $\beta = 0.547$). The main effect of Tense was not significant. The interaction of Current Condition (intermittent) and Tense was significant, but in the continuous condition for Current Condition, urinary cortisol levels stayed relatively low at all Tense ratings, while in the intermittent condition, urinary cortisol levels increased by 1.23 times as pair Tense rating increased ($\beta = 0.262$) (See Figure 3).

4 | DISCUSSION

Our study aimed to explore the impact of temporary overnight separations due to intermittent pair-housing on adult female rhesus macaques' HPA axis activity, indexed through urinary cortisol concentrations. In addition to stress, other factors such as activity level and ambient temperature can affect cortisol secretion. For this reason, it is not possible to identify a normal cortisol range for a species, population, or even an individual that is indicative of distress, eustress, or lack of stress. Our results showed that overnight separations were associated with higher concentrations of urinary cortisol, but that this association was dependent on key characteristics of the pair relationship and occurred even when accounting for other variables known to influence the production of cortisol. Most interestingly, pairs rated as having more tense relationships had higher urinary cortisol levels, but only when they were intermittently paired. Additionally, dominance status and greater rates of affiliation were associated with lower urinary cortisol.

4.1 | Intermittent pairing, relationship quality, and urinary cortisol

Females that had more tense relationships with their partners had urinary cortisol levels 1.5–3 times higher, depending on the tense rating and variability, when intermittently paired than when continuously paired. A high pair rating for Tense may indicate that the relationship is tenuous and overnight separation may be introducing uncertainty in re-establishing the relationship when reunited. Uncertainty in dominance relationships has been associated with higher levels of pro-inflammatory proteins and greater risk for diarrhea for rhesus macaques living in large outdoor social groups (Vandeleest et al., 2016). This measure of uncertainty may indicate that a poor fit in the social group is associated with poorer health outcomes. Although cortisol is not a direct measure of health (cortisol values can have implications for health, but can also vary for reasons that have nothing to do with health outcomes), it is often used as a biomarker for increased health risk due to its responsiveness to stressors and role in regulating immune function (Sapolsky et al., 2000). Our findings are also consistent with a study in wild hamadryas baboons (*Papio hamadryas ursinus)* where relationship quality (measured as a grooming diversity index) was related to HPA axis activity (Crockford, Wittig, Whitten, Seyfarth, & Cheney, 2008).

Although we did not find a difference in urinary cortisol concentration between intermittent and continuous housing conditions among pairs who did not have a Tense relationship, we caution against interpreting this as evidence that overnight separation does not cause distress or impact research outcomes. There may be differences among less Tense pairs that could not be detected in the sample used in this study. We suspect that a larger sample would find an effect, albeit a smaller one than that seen in Tense pairs.

When making decisions about pairing laboratory NHPs, behavioral, and facility managers often have a limited number of potential partners to select from and attempt pair introductions depending on factors such as indoor population size, study needs, and breeding needs. While some of these potential pairs do not remain paired past the introduction period due to conflict, those that do and become established pairs usually remain paired until there is a management reason to separate them. Therefore, it is not surprising that we found variation in relationship quality in our sample. Since it is likely that other laboratory NHP facilities have pair-housed populations with similar variation in the quality of pair relationships, our results suggest that when pairs show signs of being tolerably, but not ideally, compatible (e.g., absence of physical affiliative social interaction or sitting in proximity to one another), it is best to avoid overnight separations to prevent uncertainty at reintroduction and unusual disruptions in their physiology.

In our study, continuous pair-housing provided near constant social interaction and was associated with reduced HPA activation, regardless of pair quality. However, continuous housing is not compatible with some research objectives. For example, biological sample collection (e.g., feces, urine) often requires that pair-mates are separated for some time (e.g., overnight) to acquire samples from the correct subject. In these situations, providing some contact could limit unintended social consequences or changes to physiology. For example, when overnight separation is necessary, a grate or bar (as opposed to solid) divider that allows some visual and tactile access to pair-mates may be preferable.

To our knowledge, our study is the first to use subjective ratings to assess pair compatibility after pair introduction. Subjective rating assessment is an underutilized tool within the field of captive NHP welfare, despite the potential utility of animal caretaker knowledge. Furthermore, ratings are less time intensive than formal behavioral observations, are non-invasive unlike some physiological measurement techniques, and are scientifically valid when appropriately designed (Meagher, 2009). The interaction between housing condition (during intermittent pairing) and the quality of the pair relationship provides further support that ratings are associated with biological phenomena, in this case, changes in HPA activity. Interestingly, the IC pair we excluded from our analyses due to aggression and subsequent separation during the continuous pairing phase was rated as having a very tense relationship. These females previously knew each other from a large outdoor social group, but familiarity does not always translate to compatibility. Therefore, pair adjective ratings such as high Tense scores may act as useful guidelines for re-evaluating pair compatibility and guiding social management decisions.

4.2 | Dominance rank and urinary cortisol concentration

Our study found that urinary cortisol was lower in dominant females than in subordinate females. Therefore, including dominance status in the model was important for interpreting the association between housing condition and HPA axis activity. Primate studies of cortisol usually find an effect of social rank, but the direction of the effect is not consistent across studies (e.g., Abbott et al., 2003; Muller & Wrangham, 2004; Shively, 1998). However, it is important to note that social status or high cortisol values alone cannot be interpreted as distressing. Generally, if an individual is maintaining a healthy weight and social injuries are rare and minor, there is no reason to interpret their situation as deleterious.

4.3 | Affiliation and urinary cortisol concentration

Greater frequency of affiliative behavior with a pair-mate was also associated with lower urinary cortisol in our study. This is consistent with previous findings that affiliative social partners dampen behavioral and physiological stress responses (Hennessy et al., 2009; Kikusui et al., 2006; Wooddell et al., 2017), but like dominance status, this cannot be used to make direct inferences about stress levels in this study sample. Because affiliation was an important predictor of urinary cortisol levels, including it in our multivariate analysis was necessary to understand any association with housing condition.

Pair compatibility criteria during pair introduction vary by facility (Baker, Coleman, Bloomsmith, McCowan, & Truelove, 2014), but generally the absence of deleterious aggression, wounding, food monopolization, and presence of status signals establishing dominance are prioritized overrates of affiliative behaviors between pair-mates. In NHPs, affiliative behaviors reinforce social bonds and frequent affiliation between individuals indicates the strength of the relationship (Silk, Altmann, & Alberts, 2006). The absence or reduced frequency of affiliation may not cause external injury, but may indicate the pair is not experiencing the full benefits of social housing.

5 | SUMMARY

Overall, our results emphasize that changes to the pair-housing arrangement, in combination with aspects of a pair's social relationship, can modulate urinary cortisol concentration in pair-housed adult female rhesus macaques. Importantly, although intermittent pair-housing provides superior welfare over single-housing, our results indicate that it may be associated with increased HPA-axis activity when the relationship between the two pair-mates is tense. Our findings support the importance of assessing compatibility between pair-mates beyond the current minimum criteria of the absence of serious injury and repeated fighting. We also caution against interpreting the lack of an effect found for Tense pairs in this study as evidence that overnight separations do not have an impact on welfare or research as this may have been detected if a larger sample was possible.

We propose a continuum composed of three different aspects of compatibility. First, and as a bare minimum, the absence of serious aggression or injury demonstrates that pair-mates at least tolerate each other, and is a baseline feature of determining pair compatibility in most pairing programs at research facilities across the United States. Second, clear directionality in dominance signals between pair-mates indicates a certain and well established relationship (Pomerantz & Baker, 2017). Strongly compatible pair-mates will display these first two traits, as well as high levels of affiliative interaction, and score low on Tense as a pair when evaluated by staff with species-specific behavioral knowledge. We recommend, when possible, that behavioral management teams strive to match optimal pair-mates together but, when restricted, allow pair-mates to maintain consistency in their social interactions via continuous pair-housing, and use grates (if possible) when temporarily separating pairs overnight.

Research guiding the proper implementation of social housing is especially important for refining NHP welfare in the context of biomedical and basic research. Further research can improve biomedical and basic research project planning to mitigate physiological changes that may result from manipulations of the social environment, while maximizing the quality of life of the NHPs involved.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Cohort number	Experimental Group	Pairing change	Number of subjects: In study	Number of subjects: In analysis	Study week number: Initial 1	Initial 2	Experimental 3	Experimental 4	Experimental 5
1	IC	Intermittent (I) → Continuous (C)	6	5	I	I	C	C	C
	CI	Continuous (C) → Intermittent (I)	6	6	C	C	I	I	I
2	IC	Intermittent (I) → Continuous (C)	6	4	I	I	C	C	C
	CI	Continuous (C) → Intermittent (I)	6	5	C	C	I	I	I

FIGURE 1.

Experimental design

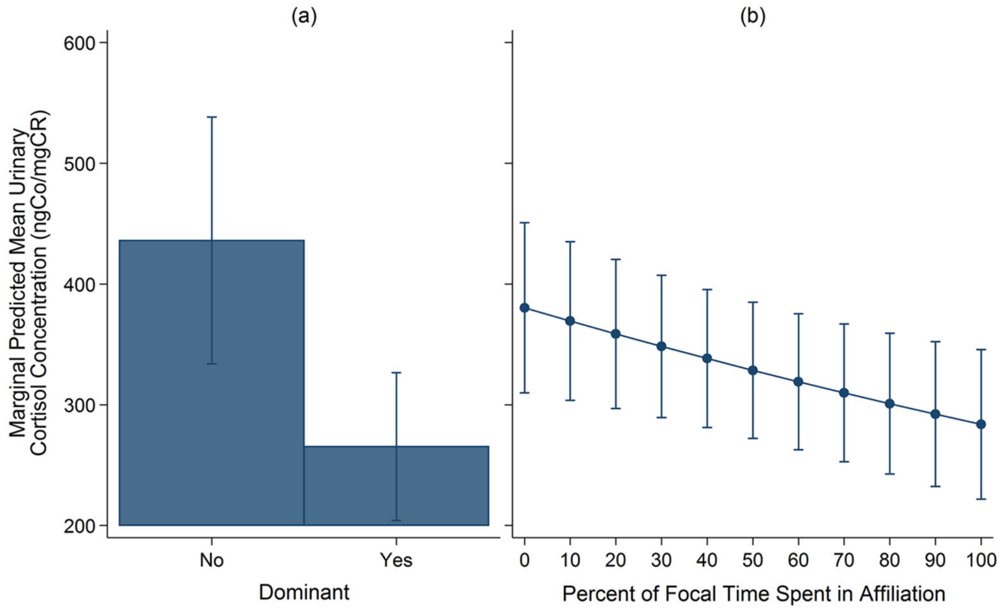

FIGURE 2.

Model 1 marginal plots for the main effects of: (a) Dominant and (b) Affiliation

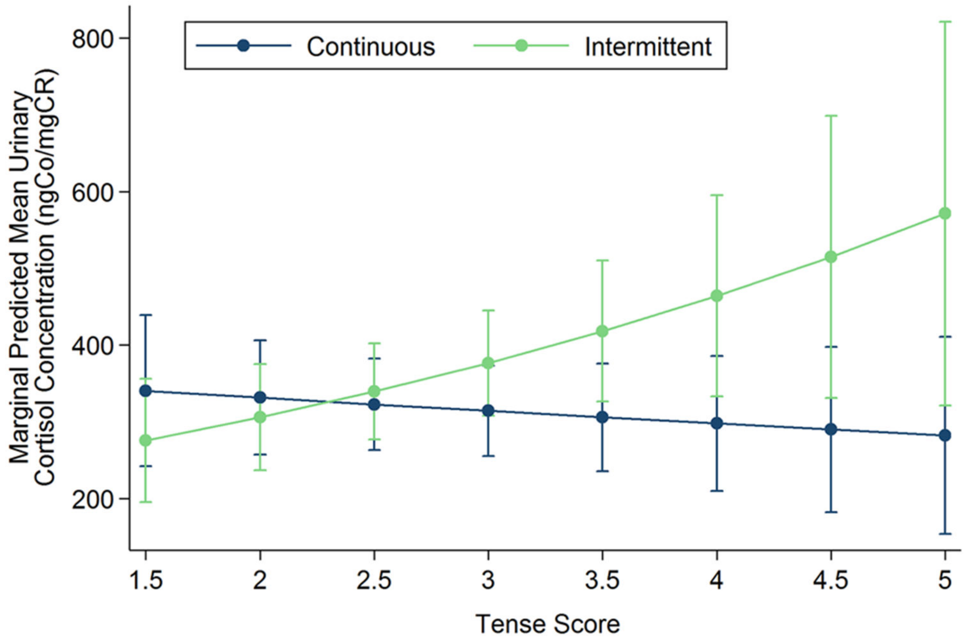

FIGURE 3.

Model 1 margins plot of the interaction of current condition and tense

TABLE 1

Variables included in model selection analyses, sorted in alphabetical order

Variable	Description
Abnormal	Proportion of focal intervals that included at least one instance of the following abnormal behaviors: regurgitate, urine/feces ingest, floating limb, self-strumming, leg lift, eye poke, suck (self or other), self-clasp, cheek biting, self-bite, threat-bite, self-hit, self-injurious behavior, hair pluck (self or other), hair ingest, pacing, swinging, flipping, twirling, rocking, bouncing, head twist, withdrawn
Affiliation	Proportion of focal intervals that included at least one instance of the following dyadic affiliative or prosocial behaviors: co-threat, recruit, recruit join, present ventrum/ body, present rump, mount, mount solicited, anogenital exploration, play, huddle, reconcile, groom given, groom receive, mutual groom
Agonistic	Proportion of focal intervals that included at least one instance of the following agonistic behaviors: non-contact aggression (threat, lunge, cringe, display, redirect, response non-contact aggression), contact aggression (push, pull, slap, wrestle, grapple, chase, bite, hair pull, pin, response contact aggression), trauma (mild or severe),
Cohort	Whether the subject was in the first or second cohort
Current Condition	Current pairing condition (continuous or intermittent)
Dominant	Whether the animal is dominant to their pair-mate based on receiving the greatest proportion of status signaling behaviors (move away, turn away, silent bared teeth) displayed between them
Experimental group	Began as intermittent and then experimentally changed to continuous (IC), or began as continuous and then experimentally changed to intermittent (CI)
Foraging enrichment	Whether the subjects received foraging enrichment prior to focal
Groom given	Subject picks, scrapes, spreads, mouth picks, and/or licks partner's hair or skin (not included in the same model with other groom variables or affiliation variable)
Groom mutual	Subjectand partner picks, scrape, spread, mouth pick and/or lick each other's hair or skin (not included in the same model with other groom variables or affiliation variable)
Groom received	Partner picks, scrapes, spreads, mouth picks and/or licks subject's hair or skin (not included in the same model with other groom variables or affiliation variable)
Grooming	Proportion of focal intervals that included at least one instance of the following grooming behaviors: groom given, groom receive, mutual groom (not included in the same model with other groom variables or affiliation variable)
Inactive	Subject is not active for more than 5 s
Initial pairing condition	Subject's pairing condition at the beginning of the study
Initial pairing condition time	Total time in months that the subject was living with current pair-mate in the initial housing condition before study
Menses	Subject's menstrual blood observed by husbandry staff.
Pair ID	The unique identification number for each pair to assess as a random effect
Pair rating anxious[a]	Score on pair rating measure “anxious” (seven-point scale): Animals seek proximity when un-paired; pair is impatient during separation by vocalizing, manipulating pairing door, or being very eager to be re-paired
Pair rating friendly[a]	Score on pair rating measure “friendly” (seven-point scale): dyad enjoys the company of each other; both animals seek out social contact with partner; for example, playing, walking next to, or sitting with another monkey
Pair rating tense[a]	Score on pair rating measure “tense” (seven-point scale): pair is sociable to each other, but posture is rigid and not relaxed
Pair rating well-meshed[a]	Score on pair rating measure “well-meshed” (seven-point scale): animals are sensitive to each other in a non-anxious way
Project phase	Current phase of the study (initial or experimental)
Related	Whether the subject's pair-mate is from the same matriline

Variable	Description
Same social group	Whether the subject was reared in the same outdoor social group with its pair-mate
Status signals dominant	Subject approaches, sniffs the mouth of, or takes the resource (e.g., food or toy) of their pair-mate
Status signals subordinate	Subject moves away, turns away, averts eyes, freezes, or gives a silent bared teeth signal to their pair-mate
Study week	The current week, out of 5, of the study
Total pairing time	Total time in months that the subject was living with current pair-mate before study
Total time indoors	Total time in months that the subject was living in indoor housing before study

[a]Definition developed by K. Chun.

TABLE 2

Best model set

	Model parameters	AICc	ΔAICc	*L*	*w*	Cumulative *w*	ER
M1	*Y* = Dominant + Affiliation + Experimental Group + Current condition + Tense + Current Condition*Tense	3063.47	0.00	1.00	0.48	0.48	1.00
M2	*Y* = Dominant + Affiliation + Project Phase + Current condition + Tense + Current Condition*Tense	3064.25	0.78	0.68	0.32	0.81	1.48
M3	*Y* = Dominant + Affiliation + Current Condition + Tense + Current Condition*Tense	3066.19	2.72	0.26	0.12	0.93	3.91
M4	*Y* = Dominant + Affiliation + Project Phase + Current condition + Total Pairing Time + Inactive	3067.30	3.83	0.15	0.07	1.00	6.79

AICc, Corrected Akaike Information Criterion; ΔAICc, Difference in AICc value from that of M1; L, Model likelihood calculated from the formula $L(g_i|\text{ data}) = \exp(-(1/2)\Delta AICc_i)$; *w*, The Akaike model weight $\left(L_i / \Sigma_{j=1}^{R} = L_j\right)$, which is a measure of the strength of the evidence represented as a probability it is the best model; ER, The evidence ratio, which is calculated by the weight of the best model divided by the weight of the given model.

TABLE 3

Variate weights for best model set

Variates	# of models	$w_{+}(j)$[a]	Mean $w_{+}(j)$[b]
Dominant	4	1.00	0.25
Affiliation	4	1.00	0.25
Current Condition	4	1.00	0.25
Tense	3	0.93	0.23
Project Phase	2	0.40	0.10
Experimental Group	1	0.48	0.12
Total Pairing Time	1	0.07	0.02
Inactive	1	0.07	0.02
Current Condition*Tense	3	0.93	0.23

[a]The sum of model weights that include the variate.

[b]The proportion of the sum of the weights to the total number of models in the best model set.

TABLE 4

Model 1 results

Variables included in Model 1	β	β SE	exp (β)	exp (β)SE	exp (β) LBCI	exp (β) UBCI	p [a]	
Dominant	−0.497	0.165	0.608	0.101	0.440	0.841	0.003	**
Affiliation	−0.003	0.001	0.997	0.001	0.995	0.999	0.008	**
Experimental Group (IC)	−0.407	0.173	0.666	0.115	0.474	0.934	0.019	*
Current Condition (intermittent)	−0.604	0.183	0.547	0.100	0.382	0.782	0.001	**
Tense	−0.054	0.094	0.948	0.089	0.789	1.139	0.568	
Current Condition *Tense	0.262	0.063	1.299	0.082	1.147	1.471	<0.001	***

Significance denoted by:

*** $p < 0.001$

** $p < 0.01$

* $p < 0.05$.

[a] Provided as probability information only, not as accept/reject criteria, which is not appropriate for an IT approach.