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

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3 1 **Intermittent pair-housing, pair relationship qualities, and HPA activity in adult female**
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6 **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 part way through the study. Each study subject was observed for five 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 3 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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3 46 Keywords: pair-housing; overnight separation; peer interaction; cortisol
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5 47 **INTRODUCTION**
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7
8 48 It is well established that social environments, compared to solitary housing, significantly
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10 49 improve captive non-human primate (NHP) welfare and health (Olsson & Westlund, 2007). For
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12 50 example, single-housing has been associated with physiological changes, such as higher blood
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14 51 pressure (Coelho, Carey, & Shade, 1991) and immunosuppression (Lilly, Mehlman, & Higley,
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16 52 1999), that increase the risk of acquiring pathological health conditions (e.g., cardiovascular
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18 53 disease or infection). Furthermore, studies in laboratory rodents have demonstrated that
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20 54 environments lacking complexity, such as limited cage features and insufficient outlets for
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22 55 expressing species adaptations, can have deleterious effects on biomedical research results (e.g.,
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24 56 Richter et al., 2011). Consequently, regulatory pressure has increased on research facilities to
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26 57 socially house NHPs (Hannibal, Bliss-Moreau, Vandeleest, McCowan, & Capitanio, 2017).
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28 58 Although social housing is the expected and enforced norm, laboratory NHPs may experience
29
30 59 extended periods of social separation due to colony or study protocols. For example, pair-mates
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32 60 may be separated to prevent a social partner from picking at and removing surgical sutures,
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34 61 confirm diarrhea or menses after overnight separation, or collect overnight urine or fecal
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36 62 samples. The effects of these separations on the welfare, physiology, and health of laboratory
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38 63 NHPs are not well understood. In this paper, we investigate the effects of daily, overnight
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40 64 separations of paired adult female rhesus macaques (*Macaca mulatta*) on urinary cortisol, a
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42 65 hormonal measure that is sensitive to environmental changes and reflects physiological states
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44 66 that may impact research outcomes.
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51 67 Among all research facilities in the United States, laboratory NHPs are primarily housed in
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53 68 social groups (61.51%), less often in pairs (22.84%), or singly-housed (15.65%) (Bennett, 2016).
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3 69 Pair-housing, the cohousing of two individuals by connected adjacent cages, has been developed
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5 70 and refined to maximize social contact for laboratory NHPs in a manner compatible with many
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7 71 research objectives (Baker, Crockett, et al., 2012). Single-housing facilitates specific research
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9 72 objectives, but maintains individuals in separate cages. Although this allows auditory, visual, and
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11 73 olfactory contact with conspecifics, tactile contact is restricted to varying degrees depending on
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13 74 whether the separating door is solid metal, bars, grate, or mesh (Baker, Bloomsmith, et al., 2014;
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15 75 Bennett, 2016). Single-housing, however, is prohibited by regulations, unless justified by clinical
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17 76 or behavioral findings that require pair separation or research needs that have been reviewed and
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19 77 approved by the institutional oversight office (United States Department of Agriculture, 2013).
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21 78 Modified forms of pair-housing are often used to accommodate research or management needs.
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23 79 Intermittent pair-housing involves temporary daily or weekly separations that last 12 or more
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25 80 hours, including overnight (Baker, 2016; Capitanio, Blozis, Snarr, Steward, & McCowan, 2017).
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27 81 In contrast, continuous pair-housing allows complete visual and physical access to a pair-mate,
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29 82 with infrequent and brief separations.
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35 83 Several studies have demonstrated welfare improvements for NHPs that are pair-housed as
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37 84 compared to those that are singly-housed. For example, pair-housing has been associated with
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39 85 improved behavioral welfare indices, including reduced levels of abnormal and anxiety-related
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41 86 behaviors (e.g., Baker, Bloomsmith, et al., 2012; Gottlieb, Maier, & Coleman, 2015), enhanced
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43 87 repertoires of species-specific behaviors (e.g., Baker, Bloomsmith, et al., 2014), and decreased
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45 88 self-injurious behavior (SIB) (e.g., Rommeck, Anderson, Heagerty, Cameron, & McCowan,
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47 89 2009; Weed et al., 2003). Another study found that pair-housed NHPs had better immune
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49 90 function than single-housed NHPs (Schapiro, Nehete, Perlman, & Sastry, 2000). While the
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51 91 benefits of pair-housing are now well established, pairing laboratory macaques with compatible
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3 92 companions is challenging and requires knowledge of and experience with species-specific
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5 93 social behavior (Truelove, Martin, Perlman, Wood, & Bloomsmith, 2017). Thus, research on
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8 94 laboratory macaque pair-housing has shifted focus to refining pairing practices to improve
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10 95 partner compatibility, welfare, and pairing success (e.g., Capitanio et al., 2017; Pomerantz &
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12 96 Baker, 2017; Truelove et al., 2017). Relatively little progress has been made, however, to
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15 97 improve our understanding of how frequent changes to pair-housing affect NHP physiology,
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17 98 despite the implications for biomedical research (reviewed in Hannibal et al., 2017).

19 99 Captive NHPs tend to have better welfare measures when they are able to express key
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21 100 species-specific behaviors (Lutz & Novak, 2005). Although most primate species spend a
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23 101 significant amount of their activity budget engaged in social behavior (Dunbar, 1991), captive
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25 102 pair-housed NHPs spend even more time doing so (Crockett, Bowers, Bowden, & Sackett,
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27 103 1994), likely due to a limited repertoire of other activities. For both wild and captive NHPs, the
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29 104 longest bouts of affiliation occur when they are huddled together overnight (Anderson, 1998;
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31 105 Eaton, Kelley, Axthelm, Iliff-Sizemore, & Shiigi, 1994). Furthermore, NHPs actively prefer the
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33 106 proximity of a social partner even when there are costs associated with that choice. For example,
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35 107 adult rhesus macaques chose to remain in the same cage as their social companions despite
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37 108 tradeoffs in available space (Basile, Hampton, Chaudry, & Murray, 2007). Also, captive tufted
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39 109 capuchin monkeys (*Cebus apella*) often chose their companions over food, even several hours
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41 110 after food deprivation (Dettmer & Fragaszy, 2000). Lastly, access to social partners buffers
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43 111 physiological stress during stressful procedures in captivity (Hennessy, Kaiser, & Sachser, 2009;
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45 112 Kikusui, Winslow, & Mori, 2006; Truelove et al., 2017), such as witnessing the anesthesia of
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47 113 another animal in the room (Gilbert & Baker, 2011).

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3 114 In contrast, separations from conspecifics can negatively impact NHP behavior and
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5 115 physiology. Physiological disruptions associated with permanent social group removal are
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7 116 “substantial” and take about 3-months to return to baseline, thus a 3-month conditioning period
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10 117 is recommended when previously outdoor housed NHPs are moved into indoor research settings
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12 118 (reviewed in Capitanio, Kyes, & Fairbanks, 2006). Temporary separations from social contact
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14 119 for greater than 10 hours to several days, are also known to increase negative indices of welfare
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16 120 in captive NHPs. For example, adolescent rhesus macaques displayed higher levels of abnormal
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18 121 and depressive behaviors in response to a 4-day social separation, increasing further after
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20 122 repeated separations (Mineka, Suomi, & DeLizio, 1981). Also, an 11-hour period of social
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22 123 isolation in Wied’s black tufted-ear marmoset monkeys (*Callithrix kuhli*) was associated with
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24 124 increased urinary cortisol concentration (Smith & French, 1997).
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28 125 While the implementation of intermittent pair-housing varies among facilities, all cases
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30 126 involve at least some overnight separation, as previously mentioned (Baker, 2016; Capitanio et
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32 127 al., 2017; Roberts & Platt, 2005; Rommeck, Capitanio, Strand, & McCowan, 2011; Tardif,
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34 128 Coleman, Hobbs, & Lutz, 2013). Continuously paired animals still experience short daytime
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36 129 separations for sample collection, health checks, and husbandry procedures, but spend more than
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38 130 half of every day together, with the exception of serious, albeit rare, health issues. At the
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40 131 California National Primate Research Center (CNPRC), intermittently housed monkeys are
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42 132 separated from about 14:00 (just prior to the afternoon feeding) until 08:00 (after the morning
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44 133 feeding) the following day, providing a maximum of 6 hours of daily socialization and physical
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46 134 contact. These separations remove the opportunity for these individuals to receive the benefits of
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48 135 overnight social contact (Eaton et al., 1994; Kikusui et al., 2006). Therefore, the welfare of
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3 136 intermittently pair-housed NHPs needs to be characterized by incorporating indices of welfare
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5 137 that can capture the lasting effects of overnight separations.
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8 138 Physiological indices of welfare, specifically the measurement of hypothalamic-pituitary-
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10 139 adrenal (HPA) axis activity, can provide insight into the impacts of overnight social separation.
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12 140 The main output of the HPA axis is cortisol, a glucocorticoid that can influence a variety of
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14 141 physiological systems, especially those involved in stress response and immune functioning
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16 142 (Sapolsky, Romero, & Munck, 2000). Depending on the biological source, elevated HPA axis
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18 143 activity can be detected several minutes (blood), hours (urine), days (feces), or months (hair)
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20 144 after a stressor has occurred (Novak, Hamel, Kelly, Dettmer, & Meyer, 2013). Activity of the
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22 145 HPA-axis is known to be highly sensitive to environmental influences (e.g., temperature, stress)
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24 146 (Herman et al., 2003; Vandeleest, Blozis, Mendoza, & Capitanio, 2013) including the social
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26 147 environment (Mendoza, Capitanio, & Mason, 2001). Social isolation and unstable social
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28 148 relationships can lead to elevated cortisol levels and, when chronic, can eventually lead to altered
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30 149 regulation of the HPA axis (Capitanio, Mendoza, Lerche, & Mason, 1998; Dettmer, Novak,
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32 150 Meyer, & Suomi, 2014). For example, wild male olive baboons (*Papio anubis*) that were about
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34 151 to lose rank had higher cortisol levels than similarly ranked males that were about to gain rank
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36 152 (Sapolsky, 1992). On the other hand, higher rates of positive social interactions, like grooming,
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38 153 have been associated with lower fecal cortisol concentrations in Barbary macaques (*Macaca*
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40 154 *sylvanus*) (Shutt, MacLarnon, Heistermann, & Semple, 2007) and with lower hair cortisol
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42 155 concentrations in rhesus macaques (Wooddell et al., 2017). Relative cortisol levels, thus, are only
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44 156 useful when informed by the context (climate, activity, rank relationships, and other social and
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46 157 environmental variables) and perturbations associated with changes in levels.
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3 158 Research on the impact of social housing (pair- vs single-housing) on cortisol levels has
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5 159 yielded mixed results. Although some previous studies found no differences in serum cortisol
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7 160 concentrations between single- and pair-housed macaques (e.g., Baker, Bloomsmith, et al., 2012;
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9 161 Gust, Gordon, Brodie, & McClure, 1994; Schapiro, Bloomsmith, Kessel, & Shively, 1993),
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11 162 others have found higher cortisol levels in singly-housed animals (Doyle, Baker, & Cox, 2008).
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13 163 These studies, however, vary in a couple of potentially important ways. First, they differ in the
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15 164 sampling matrix used to measure cortisol levels. All of the studies failing to find a relationship
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17 165 between cortisol and pairing status measured serum cortisol levels, whereas the Doyle et al.
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19 166 (2008) study measured fecal cortisol levels. These sampling matrices reflect HPA-axis activation
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21 167 on a scale of minutes (serum) to days (feces) which may have impacted the measured
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23 168 relationships. Secondly, these studies varied in whether, or the degree to which, they pre-selected
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25 169 potential pair-mates based on criteria that tend to maximize compatibility (e.g., body weight
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27 170 disparity). Since positive and negative social interactions can alter HPA axis activation, the
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29 171 qualities of the pair relationship may be critical to the ability to detect differences in cortisol
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31 172 levels. Overall, the consequences of manipulating a NHPs' social environment (e.g., switching
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33 173 between pair-housing conditions) on their behavior and physiological functioning remain largely
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35 174 unknown (Hamel et al., 2017; reviewed in Hannibal et al., 2017). Pair-mate compatibility may
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37 175 alter the magnitude of the stress response to pair separations and reunions. Therefore,
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39 176 investigating the pair relationship could uncover behavioral compatibility metrics that are likely
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41 177 to facilitate less stressful separations and reunions. It is unlikely that there is a single metric of
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43 178 pair compatibility, but converging evidence from more than one behavioral or physiological
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45 179 metric would allow managers to use the metrics they have access to and that have predictive
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47 180 power.
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3 181 In this study, we investigate whether changes in intermittent versus continuous pair-housing
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5 182 condition of adult female rhesus macaques impacts the HPA axis as measured by urinary cortisol
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7 183 concentrations. We further explore the impact of pair relationship quality and whether it
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9 184 modulates the effect of housing condition, while controlling for other aspects of the social
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11 185 environment, such as dominance status and affiliation rates. For NHPs adapted for a rich social
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13 186 life, long periods of social isolation have the potential to produce physiological variability with
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15 187 implications for the external validity of biomedical research conducted with such animals
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17 188 (Hannibal et al., 2017). If overnight separation is associated with substantial changes in HPA
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19 189 axis activity, then modifications of this practice should be considered for the benefit of both
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21 190 animal welfare and research.
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26 191 **METHODS**

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28 192 This research was conducted from March to May 2015 at the California National Primate
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30 193 Research Center (CNPRC) in Davis, California. Animal care and research protocols for this
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32 194 study were approved by the Institutional Animal Care and Use Committee at the University of
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34 195 California Davis. This research was conducted in accordance with United States federal
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36 196 regulations and adhered to the American Society of Primatologist Principals for the Ethical
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38 197 Treatment of Animals.
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42 198 **Subjects**

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44 199 In order to limit physiological variability of the study sample as much as possible, subject
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46 200 selection criteria included: (a) only females due to sex differences in physiology and the fact that
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48 201 most adults in the indoor colony are female; (b) a minimum three months indoors and in their
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50 202 pair-housing condition, without repeated incidents of serious physical aggression and wounding;
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52 203 (c) no history of conception during the past breeding season, (d) reared in an outdoor social
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3 204 group, and (e) between 4 to 11 years old, (criteria based on findings and recommendations by
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5 205 Capitanio et al., 2006; Cavigelli & Caruso, 2015; Reeder & Kramer, 2005). Subjects were
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7 206 enrolled as pairs as much as possible to avoid pair separations for other colony or project needs
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9
10 207 not related to this study. Random selection and assignment of animals was not possible because
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12 208 the purpose of the study was to understand impact of indoor pairing practices on physiology and
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14 209 the pool of animals that fit our selection criteria was very small.

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16
17 210 The study began with 24 adult female rhesus macaques. Due to our subject criteria, 2
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19 211 females were enrolled in the study while their pair-mates were not. To maintain consistency in
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21 212 behavioral data collection and conduct pair-adjective ratings, these data were collected on both
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23 213 pair-mates for all subjects, but data from the 2 non-study pair-mates of subjects was not included
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25 214 in individual level analyses. Two study subjects, who were paired together, were dropped during
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27 215 the study due to intra-pair conflict and another two were dropped from analyses due to poor or
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29 216 insufficient urinary samples, leaving 20 subjects. Subjects were ages 4.9 to 10.9 years
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31 217 (mean=6.7, SD=1.8), confirmed non-pregnant by ultrasound, and were not observed to have a
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33 218 consistent pattern of menstrual synchronization within pairing groups (i.e., females cycled at
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35 219 different times throughout the study). All subjects were born and raised in outdoor large (0.2-
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37 220 hectare outdoor enclosures containing up to 180 NHPs) or small (43.7 m² outdoor enclosures
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39 221 containing up to 30 NHPs) social groups comprised of all age and sex classes at the CNPRC for
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41 222 at least the first 2.5 years of life. Subjects selected for the study had been relocated for
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43 223 management reasons to indoor housing at least four months prior to the study (mean=20.7,
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45 224 SD=20.0). All subjects had been housed successfully (without persistent agonism or wounding)
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47 225 with another female in their baseline condition (intermittent or continuous) for at least three
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3 226 months prior to the study (mean=11.0, SD=6.4). The baseline pairing condition was intermittent
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5 227 for 9 subjects and continuous for 11 subjects.
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7 228 **Housing and Pairing**

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10 229 Animal housing consisted of pairs of stainless steel cages (floor space 0.4 m², height 0.8 m).
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12 230 The cages of paired animals were joined by an opening (approximately 30 cm by 30 cm) with a
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14 231 sliding solid stainless-steel partition that prevented physical contact. Per management practice,
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16 232 intermittently paired animals were separated by the partition prior to afternoon feeding
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18 233 (approximately 14:00) until after morning feeding (approximately 08:00) the following day.
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20 234 Therefore, intermittent pairs had about six hours of co-housing each day. Conversely,
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22 235 continuously paired animals were co-housed for at least 18 hours daily and were always together
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24 236 overnight. All socially housed animals in the colony, regardless of housing condition, experience
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26 237 occasional separations for minutes, hours, or even days for sample collections, veterinary exams
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28 238 or treatments, and husbandry procedures. However, unless intermittently-housed, the majority of
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30 239 their 24-hour days are spent in social contact. For the purposes of urine sample collection and
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32 240 feeding regime consistency across the experimental groups, continuous pairs were separated
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34 241 during each feeding time (two bouts) for about an hour in the morning, and one to three hours in
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36 242 the afternoon (cumulative maximum of four hours per day). Afternoon feeding time coincided
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38 243 with urine sample collection for all pairs, to ensure correct identification and prevent cross-
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40 244 contamination of samples. Continuous pairs were re-paired immediately after an adequate
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42 245 sample was obtained or as soon as the 4-hour mark was reached. Intermittent pairs remained
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44 246 separated overnight, consistent with the colony management protocol for this housing category.
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46 247 The short separations of continuously housed subjects for sample collection is not of long
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48 248 enough duration to be considered intermittent because they were only long enough to obtain
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3 249 samples and they were not separated overnight. Subjects were fed a standard monkey chow diet
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5 250 and a forage mixture of rice, split peas, and oats twice daily by animal care staff, with fresh
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7 251 water available *ad libitum*. Regular facility enrichment (e.g., mirror, chew toy, forage board,
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9 252 metal perch, puzzle feeders) was provided to each subject according to CNPRC standard
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11 253 operating procedures (SOPs) throughout the study.
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14 254 **Experimental Design**

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17 255 To compare the behavior and urinary cortisol concentration of continuously (C) versus
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19 256 intermittently (I) pair-housed female rhesus macaques, subjects were assigned to one of two
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21 257 experimental groups (i.e., CI or IC) based on their pairing condition at the beginning of the study
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23 258 (i.e., initial pairing condition; variable definitions are listed in Table 1). Pairs were in their initial
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25 259 pairing condition for two weeks (i.e., initial project phase), and then switched to their
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27 260 experimental condition for three weeks (i.e., experimental project phase) (Fig 1). Because it was
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29 261 not possible to complete data and sample collection on all subjects in one five-week study
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31 262 period, subjects were studied in two cohorts, balanced by experimental group so that there were
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33 263 about equal numbers of CI and IC subjects in each cohort. The first cohort was studied March 23
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35 264 to April 24, 2015 and the second cohort was studied April 27 to May 29, 2015. All data
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37 265 collection occurred on weekdays (initial project phase: 9-10 days; experimental project phase:
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39 266 14-15 days).
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44 267 **Behavioral Data Collection**

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47 268 Two eight-minute focal observations were conducted on each pair per observation day,
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49 269 between 11:15 and 13:45 hours in a randomized order. Affiliative, agonistic, status, activity, self-
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51 270 directed, and abnormal behaviors (see Table 1 for variables comprised of these behaviors) were
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53 271 recorded using the HanDBase application (DDH Software, Wellington, Florida, USA) on an
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3 272 Android tablet. Observations were conducted solely by co-author L. C. Cassidy, who was
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5 273 previously trained as a CNPRC behavioral management staff member and reliable on all
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7 274 observation ethograms. For each pair, 18-20 observations were conducted during the initial
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9 275 pairing condition and 26-28 observations were conducted during the experimental pairing
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11 276 condition. Behaviors were recorded using one-zero sampling with 20-second sample intervals,
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13 277 except for self-directed behaviors which were recorded using all occurrences event sampling. For
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15 278 each observation day, proportions and frequencies were calculated for behaviors recorded with
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17 279 one-zero sampling and event sampling, respectively.
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22 280 [INSERT TABLE 1 HERE]
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24 281 **Pair Relationship Adjective Ratings**

25
26 282 Four pair rating adjectives, “Anxious,” “Tense,” “Well-meshed,” and “Friendly” (see Table
27
28 283 1, Pair Rating variables), defined by co-author K. Chun, were used to evaluate the relationship of
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30 284 each pair on a seven-point scale. Adjective ratings allow observers to integrate multi-modal
31
32 285 information about animals across time and experiences, and can be scientifically tested for
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34 286 reliability and validity (Meagher, 2009). Dyad ratings have been used to assess social
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36 287 interactions between amygdala lesioned vs. control animals (Emery et al., 2001). Like
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38 288 personality ratings, these adjectives likely remain relatively constant across different contexts
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40 289 (Capitanio, 1999). It was not our aim to use adjective ratings to assess possible changes to pair
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42 290 relationships between the initial and experimental project phases. Rather, we incorporated them
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44 291 to have an overall assessment of qualities of the pair relationship, irrespective of Project Phase,
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46 292 to assess whether this had an impact on potential changes in physiological responses to the
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48 293 experiment. Pair adjective ratings for the current study were assessed based on the behavioral
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50 294 observer’s (co-author L.C. Cassidy) direct experience with the subjects over the study period.
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3 295 Ratings were conducted one to two days after the data collection period for each cohort
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5 296 concluded, and again nine days later to assess intra-observer reliability using Krippendorff's α for
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7 297 interval metrics (Anxious $\alpha=0.92$; Tense $\alpha=0.88$; Well-meshed $\alpha=0.93$; Friendly $\alpha=0.84$) (Hayes
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9 298 & Krippendorff, 2007). The mean of the two observations for each reliable pair adjective rating
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11 299 was used in analyses.

14 300 **Urine Sample Collection**

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17 301 Throughout the five-week study period, urine samples were collected from each subject
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19 302 between the hours of 14:00 and 17:00 each weekday until up to three urine samples over 3mL in
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21 303 volume (considered an adequate sample) were collected for that week. The limited and consistent
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23 304 collection period allowed minimal separation of the pairs and reduced variation in cortisol levels
24
25 305 due to diurnal variation in primates (Novak et al., 2013). Urine was collected from clean stainless
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27 306 steel cage pans placed underneath each subject's cage. The pans were periodically checked for
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29 307 urine and a maximum volume of 45mL was transferred into a 50mL polypropylene vial. 351
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31 308 samples were collected from 22 animals. On the day of collection, urine samples were stored at
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33 309 room temperature until 18:00. Lastly, the samples were centrifuged at 2500 RPM for five
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35 310 minutes to remove impurities (e.g., food particles), and the supernatant transferred to 5 mL
36
37 311 polypropylene vials and stored at -80° C until assay.

42 312 **Cortisol Assays**

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45 313 Urinary free cortisol (Co) was measured using a quantitative competitive immunoassay and
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47 314 direct chemiluminescent technology developed and conducted by the CNPRC Primate Assay
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49 315 Laboratory Core. A total of 313 urine samples were assayed in duplicate for this study.
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51 316 Analytical sensitivity of the cortisol immunoassays was 2 ng Co/mL. Inter-assay coefficient of
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53 317 variation (CV) was 3.1% and intra-assay CV was 1.6%. Creatinine (Cr) was measured by a

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3 318 colorimetric assay to control for variations in subject body weight, urine output, and water
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5 319 content in each sample (Novak et al., 2013). Analytical sensitivity was 0.05 mg Cr/mL, inter-
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7 320 assay CV was 1.2%, and intra-assay CV was 0.5% for the creatinine assays. Urine sample
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9 321 concentration was normalized by dividing the cortisol concentration by the corresponding
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11 322 creatinine concentration. Urine samples with a creatinine concentration of 0.20 mg Cr/mL and
12
13 323 below were excluded (n=55) from our analysis as they could have resulted in falsely elevated
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15 324 normalized cortisol concentrations. Of 258 urine samples that met our analysis criteria, urinary
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17 325 cortisol per creatinine ranged from 32.32 ng Co/mg Cr to 1617.73 ng Co/mg Cr (mean=362.29,
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19 326 SD=283.34 ng Co/mg Cr).

23 24 327 **Data Analysis**

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26 328 Data were analyzed in Stata 14.1 using a generalized linear mixed model (GLMM) for a
27
28 329 gamma distribution (meglm command) (Hardin & Hilbe, 2007). Both subject and pair identity
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30 330 were considered as potential random effects. An information theoretic (IT) approach was used to
31
32 331 evaluate models based on goodness-of-fit, sample-size-corrected Akaike Information Criterion
33
34 332 (AICc) scores, and differences in AICc scores (Δ AICc) following methods described by
35
36 333 Burnham and Anderson (2002) and Burnham, Anderson, and Huyvaert (2011). We included
37
38 334 variables in the models that the literature indicates may have an impact on HPA axis activity
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40 335 (e.g., menses and activity) or pair compatibility (e.g., affiliation and agonistic behavior), as well
41
42 336 as the specific variables (Current Condition and pair adjective ratings) of interest to our research
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44 337 questions (see Table 1 for a list of all variables). The random effects were evaluated before
45
46 338 considering models with fixed effects and only subject ID alone was retained as a random effect.
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48 339 Collinear variables were not used in the same model and among collinear variables, the variable
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50 340 with the lowest AICc score was retained for further model comparison.
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3 341 Supplementary Table 1 contains a list of all models tested and the reasons these models were
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5 342 rejected from consideration. We considered all models that had both a model chi-square
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7 343 indicating a minimally good model and an AICc score less than the random effects only model,
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9 344 which indicates whether a model is better than a model with no predictors. Models violating the
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11 345 principal of parsimony were excluded (Burnham & Anderson, 2002). Model likelihoods, Akaike
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13 346 weights, and evidence ratios, which measure the strength of the evidence for these models, were
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15 347 calculated for a candidate set of models with a $\Delta AICc \leq 7.0$ (Burnham & Anderson, 2002;
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17 348 Burnham et al., 2011; Grueber, Nakagawa, Laws, & Jamieson, 2011; Symonds & Moussalli,
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19 349 2011). From this candidate model set, a best model set was then selected based on evidence
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21 350 ratios ≤ 10 and weights were then renormalized (Burnham & Anderson, 2001, 2002). The
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23 351 Akaike weights for the best model set were used to calculate variate weights by summing the
24
25 352 model weights for each variate across all models in which it was included (Burnham &
26
27 353 Anderson, 2002). Variate weights measure the relative importance of each variate for
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29 354 understanding the outcome, with 1 indicating it has the highest possible certainty of being
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31 355 important. Marginal effects (margins command) and plots (plot command) were produced from
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33 356 the top model for predictors of interest (Hardin & Hilbe, 2007).

39 357 **RESULTS**

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42 358 Of the models predicting urinary cortisol levels in our study animals, nine had at least some
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44 359 support with $\Delta AIC \leq 7$ (Burnham & Anderson, 2002) and were further examined as the
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46 360 candidate set of models (see Supplementary Table 2). From this set of candidate models, a set of
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48 361 best models with evidence ratios < 10 were selected and the model weights renormalized (see
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50 362 Table 2) (Burnham & Anderson, 2001). The Akaike weight of the best model (M1) was 0.481;
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52 363 therefore, there was not strong enough evidence to rely on this as the single best model and
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3 364 information from other models in the best model set should also be considered. All models in the
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5 365 best model set contained main effects for Dominant (descriptives of categories:
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7 366 dominant=50.0%, subordinate=50.0%), Affiliation (descriptives of percent of observation
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9 367 period: mean=36.7, min=0.0, max=100.0, sd=30.5), and Current Condition (descriptives of
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11 368 categories: continuous=47.9%, intermittent=52.1%). In addition, the three models with the
12
13 369 highest weight (M1, M2, and M3, $w=0.929$) also contained a main effect for Tense (descriptives
14
15 370 of score: mean=2.7, min=1.5, max=5, sd=1.4) and an interaction between Tense and Current
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17 371 Condition. The cumulative weight of Models 1 and 2 was 0.81 and the only differences between
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19 372 models 1 and 2 were the main effects of Experimental Group (seen in model 1, but not 2)
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21 373 (descriptives of categories: CI=55.0%, IC=45.0%) and Project Phase (seen in model 2, but not 1)
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23 374 (descriptives of categories: initial=42.5%, 57.5%). Model 4 included Project Phase, which also
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25 375 occurred in model 2, as well as Total Pairing Time (descriptives of months: mean=11.5,
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27 376 min=3.6, max=24.8, sd=6.4) and Inactivity (descriptives of percent of observation period:
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29 377 mean=38.6, min=0.0, max=100.0, sd=24.3), which occurred in no other models in the best model
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31 378 set.

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38 379 [INSERT TABLE 2 HERE]

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40 380 The predictors in the best models are listed by order of importance based on their
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42 381 corresponding variate weights (the sum of the model weights for the models containing variate j
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44 382 and denoted as $w_+(j)$) in Table 3. All models included the main effects of Dominant, Affiliation,
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46 383 and Current Condition and thus all had $w_+(j)=1$. Tense and the interaction of Current Condition
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48 384 and Tense occurred in the top three models and had $w_+(j)=0.93$. Experimental Group
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50 385 ($w_+(j)=0.48$) only occurred in Model 1, Project Phase ($w_+(j)=0.40$) only occurred in Model 2 and
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3 386 Model 4, and both Total Pairing Time and Inactivity (both had $w_+(j)=0.07$) only occurred in
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5 387 Model 4.

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8 388 [INSERT TABLE 3 HERE]

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10 389 The results of the best model (Model 1) are presented in Table 4. Dominant animals had
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12 390 urinary cortisol levels that were nearly half of those in subordinate animals ($\beta=-0.497$) (see Fig
13
14 391 2a). An increase in affiliation by ten percentage points was associated with 0.029 times lower
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16 392 (about three percent lower) cortisol levels ($\beta=-0.003$) (See Fig 2b). Although significant, the
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18 393 main effect of Current Condition was relatively small with an increase in urinary cortisol of
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20 394 about 0.12 times when Tense was at the mean value (2.73) for the sample ($\beta=-0.604$,
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22 395 exponentiated $\beta=0.547$). The main effect of Tense was not significant. The interaction of
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24 396 Current Condition (intermittent) and Tense was significant, but in the continuous condition for
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26 397 Current Condition, urinary cortisol levels stayed relatively low at all Tense ratings, while in the
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28 398 intermittent condition, urinary cortisol levels increased by 1.23 times as pair Tense rating
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31 399 increased ($\beta=0.262$) (See Fig 3).

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33 400 [INSERT TABLE 4 HERE]

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35 401 [INSERT FIG 1 HERE]

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37 402 [INSERT FIG 2 HERE]

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39 403 [INSERT FIG 3 HERE]

40 404 **DISCUSSION**

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42 405 Our study aimed to explore the impact of temporary overnight separations due to
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44 406 intermittent pair-housing on adult female rhesus macaques' HPA axis activity, indexed through
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46 407 urinary cortisol concentrations. In addition to stress, other factors such as activity level and
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48 408 ambient temperature can affect cortisol secretion. For this reason, it is not possible to identify a

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3 409 normal cortisol range for a species, population, or even an individual that is indicative of distress,
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5 410 eustress, or lack of stress. Our results showed that overnight separations were associated with
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7 411 higher concentrations of urinary cortisol, but that this association was dependent on key
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9 412 characteristics of the pair relationship and occurred even when accounting for other variables
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11 413 known to influence the production of cortisol. Most interestingly, pairs rated as having more
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13 414 tense relationships had higher urinary cortisol levels, but only when they were intermittently
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15 415 paired. Additionally, dominance status and greater rates of affiliation were associated with lower
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17 416 urinary cortisol.
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22 417 **Intermittent pairing, relationship quality, and urinary cortisol**

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24 418 Females that had more tense relationships with their partners had urinary cortisol levels 1.5-
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26 419 3 times higher, depending on the tense rating and variability, when intermittently paired than
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28 420 when continuously paired. A high pair rating for Tense may indicate that the relationship is
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30 421 tenuous and overnight separation may be introducing uncertainty in re-establishing the
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32 422 relationship when reunited. Uncertainty in dominance relationships has been associated with
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34 423 higher levels of pro-inflammatory proteins and greater risk for diarrhea for rhesus macaques
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36 424 living in large outdoor social groups (Vandeleest et al., 2016). This measure of uncertainty may
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38 425 indicate that a poor fit in the social group is associated with poorer health outcomes. Although
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40 426 cortisol is not a direct measure of health (cortisol values can have implications for health, but can
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42 427 also vary for reasons that have nothing to do with health outcomes), it is often used as a
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44 428 biomarker for increased health risk due to its responsiveness to stressors and role in regulating
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46 429 immune function (Sapolsky et al., 2000). Our findings are also consistent with a study in wild
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48 430 hamadryas baboons (*Papio hamadryas ursinus*) where relationship quality (measured as a
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3 431 grooming diversity index) was related to HPA axis activity (Crockford, Wittig, Whitten,
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5 432 Seyfarth, & Cheney, 2008).

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7 433 Although we did not find a difference in urinary cortisol concentration between intermittent
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9 434 and continuous housing conditions among pairs who did not have a Tense relationship, we
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11 435 caution against interpreting this as evidence that overnight separation does not cause distress or
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13 436 impact research outcomes. There may be differences among less Tense pairs that could not be
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15 437 detected in the sample used in this study. We suspect that a larger sample would find an effect,
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17 438 albeit a smaller one than that seen in Tense pairs.

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19 439 When making decisions about pairing laboratory NHPs, behavioral and facility managers
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21 440 often have a limited number of potential partners to select from and attempt pair introductions
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23 441 depending on factors such as indoor population size, study needs, and breeding needs. While
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25 442 some of these potential pairs do not remain paired past the introduction period due to conflict,
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27 443 those that do and become established pairs usually remain paired until there is a management
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29 444 reason to separate them. Therefore, it is not surprising that we found variation in relationship
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31 445 quality in our sample. Since it is likely that other laboratory NHP facilities have pair-housed
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33 446 populations with similar variation in the quality of pair relationships, our results suggest that
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35 447 when pairs show signs of being tolerably, but not ideally, compatible (e.g., absence of physical
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37 448 affiliative social interaction or sitting in proximity to one another), it is best to avoid overnight
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39 449 separations to prevent uncertainty at reintroduction and unusual disruptions in their physiology.

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41 450 In our study, continuous pair-housing provided near constant social interaction and was
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43 451 associated with reduced HPA activation, regardless of pair quality. However, continuous housing
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45 452 is not compatible with some research objectives. For example, biological sample collection (e.g.,
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47 453 feces, urine) often requires that pair-mates are separated for some time (e.g., overnight) to
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3 454 acquire samples from the correct subject. In these situations, providing some contact could limit
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5 455 unintended social consequences or changes to physiology. For example, when overnight
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7 456 separation is necessary, a grate or bar (as opposed to solid) divider that allows some visual and
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9 457 tactile access to pair-mates may be preferable.

11
12 458 To our knowledge, our study is the first to use subjective ratings to assess pair compatibility
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14 459 after pair introduction. Subjective rating assessment is an underutilized tool within the field of
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16 460 captive NHP welfare, despite the potential utility of animal caretaker knowledge. Furthermore,
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18 461 ratings are less time intensive than formal behavioral observations, are non-invasive unlike some
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20 462 physiological measurement techniques, and are scientifically valid when appropriately designed
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22 463 (Meagher, 2009). The interaction between housing condition (during intermittent pairing) and
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24 464 the quality of the pair relationship provides further support that ratings are associated with
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26 465 biological phenomena, in this case, changes in HPA activity. Interestingly, the IC pair we
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28 466 excluded from our analyses due to aggression and subsequent separation during the continuous
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30 467 pairing phase was rated as having a very tense relationship. These females previously knew each
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32 468 other from a large outdoor social group, but familiarity does not always translate to
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34 469 compatibility. Therefore, pair adjective ratings such as high Tense scores may act as useful
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36 470 guidelines for re-evaluating pair compatibility and guiding social management decisions.

41 42 471 **Dominance rank and urinary cortisol concentration**

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44 472 Our study found that urinary cortisol was lower in dominant females than in subordinate
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46 473 females. Therefore, including dominance status in the model was important for interpreting the
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48 474 association between housing condition and HPA axis activity. Primate studies of cortisol usually
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50 475 find an effect of social rank, but the direction of the effect is not consistent across studies (e.g.,
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52 476 Abbott et al., 2003; Muller & Wrangham, 2004; Shively, 1998). However, it is important to note
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3 477 that social status or high cortisol values alone cannot be interpreted as distressing. Generally, if
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5 478 an individual is maintaining a healthy weight and social injuries are rare and minor, there is no
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8 479 reason to interpret their situation as deleterious.

9 10 480 **Affiliation and urinary cortisol concentration**

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12 481 Greater frequency of affiliative behavior with a pair-mate was also associated with lower
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14 482 urinary cortisol in our study. This is consistent with previous findings that affiliative social
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17 483 partners dampen behavioral and physiological stress responses (Hennessy et al., 2009; Kikusui et
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19 484 al., 2006; Wooddell et al., 2017), but like dominance status, this cannot be used to make direct
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21 485 inferences about stress levels in this study sample. Because affiliation was an important predictor
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23 486 of urinary cortisol levels, including it in our multivariate analysis was necessary to understand
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25 487 any association with housing condition.

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28 488 Pair compatibility criteria during pair introduction vary by facility (Baker, Coleman,
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30 489 Bloomsmith, McCowan, & Truelove, 2014), but generally the absence of deleterious aggression,
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32 490 wounding, food monopolization, and presence of status signals establishing dominance are
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34 491 prioritized over rates of affiliative behaviors between pair-mates. In NHPs, affiliative behaviors
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36 492 reinforce social bonds and frequent affiliation between individuals indicates the strength of the
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38 493 relationship (Silk, Altmann, & Alberts, 2006). The absence or reduced frequency of affiliation
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40 494 may not cause external injury, but may indicate the pair is not experiencing the full benefits of
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42 495 social housing.

43 44 45 496 **Summary**

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49 497 Overall, our results emphasize that changes to the pair-housing arrangement, in combination
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51 498 with aspects of a pair's social relationship, can modulate urinary cortisol concentration in pair-
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53 499 housed adult female rhesus macaques. Importantly, although intermittent pair-housing provides
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3 500 superior welfare over single-housing, our results indicate that it may be associated with increased
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5 501 HPA-axis activity when the relationship between the two pair-mates is tense. Our findings
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8 502 support the importance of assessing compatibility between pair-mates beyond the current
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10 503 minimum criteria of the absence of serious injury and repeated fighting. We also caution against
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12 504 interpreting the lack of an effect found for Tense pairs in this study as evidence that overnight
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14 505 separations do not have an impact on welfare or research as this may have been detected if a
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17 506 larger sample was possible.

19 507 We propose a continuum composed of three different aspects of compatibility. First, and as a
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21 508 bare minimum, the absence of serious aggression or injury demonstrates that pair-mates at least
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23 509 tolerate each other, and is a baseline feature of determining pair compatibility in most pairing
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25 510 programs at research facilities across the United States. Second, clear directionality in dominance
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27 511 signals between pair-mates indicates a certain and well established relationship (Pomerantz &
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29 512 Baker, 2017). Strongly compatible pair-mates will display these first two traits, as well as high
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31 513 levels of affiliative interaction, and score low on Tense as a pair when evaluated by staff with
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33 514 species-specific behavioral knowledge. We recommend, when possible, that behavioral
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35 515 management teams strive to match optimal pair-mates together but, when restricted, allow pair-
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37 516 mates to maintain consistency in their social interactions via continuous pair-housing, and use
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40 517 grates (if possible) when temporarily separating pairs overnight.

44 518 Research guiding the proper implementation of social housing is especially important for
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46 519 refining NHP welfare in the context of biomedical and basic research. Further research can
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48 520 improve biomedical and basic research project planning to mitigate physiological changes that
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50 521 may result from manipulations of the social environment, while maximizing the quality of life of
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53 522 the NHPs involved.

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534 collected the biological samples. LC prepared, processed, and stored the biological samples. BM
535 provided laboratory supplies, equipment, and laboratory space for collecting, processing, and
536 storing samples. DH performed and interpreted the statistical analyses. DH, LC, JV, and SW
537 wrote and revised the manuscript with additional edits and revisions by BM, SS, KC, and AB.

538 FIGURE LEGENDS

539 Figure 1. Experimental design

540 Figure 2. Model 1 marginal plots for the main effects of: a) Dominant and b) Affiliation.

541 Figure 3. Model 1 margins plot of the interaction of Current Condition and Tense

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For Peer Review

RESEARCH HIGHLIGHTS

- Overnight separations of tense pairs are associated with increased HPA-axis activity
- Continuous and compatible pair-housing are recommended
- When separating overnight, contact via bars or grates may improve welfare and alleviate unintended effects

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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)

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4	Groom Mutual	Subject and 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)
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7	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)
8		
9		
10	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)
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14	Inactive	Subject is not active for more than 5 seconds
15		
16		
17	Initial Pairing Condition	Subject's pairing condition at the beginning of the study
18		
19	Initial Pairing Condition Time	Total time in months that the subject was living with current pair-mate in the initial housing condition before study
20		
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22	Menses	Subject's menstrual blood observed by husbandry staff.
23		
24	Pair ID	The unique identification number for each pair to assess as a random effect
25		
26	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
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31	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
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35	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
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39	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
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4	Project Phase	Current phase of the study (initial or experimental)
5		
6	Related	Whether the subject's pair-mate is from the same matriline
7		
8	Same Social Group	Whether the subject was reared in the same outdoor social group with its pair-mate
9		
10	Status Signals Dominant	Subject approaches, sniffs the mouth of, or takes the resource (e.g., food or toy) of their pair-mate
11		
12	Status Signals Subordinate	Subject moves away, turns away, averts eyes, freezes, or gives a silent bared teeth signal to their pair-
13		mate
14		
15	Study Week	The current week, out of 5, of the study
16		
17	Total Pairing Time	Total time in months that the subject was living with current pair-mate before study
18		
19	Total Time Indoors	Total time in months that the subject was living in indoor housing before study
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Table 2. Best model set

Model parameters	AICc	Δ AICc	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

Δ AICc: Difference in AICc value from that of M1

L: Model likelihood calculated from the formula $L(g_i|\text{data}) = \exp(-(1/2)\Delta\text{AICc}_i)$

w: The Akaike model weight ($L_i / \sum_{j=1}^R L_j$). 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	$\exp(\beta)$	$\exp(\beta)$ SE	$\exp(\beta)$ LBCI	$\exp(\beta)$ 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	***

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

Significance denoted by: *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$

Cohort number	Experimental Group	Pairing change	Number of subjects		Study week number				
			In study	In analysis	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

44x10mm (600 x 600 DPI)

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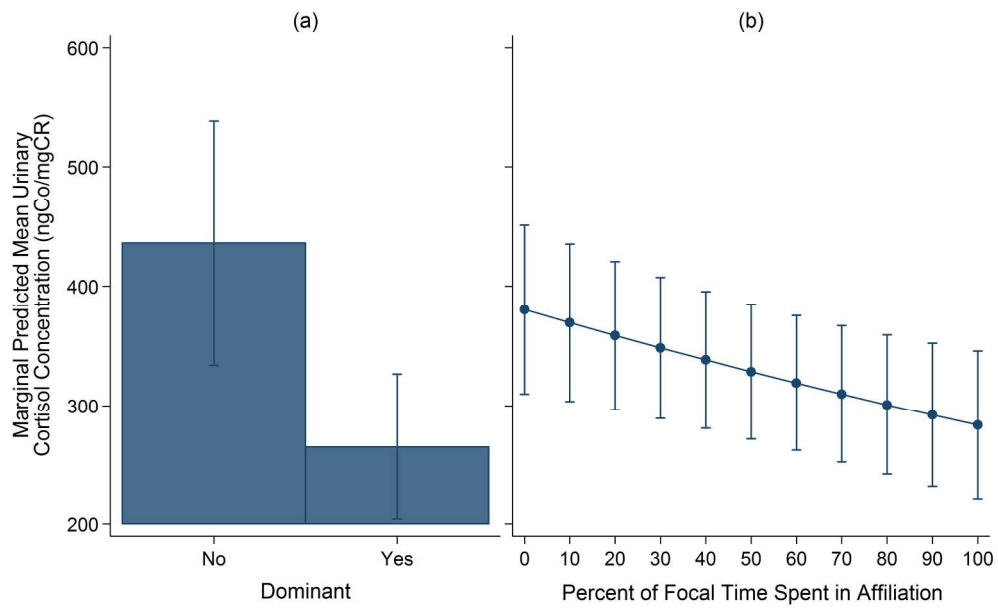


Figure 2. Model 1 marginal plots for the main effects of: a) Dominant and b) Affiliation.

111x69mm (600 x 600 DPI)

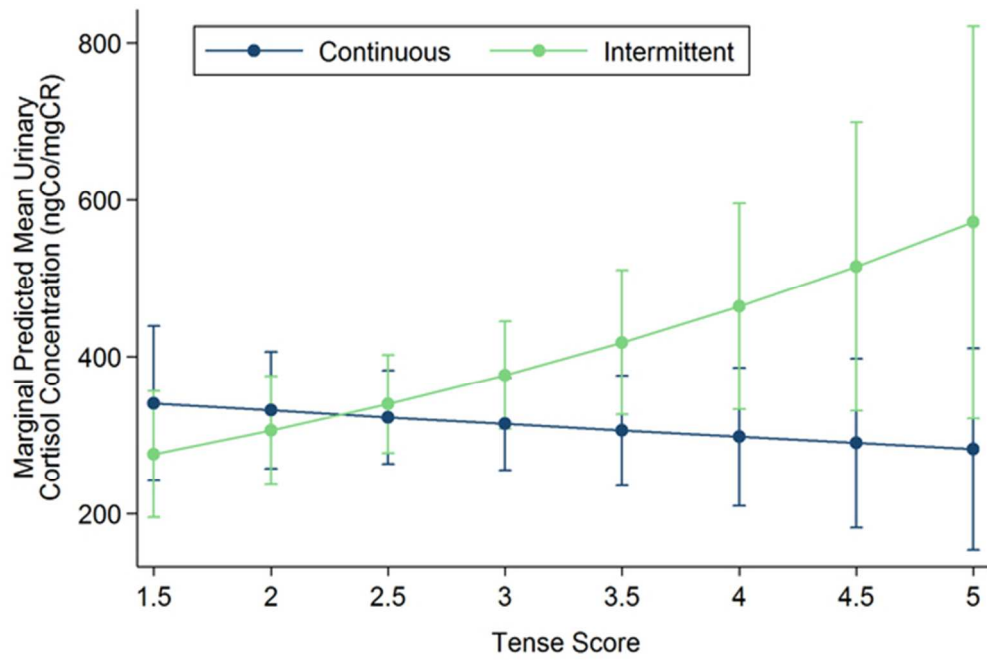


Figure 3. Model 1 margins plot of the interaction of Current Condition and Tense.

55x36mm (300 x 300 DPI)

Supplementary Table 1. Complete model set

Model	Retained	Reason for not Retaining	Independent Variables	Model Wald χ^2	Model P-value	AIC	AICc	Δ AIC	Δ AICc
M1	Yes		Dominant + Experimental Group + Affiliation + Current Condition + Tense + Current Condition*Tense	42.4	<0.0001	3062.84	3063.47	0.00	0.00
M2	Yes		Dominant + Project Phase + Affiliation + Current Condition + Tense + Current Condition*Tense	39.08	<0.0001	3063.62	3064.25	0.78	0.78
M3	Yes		Dominant + Affiliation + Current Condition + Tense + Current Condition*Tense	34.68	<0.0001	3065.71	3066.19	2.87	2.72
M4	Yes		Dominant + Project Phase + Affiliation + Total Pairing Time + Current Condition + Inactive	37.27	<0.0001	3066.67	3067.30	3.83	3.83
M5	Yes		Dominant + Experimental Group + Current Condition + Tense + Current Condition*Tense	34.44	<0.0001	3067.83	3068.31	4.99	4.84
M6	Yes		Dominant + Experimental Group + Affiliation + Total Pairing Time + Well-meshed + Current Condition + Current Condition*Well-meshed	41.46	<0.0001	3067.80	3068.59	4.96	5.12
M7	Yes		Dominant + Project Phase + Current Condition + Tense + Current Condition*Tense	30.46	<0.0001	3069.13	3069.61	6.29	6.15
M8	Yes		Dominant + Project Phase + Affiliation + Total Pairing Time + Current Condition + Well-meshed	36.45	<0.0001	3069.20	3069.83	6.36	6.36
M9	Yes		Dominant + Experimental Group + Total Pairing Time + Current Condition + Tense + Current Condition*Tense	35.22	<0.0001	3069.39	3070.02	6.55	6.55
	Yes		Dominant + Project Phase + Affiliation + Total Pairing Time + Current Condition	30.83	<0.0001	3070.34	3070.82	7.50	7.36
	Yes		Dominant + Current Condition + Tense + Current	26.47	<0.0001	3070.96	3071.33	8.13	7.86

		Condition*Tense							
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5									
6	No	Not parsimonious	Dominant + Project Phase + Affiliation + Total Pairing Time + Current Condition + Tense	32.67	<0.0001	3071.25	3071.88	8.41	8.41
7									
8									
9	No	Not parsimonious	Dominant + Project Phase + Affiliation + Total Pairing Time + Current Condition + Foraging enrichment	31.44	<0.0001	3071.67	3072.29	8.83	8.83
10									
11									
12	Yes		Dominant + Project Phase + Affiliation + Total Pairing Time	26.49	<0.0001	3071.97	3072.33	9.13	8.86
13									
14	No	Not parsimonious	Dominant + Project Phase + Affiliation + Total Pairing Time + Current Condition + Agonistic	31.15	<0.0001	3071.93	3072.56	9.09	9.09
15									
16									
17	No	Not parsimonious	Dominant + Project Phase + Affiliation + Total Pairing Time + Current Condition + Friendly	31.45	<0.0001	3071.97	3072.60	9.13	9.13
18									
19									
20	No	Not parsimonious	Dominant + Project Phase + Affiliation + Total Pairing Time + Current Condition + Anxious	30.89	<0.0001	3072.29	3072.92	9.45	9.45
21									
22									
23	No	Not parsimonious	Dominant + Project Phase + Affiliation + Total Pairing Time + Current Condition + Abnormal	30.83	<0.0001	3072.32	3072.94	9.48	9.48
24									
25									
26	No	Not parsimonious	Dominant + Project Phase + Affiliation + Total Pairing Time + Experimental Group	28.94	<0.0001	3072.50	3072.98	9.66	9.52
27									
28									
29	No	Not parsimonious	Dominant + Project Phase + Affiliation + Total Pairing Time + Tense	28.78	<0.0001	3072.59	3073.07	9.75	9.61
30									
31									
32	No	Not parsimonious	Dominant + Project Phase + Affiliation + Total Pairing Time + Cohort	28.6	<0.0001	3072.73	3073.22	9.89	9.75
33									
34	Yes		Dominant + Project Phase + Affiliation	21.6	0.0001	3073.30	3073.55	10.46	10.09
35									
36									
37									
38	No	Not parsimonious	Dominant + Project Phase + Affiliation + Total Pairing Time + Current Condition + Friendly + Current Condition*Friendly	32.76	<0.0001	3072.90	3073.69	10.07	10.22
39									
40	No	Not	Dominant + Project Phase + Affiliation + Total Time	24.29	0.0001	3073.39	3073.75	10.55	10.29
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3		parsimonious	Indoors						
4									
5	Yes		Current Condition + Tense + Current Condition*Tense	20.43	<0.0001	3074.01	3074.26	11.17	10.80
6									
7	No		Dominant + Project Phase + Affiliation + Cohort	21.76	0.002	3075.18	3075.55	12.34	12.08
8									
9		Not	Dominant + Experimental Group + Affiliation + Total						
10	No	parsimonious	Pairing Time + Current Condition + Well-meshed	29.48	<0.0001	3076.90	3077.52	14.06	14.06
11									
12	Yes		Dominant + Project Phase	14.64	0.0007	3077.67	3077.84	14.83	14.37
13									
14	Yes		Dominant + Experimental Group + Affiliation	19.3	0.0002	3077.62	3077.87	14.78	14.41
15									
16		Not	Dominant + Project Phase + From Same Social Group						
17	No	parsimonious		17.18	0.0007	3077.83	3078.08	14.99	14.62
18									
19		Not	Dominant + Project Phase + Total Time Indoors						
20	No	parsimonious		16.87	0.0008	3078.03	3078.29	15.19	14.82
21									
22		Not	Dominant + Experimental Group + Affiliation + Total						
23	No	parsimonious	Pairing Time + Current Condition	23.3	0.0003	3078.14	3078.62	15.30	15.16
24									
25		Not	Dominant + Experimental Group + Groom Given						
26	No	parsimonious		17.52	0.0006	3078.75	3079.01	15.91	15.54
27									
28		Not	Dominant + Experimental Group + Affiliation + Total						
29	No	parsimonious	Pairing Time + Current Condition + Tense	25.89	0.0002	3078.72	3079.35	15.88	15.88
30									
31			Dominant + Experimental Group + Affiliation + Total						
32	No		Pairing Time + Current Condition + Inactive	24.65	0.0004	3078.83	3079.35	15.99	15.88
33									
34		Not	Dominant + Project Phase + Menses						
35	No	parsimonious		14.95	0.0019	3079.42	3079.68	16.58	16.21
36									
37		Not	Dominant + Experimental Group + Grooming						
38	No	parsimonious		17.22	0.0006	3079.48	3079.74	16.64	16.28
39									
40		Not	Dominant + Experimental Group + Affiliation + Cohort						
41	No	parsimonious		19.69	0.0006	3079.41	3079.77	16.57	16.30
42									
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4	No	Not parsimonious	Dominant + Experimental Group + Affiliation + Total Pairing Time	19.55	0.0006	3079.48	3079.84	16.64	16.37
5									
6									
7	No	Not parsimonious	Dominant + Project Phase + Cohort	14.69	0.0021	3079.63	3079.89	16.79	16.42
8									
9	Yes		Dominant + Affiliation	12.88	0.0016	3079.77	3079.94	16.93	16.48
10									
11	No	Not parsimonious	Dominant + Experimental Group + Affiliation + Total Pairing Time + Tense	22.76	0.0004	3079.69	3080.17	16.85	16.71
12									
13	Yes		Dominant + Groom Given	11.86	0.0027	3080.61	3080.78	17.77	17.31
14									
15	Yes		Project Phase	8.52	0.0035	3081.02	3081.13	18.18	17.66
16									
17									
18	No	Not parsimonious	Dominant + Affiliation + Menses	13.96	0.003	3080.89	3081.15	18.05	17.68
19									
20	No	Not parsimonious	Dominant + Affiliation + Agonistic	13.41	0.0038	3081.23	3081.28	18.39	17.81
21									
22	Yes		Dominant + Grooming	11.04	0.004	3081.53	3081.70	18.69	18.24
23									
24	Yes		Dominant + Experimental Group	12.49	0.0019	3081.83	3082.00	18.99	18.54
25									
26	No	Not parsimonious	Project Phase + Menses	8.66	0.0132	3082.89	3083.06	20.05	19.59
27									
28	No	Not parsimonious	Dominant + Experimental Group + Agonistic	12.91	0.0048	3083.27	3083.53	20.43	20.06
29									
30	No	Not parsimonious	Dominant + Experimental Group + Menses	13.15	0.0043	3083.32	3083.58	20.48	20.11
31									
32	Yes		Affiliation (all dyadic affiliative behavior and recruit and cothreat behaviors)	6.02	0.0141	3083.54	3083.64	20.70	20.18
33									
34	No	Not parsimonious	Dominant + Experimental Group + From Same Social Group	12.72	0.0053	3083.70	3083.70	20.86	20.23
35									
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4	No	Not parsimonious	Dominant + Experimental Group + Total Pairing Time	12.74	0.0052	3083.68	3083.93	20.84	20.47
5									
6									
7	No	Not parsimonious	Dominant + Experimental Group + Cohort	12.64	0.0055	3083.75	3084.00	20.91	20.54
8									
9	Yes		Dominant	6.33	0.0119	3083.97	3084.07	21.13	20.60
10									
11	Yes		Groom Given	5.66	0.0174	3084.01	3084.11	21.17	20.65
12									
13									
14									
15	Yes		Study Week (not used further because Project Phase performed better and is essentially a coarser recode of this variable)	11.79	0.019	3083.76	3084.12	20.92	20.65
16									
17									
18	No	Not parsimonious	Dominant + Current Condition	8.44	0.0147	3083.98	3084.15	21.14	20.69
19									
20									
21	No	Not parsimonious	Dominant + From Same Social Group	8.64	0.0133	3084.31	3084.32	21.47	20.86
22									
23									
24	No	Not parsimonious	Affiliation + Menses	6.74	0.0344	3084.88	3085.05	22.04	21.58
25									
26	Yes		Grooming	4.39	0.0361	3085.15	3085.25	22.31	21.78
27									
28									
29	No	Not parsimonious	Dominant + Agonistic	7.06	0.0293	3085.21	3085.38	22.37	21.91
30									
31									
32	No	Not parsimonious	Affiliation + Cohort	6.33	0.0422	3085.24	3085.41	22.40	21.94
33									
34									
35	No	Not parsimonious	Dominant + Menses	7.02	0.0298	3085.38	3085.55	22.54	22.08
36									
37	Yes		Related	7	0.03036	3085.47	3085.64	22.63	22.17
38									
39	No	Not parsimonious	Dominant + Tense	6.85	0.0325	3085.57	3085.74	22.73	22.27
40									
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4	No	Not parsimonious	Dominant + Current Condition + Tense	8.85	0.0313	3085.68	3085.93	22.84	22.47
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6									
7	No	Not parsimonious	Dominant + Cohort	6.42	0.0404	3085.90	3086.07	23.06	22.61
8									
9									
10	No	Model χ^2 too low	Total Time Indoors	3.04	0.0811	3086.63	3086.73	23.79	23.27
11									
12									
13	No	Model χ^2 too low	Total Pairing Time	2.37	0.124	3087.22	3087.22	24.38	23.76
14									
15									
16	No	Model χ^2 too low	Experimental Group	2.31	0.1282	3087.27	3087.27	24.43	23.80
17									
18									
19	No	Model χ^2 too low	Current Condition	2.05	0.1519	3087.41	3087.41	24.57	23.94
20									
21	Yes		AnimalID random effects only (empty model)	.	.	3087.45	3087.50	24.62	24.04
22									
23									
24	No	Model χ^2 too low	Foraging enrichment	1.59	0.2077	3087.85	3087.85	25.01	24.38
25									
26									
27	No	Model χ^2 too low	Mutual Groom	1.43	0.2312	3088.12	3088.12	25.28	24.66
28									
29									
30	No	Model χ^2 too low	From Same Social Group	1.28	0.2581	3088.12	3088.12		
31									
32	No	Model χ^2 too low	Initial Pairing Condition (Not used further because similar to Total Pairing Time)	1.08	0.2995	3088.41	3088.41	25.57	24.94
33									
34									
35	No	Model χ^2 too low	Well-meshed	0.95	0.3308	3088.53	3088.53	25.69	25.06
36									
37									
38	No	Model χ^2 too low	Agonistic	0.5	0.4783	3088.94	3088.94	26.10	25.47
39									
40	No	Model χ^2 too low	Tense	0.42	0.5149	3089.03	3089.03	26.20	25.57
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3		low							
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5		Model χ^2 too							
6	No	low	Experimental Group + Menses	2.68	0.262	3088.92	3089.09	26.08	25.62
7									
8		Animal ID	PairID + AnimalID nested random effects only (empty						
9	No	alone is better	model)	.	.	3088.99	3089.10	26.16	25.63
10									
11		Model χ^2 too							
12	No	low	Menses	0.43	0.5144	3089.04	3089.14	26.20	25.67
13									
14		Model χ^2 too	Groom Received (not used further because of other						
15	No	low	groom variables)	0.3	0.5808	3089.15	3089.15	26.31	25.69
16									
17		Model χ^2 too							
18	No	low	Experimental Group + Cohort	2.55	0.2796	3089.06	3089.23	26.22	25.77
19									
20		Model χ^2 too							
21	No	low	Current Condition + Tense	2.4	0.3016	3089.08	3089.25	26.24	25.78
22									
23		Model χ^2 too							
24	No	low	Inactive	0.17	0.6816	3089.29	3089.29	26.45	25.82
25									
26		Model χ^2 too							
27	No	low	Abnormal	0.08	0.7788	3089.38	3089.38	26.54	25.91
28									
29		Model χ^2 too							
30	No	low	Cohort	0.18	0.675	3089.28	3089.38	26.44	25.92
31									
32		Model χ^2 too							
33	No	low	Total Submissive Behaviors	0.06	0.8048	3089.39	3089.39	26.55	25.93
34									
35		Model χ^2 too							
36	No	low	Friendly	0.05	0.8195	3089.40	3089.40	26.56	25.94
37									
38		Model χ^2 too							
39	No	low	Total Dominant Behaviors	0.11	0.7431	3089.35	3089.45	26.51	25.99
40									
41	No	Model χ^2 too	Anxious	0	0.9937	3089.45	3089.45	26.62	25.99
42									
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low

No	Animal ID alone is better	PairID random effects only (empty model)	.	.	3142.87	3142.92	80.03	79.46
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For Peer Review

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Supplementary Table 2. Candidate model set

	Model parameters	AICc ^a	Δ AICc ^b	L ^c	w ^d	Cumulative w ^e	ER ^f
M1	Y = Dominant + Affiliation + Experimental Group + Current Condition + Tense + Current Condition*Tense	3063.47	0.00	1.00	0.42	0.42	
M2	Y = Dominant + Affiliation + Project Phase + Current Condition + Tense + Current Condition*Tense	3064.25	0.78	0.68	0.28	0.71	1.48
M3	Y = Dominant + Affiliation + Current Condition + Tense + Current Condition*Tense	3066.19	2.72	0.26	0.11	0.81	3.91
M4	Y = Dominant + Affiliation + Project Phase + Current Condition + Total Pairing Time + Inactive	3067.30	3.83	0.15	0.06	0.88	6.79
M5	Y = Dominant + Experimental Group + Current Condition + Tense + Current Condition*Tense	3068.31	4.84	0.09	0.04	0.91	11.27
M6	Y = Dominant + Affiliation + Experimental Group + Total Pairing Time + Current Condition + Well-meshed + Current Condition*Well-meshed	3068.59	5.12	0.08	0.03	0.95	12.95
M7	Y = Dominant + Project Phase + Current Condition + Tense + Current Condition*Tense	3069.61	6.15	0.05	0.02	0.97	21.64
M8	Y = Dominant + Affiliation + Project Phase + Total Pairing Time+ Current Condition + Well-meshed	3069.83	6.36	0.04	0.02	0.98	24.06
M9	Y = Dominant + Experimental Group + Total Pairing Time + Current Condition + Tense + Current Condition*Tense	3070.02	6.55	0.04	0.02	1.00	26.50

^aAICc: Corrected Akaike Information Criterion

^b Δ AICc: Difference in AICc value from that of M1

^cL: Model likelihood calculated from the formula $L(g_i|data) = \exp(-(1/2)\Delta AICc_i)$

^dw: The Akaike model weight ($L_i / \sum_{j=1}^R L_j$). A measure of the strength of the evidence for that model, represented as a probability.

^eER: The evidence ratio, which is calculated by the weight of the best model divided by the weight of the given model