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Benefits of pair housing are consistent across a diverse population of rhesus macaques

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Abstract

Introducing singly housed rhesus macaques (*Macaca mulatta*) into isosexual pairs is widely considered to improve welfare. The population of laboratory rhesus macaques is heterogeneous on a variety of factors and there is little literature available to directly evaluate the influence of many of these factors on the benefits of pair housing. Subjects were 46 adult female and 18 adult male rhesus macaques housed at the Tulane National Primate Research Center and the Yerkes National Primate Research Center. Behavioral data totalling 859 h and serum cortisol levels derived from 312 serum samples were analyzed for main effects of housing condition, comparing single housing to pair housing. In addition, a series of analyses were performed to test for interactions between housing condition and seven independent variables: sex, age, prior duration of single housing, presence or absence of a history of self-injurious behavior, and dominance rank, levels of affiliation and agonism in the paired setting. After the collection of 4–8 h of baseline data and three serum cortisol samples, pairs of individuals were introduced to one another and data collection was repeated, no earlier than 4 weeks after introduction. In pair housing both female and male subjects showed decrease in abnormal behavior (females: 54% reduction; $P = 0.001$; males: 18% reduction; $P = 0.0007$) and anxiety-related behavior (females: 35% reduction; $P = 0.0001$; males: 41% reduction; $P = 0.0001$), and increases in locomotion (females: 41% increase; $P = 0.0001$; males: 76% increase: $P = 0.002$). In pair housing, there were no significant sex differences in social behavior. Descriptively, paired females spent 12% of samples engaged in affiliative behavior and 0.5% engaged in agonistic behavior (back-transformed arcsin square root means). The corresponding values for males were 12% and 0.3%. No interaction effects were detected with any of the independent variables tested in this study. Cortisol values varied with sex but did not differ between housing conditions; no differences were detected when any of the above variables were included in the statistical model. Results support the general consensus among those studying the welfare of captive primates that social housing is a potent means for promoting

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Conflicts of interest

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behavioral indicators of the psychological well-being of laboratory primates. These results are of considerable practical significance and include information that refutes common perceptions about the unsuitability of males as socialization candidates, perceived negative

Keywords

Primate; Behavioral management; Social; Welfare; Cortisol

1. Introduction

There is a general consensus among those studying the welfare of captive primates that social housing is the most important means of promoting the psychological wellbeing of laboratory primates (e.g. Committee on Well-being of Nonhuman Primates, 1998; Reinhardt and Reinhardt, 2000; Wolfensohn and Honess, 2005). Psychological wellbeing has been defined as a positive mental state, with freedom from psychological and physiological pain and distress (National Research Council, 1998). Multiple criteria are recommended for the evaluation of psychological well-being, including measures of species-typical behavior, coping, maladaptive behavior, and physiological adaptation to the environment (National Research Council, 1998). The term welfare is sometimes used interchangeably with psychological well-being, but it is often related to the long-term status of the animals, including how they are coping with their living conditions (American Veterinary Medical Association, 2011), while psychological well-being may be related more to the current behavioral or psychological status of the animal (Morton and Hau, 2010). It is well established that socially housed rhesus macaques (*Macaca mulatta*) demonstrate well-being that is superior to that of singly housed primates; pair housed rhesus macaques display more affiliative interactions, physical activity, play, and exploration than singly housed individuals (e.g. Eaton et al., 1994; Schapiro et al., 1996) and less abnormal, stereotyped, and self-injurious behavior (Lutz et al., 2003; Novak, 2003; Schapiro et al., 1996). Social housing may act as a buffer to stress for rhesus macaques as they are exposed to research events (Gilbert and Baker, 2011), removed from social groups (Gust et al., 1994), or transported to different facilities (Fernström et al., 2008).

In the United States, approximately three-quarters of laboratory primates are housed socially (Baker et al., 2007). About half of the rhesus macaques living indoors in laboratories are housed in pairs or small groups, representing nearly 5000 animals (Baker et al., 2007). Pairs assigned to research projects are commonly isosexual because studies often employ a single sex and/or because breeding must be avoided. This population is heterogeneous on a number of factors that may influence the way individuals respond to social housing but the possible effects have not been measured. For example, prior research has demonstrated improved welfare in both female (Eaton et al., 1994) and male adult rhesus macaques (Doyle et al., 2008) when placed in isosexual pairs, but these studies vary in design so that effects of pair housing cannot be directly compared across sex. A more complete understanding of how the benefits of pair housing may differ between the sexes would be valuable because there remains a perception, unsupported by research findings, that males do not derive benefit from isosexual pair housing, if they can be paired at all.

Other factors that may influence response to social housing are prior tenure in single housing and animals' age. One study found that a history of longer duration of single housing led to lower levels of affiliation in a social setting (Taylor et al., 1998). The effect of such tenure in single housing on abnormal behavior has been thoroughly documented. It is positively correlated with the expression of abnormal behavior (Lutz et al., 2003; Rommeck et al., 2009) and the likelihood of engaging in self-injurious behavior (Novak, 2003). Given that behavior changes with time spent living in single caging, it is plausible that the duration of single housing prior to social introduction could affect an individual's response to pair housing.

For those macaques that engage in self-injurious behavior (SIB), it is especially critical to determine the social setting that best controls the expression of this abnormal behavior. Social housing is one of the few potentially effective approaches to treat SIB (Weed et al., 2003). However, self-injurious behavior may function as a coping strategy to reduce arousal (Tiefenbacher et al., 2000). Because some social interactions are stressful, it is possible that interaction with another monkey could elicit self-injury or interfere with normal social responses. For this reason, some people managing macaques may be hesitant to socialize individuals with histories of self-injury for fear of triggering the behavior.

The effects of introduction into pairs may be influenced not only by individual characteristics and history, but also by aspects of the new social relationship as well. First, it is reasonable to question whether pair housing benefits the subordinate member of a pair to the degree that it does the dominant. Behavioral and health measures suggest that this is the case among female pairs (Eaton et al., 1994). In addition, the response to stressful conditions suggest that social buffering occurs for both dominant and subordinate members of male pairs (Gilbert and Baker, 2011), but the interaction between rank and housing has not been fully explored. It is also not known whether the benefits of pair housing are influenced by variation in relationship quality. Across pairs that are deemed successful in terms of rarity of injurious aggression and lack of food monopolization, grooming or fighting may be seen at very different levels. Some pairs may be considered as 'unfriendly' or 'uninterested'. Managers must decide whether to maintain pairs that affiliate rarely or show relatively high levels of agonism. When alternative partners are available, this decision may be easy to make, but this is not always the case. Within a biomedical research project, the composition of pairs is often dictated by treatment group, each of which may consist of a small number of individuals. Therefore, it is important to determine whether relationships judged to be of relatively low quality benefit animals in the same way as those housed in well 'bonded' pairs, or whether partners which affiliate rarely or show high levels of agonism derive any benefit over living alone.

While psychological well-being may be measured by means of multiple axes, such as reproductive success, physical health and responses to stressors (e.g. Novak and Drewsen, 1989; Novak and Suomi, 1988), behavior has been the focus of most of the work evaluating primate socialization. Cortisol is a less common measurement employed in such studies. Several prior studies have compared cortisol levels in singly and pair housed rhesus macaques. Most have not detected differences in cortisol measures or corticoadrenal functioning between macaques housed long term in single versus pair housing (e.g.

Reinhardt et al., 1991a; Schapiro et al., 1993), but at least one study found cortisol levels were lower among adult male rhesus housed in compatible pairs compared to when they were housed singly (Doyle et al., 2008). The current study assesses serum cortisol levels to discern whether pair housing had a measurable effect on hypothalamic–pituitary–adrenal (HPA) axis activity.

Prior studies concerning pair housing of adult rhesus macaques have differed in design, sample size, and diversity of subjects (Doyle et al., 2008; Eaton et al., 1994; Lutz et al., 2003; Novak, 2003; Schapiro et al., 1996). A subject pool of sufficient size and variability with respect to sex, age, and history is necessary for determining how broadly the benefits of pair housing detected in prior research will be garnered across the many sources of variability within the population of caged rhesus macaques. It is necessary for determining whether there are subsets of the singly housed rhesus macaque population that do not experience improved well-being to the same extent as others. The objective of the current study was to permit these assessments, using behavioral and serum cortisol measures on a large sample of adult rhesus macaques housed at two laboratory primate facilities. The following hypotheses, based on the literature reviewed above, were tested. (1) Subjects' sex and age will not affect their behavioral response to pairing. (2) Those subjects with longer histories of single housing will show fewer behavioral benefits (i.e., smaller reductions in abnormal behavior, in anxiety-related behavior, and reduced levels of affiliative behavior) with pair housing than those with shorter histories. (3) Those subjects with histories of self injury will show fewer behavioral benefits (i.e., smaller reductions in abnormal behavior, in anxiety-related behavior, and reduced levels of affiliative behavior) with pair housing than those without such histories. (4) Subordinate and dominant members of pairs will show similar behavioral benefits (i.e., reductions in abnormal behavior, in anxiety-related behavior, and increases in affiliative behavior) with pair housing. (5) Relationship quality, as defined by levels of affiliative and agonistic behavior within the pairs, will not affect the subjects' responses to pairing. (6) Pair housing will not alter cortisol values when compared to single housing.

2. Materials and methods

This study was conducted between September 2001 and May 2008. It was designed as a collaborative research project between the Tulane National Primate Research Center (TNPRC) and the Yerkes National Primate Research Center (YNPRC), United States. Methodologies at the two centers were identical except stated in Section 2.2.

2.1. Subjects and housing

Subjects included 46 female and 18 male rhesus macaques from the TNPRC and the YNPRC (see Table 1). Most subjects were mother reared in breeding groups; however, six experienced nursery rearing with or without social companions, and/or housing with just the mother in a cage.

Females ranged in age from 4.7 to 14.7 years (mean 9.3 years [standard error = 0.4]) and males from 3.7 to 7.1 years (mean 5.2 years [standard error = 0.4]). While all subjects had been housed socially at some point in their lives, the duration of the current period of single

housing at the onset of the study ranged from 4 months to 9.7 years among females (mean 3.0 years [standard error = 0.3]) and from 1 month to 4.9 years among males (mean 1.4 years [standard error = 0.4]). The variability in these durations reflects the different histories of assignment to past research protocols. No socially housed animals were placed into single housing for purposes of this study. Among the study animals, nine females and four males had been observed performing SIB prior to or during the study period. During the study, SIB consisted of self-directed biting and slapping, but no self-wounding occurred.

All aspects of management and research use conformed to applicable United States federal regulations and the guidelines described in the *Guide for the Care and Use of Laboratory Animals* (National Research Council, 1996) and the United States Department of Agriculture's Animal Welfare regulations (1991) and adhered to the study's protocol as approved by the TNPRC and YNPRC Institutional Animal Care and Use Committees. The subjects were housed indoors in rooms maintained on a 12:12-h light:dark cycle and ambient temperature between 18 and 22 °C with a relative humidity of 30–70%. Stainless steel cages had a height of 0.8–0.9 m and floor space of 0.4–0.8 m² depending on body weight and social housing status and in accordance with federal animal welfare regulations. All subjects weighed less than 10 kg and were singly housed in cages with 0.4 m² of floor space cage; following introduction, pairs were housed in twice that amount of space. During the single housing phase of the study, subjects were housed in the same room and next to their future social partner to reduce potential confounds relating to variations in the external environment (e.g. number of animals in the room, identity of caregiver or animals in visual contact).

Animal care staff provided nutritionally complete food biscuits twice daily, and fresh water was available *ad libitum*. Three to five times per week, husbandry staff handed out fruits, vegetables, and other food treats as feeding enrichment. Each cage included a perch and a manipulable object such as a toy, PVC piece or hardwood segment. Animals receiving intervention for abnormal behavior were housed in cages equipped with foraging or grooming devices at the time of enrollment. Devices were not removed during data collection; and were not added to cages for other animals once data collection had commenced.

2.2. Pairing process

For over half of the pairs studied, the identity of the pair-mate was entirely dictated by research constraints (i.e., pairs could only be derived from the same research project and treatment group). When there were options for the composition of potential pairs, no more than two possible pair-mates were available. Pairs were chosen so that individuals were not closely matched in body weight, since greater weight disparities have been associated with a higher success rate at the TNPRC (personal observation). Individuals were also not matched for similarity in temperamental characteristics such as aggressive or fearful responses to humans. All pairs were isosexual.

The current study assessed two housing conditions, a baseline condition involving single housing and an experimental paired condition, each lasting at least 8 weeks. A within-subjects design was used to compare the baseline condition to the continuous full-contact

pair housing condition (hereafter termed ‘pair housing’). Social introductions between potential pair mates began with subjects being placed into protected contact by replacing the solid panel separating them with a panel permitting limited contact. This introductory phase lasted for 1 or 2 weeks. At the TNPRC, protected contact involved 7 consecutive days in which individuals could touch through a panel with 12 oblong holes measuring 5.1 cm × 8 cm. At the YNPRC, subjects spent one 24-h period with a panel with a large number of 1-cm holes (permitting visual access and only fingertip contact), 6 days with a panel with 3 cm × 15 cm oblong holes, and 7 days with a panel with 5 cm × 15 cm oblong holes. Most monkeys were subsequently provided entry into one another’s cages (i.e., pair housing) by removing the panel immediately after the introductory protected contact period. Twelve subjects were chosen at random to remain in protected contact for up to 8 weeks to evaluate that form of housing before the panel was removed and subjects attained access to each others’ cages.

Pairs were closely monitored in person and via videotape for injurious or escalating aggression that would have resulted in separation. Introductions proceeded only for pairs that did not display these problems. Not all of the introductions implemented for this study were successful; 21% of female–female introductions were terminated because of persistent agonism, food monopolization, or wounding. All male–male introductions were successful. The present study describes the behavior of the 64 subjects that were maintained in pair housing without evidence of incompatibility. Rank within each pair was determined by the directionality of fear-grimaces and supplants. After completion of the study, animals remained in pair housing to support their well-being; study aims did not include an assessment of separation stress or an attempt to recreate baseline conditions.

2.3. Data collection

2.3.1. Behavioral data—Data were collected and coded by four individuals after the establishment of inter-observer reliability among at least two observers at different facilities, with a minimum of 85% agreement. After the collection of baseline data, the social introductions were performed. Data collection on pair housing began between 4 weeks and 4 months after the introduction process was completed. Videotaping was employed to collect 60-min focal observations with start times held steady across conditions for each individual, in recognition of the effect of time of day on behavior. Data collection was scheduled to avoid daily feedings, routine husbandry, and research procedures. In each phase, 4–8 h of data were collected per animal, for a total of 859 h of observational data. Data were coded with an ethogram including 62 behaviors, using instantaneous sampling with a 15-s inter-sample interval. Predetermined decision rules were applied for priority of data entry for samples in which more than one behavior occurred. Point samples for individual subjects were pooled across observation periods, and statistical analyses were performed using percentage of samples for each behavior in each study phase. Behaviors of interest in the current study were collapsed into seven categories for analysis (see Table 2 for operational definitions).

2.3.2. Serum cortisol data—Serum cortisol assays were performed on blood collected from 52 of the study animals, including 39 from the TNPRC subjects and 13 from the

YNPRC. Assays were performed by collecting blood samples (3 mL) from the femoral vein of anaesthetized monkeys (ketamine 10 mg/kg) according to TNPRC and YNPRC standard operating procedures. Three blood samples were collected from each subject during the same 2-h interval of the morning, generally 1–2 weeks apart within each phase, with the first sample in pair housing collected no earlier than 4 weeks after introduction.

Blood samples were immediately injected into coded vacutainer tubes containing EDTA and centrifuged at 2500–3000 rpm for 8 min. The clear plasma layer was transferred to a second coded eppendorf tube and frozen at -80°C for subsequent cortisol assay using a volume of 100 μL serum and 1 mL of radioassay. Different commercially available kits were used at the TNPRC (Siemens Healthcare Diagnostics [SHD]) and YNPRC (Diagnostic Systems Laboratories [DSL]). Extensive inter-kit comparisons made by the Tulane Endocrine Laboratory demonstrated that values derived from SHD kits will vary from those derived from DSL kits (Mark Wilson, personal communication). Therefore, values were converted to Z-scores for statistical analysis.

Values from the three samples per animal per study phase were averaged for analysis. However, only samples drawn less than 15 min after ketamine injection were used because animals were not trained for cooperation with injection. Therefore results after this window of time could be expected to be influenced by the stress of the injection. On this basis, 3% of samples were excluded, providing only two data points per phase for a small subset of subjects.

2.4. Statistical analysis

All statistics were calculated using Statistica 9.0 for Windows. For all categories of behavioral data analyzed, measures of skewness, kurtosis, and homogeneity of variance failed to meet required assumptions for parametric tests, so data were transformed using an arcsin square root transformation prior to analysis. Multivariate analyses of variance (MANOVA) for repeated measures with sex as a grouping factor were used for the analysis of the five non-social behavioral categories (abnormal, anxiety-related, enrichment-directed, inactivity, and locomotion). Alpha was set at 0.0007 (two tailed), using a Bonferroni adjustment to correct for multiple comparisons. Social behaviors were not included in the MANOVA test because their expression was almost entirely constrained in single housing and these data would have therefore skewed the analysis. Significant sex differences were detected across the nonsocial behaviors (see Section 3.1) so these behavioral changes were analyzed separately for each sex. Following a significant overall MANOVA, its univariate results identified which behavioral categories differed significantly.

Within each sex, subjects were classified in the following ways: (1) age, (2) duration of prior single housing, (3) history of SIB, (4) dominance rank in pair housing, (5) percent samples engaged in affiliative behavior in pair housing, and (6) percent samples engaged in agonistic behavior in pair housing. A series of multivariate analysis of covariance (MANCOVA) or MANOVAs were performed using housing condition and each of the six subject classifications as independent variables. MANCOVAs were applied to the continuous variables (age, duration of single housing, levels of affiliative and agonistic behavior) and MANOVAs to the categorical variables (SIB, dominance rank).

Housing condition was not employed as an independent variable in analyzing social behavior. In addition, sex was not included as an independent variable in analyses of social behavior since there were no sex differences found (see Section 3.1). Therefore a second set of analyses was conducted on the two social behaviors, using as independent variables age, duration of prior single housing, and history of SIB as independent variables, with Pearson correlations used for the two continuous independent variables (age and duration of prior single housing) and ANOVAs for the categorical variable (history of SIB).

Significant sex differences were detected across cortisol values (see Section 3.2). Therefore, analyses of cortisol were explored for each sex separately. These analyses involved MANCOVAs and MANOVAs using housing as an independent variable as well as the five subject classifications listed above.

3. Results

3.1. Behavior

3.1.1. Nonsocial behaviors—A MANOVA applied to the five categories of nonsocial behavior showed significant sex differences ($F_{5,58} = 3.70$; $P = 0.006$) so each sex was examined separately for analyses involving these behaviors. Among females, the overall MANOVA yielded significant results ($F_{5,41} = 88.81$, $P = 0.001$) due to changes in levels of several behaviors (see Table 3). Subjects in pair housing spent less time performing abnormal and anxiety-related behaviors and more time locomoting compared to when they were housed individually.

Age ($F_{5,40} = 1.54$, $P = 0.20$) showed no interaction effect with housing condition. Duration of prior single housing, which was not significantly correlated with age ($r = -0.007$; $P = 0.96$), also had no interaction effect with housing condition ($F_{5,35} = 1.57$, $P = 0.19$). History of SIB ($F_{5,40} = 2.78$, $P = 0.03$) did not show an interaction effect with housing condition. Eventual dominance rank showed no interaction effect with housing condition ($F_{5,40} = 0.32$, $P = 0.90$). Level of affiliative behavior did not correlate with levels of agonistic behavior ($r = -0.23$, $P = 0.13$) and neither factor showed an interaction effect with housing condition (affiliative behavior: $F_{5,40} = 0.76$; $P = 0.59$; agonistic behavior: $F_{5,40} = 1.46$, $P = 0.23$).

Among males, the overall MANOVA applied to the five nonsocial categories of behavior showed a main effect of housing ($F_{5,13} = 15.98$, $P = 0.0001$; see Table 4). Abnormal and anxiety-related behaviors decreased in pair housing as compared to single housing, and locomotion increased.

As with females, age and prior tenure in single housing were not significantly correlated ($r = 0.41$; $P = 0.09$), and no interaction effects were detected with any of the independent variables (age: $F_{5,12} = 1.52$, $P = 0.26$; duration of prior single housing: $F_{5,12} = 0.95$, $P = 0.48$; history of self-injurious behavior: $F_{5,12} = 1.55$, $P = 0.27$; dominance rank: $F_{5,12} = 2.63$, $P = 0.08$; level of affiliative behavior in full contact: $F_{5,12} = 3.92$; $P = 0.03$; level of agonistic behavior in full contact: $F_{5,12} = 7.24$, $P = 0.002$).

3.1.2. Social behaviors—During pair housing, there were no differences between the sexes with respect to social behavior ($F_{2,61} = 0.86$; $P = 0.43$) so the sexes were analyzed together. Descriptively (all values consisting of backtransformed arcsin square root means), paired females spent 12.41% of samples engaged in affiliative behavior and 0.52% engaged in agonistic behavior. The corresponding value for males was 12.42% and 0.33%. Across the sexes combined, individuals spent 0.005% of time behaving affiliatively toward their future partners, and 12.41% of time affiliating in pair housing. Agonistic behavior was directed at partners in 0.04% of samples in single housing and 0.47% in pair housing (levels of agonism were not considered problematic and no pairs were separated due to fighting). No significant effects were found for age (affiliation: $r = -0.11$, $P = 0.38$; agonism: $r = 0.0004$, $P = 0.99$), or duration of prior single housing (affiliation: $r = 0.03$, $P = 0.81$; agonism: $r = -0.21$, $P = 0.10$). History of SIB did not affect either category of social behavior (affiliation: $F_{1,62} = 0.80$, $P = 0.38$; agonism: $F_{1,62} = 0.68$, $P = 0.41$).

3.2. Cortisol

Serum cortisol values varied significantly between females and males ($F_{1,50} = 8.14$, $P = 0.006$) so the sexes were analyzed separately. Among females, z -transformed serum cortisol values did not differ between single and pair housing (single housing: $z = 0.24$, raw score 34.47 ± 2.37 $\mu\text{g/dL}$; pair housing: $z = 0.13$, raw score 31.57 ± 2.06 $\mu\text{g/dL}$; $F_{1,37} = 0.22$, $P = 0.54$). No interaction effects were detected with any of the independent variables (age: $F_{1,36} = 0.73$, $P = 0.27$; duration of prior single housing: $F_{1,36} = 0.07$, $P = 0.73$; history of SIB: $F_{1,36} = 0.61$, $P = 0.31$; dominance rank: $F_{1,36} = 0.05$, $P = 0.82$; level of affiliative behavior in full contact: $F_{1,36} = 0.35$; $P = 0.45$; level of agonistic behavior in full contact: $F_{1,36} = 0.64$, $P = 0.30$). Among males, z -transformed serum cortisol values did not differ between single and pair housing (single housing: $z = -0.66$, raw score 25.01 ± 3.01 $\mu\text{g/dL}$; pair housing: $z = -0.38$, raw score 26.27 ± 3.64 $\mu\text{g/dL}$; $F_{1,13} = 0.158$, $P = 0.23$). No interaction effects were detected with any of the independent variables (age: $F_{1,12} = 0.20$, $P = 0.49$; duration of prior single housing: $F_{1,12} = 0.49$, $P = 0.28$; history of SIB: $F_{1,12} = 0.20$, $P = 0.49$; dominance rank: $F_{1,12} = 0.05$, $P = 0.74$; level of affiliative behavior in full contact: $F_{1,12} = 0.01$; $P = 0.86$; level of agonistic behavior in full contact: $F_{1,12} = 0.04$, $P = 0.77$).

4. Discussion

Consistent with previous research, this study clearly demonstrated improved behavioral indicators of wellbeing among singly housed rhesus macaques when they were provided a same-sex social partner. Once paired, levels of abnormal and anxiety-related behavior fell and locomotion increased, all widely accepted as indicators of improved welfare. Despite significant main effects of sex on nonsocial behavior, response to pairing was similar in both sexes, supporting our hypothesis. This finding provides scientific validation of the benefits of pair housing for both sexes, without using a patchwork of results from several studies with considerable differences in study design. While the behavioral data indicated improved well-being, the cortisol findings revealed no differences associated with pair or single housing. While this finding was expected based on previous studies (Crockett et al., 1994; Reinhardt et al., 1991a; Schapiro et al., 1993), it is interesting that even across the subgroups of subjects with varying characteristics (e.g. histories of SIB, duration singly housed) there

was no impact on cortisol values, so this measure did not indicate that subgroups were experiencing high levels of stress.

Females and males spent the same amount of time engaged in both affiliative contact and agonism. The fact that females and males performed these behaviors at the same level may be surprising given sex differences among wild rhesus macaques (Bernstein, 1970; Kaufman, 1967) and variation in stress response by the sexes to social separation and reunion in group housed rhesus macaques (Gust et al., 1993). In large captive groups, male rhesus macaques spend about 20% of their time engaged in grooming, but little of this time is directed toward other adult males (Post and Baulu, 1978). It appears that males' need for grooming can be met with pair housing, although they shift the focus of this activity to other males when female partners are not available. This finding is particularly important because of the false perception that male rhesus macaques would not benefit from isosexual pair housing or simply cannot live in a pair housed situation without injury and disruption to research protocols. Behavioral management programs for rhesus macaques should not exclude males *a priori* from pair housing.

This study found no evidence that age impacted the behavioral outcomes of pairing among these adult subjects, supporting our hypothesis. While subadult and adult rhesus macaques show differences in aggressive and prosocial responses in large social groups of rhesus macaques (Schwandt et al., 2010), differences were not found within the subjects' age range of 4–17 years. However, because the ages of female subjects ranged up to 17 years, possible effects of senescence could have been detected; that none were found contrasts with age-associated social changes in captive and naturalistic groups (Gust et al., 2000; Hoffman et al., 2010). Also, in contrast to a previous study of bonnet macaques (Taylor et al., 1998), this study found no effects of the duration of prior single housing on levels of affiliative behavior, or any other behavior, in either females or males. This variable did not interact with housing condition for cortisol values. These results should dispel any perception that monkeys become accustomed over time to single housing and therefore would have little to gain from pairing, or that tenure in single housing would perturb the ability of monkeys to respond appropriately to social companions. It is also interesting in light of the fact that increases in the duration of single housing is certainly known to alter behavior, but these changes do not interfere with the efficacy of pair housing.

Another key finding of this study is that a history of SIB did not diminish the benefits of pair housing. Individuals with SIB responded to the stimulation of a social partner in the same manner as animals without this pathology and did not self-injure in pair housing, which suggests that they are not a more vulnerable or less appropriate population for pairing. This finding contradicts our hypothesis that differences would be revealed based on a history of SIB. Since social housing shows promise as an intervention for treating this behavior, these results are encouraging. However, it must be noted that pair housing did not extinguish self-biting; three subjects were recorded self-biting at least once during the full-contact phase. Pair housing is not a cure for SIB (e.g. Reinhardt and Rossell, 2001).

There is physiological and behavioral evidence that social subordination is a chronic stressor in group-housed rhesus macaques (e.g. Wilson et al., 2005, 2008). Therefore, it is legitimate

to ask whether both the subordinate and the dominant animals benefit from pair housing when living in indoor caging, and, if so, to the same degree. The current study found, among both sexes, that subordinate individuals show the same reductions in abnormal and anxiety-related behavior and the same increases in activity as dominant individuals. This finding supports our hypothesis. In addition, rank did not interact with housing condition with respect to cortisol values. These findings suggest that perhaps the stress of single housing outweighs the stress associated with subordinate rank, if any. This point is of considerable practical importance because primate caregivers are often concerned about the welfare of subordinate members of pairs. For example, subordinate animals may be unable or unwilling to take prized feeding enrichments from staff, who infer that the subordinate animal is distressed and would be better off alone. The findings of this study can allay that concern and emphasize the value of a social companion despite the monopolization of food treats.

This study found no evidence that behavioral benefits of pair housing were dampened for pairs that showed relatively low levels of affiliation or high levels of agonism. Pairs that appeared to have a less positive relationship still benefitted from the presence of a companion. It must be noted, however, that this negative finding pertained only to the variability in social measures among the pairs studied here. Results do not suggest that alternative partners should not be tested when available. There is certainly a threshold of relationship quality that could dampen the benefits of pair housing (e.g. pairs in which subordinates are harassed or persistently fearful). That threshold was not reached in this study. Some negative social dynamics should be tolerated if the only alternative is returning animals to single housing.

One might predict that restricted rearing backgrounds would result in a poorer outcome than mother-rearing in a social setting due to the well-documented social and cognitive deficits, behavioral abnormalities, and neophobic and fear-related responses associated with restricted early histories (e.g. Kempes et al., 2008, 2009; Mason, 1963; Rosenblum et al., 2001). While rearing background is a source of potential variability in response to pair housing, the background of study subjects did not lend itself exploring rearing as an independent variable; the sample size was small and the variability in rearing environments was large (see Section 2.1). Descriptively, among the subjects (all females) reared in restrictive settings, levels of abnormal behavior dropped 72% and anxiety-related behavior by 17%; locomotion increased by 30%. These values hint that at least some degree of rearing impoverishment may not detract from the benefits of pair housing.

It is also interesting to note that, descriptively, levels of abnormal behavior decreased dramatically in females but more modestly in males. There is some evidence that the effects of pair housing on males may differ between the initial weeks after introduction versus several months later. Doyle et al. (2008) found that in the first few weeks after introduction, abnormal behavior fell dramatically, but this reduction did not remain statistically significant 3–5 months later. Nonetheless, in both the current study and Doyle et al. (2008), males showed improved welfare by several measures other than reductions in abnormal behavior. However, when focused on producing substantial reductions in abnormal behavior over the long-term, additional intervention may be required.

As in several previous studies (Crockett et al., 1994; Reinhardt et al., 1991a; Schapiro et al., 1993), the current study failed to detect changes in serum cortisol due to housing condition, supporting our hypothesis. Notably, one study that did find changes measured fecal rather than serum cortisol (Doyle et al., 2008). Fecal cortisol is a superior method for measuring long-term stress since its collection involves no short-term stressors. With serum, detection of long-term effects is vulnerable to the short-term stress associated with sample collection. While studies can attempt to reduce that confound using only those blood samples that were drawn within a short window of time after ketamine injection, individual subjects may have anticipated that they were soon to be injected, which may have increased the variability of samples and hampered the ability of statistical testing to detect differences. For future studies it may be preferable to assay cortisol from feces, hair, or urine samples. Positive reinforcement training for cooperation with receiving an injection is also a possible strategy for avoiding confounds associated with coercive and aversive procedures (Reinhardt et al., 1991b).

In a laboratory setting, compatible pairs may be separated due to research protocol requirements (e.g. onset of study phases requiring single housing or experimental sacrifice of one member of the pair). As facilities increase the use of pair housing, it becomes more important to document the short- and long-term effects of social separation and explore techniques for reducing the stress of separation. One challenge of implementing a socialization program is deciding whether to introduce animals that are known to require single housing in the near future. The effects of separation should be measured against the benefits to the animal of pair housing, and more research is needed to make this comparison. Such studies could be conducted opportunistically without imposing separation or single housing solely for answering these questions.

This study adds to the scientific literature which should guide the use of pair housing to improve behavioral indicators of well-being for captive rhesus macaques. Nonetheless, it is important to note that this study assessed well-being in successfully introduced individuals, and that there are a multitude of questions related to the introduction process that were not addressed. Examining the outcome of the introduction process has not been analyzed across a diverse pool of subjects and the variables associated with successful (and unsuccessful) introductions must be determined to design effective socialization programs, to provide maximum benefit with minimal risk, and to potentially tailor methodology to individual animals.

5. Conclusion

Introducing singly housed adult rhesus macaques into isosexual pairs reduced expression of abnormal behaviors and anxiety-related behaviors, increased locomotion, and permitted the expression of a full range of social interaction. These consequences of pairing did not differ across the variability in the study population; sex, age, prior tenure in single housing, history of self-injurious behavior, or the eventual rank and amount of affiliation and agonism moderated these findings. These results are of considerable practical significance given skepticism regarding the suitability of some animals as candidates for pairing for a variety of reasons. Concerns for individuals that are subordinate and pairs that affiliate relatively rarely

or evince a small degree of conflict should not lead to social separation without recognition of the benefits that they derive from social housing. Restricting or tailoring socialization programs according to these variables may not be necessary in order to impart improved welfare across the diverse population of singly housed rhesus macaques housed in laboratories.

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Table 1

Study subjects.

Rearing	TNPRC			YNPRC		
	Mother-rearing	Other rearing	Unknown	Mother-rearing	Other rearing	Unknown
Females	22	4	6	12	2	0
Males	12	0	0	6	0	0

Table 2

Behavioral categories analyzed (in bold).

Abnormal

Appetitive: coprophagy, feces paint, regurgitate, urine drink
 Locomotor stereotypes: bizarre posture, flip, floating limb, head toss, jump, pace, rock
 Non-injurious self-directed: eye-poke, self-clasp, self-mouth
 Overgroom: pluck hair from self, using hands or teeth; may include ingesting hair
 Self-injurious: self-bite, self-slap (no self-wounding occurred during the study)

Anxiety-related

Body shake: rapid shaking of head and shoulders
 Scratch: vigorous strokes of the hair
 Self-groom: any picking, stroking and/or licking of one's own body hair, including biting or chewing on nails
 Yawn: monkey opens mouth wide, often exposing teeth

Affiliative

Contact affiliative: groom, rest in contact, social play, cling, mount, genital explore
 Non-contact affiliative: attempt to touch, lip-smack, present

Agonistic

Contact aggression: moderate aggressive contact (pushing, pulling, grabbing, minor scratching), severe aggressive contact (biting with injury)
 Non-contact aggression: bob, cage shake, cringe, crook tail, ear flick, fear grimace, flee, grab, jaw snap, lunge, open-mouth stare, rapid glances, rump present, stare, teeth grinding, attempt to bite

Enrichment-directed

Attack enrichment: bite or grab manipulable object (e.g. toy) in an aggressive manner
 Display with enrichment: use manipulable object to threaten by shaking, throwing, etc.
 Manual/oral enrichment manipulation: Touch, handle, forage, chew, lick, suck manipulable object

Inactive: passive, awake or asleep**Locomotion:** walk, climb, jump

Table 3

Effects of housing condition on nonsocial behavior among female rhesus macaques: backtransformed arcsin square root mean% samples (with transformed means and standard errors in parentheses).

	<i>F</i> _{1,45}	<i>P</i>	Single housing	Pair housing
Abnormal	48.21	0.00	8.73 (30.00 ± 3.76)	3.02 (17.47 ± 2.84)
Anxiety	159.11	0.00	9.79 (31.82 ± 1.70)	6.13 (25.02 ± 1.52)
Enrichment	2.90	0.10	1.19 (10.95 ± 1.33)	1.12 (10.61 ± 1.16)
Inactivity	8.84	0.005	43.31 (71.83 ± 3.14)	35.65 (63.98 ± 2.53)
Locomotion	104.24	0.00	1.51 (12.34 ± 1.07)	2.46 (15.76 ± 0.92)

Table 4

Effects of housing condition on nonsocial behavior among male rhesus macaques: backtransformed arcsin square root mean% samples (with transformed means and standard errors in parentheses).

	$F_{1,17}$	P	Single housing	Pair housing
Abnormal	16.99	0.001	4.35 (21.00 ± 5.61)	3.06 (17.57 ± 4.89)
Anxiety	51.66	0.000	13.63 (37.81 ± 2.80)	7.26 (27.28 ± 3.09)
Enrichment	0.65	0.43	3.38 (18.50 ± 3.38)	2.89 (19.86 ± 3.27)
Inactivity	0.001	1.00	36.63 (65.00 ± 3.79)	27.95 (55.70 ± 3.79)
Locomotion	12.89	0.002	1.70 (13.06 ± 2.02)	3.39 (18.51 ± 2.25)