Pair Housing of Macaques: A Review of Partner Selection, Introduction Techniques, Monitoring for Compatibility, and Methods for Long-Term Maintenance of Pairs

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Abstract

Pair housing of macaques has become a widely implemented compromise between meeting the social needs of the monkeys and allowing for their use in biomedical research. While beneficial to the animals, pair housing can provide challenges for those caring for them. Drawing from both scientific literature and direct experience, this paper provides a review of practical aspects of pair housing including partner selection, pairing methodologies, staff education, and equipment considerations. Recommendations include selecting a pairing method appropriate to the facility and the individual animals being paired, educating staff on social behavior, and establishing a pair monitoring program to facilitate long-term pair maintenance. Assessment of behavior is essential in determining the compatibility of new pairs and in identifying established pairs that may need interventions to enhance their long-term compatibility. The pair housing program at the Yerkes National Primate Research Center is described as one model of a successful program.

Keywords

Socialization; pairing; captive social management; welfare

INTRODUCTION

The Importance of Pair Housing

Providing laboratory primates with an appropriate social environment is the best technique for fostering species-typical behavior, which is a commonly used marker of welfare [Novak & Suomi, 1988; Snowdon & Savage, 1988]. Macaques that spend extensive periods housed alone often express behavioral problems such as incompetent sexual and maternal behavior [Suomi et al., 1971; Goy et al., 1974; Suomi, 1978], hyperemotional behavior [Capitanio, 1986; Suomi, 1991], and a variety of abnormal behavior patterns [Lutz et al., 2003]. Infants raised without conspecifics are especially vulnerable to these effects [Suomi, 1997; Gottlieb et al., 2013].

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Housing macaques in large social groups that mimic their social organization in the wild is desirable, but is often not practical within the biomedical research setting. Pair housing of macaques has become a widely implemented compromise, as pairs can be maintained in standard indoor caging, allowing access for research procedures while meeting the requirements for social housing as set by some standards [e.g., NRC, 2011]. Pair-housed macaques experience many advantages over singly-housed monkeys, including higher levels of affiliative interactions, physical activity, play, and exploration [Eaton et al, 1994; Schapiro et al., 1996; Baker et al., 2012a], and fewer abnormal behaviors [Schapiro et al., 1996; Lutz et al., 2003; Baker et al, 2012a].

Many publications describe pair housing, and we direct the reader to this literature [for a list of relevant references see ASP, 2015], including particularly important reviews by Seelig [2007] and DiVincenti and Wyatt [2011]. However, there is little published literature that addresses the practical elements of pair housing, such as the selection of potential partners, introduction techniques, methods for monitoring pairs for compatibility, and procedures for maintaining pairs over long periods. The focus of this manuscript is to describe these practices, to relate them to the scientific literature, and to provide a working example of these practices within an established pair housing program.

Identifying Social Partners

The goal of pair housing is to find compatible partners that can enhance the psychological well-being of both animals, while maintaining their physical health and meeting research objectives. Many variables should be considered when identifying potential partners, including individual characteristics such as sex, age, and weight; study assignment; viral status; rearing history; abnormal behavior history; and temperament. Other factors such as caging availability, personnel time, research timelines, and planned anesthetic accesses must also be considered when choosing which animals to introduce to one another. Some variables influence the prioritization of introductions (e.g., age, research timelines, behavioral problems) while others influence whether introductions take place at all (e.g., availability of same-sex partner or partner with compatible viral status). Potential pairs should be evaluated on a case-by-case basis, and variables will differ between institutions and research projects.

Sex, Age, and Weight.—Sex is often the first characteristic considered when choosing potential partners, and same-sex pairs tend to be the norm [e.g., Reinhardt, 1994b; Byrum & St. Claire, 1998; Lynch, 1998], as reproduction is often not desired. Assigning equal numbers of males and females to a study will facilitate forming same-sex pairs.

Heterosexual pairings both within and across macaque species have also been accomplished successfully [Rehrig et al., 2014] and can be especially useful in providing socialization for prepubescent monkeys. If opposite sex, sexually mature macaques are to be paired, contraception may be required. All contraception options (gonadectomy, vasectomy, tubal ligation, or chemical methods) carry risk or may have potentially adverse side effects that could compromise an active study or prevent future animal assignment to certain protocols [e.g., Hild-Petito et al., 1998; LaCreuse et al., 2009; Cruzen et al., 2011]. Surgical

contraception is not ideal as it is generally irreversible, causes permanent hormonal changes, and can cause pain even if appropriately treated with analgesics.

Investigators often request animals similar in age and weight for study assignment due to the nature of the research. Experimental materials (e.g., medications, antibodies, or immunizations) can be expensive or of limited availability, in which case younger animals of smaller size and body weight may be preferred. Older and/or larger animals may be needed if a study requires sexually mature animals, if large amounts of biological samples are to be collected, or if certain biomedical implants are being used. When similar weights are not required for research purposes, pairings of adult rhesus males with greater weight disparities are more likely to be successful than pairs with more similar weights [Doyle et al., 2008; Capitanio et al., 2015]. This may indicate that a difference in body mass may act to reduce dominance contests involving physical contact [e.g., Preuschoft & van Schaik, 2000]. In contrast, recently presented findings indicate that weight disparities do not impact pairing success in adult male cynomolgus macaques [Abney et al., 2014], nor for juvenile or young adult pairs of rhesus macaques [West et al., 2009; Maguire-Herring et al., 2013]. Pairings of an adult with an infant or juvenile have high rates of success [Reinhardt 1994a; 93.8% female/infant pairs, 92.3% male/infant pairs] perhaps because they take advantage of the inhibition of aggression towards younger animals by adults [Deag & Crook, 1971; Redican & Mitchell, 1973], and may be good options for housing surplus infants or long-term singly-housed adults [Champoux et al., 1989; Reinhardt 1994a].

Study assignment.—Once a macaque is assigned to a particular research protocol, the pool of potential partners may be limited to others assigned to that study or to those within a study treatment group. Some investigators seek exemption from social housing through the Institutional Animal Care and Use Committee (IACUC) with scientific justification, or animals might be exempted for behavioral reasons or due to veterinary necessity for clinical management related to research interventions [AWR, 2008]. However, with coordination between behavioral specialists, veterinarians, and researchers, monkeys can often still be paired according to study assignment constraints [Tardif et al., 2013]. There are numerous published reports of successful pair housing of animals on water restriction, those with cranial devices or collars (used for pole and collar restraint), and animals participating in behavioral testing [e.g., Hotchkiss & Paule, 2003; Roberts & Platt, 2005; DiVincenti & Wyatt, 2011; Gazes et al., 2012]. Even in infectious disease studies, including some Acquired Immune Deficiency Syndrome (AIDS) related studies, it may be possible to pair house macaques up until the time of inoculation, and then reunite them once infection is established [e.g., Byrareddy et al., 2014].

Viral status.—Potential partners may need to be matched according to their viral status, including their specific pathogen-free (SPF) status. Many primate breeding institutions have established colonies of SPF animals to provide research subjects that are void of certain viral and bacterial diseases of concern. This requires strict separation of SPF-positive and negative animals to maintain the required pathogen exclusions [Tardif et al., 2012].

Rearing history.—Social rearing history (e.g., mother- or peer-reared) must be considered when choosing partners because it influences the expression of social behavior. Animals

with similar rearing histories are often housed together because of presumed similarities in ability of the monkeys to interpret social cues. Young rhesus monkeys prefer those with a similar rearing history, even when unfamiliar [Pratt & Sackett, 1967]. However, this does not preclude the co-housing of animals with mixed rearing histories, particularly early in life [Champoux et al., 1991; Suomi, 1991]. Caution should be taken when pairing adults with different rearing histories because of the potential for reactivity and poor social responses from peer-reared animals [Chamove et al., 1973; MacDonald, 1985].

Abnormal behavior history.—Determining individuals' histories of abnormal behavior is also useful, but existing behavioral problems should not eliminate the opportunity for social housing. Social housing is beneficial for those with abnormal behavior [Baker et al., 2012a] and may mitigate established behavioral problems such as self-biting or stereotypic locomotion [Schapiro et al., 1996; Reinhardt, 1999; Weed et al., 2003; Doyle et al., 2008; Rommeck et al., 2009; Baker et al., 2014a], although it is not guaranteed to be effective in all cases [e.g., Eaton et al., 1994].

Temperament.—Partner temperament impacts macaque introductions [Coleman, 2012]; measurement of individual temperaments shows promise as a partner selection tool. Simple temperament assessments can be completed fairly quickly at cage side by measuring the monkey's response to the presentation of a novel food and/or object, or to a human "intruder" [Kalin & Shelton, 1989] [for methodologies see: Coleman et al., 2005; Coleman et al., 2011; Fairbanks & Jorgensen, 2011]. The resulting temperament rating is matched to similar ratings of potential partners, as adult macaque pairs with similar temperaments seem to be more successful and engage in more affiliative and less aggressive interactions than those with dissimilar temperaments [McMillan et al., 2003]. Young rhesus macaques also prefer the company of others with similar temperaments [Weinstein & Capitanio, 2008]. Patterns of activity and emotionality can also be evaluated and matched to increase pair housing success [Capitanio et al., 2015].

Methods for Introducing Social Partners

Once potential partners are identified, the introduction technique must be selected. Introduction techniques vary by facility and can be influenced by animal-specific variables such as age and familiarity prior to introduction. Some techniques are known by many names and have been employed for many years; others are less widely used. The methods include gradual steps (GS), cage-run-cage (CRC), rapid steps (RS), transport (TR), and anesthetization (AN) introductions. Some methods (e.g., GS, CRC, RS) involve allowing a form of protected contact between potential pair members (e.g., visual or physical contact through physical barriers such as mesh or plastic dividers with holes of various sizes) prior to moving to full contact. Other methods (e.g., TR, AN) move animals directly to full physical contact with a potential partner. Introductions also differ with the type and location of the caging used. Each method has associated pros and cons, including different levels of staff time required and different caging requirements (see Table 1). For all methods involving multiple steps, we advise that progression through steps be based upon observed social interactions of the animals rather than following a predetermined schedule.

Comparing introduction methods.—Choosing the optimal introduction method for a potential pair may depend on factors such as the monkeys' age, sex, and behavioral history, as well as available facility resources such as caging and trained personnel. Understanding the needs of the individuals, available resources, and the strengths and limitations of each method will help you determine how to proceed with an introduction.

The progressive limited contact approach of the GS method provides several benefits: increased observation time that allows staff to assess compatibility, limited wounding capabilities during the initial phases, and promotion of confidence in some monkeys as they have the choice to approach their prospective partner. This method is also flexible – variations can be applied according to available caging (e.g., provision of visual contact through a combination of fine and wide mesh grating, then increasing amount of physical contact until fully paired [Worlein et al., 2015]). The GS method has been successfully applied to both sexes of varying ages and macaque species [e.g., Lynch 1998; Doyle et al., 2008] and to animals with behavioral problems such as self-biting and locomotor stereotypies [Baker et al., 2014a].

In addition to these benefits, the CRC method also provides additional flight space and may be especially useful for large macaques. In this method, progressing to full contact housing occurs outside of the home room which can be beneficial as animals are not likely to redirect aggression resulting from interactions with in-room neighbors to the new partner [Reinhardt, 1994a, b]. These factors make the GS and CRC introduction methods appropriate for consideration even with high-risk animals such as adult males.

However, both the CRC and GS methods require more staff time because of the multiple steps, and CRC requires additional monitoring once monkeys return to their home room. CRC introductions may involve further demands of staff should there be any social discord (e.g., efforts to separate a pair in a run). These gradual introduction methods can possibly create a false sense of security in staff members as contact aggression may not occur until the animals are fully paired, and they may create frustration between the animals due to the delay before full contact.

Fewer steps in the RS method has the benefit of requiring less staff time to introduce a new pair, and staff are more likely to see a full range of dominance-subordinate behaviors earlier in the introduction process, which can improve compatibility assessment. Potential disadvantages are increased short-term distress (due to the sudden change in housing and contact with an unfamiliar monkey) and less time for staff observation of interactions. The RS method may be more appropriate for young and/or female macaques due to the lessened risk of injury.

During transport, monkeys exhibit behavioral and physiological changes reflective of stress [Davenport et al., 2008; Fernström et al., 2008]. This stress may cause monkeys introduced using the TR method to spend more energy coping with environmental changes rather than directing energy toward the new partner, increasing the likelihood of success. If compatible, the new partner may blunt the immediate stress response, playing a social buffering role [Meyer & Hamel, 2014]. TR is a quick method for pairing [e.g., Jorgensen et al., 2015], and

may result in limited *initial* wounding [M. Truelove, personal observation]. This method may be appropriate for macaques of both sexes of any age who have lived together in group housing and are being moved to pair housing, although these pairs do not always work out [Schino et al., 1990]. It can also be useful with unfamiliar young males and with any aged females [e.g., Goosen et al., 1984].

The AN method is relatively quick, is believed to limit *initial* wounding, and is sometimes used to socialize difficult-to-pair individuals such as adult males [e.g., Bourgeois & Brent, 2005; Nelsen et al., 2014]. However, it is not widely used and is somewhat controversial due to concerns about one monkey recovering more quickly and harming the still-anesthetized partner. In addition, post-anesthetic sedation may impact early interactions of the pair. A potential drawback of both the TR and AN methods is that the initial inhibition of wounding, akin to that which may occur during group formation of unfamiliar macaques [Gust et al., 1991], can lead to a false sense that the animals are compatible, when they may not be. More staff time may be required to assess compatibility *after* the pair is established when using this method.

Comparing success rates.—The question that anyone conducting socializations wants answered is, "Which technique is best?" A few studies have compared introduction methods and are informative. Two methods within a single facility were compared across female rhesus and cynomolgus macaques [Sullivan et al., 2009]; researchers found that introducing animals with multiple, full contact sessions of increasing duration had a higher success rate (73%) than the more gradual method using successive placement of dividers (53% success rate). Another study compared a single method across two facilities, one with female rhesus and one with female cynomolgus [Baker et al., 2012b]. Subjects living in protected contact (separated with a divider) were moved into full contact housing with varying success (64% for rhesus pairs, 86% for cynomolgus pairs). Another study retrospectively examined three pairing methodologies used with rhesus macaques at four facilities and determined that success rates ranged from 52–65% for female pairs and 32–69% for male pairs [Baker et al., 2014b]; from this study, it was clear that no one method resulted in the highest success rate for all macaques.

These three studies illustrate the variability of introduction techniques within and across facilities and the value of defining success and reporting success rates to facilitate such valuable comparisons across facilities. Additional studies have reported a range of success rates for adult male macaques: 53% [Crockett et al., 1994] to 94% [Lynch, 1998] for cynomolgus, and 83% [Reinhardt, 1998] to 100% [Doyle et al., 2008] for rhesus, further emphasizing the variability across species and sex. Adult female cynomolgus have had reported success rates ranging from 81% [Kurth & Bryant, 1998] to 100% [Crockett et al., 1994]. Adult female stumptail macaque (*Macaca arctoides*) pairs have a reported success rate of 80% and males, 100% [Reinhardt, 1998]. Differences in success rates across facilities should be carefully scrutinized as they may be due to any number of complicating factors that influence socializations including personnel experience, characteristics of the animal population being introduced, differences in caging or in practices (e.g., avoiding "risky" introductions), differing criteria for introduction success (both in defining and reporting), and different decisions on when to halt introductions [e.g., Hannibal et al., 2015].

Pairing situations perceived as challenging.—Successfully introducing some demographics of macaques can be easier than others; however, pair housing individuals traditionally deemed difficult to socialize can be achieved. Challenging introductions may include animals thought of as too old, singly housed for too long, too behaviorally compromised, or too risky because of their age/sex class or presence of an embedded appliance.

Older macaques (> 17 yrs; [Williams, 1986]) can be successfully paired with one another (although success rates may be relatively low), and with younger animals [Reinhardt & Hurwitz, 1993]. Health considerations such as reduced mobility and delayed wound healing secondary to chronic diseases (e.g., diabetes mellitus) should be taken into account. Although macaques with histories of single housing may have behavioral abnormalities [Lutz et al., 2003] and/or social inhibition [Taylor et al., 1998], they can benefit from pair housing [Baker et al, 2012a; Reinhardt & Hurwitz, 1993; Taylor et al., 1998], as can monkeys with physical [Turner et al., 2012] or psychological [e.g., Weed et al., 2003] impairments. Macaques with these backgrounds may require slower methods of introduction (e.g., GS, CRC) combined with techniques (e.g., visual barriers, social proximity training) to combat heightened reactivity to unfamiliar conspecifics.

Even though adult male macaques pose a challenge because of their large size and the risk of severe injury by their canine teeth, they can be successfully paired, although reduced success rates should be expected. Providing adequate cage space and a period of non-contact familiarization, as well as observing a clear dominant-subordinate relationship prior to moving animals to full contact, is crucial for these pairings to succeed [Reinhardt 1994b; Watson, 2002; Doyle et al., 2008].

Macaques with embedded appliances, such as cranial implants, can also be pair-housed with success rates of 90% or more and maintained on active studies with risks to the appliances being no greater than among singly-housed subjects [e.g., Roberts & Platt, 2005; Doyle et al., 2008; DiVincenti & Wyatt, 2011; APV, 2012; Galvan et al., 2014]. Based on our experience, we recommend that monkeys with embedded biomedical devices be paired *prior to* implantation to minimize risk to appliances.

If needed, there are also alternatives to pairs being fully housed together at all times. Macaques can live long-term with protected contact housing, although there are species differences in the behavioral benefits when compared to full pair housing [Baker et al., 2014a; Lee at al., 2012]. For example, while living in protected contact, abnormal and tension-related behaviors were higher for rhesus than for cynomolgus suggesting that the behavior of female rhesus is more impacted by single housing than cynomolgus females [Baker et al., 2012b]. Macaques can also be maintained in intermittent full contact housing (i.e., full contact with periods of separation) and still experience the benefits of full contact pair housing, so this method is preferred to protected contact housing [Baker et al., 2014a]. The AAALAC website indicates that intermittent full contact housing is a form of social experience that should be considered when full time housing with conspecifics is not possible [AAALAC, 2011a].

Monitoring of Pairs

Assessment for compatibility during pair formation is key to minimizing risk. Per the *Guide for the Care and Use of Laboratory Animals* [NRC, 2011, p.64], "Animals…should be monitored closely during [the] introductory period and thereafter to ensure compatibility." Monitoring macaques requires a significant time investment, including the time to train personnel to accurately recognize and record relevant behaviors and the time to conduct observations. Important considerations for monitoring are: who will do it, which data collection method to use, what behaviors to note, and how much time to observe. It is also advisable to establish guidelines for permissible levels of conflict or injury during the pairing attempt. Macaques vary in dominance style, and conflict is not unlikely during an introduction, so it is important to know how social interactions reflect the dominance relationship between two monkeys when pairing them. For example, rhesus have a despotic dominance style – individuals engage in subordinate-oriented unidirectional aggression, severe aggression occurs frequently, and post-conflict affiliation is infrequent [Preuschoft & van Schaik, 2000]; when pairing this species, one would look for evidence of unidirectional dominance.

Who will monitor the animals.—Knowledgeable personnel are needed to monitor pairs during social introductions. Staff should be trained to recognize and interpret macaque facial expressions, vocalizations, gestures, social interactions, and abnormal and stress-related behaviors and should be familiar with facility introduction and separation procedures. Misinterpretation of behaviors observed during an introduction could result in the premature separation of a new pair or failure to separate a pair that should be split up [Jennings et al., 2009]. Additionally, a shift in an individual monkey's usual behavior during an introduction can indicate pair incompatibility, underscoring the importance of staff familiarity with each individual monkey. Staff should also understand the social dynamics of the species, criteria for intervention in social interactions, and implementation of animal training to ameliorate social problems. Education can be sought by reading published literature, attending laboratory animal and nonhuman primate conferences and workshops, or through in-house behavioral management instruction.

Data collection method.—Data collection techniques can range from informal (e.g., recording observations in a notebook) to formal (e.g., focal-animal observations conducted using a handheld computer and software such as Noldus's Observer©). Determining the level of complexity of the behavioral data is a trade-off between the amount of detail collected and the training time required to gather that information reliably [Martin & Bateson, 1993]. One compromise could be using a simple sampling method of one-zero recording (i.e., indicating if the behavior occurred during the specified time interval or not) with a checklist of target behaviors. This may provide adequate information while allowing for good inter-observer reliability.

Behaviors to be recorded.—Target behaviors should be clearly and objectively defined. For examples of ethograms, see Augustsoon & Hau [1999], Ha et al. [2011], and Xu et al. [2012], and see "The Macaque Website" (www.nc3rs.org.uk/macaques) for video and photographic examples of behavior and thorough information on macaque behavior and

welfare. Since the primary purpose of these data is to monitor social relationships, behaviors such as proximity, threats, aggressive contact, affiliative interaction (e.g., grooming), avoidance, and fear behaviors are important to include. Enlisting and co-threatening (i.e., the recruitment for and engagement of two or more animals displaying simultaneous threats toward a common opponent) should also be recorded because these behaviors are especially good indicators of social compatibility [Watson, 2002; Baker, 2010; Truelove et al., 2014]. It is also useful to monitor animals for increases in abnormal or stress-related behaviors as a result of the socialization process. Changes to baseline levels of behaviors are expected to occur during an introduction; however, any increases in existing problem behaviors should subside shortly after the introduction, or this may point to incompatibility.

Duration of observation periods.—Determining the duration and the timing of observations is key, and balancing this with other personnel responsibilities can be difficult. It is essential to monitor animals during the initial phases of an introduction, and as the social relationship stabilizes over time, fewer/shorter observations are required. Of the primate housing facilities polled by Baker and colleagues [2007], 95% of the responding facilities monitored new pairs beyond the first day. The number of additional days with observation varied depending on factors such as species, social experience, group size, and observed interactions, but most (64%) of these facilities implemented heightened monitoring for over a week [Baker et al., 2007]. Pairs also require some level of continued monitoring following their introduction for on-going assessment of compatibility. Should problems be observed or reported, more in-depth assessments are recommended.

Techniques for Maintaining Pairs

Several behavioral management techniques can be employed to aid in the maintenance of pairs and to promote their long-term success. The primary issues that influence decisions about keeping pairs together include problems with pair separation and reintroduction, resource guarding, and aggression or extreme submission, especially during activities such as feeding.

Positive reinforcement training.—Positive reinforcement training (PRT) can be a powerful technique for addressing difficulties within pairs. Using PRT, pairs can be trained to separate into their individual home cages for research or husbandry needs. Steps involve movement of individuals into separate cages, to designated targets (e.g., small piece of plastic pipe hung on the cage), and then remaining at the targets while a cage divider is placed to separate the animals. Home cage pair separation can be readily used for studies that require pairs to undergo daily separations and re-introductions for sample collection, injections, or cognitive testing, reducing the need for individual housing of animals.

Cooperative feeding training can be employed for macaques experiencing feeding competition [Bloomsmith et al., 1994]. The goal is to reduce aggression during feeding times by reinforcing calm behavior in the food-dominant animal while the subordinate animal receives and consumes food in their presence. Behaviors incompatible to aggressing upon or stealing food from the subordinate (e.g. sitting and staying in one place) are

reinforced in the food-dominant animal, while the subordinate's behavior of taking food in the dominant's presence is reinforced.

Social proximity and positive social interactions between partners can be promoted using PRT, which may be useful in pairs that rarely interact or that have negative interactions; this is especially helpful when partner options are limited. Social proximity is reinforced, encouraging the subordinate animal to initiate proximity and the dominant animal to tolerate that proximity without aggressive behavior. After proximity is accomplished, gentle touching of a partner is shaped using successive approximation [Schapiro et al., 2001, 2003]. The benefit of this type of training has been observed both during and outside of training sessions, suggesting it has a positive effect on animal welfare [Desmond & Laule, 1994; Schapiro et al., 2001].

Provision of resources.—The addition of food or other enrichment items can trigger competition [Honess & Marin, 2006]. To minimize resource-oriented aggression, provide two enrichment devices per pair, with one attached to each home cage to allow space between the two individuals [Schapiro & Bloomsmith, 1994]. Enrichment devices (e.g., puzzle feeders) should be filled simultaneously, and if a food resource is being given to one pair member, it is a general rule-of-thumb to provide that resource to both animals so as to not create competition. Other food-related considerations include using feeding boxes (commercially available feeders, such as the Prima-Forager from Primate Products, Inc.) to increase space between animals during feeding [e.g., Boccia et al., 1988; Schaub, 1995]; distributing smaller pieces of food to provide an opportunity for access by the subordinate [Chancellor & Isbell, 2008]; increasing the frequency of meals to discourage food aggression [Taylor et al., 1997]; and temporarily separating partners for daily feedings to ensure access to food by each animal.

Visual barriers.—Providing a visual break from nearby conspecifics via a barrier located within or on the front of caging can reduce aggression in group-housed monkeys and is thought to also be helpful for pair-housed monkeys [Honess & Marin, 2006]. Increases in proximity between monkeys have been observed when barriers were given, but impacts on affiliative behavior have varied [Reinhardt & Reinhardt, 1991; Basile et al., 2007].

Social space.—When pair housing macaques, one should provide enough physical space [NRC, 2011], and consider the typical activity of the age- and sex-class with which one is working [Buchanan-Smith et al., 2004] to ensure adequate social space, or "the space required by a subordinate partner to buffer potential social tension, by increasing the distance to a dominant counterpart" [Reinhardt & Reinhardt, 2000]. For example, to meet the needs of adult macaques, caging should provide adequate flight space, as well as adequately-sized doorways between adjoining cages. A concern specific to large monkeys is providing a sufficiently large open, pass-through space between adjoining caging to prevent one individual from restricting the other's access to and use of all available space. It is important to identify monkeys who are not freely using their provided space as they are potential targets for future aggression by cage mates. To maximize its usefulness at a facility, caging should be configurable in different ways so that staff can decide how best to

introduce and maintain animals based on their individual characteristics [Jennings et al., 2009].

Providing additional space is another way to help pairs stay together as this supplies the animals with more places to withdraw and engage in solitary activities [Jennings et al., 2009]. This can be achieved by removing a cage floor in adjacent cages to provide more vertical space [e.g., Clarence et al., 2006]; using connecting tunnels between cages to increase complexity and space [e.g., Salzen & Marriott, 1989; Albanese, 2015]; or using exercise cages or multiple racks and cages linked together [e.g., Seier & de Lange, 1996; Martin et al., 2002]. If additional cage space is limited, the decision to provide extra space should be made on a case-by-case basis by evaluating the size, activity, and social interactions of each pair.

Temporary pair separations.—Minimizing unnecessary separations of pairs may help keep them together longer. Allowing newly formed pairs to remain together, undisturbed, for the first few weeks promotes the establishment of the dominance relationship and cementing of the animals' bond [e.g., Lynch, 1998; Reinhardt, 1998]. Avoiding frequent, extended separation of pairs when possible to minimize the risk of aggression at reintroduction is good practice, particularly for high-risk pairs, such as adult males [Lynch, 1998]. However, one study has shown that adult rhesus macaques can be maintained in intermittent full contact housing (separated for two, 24-hour periods per week) without any problems [Baker et al., 2014a], so the need for frequent separations is not sufficient cause for permanent separation. When intermittent full contact housing is not possible, one may mitigate the impact of separations by minimizing the separation duration, permitting visual contact while separated [see Kikusui et al., 2006 and Hennessy et al., 2009 for reviews on social buffering and its benefits], and using PRT for the separation and subsequent re-introduction procedures to reduce the likelihood of aggression. Some macaques may be stressed by visual contact (e.g., clear divider) with a lack of physical contact with their partner, and may do better with protected contact or temporary single housing, so the best arrangement should be determined on a case-by-case basis.

Behavioral assessment.—Behavioral assessment is the foundation of a behavioral management program. With objective, quantitative observations, the most informed management decisions for the animals' welfare can be made. In a pair's lifespan, physical or behavioral symptoms may indicate a social problem; behavioral assessment is a tool that can be used to further discern etiology when it is not apparent. For example, excessive alopecia is sometimes a reason for pair separation, but a veterinary examination should be carried out to eliminate an underlying medical cause of hair loss before it is assumed to be due to hair-pulling, unless hair-pulling has been observed. In a rhesus troop, hair-pulling and ingestion occurred almost unidirectionally (dominant to subordinate animal, 95% of observed occurrences); researchers interpreted this as an aggressive behavior that reflects a maladaptive environmental stress-response [Reinhardt et al., 1986]. This suggests that, when hair-pulling is observed in a pair, closer examination is warranted to determine whether the recipient is exhibiting distress; also, additional observation could reveal patterns of over-grooming (i.e., excessive allogrooming) between pair members. Behavioral management

techniques could be applied in lieu of separation to alleviate on-going environmental stressors or to redirect grooming efforts.

Since the presence of a human observer can influence macaque behavior [Clark & Mason, 1988; Iredale et al., 2010], videotaping can provide valuable insight into the social dynamics of a pair. This may be especially useful when animals show limited interaction during the initial phases of an introduction. A lack of social interaction can make the decision of whether or not to leave two animals paired particularly difficult. Macaques that have an affiliative relationship might huddle together at night [Erffmeyer, 1982; Eaton et al., 1994], even if they have not been observed in relaxed close proximity during daytime observations. If a thorough assessment indicates the pair is not engaging in substantial positive interactions, finding different partners for each should be considered to increase welfare. Partnerships that are only tolerable may not serve as a buffer against potential physiological responses to environmental stressors [Hannibal et al., 2015]; however, pairs who show even low levels of affiliation benefit from having a partner [Baker et al., 2012a].

Permanent Pair Separations.

While social housing generally improves welfare, there are circumstances that warrant consideration of permanent separation of a pair. Reasons to consider pair separation include: persistent and increasing aggression (noncontact or contact) and wounding; alopecia resulting from allogrooming; excessive fear or extreme levels of submissive behavior; resource monopolization (e.g., food); and weight loss and/or decreased appetite. While facilities may set uniform thresholds for when pairs should be separated (e.g., one major wounding event results in a separation), an individual cost-benefit analysis for the welfare of each member of a pair should be part of the decision. For example, even if significant alopecia develops as a result of allogrooming, the welfare of the animal may still be better socially-housed than singly-housed if the hair pulling interactions are not rough or painful. Similarly, the risk of injury must be balanced with the long-term benefits of social housing, and the recognition that single housing carries many risks to welfare. The expectation of injury-free pairings for macaques is not realistic [see Hannibal et al., 2015]. Many times, behavioral management interventions such as those described in the Techniques for Maintaining Pairs section can help alleviate problems between social partners. However, animals who are unable to use available space, cannot freely access food or water because of excessive fear, cannot rest because of constant supplanting, or are losing weight due to food monopolization by their partner, are candidates for separation. These examples highlight the need for ongoing compatibility monitoring of pairs. It is important to remember that the goal of socialization is to improve the welfare for all animals; the welfare of the subordinate is an equally important consideration when evaluating pairing success.

Reporting and Documentation Related to Housing Status

Single housing of social primates is considered an exception to the standards under 9 CFR, and must be reported in research facility's annual reports (see 9 CFR 2.36(b)(3)) [OLAW, 2012]. The reason for each primate's exemption from social housing must be reviewed by the Attending Veterinarian at least every 30 days, unless the exemption is permanent [AWR, 2008, p.84], and a record of all exemptions must be maintained [AWR, 2008, p.85]. Animals

housed in protected contact should be included in this exemption process since protected contact housing is considered a form of single housing [AAALAC, 2011b; OLAW, 2012].

Information should also be maintained that can be compiled at a programmatic level including the outcome of each attempted introduction, the housing that was used (e.g., dividers, novel or familiar caging), and the total number of socially- and singly-housed primates within the colony. Tracking this information will allow one to (1) identify trends (e.g., higher success rate for female pairs than for male pairs), which could lead to program improvements; (2) compare data to other facilities or to published data; and (3) illustrate progress in the program during assessments by external groups (e.g., IACUC, USDA, AAALAC).

Pair Housing Program at the Yerkes National Primate Research Center

This description of the program at the Yerkes National Primate Research Center (YNPRC) is intended to serve as one possible model of a successful program, and some of the decisions we have made are ones that others may also face.

Introduction methods.—The primary mode used when introducing caged pairs at YNPRC is the GS method. The TR method is used when group-housed macaques are transferred from our breeding facility to our indoor housing facility. We occasionally use the RS method with young monkeys and adult females, and we have used the CRC method sparingly due to infrequent access to run space for introductions. Introductions primarily occur in mixed-sex rooms. Same-sex rooms are sometimes advocated [e.g., Reinhardt et al., 1995]; however, based on our experience, this does not seem to be a requirement for success. We try to minimize stressors such as sanitization and construction near rooms where introductions are taking place, as these activities influence behavior [e.g., Begnoche, 2014]. Monkeys are generally given a period of acclimation following relocation to a new cage or room prior to an introduction [Capitanio et al., 2006], allowing behavioral and physiological disturbances to settle; this acclimation time may vary based on an individual's health status and study timelines.

Defining introduction success.—We have defined successful social introductions as monkeys living together for a minimum of 14 consecutive days following the last step in the process. Social introductions continue as long as the following indicators of compatibility are observed [e.g., Reinhardt et al., 1995]: no occurrence of serious wounding or frequent minor wounding; no food monopolization; both monkeys make use of available cage space; no signs of depression or excessive fear; no health problem likely to be caused by social stress (e.g., poor appetite, diarrhea, or poor coat condition); and some positive social interaction (e.g., relaxed close proximity, grooming).

Success rate.—Our success rate for macaque introductions in caged pairs for 2010–2014 was 92.1% (1147 introductions, N =1934 macaques; 1128 *M. mulatta*, 8 *M. nemestrina*, 13 *M. fascicularis* pairs; M=5.4 years; range of 0.02–28.4 years). Pairs were less successful among older subjects (see Table 2). During this five-year period, 6.3% of the introductions (n=98 animals) included some type of wound in the first 14 days (the majority of which

were deemed by veterinary personnel to be minor). In only 1% of the introductions (n =12 animals) was a monkey anesthetized to determine the extent of an injury and/or the need for treatment. Of those introductions that were ultimately successful (as previously defined), 3.7% included a wound, and among those introductions that were ultimately unsuccessful, there was a tenfold higher wounding incidence of 37.5%. Of the 1059 pairs successfully introduced during this period, 41 pairs were later separated permanently for incompatibility (see Table 2).

Behavioral monitoring system.—The pair formation monitoring system at the YNPRC consists of a one-zero style datasheet on which the presence/absence of 55 target behaviors are noted during each observation period; these include social, abnormal, and anxiety-related behaviors. These data provide an objective assessment of social interactions expressed during an introduction and serve as a foundation on which to base our pair management decisions [e.g., Truelove et al., 2014]. During the familiarization stage on day one, adults are monitored for a minimum of 30 minutes; younger animals may be observed for less time. Monitoring times vary throughout the pairing process depending on the social interactions observed, but an average is about 45 minutes at each stage of protected contact (each time a divider is changed allowing more contact between the pair) and 75 minutes at full contact. Once the housing goal is met (e.g., protected contact, full contact), animals are monitored for an additional five weekdays, for a minimum of 10 minutes per day. Subsequently, they are observed three times weekly by behavioral management staff.

Staff communication.—Communication to alert staff of newly formed pairs is vital. Signs are posted on animal room doors to indicate to all staff that an introduction is on-going. Electronic messages are sent to all staff working with or around the new social pair.

Staff is directed to report any behaviors of concern (e.g., aggression, fear, abnormal behaviors, over-grooming) on a document located outside of each room. Behavioral Management staff follows up on all reports and implements any necessary treatment. Over a period of 24 months, we received 127 such reports related to social behavior (15% of total reports), so we feel this method has effectively increased communication regarding social concerns.

Pairs requiring additional assessment.—Beyond the initial introduction, some pairs have possible compatibility problems (e.g., fighting, food-related aggression) and receive an in-depth "pair assessment." Some pairs maintained at protected contact are also given these assessments to ascertain whether we should move them to full contact pairing. Each pair being assessed is observed for a minimum of 2.5 hours over five days during various daily activities. A summary of observations and recommended treatments are communicated to affected staff and are implemented by a behavioral specialist (treatments as described in Techniques for Maintaining Pairs section).

Of the 100 pair assessments conducted to date, 14% of the pairs were deemed incompatible and were moved to protected contact or individual housing. Outcomes varied for the remaining pairs: 67% stayed together or moved from protected to full contact housing; 36% received a treatment and/or additional staff training was implemented.

DISCUSSION

The language used in the current edition of the *Guide* [NRC, 2011] illustrates a shift in thought with regard to social housing of laboratory primates, indicating clearly that social housing is the default. This change has required the development of new animal management practices, job duties, and research programs evaluating the welfare impact of these changes. It has also influenced the way in which biomedical research is being conducted with nonhuman primates, as more research projects are now performed with socially-housed subjects.

Increasing efforts for social housing involves coordination and communication among many staff members with differing roles; veterinarians, behavioral specialists, colony managers, animal care personnel, and laboratory technicians are all valuable in maximizing social housing. People in these differing roles contribute their diverse perspectives and provide unique information related to social housing. A team approach to pair housing benefits the animals, the research, and those managing their care [Rice et al., 2002].

Pair housing of macaques has been successfully accomplished for over 25 years using a variety of approaches. Arming oneself with the knowledge of what to look for when selecting potential partners; which introduction technique to choose; how to monitor a pair once introduced; and how to maintain a pair for the duration of their partnership will help optimize the process. There is no one-size-fits-all approach for all macaque pairs or for all institutions. Numerous factors need to be taken into consideration to maximize the safety and longevity of each pair, to operate within facility limitations, and to use and cultivate staff expertise. This paper compiles published literature, practical experience, and a programmatic description to serve as a resource to consult when planning for introductions or for enhancing an existing socialization program (see Table 3 for a summary of recommendations).

Future goals for social housing programs include increasing the number of successful pair introductions and improving long-term pair maintenance. Pair introduction success may be enhanced by further exploration of predictors of pairing success (e.g. temperament, weight disparity, behavior patterns of successful and unsuccessful pairs) and by additional comparisons of best introduction methods. Future improvements in pair maintenance may involve further assessment of how social dynamics are impacted by management and research decisions (e.g., pair separations or movement of animals between rooms) and evaluation of available methods to address social challenges, such as the use of PRT to reduce competition to increase the efficacy of such tools. As we move ahead, for many institutions there remain persistent roadblocks to social housing programs (e.g. money, staff time, equipment) [Baker et al., 2007]. Institution-wide understanding and implementation of scientifically- and experientially-based best practices in social housing programs will help institutions remove obstacles to advancing social housing programs and attend to the fundamental need of laboratory macaques to live socially while they are subjects of biomedical research studies.

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Table 1.

Comparing pairing methodologies for macaque introductions.

Introduction Method	Introduction Steps	Introduction Location	Potential Advantages	Potential Disadvantages
Gradual Steps (GS)	1 Visual contact ^ 2 Protected contact * 3 Full contact	Home cage	Minimization of injury risk to individuals; incremental compatibility assessment; may build confidence in some individuals.	Increased staff time for monitoring each step; some behaviors (including aggression) may not be evident until full contact; behavioral issues because of delayed full contact. Duration to complete varies widely by facility.
Cage-Run-Cage (CRC)	1 Visual contact ^ 2 Protected contact * 3 Full contact	Home cage for initial steps, run or larger caging for full contact	Minimization of injury risk to individuals; incremental compatibility assessment; may build confidence in some individuals; additional flight space for large macaques.	Increased staff time for monitoring each step; some behaviors (including aggression) may not be evident until full contact; behavioral issues because of delayed full contact. Duration is approximately 10 days.
Rapid Steps (RS)	1 Visual contact ^ 2 Full contact	Home cage	Quick (1 day); some incremental compatibility assessment; less staff time required compared to GS and CRC methods.	Increased short-term distress of individuals; less time spent observing social interactions.
Transport (TR)	Transport box to full contact	Novel cage	Quick (1 day); potentially fewer wounding events at introduction compared to other methods.	Increased cumulative stress between transport and introduction procedures.
Anesthetization (AN)	Full contact at anesthesia recovery	Home cage	Quick (1 day); potentially fewer wounding events at introduction compared to other methods.	Differing rates of anesthetic recovery may pose a danger.

^ Visual contact: provided to two individuals in adjacent caging either through fine mesh or a clear polycarbonate divider.

* Protected contact: provided by the stepwise placement of mesh or grated partitions, grooming bars, or plastic perforated dividers with holes; the width of the mesh/bars or hole size increases as the introduction progresses.

Table 2.

Success rates for macaque pair introductions at YNPRC from 2010 – 2014

	Successful* Introductions # (%)	Successful male-male introductions # (%)	Successful female-female introductions # (%)	Successful mixed-sex introductions # (%)	Successful Pairs later Separated for Social Problems # (%)
Juvenile (0–2 years)	138 (100%)	72 (100%)	35 (100%)	31 (100%)	2 (1.4%)
Subadult (2–4 years)	513 (98.3%)	332 (99.1%)	171 (96.5%)	10 (100%)	14 (2.7%)
Adult (4–16 years)	354 (84.3%)	87 (75.9%)	242 (81.4%)	25 (100%)	22 (6.2%)
Old (> = 17 years)	54 (78.2%)	9 (88.9%)	44 (70.5%)	1 (100%)	3 (5.6%)
Total	1059 (92.2%)	500 (95.2%)	492 (88.3%)	67 (100%)	41 (3.9%)

*
YNPRC defines an introduction as successful if the monkeys live together for a minimum of 14 consecutive days following the last step in the introduction process.

Table 3.

Recommendations for Developing a Pair Housing Program

Vital Program Components	Specific Considerations
Partner Selection Process	Create a process for selecting potential partners that may include considering their sex, age, weight, study assignment, viral status, rearing history, abnormal behavior history, and temperament. Extrinsic factors such as caging availability, personnel resources, research timelines, and planned anesthetic accesses should also be considered.
Introduction Techniques	A variety of introduction techniques are available, and the optimal method for each pair should be chosen depending upon characteristics of the animals, facility, and staff. Most commonly used methods involve some form of protected contact between potential pair-mates prior to moving to full contact, but other methods put macaques directly into full contact. We advise that progression through steps of the process be based upon observed social interactions of the animals rather than on a predetermined schedule.
Special Consideration Populations	Challenging introductions may include animals thought of as too old, singly housed for too long, too behaviorally compromised, too risky (e.g., adult males) or too fragile due to the presence of an embedded appliance, but macaques with all of these characteristics have been pair housed.
Staff Training	Training for those conducting introductions and for those working with established pairs should ensure knowledge of behavior. Pairs should be monitored more during the initial phases of an introduction. Less observation is required as the social relationship stabilizes. Develop a data collection method for monitoring pairs using clearly defined behaviors related to social compatibility. Staff should have expertise in and have time to implement behavioral management techniques to aid in the maintenance of pairs to promote their long-term success. Staff should be able to identify pairs who might require separation.
Equipment	Available equipment should meet the social space needs of individuals and provide maximum flexibility during pair introduction and pair maintenance.
Documentation	Documentation should be maintained on the outcome of each attempted introduction, the housing that was used, individual animal characteristics, and the total number of socially- and singly-housed primates within the colony.