Safe Pair Housing of Macaques

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Animal Welfare Institute

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Animal Welfare Institute
P.O. Box 3650
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www.awionline.org

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INTRODUCTION

It is well-accepted that one of the most important elements in addressing the psychological well-being of nonhuman primates is the provision of appropriate social companionship1. In the natural environment, most species of macaques live in large social groups, with smaller groups consisting of females and their offspring1. They develop complex relationships with one another, and lack of social interaction can have significant negative consequences, including breeding failure, inappropriate parental care, and stereotypic and/or self-abusive behavior[1, 2]. Pair housing is an effective method for the provision of social housing to nonhuman primates in research facilities. It allows for primates to exhibit a wide variety of species-typical behaviors, such as grooming and play.

However, careful planning is an important aspect of pair housing procedures, due to the potential for aggression when strange animals are introduced to one another. Macaques have a strong hierarchal social structure, and challenging of dominance can result in aggression and resultant injuries. Because of this behavior, many facilities have been reluctant to pair house macaques in a laboratory setting[3-6]. However, many reports have shown that, with careful planning and observation of behavior, a very high success rate can be achieved, with both male and female macaques of multiple species[7-13] (see pages 8-9). Indeed, macaques are very social animals who will seek out any form of contact when given the opportunity (Figure 1). The purpose of this booklet is to discuss various pairing methods and describe which behaviors can help to determine the success of a potential pair.

SELECTION OF POTENTIAL PAIRS

Selecting animals as potential cage-mates can be an intimidating task, and a number of strategies have been used to predict which animals may be compatible as a pair. One option is to use a random approach[3, 9, 14], while another option is to evaluate the behavior of the animals prior to introduction; for example, to determine whether the animals have more dominant traits (e.g. assertive behavior, cage shaking) or submissive traits (e.g. looking away, shying away to back of cage)[10, 15-18]. However, one should keep in mind that there is conflicting evidence regarding the influence of an animal's behavior on pairing success[19-21]. Additionally, some authors recommend pairing animals who have not had recent visual access to each other[11]. Other considerations include the age, gender and social history of the animal, as well as the needs of the investigators and the amount of work involved in moving animals[11, 22].

PAIR FAMILIARIZATION

Many authors describe the use of a non-contact familiarization period as a tool for establishing potential macaque pairs[7, 10, 13, 23-25]. During this period, two animals are introduced to each other in a manner that allows non-contact communication, but prevents potentially injurious tactile interactions. A number of methods have been used to allow this type of introduction, including the use of clear plastic panels (Figures 2-3)[3, 7, 26] or mesh dividers (Figures 4-5)[10, 11, 14, 23, 26], as well as utilizing the squeeze back of the cage (Figure 6)[12, 13].

Alternatively, home cages of potential cage-mates can be placed in close enough proximity to allow interactions[16, 27]. For example, two cages can be arranged in close proximity and fruit offered at an equal distance to both animals. It is expected that the more dominant animal will grab the fruit first, while the more subordinate looks away or redirects aggression to the observer (Figure 7).

The primary reason for the non-contact familiarization is to allow animals to establish a dominant-subordinate relationship[9, 23, 28, 29]. Once this

relationship is formed, there should be no reason for the animals to fight when allowed full contact with each other[30, 31]. Some authors suggest that the familiarization should occur in a neutral environment[16], while others have had success without moving the animals to an unfamiliar or neutral space[32].

Evaluating the behavior of the animals during the non-contact familiarization period is critical to the success of the pairing; without the establishment of a dominant-subordinate relationship, the pair is likely to fight following introduction. A wide range of behaviors can be used to determine whether this relationship has been formed; signs include unidirectional fear-grinning (Figure 8), withdrawing (Figure 9), yielding, looking away, and threatening away[14, 24]. Dominant animals may display assertive postures, while submissive animals may hold a more submissive posture (Figure 10), lip smack, present their rump, and facial pucker toward the dominant animal, and may spend more time in close proximity to the divider[7]. It is important that signs of submission are seen uniformly in one animal prior to introduction.

Affiliative behaviors indicative of compatibility include one or both animals spending time near the divider, and one animal mimicking the other[9, 11].

Signs indicating that the pair will likely not be compatible include “finger fighting” through mesh dividers, charging or threatening at each other across the divider, or extreme fear in one animal[10, 11, 13]. Once a clear dominance-subordinate relationship has been established, pairing can proceed, as the animals will have little reason for fighting[14, 24] (Figure 11).

Although non-contact familiarization appears to be important in the formation of most adult pairs, not all pair formations require this introductory period. For example, adult-infant pairs can be formed successfully by placing infants directly into the cages of adults without prior familiarization[31, 33] (Figure 12). Similarly, juveniles can usually be placed together directly without prior introduction (Figure 13)[31]. Another example

is a situation in which two adult macaques have recently lost their cage-mates. In this case, the two previously unfamiliar animals may be directly paired with each other, without a period of non-contact familiarization. It is thought that social experience gained from previous exposure to a companion may allow the animals in this situation to adequately cope with the pairing process in a non-aggressive manner. This has been shown to be successful in both male and female rhesus monkeys[34, 35].

THE PAIRING PROCESS

A number of successful methods for introduction have been described, although most follow similar steps with variations to accommodate facility limitations. Ideally, pairs should be transferred to a different cage in an unfamiliar room or environment for pairing, which provides a neutral space for introduction[11, 14-16]. However, some authors have reported successful pairing without the need to transfer to a new room or a new cage[13], or by transferring to a new cage in the same room[10, 15]. Once the animals are together, they should be observed to ensure that they are compatible.

POST-PAIRING MONITORING

The success of pairing is generally known within the first 30 minutes. However, close observation throughout the first 24 hours is recommended[10]. Behaviors indicating a compatible match include: sharing a perch (Figures 14-15), sharing food (Figure 16), grooming each other (Figure 17), hugging each other (Figure 18), cooperative behavior such as lip smacking, defensive vocalizations, and postures toward an observer or other monkeys in the room (Figure 19), and continued unidirectional display of submissive behavior, including presenting of the rump (Figure 20), fear grinning (Figures 21-22), looking away (Figure 23), threatening away (Figure 24), and lip smacking[11, 15].

Shortly after pairing, it is not uncommon for the dominant animal to aggressively assert his or her rank or for the occasional minor fight to ensue; however, if more than superficial wounds result, the pair should be separated[10].

A tool that may help increase the long-term success of a pair is the use of a privacy divider (Figures 25-26)[36, 37]. The divider allows animals the option to have visual isolation from each other, which can be particularly important during feeding time. Regardless of the success of the initial pairing, it is essential to remember that relationships between animals can change over time, so continuous monitoring of pairs is important to ensure that both animals are receiving benefit from the pairing[1].

SPECIAL CONSIDERATIONS

Animals with implants

Investigators may be reluctant to socially house macaques with implants, such as cranial implants, vascular access ports, tethers and eye coils. Some concerns include risk of injury to the animal or damage to the implant, fluctuations in body weight, and decreases in testing performance[4, 38]. However, several reports have demonstrated that nonhuman primates with implants can be safely pair housed while performing behavioral testing without causing damage to the implants, even when socially housed on the same day as the implant surgery[38] .

Pregnant and lactating females

In natural conditions, macaques live in elaborate matrilineal groups consisting mostly of females and their offspring[1, 43]. Similarly, in the laboratory setting, females who are pregnant and/or raising offspring can be housed together without adversely affecting their compatibility (Figure 27)[25, 31]. However, dominant females may "steal" the offspring of the subordinate companions, which may be cause for separating the pair[31]. Thus, while it is ideal and feasible to house females together while one or both are raising offspring, close monitoring of compatibility is necessary, as with any pair.

Aged macaques

Many aged macaques in biomedical research have been conventionally housed singly for many years.[44] Reluctance to socially house these elderly animals may be due to a combination of factors, including their

fragile appearance and the perception that a cage-mate may negatively affect well-being[45]. However, studies have shown that 30 to 35 years old macaques can be safely paired with each other or with younger partners, including infants, without jeopardizing general health[44, 45].

Discontinuous or partial social housing

Some experimental paradigms or health concerns may require animals to be separated for a period of time. In these situations, various methods can be employed to maximize social interactions, while allowing research or veterinary intervention to proceed as needed. Mesh dividers or clear partitions, as used for non-contact familiarization, can be used so that the animals are physically separated, but are still able to communicate visually and vocally with each other. This will help to increase the chance for successful reintroduction[46]. Similarly, cuts can be made into clear or opaque plastic dividers to allow communication and limited tactile interaction during periods of separation (Figures 28-29). Grooming-contact bars are another option, allowing the animals to groom each other when it is mutually desired (Figure 30-32)[47]. This may be an appropriate option for animals who need to remain separated for extended periods of time. Additionally, a modification of the grooming-contact bars can be utilized to allow males and females to groom each other without the risk of pregnancy[47].

It is important to note that when reintroducing pairs after periods of separation, particularly if the separation has lasted for longer than one day, a brief non-contact familiarization period is recommended to allow the two animals to recognize each other[24, 48]. There may be sex- and species-specific differences in this requirement; for example, it has been reported that adult male cynomolgus macaques may be easier to re-pair than adult female cynos, while adult male rhesus may be more prone to problems with aggression during reintroduction[48].

CONCLUSIONS

Macaques are social animals who require interaction with conspecifics in order to maintain psychological health. However, social housing has not yet become the standard of care for macaques housed in laboratory facilities. A recent survey of 22 facilities reported that only 46 percent of indoor-housed primates were socially housed[49]. While the most natural means of providing social interaction would be housing in large social groups, pair housing is an alternative method and may be the most practical and safe option in current laboratory animal facilities. Close observations to behavioral cues and a clear understanding of social communication between macaques is critical for the safe formation of pairs. With careful planning and analysis of these behavioral cues, pair housing can be safely incorporated as a standard in the laboratory facility.

COMPATIBILITY OF MACAQUES FOLLOWING PAIR FORMATION

Following is a table of available data on the compatibility of macaques following pair formation. In general, pairs are considered to be compatible on the day of pairing when: no aggressive interactions result in severe injury, no signs of depression are observed in either animal, and both are able to obtain their share of food. For the technique applied, U=unfamiliarized partners; FN=familiarized partners with undetermined dominance; FD=familiarized partners who have established dominance; GC=partners separated by grooming-contact bars; UDR=unfamiliarized partners who have recently lost a partner (direct re-pairing).

Species	Age Class	Sex	Technique Applied	Percentage of Pairs Compatible	Reference
M. arctoides	adult	F M	FD FD	100% 100%	14. Reinhardt, V., 1998.
M. fascicularis	young adult and adult	M	FD	94%	10. Lynch, R., 1998.
M. fascicularis	adult adult	F F	FN U	75% 100%	15. Kurth, B. and D. Bryant, 1998.
M. fascicularis	adult	M	FD	100%	16. Reaves, M. and J. Cohen, 2005.
M. fascicularis	adult	M	FN	100%	18. Asvestas, C., 1998.
M. fascicularis	adult	F M	FN FN	100% 40%	22. Crockett, C.M., 1994.
M. fascicularis	adult	M&F F M	GC GC GC	100% 100% 89%	47. Crockett, C.M., 1997.
M. mulatta	adult	F M	FD FD	98% 100%	14. Reinhardt, V., 1998.

Species	Age Class	Sex	Technique Applied	Percentage of Pairs Compatible	Reference
M. mulatta	adult	M	FD	100%	23. Reinhardt, V., 1989.
M. mulatta	adult	M	FD	100%	26. Abney, D.M. and J.L. Weed, 2006.
M. mulatta	adult adult, infant juvenile	M F M F M F	FD FD U U U U	88% 80% 92% 94% 100% 100%	*31. Reinhardt, V.,* 1994.
M. mulatta	adult	M	FD	100%	32. Doyle, L.A., K.C. Baker, and L.D. Cox, 2008.
M. mulatta	adult	F	U	100%	34. Reinhardt, V., 1989.
M. mulatta	juvenile to adult	M	U	96%	35. Reinhardt, V., 1991.
M. mulatta	aged adult aged adult, infant aged adult, infant	F F M	FD U U	100% 100% 100%	45. Reinhardt, V., 1991.
M. nemestrina	juvenile and adult	F	FD	100%	13. Byrum, R. and M. St. Claire, 1998.

Figure 1. Two singly housed macaques reach out for tactile social interaction.

Figure 2. This primate cage is set up with a lexan panel.

Figure 3. Two rhesus macaques are separated by a lexan panel during non-contact familiarization. The panel allows for visual and vocal communication without the risk of injury.

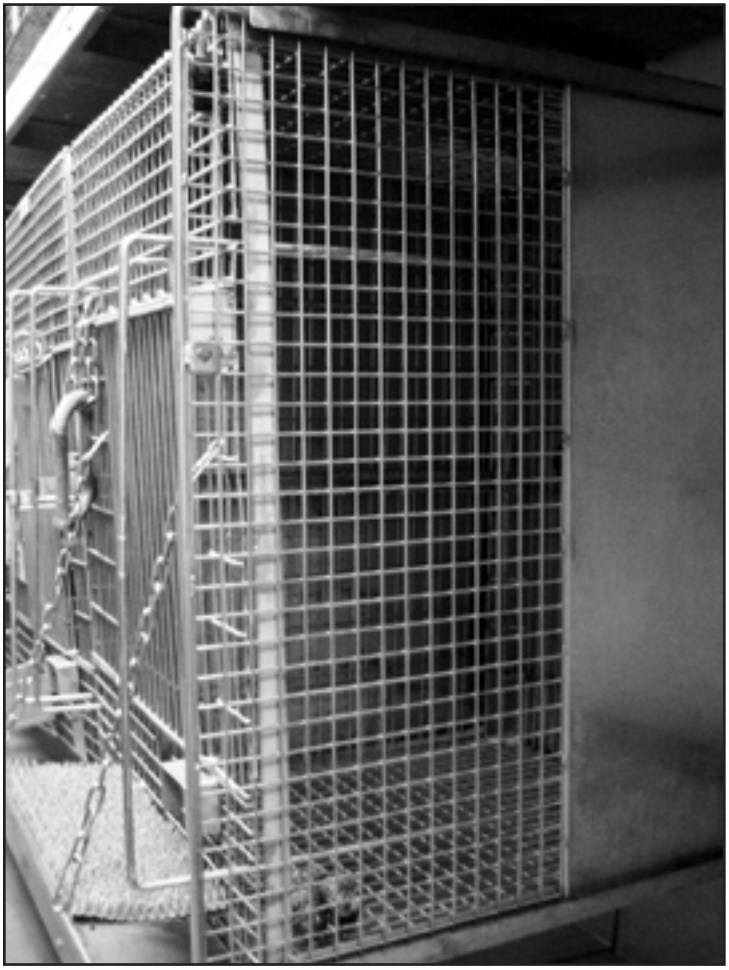

Figure 4. The mesh divider is another option for non-contact familiarization. However, care must be taken because animals can usually fit fingers through the mesh, and an adjacent animal may bite them.

Figure 5. This image illustrates the use of double-mesh, which allows olfactory communication, in addition to visual communication. Double mesh helps to prevent injuries to digits that may occur with the use of a single mesh panel.

Figure 6. Two female macaques are introduced to each other via the squeeze-back of the cage. a) The squeeze bars are pulled half way and locked into place, giving the first animal the front half of her cage. b) The window separator is opened, allowing the female in the non-restrained cage to peek at or come into the other cage safely and interact with the other female. c) The female in the non-restrained cage steps into the back of the other female's cage. d) The two females are now able to establish their dominance hierarchy. The process can be repeated to allow each animal to enter the other's cage.

Figure 7. a) A piece of fruit is presented at the same distance between the two animals. b) One animal grabs the fruit. If this is repeatable over multiple sessions, it is probable that the animal who gets the fruit is the one who is dominant. The more subordinate animal may look away or demonstrate aggression toward the observer. c) When both animals grab the treat, they most likely have not yet determined their dominance-subordinate relationship. This is particularly evident if they both demonstrate aggression toward each other.

Figure 8. A juvenile rhesus demonstrates fear-grinning. When fear-grinning occurs by only one of the animals during the non-contact familiarization process, it is an indication that the animals have established their dominance-subordinate relationship, with the fear-grinning animal being subordinate to the dominant partner.

Figure 9. Two females get to know each other through a mesh divider. Note that the animal on the left maintains a more dominant posture, while the animal on the right appears to be giving her distance (withdrawing).

Figure 10. Observation of behaviors during the non-contact familiarization period helps to determine whether a dominant-subordinate relationship has been formed. a) A dominant posture in this male is demonstrated by tall stance and direct stare at the mirror. b) In contrast, another male demonstrates submission by maintaining a lower posture and looking away from the mirror.

Figure 11. After their dominance hierarchy has been established, the two females from Figure 10 are paired. a) The mesh divider is pulled, and one animal moves into the other's cage. b) Having already determined their dominance status, the two females sit peacefully together.

Figure 12. Pairing an adult macaque with a much younger animal may not require a non-contact familiarization period.

Figure 13. Juvenile macaques usually get along well with each other even when they are strangers, so there is no need to familiarize them prior to pair formation.

Figure 14. Shortly after introduction, two males eat fruit while peacefully sharing a perch.

Figure 15. Sharing a perch in close proximity is indicative of a positive stable relationship.

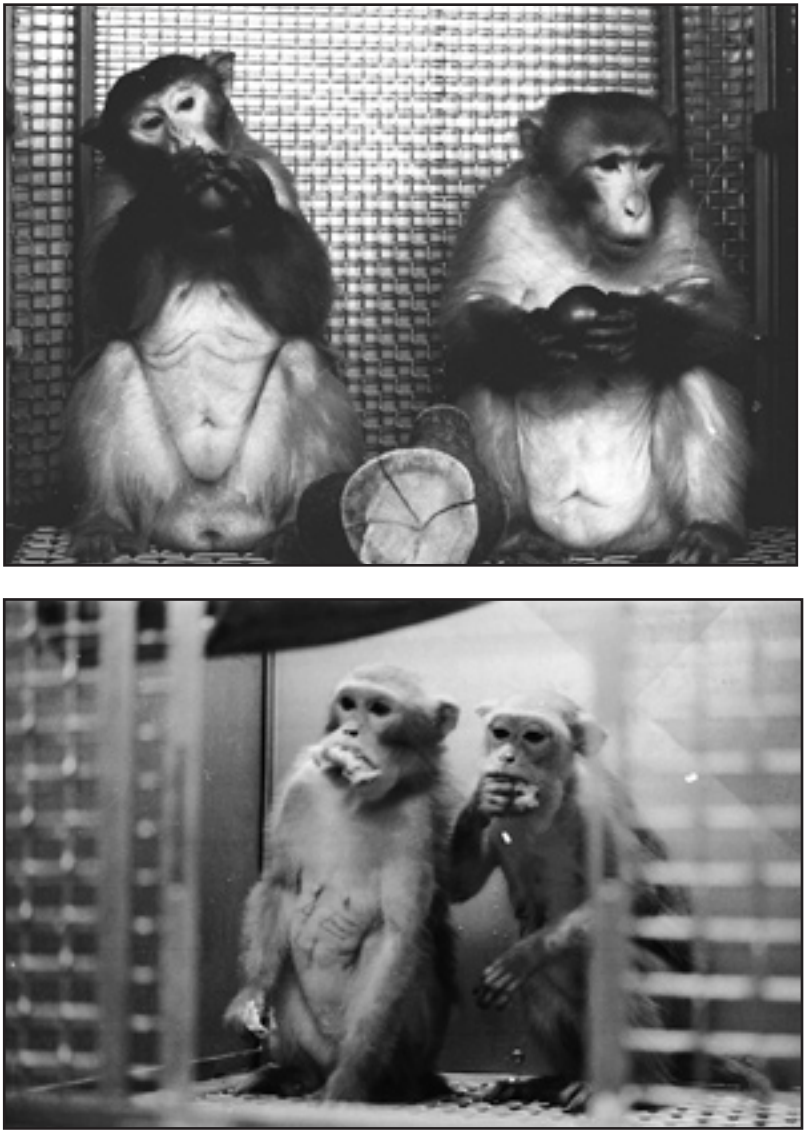

Figure 16. The partners of two different pairs of rhesus macaques demonstrate compatibility by sharing food with each other.

Figure 17. Grooming is an affiliative behavior that indicates successful pair housing in most cases.

Figure 18. Two animals who hug each other, such as these juvenile rhesus macaques, are compatible.

Figure 19. Cooperative behaviors are a sign of compatibility. a) Two rhesus are cooperatively lipsmacking. b) and c) These are cooperative demonstrations of threatening behaviors and postures.

Figure 20. A young male rhesus presents as a sign of submission.

Figure 21. Fear-grinning communicates submission.

Figure 22. Fear-grinning is demonstrated by this male rhesus as his cage-mate passes by.

Figure 23. Two rhesus display dominant and submissive behaviors while sharing a watermelon. The animal on the right is demonstrating submission by looking away from the dominant animal on the left.

Figure 24. A young adult male macaque (right) threatens a perceived intruder in front of the alpha male and alpha female, implicitly indicating his low rank in the troop.

Figure 25. Use of a privacy divider allows visual seclusion while the animals are at the front of the cage.

Figure 26. A female cynomolgus macaque looks through an opening in a privacy panel.

Figure 27. Females who are raising offspring may be kept with a cage-mate without affecting their compatibility.

Figure 28. Cut plastic panels are a lightweight option for allowing visual, olfactory and limited contact interaction.

Figure 29. Metal dividers can also be cut to allow for social interaction.

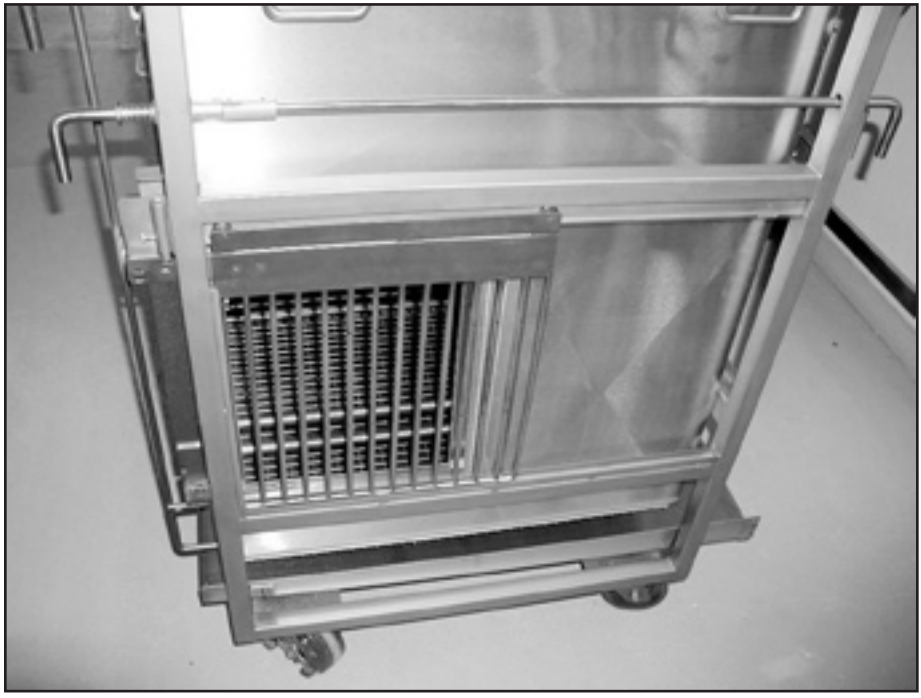

Figure 30: Grooming bars allow for grooming when mutually desired by two animals in adjacent cages. The grooming bars pictured can be repositioned to allow full contact and access to both cages.

Figure 31. These male cynomolgus macaques utilize their grooming-contact caging.

Figure 32. These male pig-tailed macaques are also housed in grooming-contact caging.

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