Considerations in the Selection and Conditioning of Old World Monkeys for Laboratory Research: Animals from Domestic Sources

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Abstract

Nonhuman primates from domestic sources constitute an important resource for the research community. The life history of the Old World monkey species that comprise the bulk of this resource is described, and issues that colony managers and researchers alike should consider regarding animal selection (e.g., species, age, sex, rearing history, temperament, genotype, viral status, geographic origin) are discussed. Preparation of domestically bred animals for research usually involves some combination of social separation, relocation, resocialization, alterations in physical space, photoperiod, and diet, as well as exposure to novel environments. The research literature that has focused on these issues is reviewed, and authors suggest that once animals have been assigned to their project housing situation, a period ranging up to 3 mo (depending on the magnitude of the change in housing) might be warranted before an experimental protocol should begin. Attention to issues of animal selection and conditioning by both researchers and colony managers can lead to the shared goal of high-quality research that utilizes the minimal number of animals.

Key Words: dominance; early experience; genetics; relocation; separation; stress; temperament

Introduction

Nonhuman primates represent a small but critical proportion (generally considered <0.3%) of animals used in laboratory research in the United States. Many of these animals originate from domestic sources, often from one of the eight National Primate Research Centers. Domestically bred animals typically provide the researcher numerous benefits including known medical, social, and dietary history; adaptation to laboratory conditions; habituation to people and colony management practices; and often, known parentage. In this article, we discuss issues pertaining to animal selection and conditioning that a researcher should consider when planning a project using primates from domestic sources. Our goal is to raise issues that might be overlooked but that could be critical to ensuring both animal health and welfare, and the validity of research results.

A number of species of nonhuman primates are used in laboratory research. By far, the greatest numbers are Old World monkeys (OWMs[1]). The genus most commonly used is *Macaca*, and of the macaques, the rhesus (*Macaca mulatta*), cynomolgus or long-tailed (*Macaca fascicularis*), and pigtailed (*Macaca nemestrina*) macaques represent the most frequently used. Other species include baboons (*Papio* spp.), other macaques (*Macaca* spp.), and vervets (*Chlorocebus* spp.), also known as African green monkeys (Carlsson et al. 2004).

Although breeding of OWMs technically requires only a single male and a single female, large-scale breeding programs often house their animals in groups that are somewhat species typical in structure and organization. In the wild, macaque, baboon, and vervet groups are multimale/multifemale, with group sizes ranging up to 100 animals or more depending on the species and locale (Melnick and Pearl 1987). Typically, the groups are organized by kinship and dominance. Females form the core of the group, typically do not emigrate during their lifetimes, and retain strong bonds with their female kin. Power relationships among animals are often manifested as dominance hierarchies, wherein individuals dominate other individuals and entire matrilines dominate other matrilines (Melnick and Pearl 1987). While many domestic breeding facilities sometimes attempt to replicate this multimale/multifemale structure and organization through use of large outdoor cages, a second configuration—one-male/multifemale groups—is also common. Because most OWM species used in the laboratory (hereafter referred to as laboratory OWM species [LOWMS[1]]) are sexually dimorphic, and males actively compete with each other for access to females and other

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[1] Abbreviations used in this article: LOWMS, laboratory Old World monkey species; OWM, Old World monkeys; SIV, simian immunodeficiency virus; SPF, specific pathogen-free; SR, separated and relocated.

resources, this "harem" configuration can result in reduced opportunities for aggression and wounding.

The primary social bond in LOWMS is between mother and infant, and there is a protracted period of immaturity. Early development for infant OWMs begins with complete dependence on the mother, which decreases over the first 6 mo of life. After weaning, both male and female juveniles continue to maintain strong social bonds with their mothers and other matrilineal kin (Nicolson 1987). Behavior changes markedly with age. Young animals spend a large percentage of their time engaged in social play, exploration of the environment, and vigorous motor activity (Fairbanks 2000). At sexual maturity, which occurs at approximately 3 to 5 yr of age for females, and 1 yr later for males (Melnick and Pearl 1987), aggressive and competitive behavior increases sharply for both sexes but particularly for males, while grooming becomes an increasingly important activity for females (Walters 1987). In the wild, males gradually separate from their mothers and form relationships with other males in preparation for emigration from their natal group, whereas females retain strong ties with their mothers and female kin for life (Pusey and Packer 1987).

As this brief description of the lifestyle of LOWMS illustrates, these species are social year-round. Continuous social housing in captivity permits the expression of the full range of behavior in these animals. Housing the animals in groups of mixed ages and sexes promotes normal development in offspring and yields animals that are competent breeders and will ultimately replenish the colony and that are healthy, both physically and psychologically, for research. In fact, social housing is considered one of the most important factors in promoting the psychological well-being of these animals (NRC 1998). In addition, social housing, especially in outdoor colonies, can be remarkably cost-effective. However, research with LOWMS is very expensive, with per animal purchase costs at the time of writing ranging from \$2500 (e.g., vervets) to \$7500 or more for a specific pathogen-free (SPF[1]) juvenile, female, Indian-origin rhesus macaque (Primate Supply Information Clearinghouse, Washington National Primate Research Center, personal communication, 2006). Animal per diem charges can also be expensive, ranging from a few dollars/animal/day for outdoor housing to more than \$20/animal/day for specialized housing for research projects. The expense of primate research is one important consideration, although not the only consideration, in trying to reduce animal numbers in laboratory research. We believe that some of the issues that we discuss below pertaining to animal selection and animal conditioning are relevant to attaining that goal.

Animal Selection

In the preceding text, we described the social organization of OWM groups and the reasons domestic breeders attempt to mimic aspects of this organization in captive colonies. Although there are many good reasons for this practice, there is also one major drawback: Colony managers cannot provide a researcher with a "random sample" of animals for his/her experimental research. In other words, not all animals are equally available for assignment to research protocols; the needs of researchers must be balanced against colony management needs. Even when researchers request animals that possess specific characteristics, the animals available for selection will likely not represent the full population of animals that possess those characteristics. In this section, we discuss issues pertaining to "inadvertent" versus "intentional" selection of potential research subjects.

Inadvertent Selection

Although a researcher may request animals with particular characteristics (e.g., specific age or sex), a number of other factors are likely considered by colony managers when an actual assignment is made. These factors, which are briefly described below, include social rank, age and sex, and behavioral criteria.

Social Rank

In a large multimale/multifemale social group, the alpha male plays a "control" role in controlling intragroup aggression (Bernstein and Sharpe 1966). His social power is often a result of alliances that he may form with other males, or with adult female members of a high-ranked matriline. Moreover, high-ranked adult females also are important influences on group stability, and this is especially true in vervets (Raleigh and McGuire 1989). High-ranked adult animals are not typically selected for studies because their removal from an existing social group could lead to instability and possible wounding in the cage. High-ranked animals, of course, generally possess particular characteristics that lower ranked animals do not have. Attainment of high rank often involves a certain degree of confidence (a commonly found personality dimension: Caine et al. 1983; Capitanio 2004) as well as social skill (Bernstein 1981). Physiological differences have also been found between high- and low-ranked animals. For example, male rank attainment depends to a certain extent on size, temperament, and serotonin and dopamine neurochemistry (Fairbanks et al. 2004), and high-ranked cynomolgus macaque males have been found to possess greater numbers (or availability) of dopamine D2 receptors in the brain (Morgan et al. 2002). Rank differences have also been found in stress physiology—lower ranked animals can show elevations in plasma cortisol concentrations in response to challenge that are not shown by higher ranked animals (Abbott et al. 2003). Similar rank-related considerations may also apply to females, although the matrilineal structure of female dominance relations complicates matters. Nevertheless, evidence exists that there are rank-related differences among female macaques in terms of dopamine function (Shively 1998) and in cellular changes that may be associated with later risk for endometrial cancer (Shively et al. 2004).

Age and Sex

If the subject's age or sex is not important to the study, the investigator may be assigned animals to accommodate aspects of colony management. In the wild, multimale/multifemale groups usually have a sex bias toward females. When attempting to create conditions in captivity that are as naturalistic as possible, and assuming the birth:sex ratio is about 1:1, as is typical, there is usually a surplus of males. Colony staff may want to assign juvenile males preferentially to avoid eventually having too many adult males in a breeding cage. Once animals are assigned to a project, they are usually relocated to individual or small group cages. Juvenile males or females may be assigned to projects based on cage size considerations. Established housing standards (e.g., NRC 1996) dictate cage sizes based on the weight of animals, and if age or sex is unimportant to the investigator, colony staff may prefer selecting animals that can be housed in the smaller cage sizes inasmuch as smaller cages are less expensive to purchase and easier to handle for sanitation, and more cages fit into a housing room.

Behavioral Criteria

Selection criteria may involve animals with particular behavioral characteristics. In some cases, animals that evidence unusual behaviors may be selected preferentially. This group could include animals that display abnormal behaviors, appear to be hyperaggressive or hyperfearful, or are very low ranked and are targeted by higher ranked cagemates. In addition, preferences that center personnel may develop to particular individuals may result in those animals being less likely to be assigned to research projects.

Conclusion

To the extent that particular types of animals are included or excluded preferentially in the selection process, any behavioral, anatomical, physiological, or genetic factors that are associated with those characteristics are also represented disproportionately in the sample. This disproportion may affect research results as well as the ability to generalize broadly to other animals or other species, such as humans. Finding the proper balance between scientific needs and maintaining the integrity of a colony of animals is difficult. One possible solution is to characterize the entire population at a facility along some basic dimensions that might be relevant to researchers, such as temperament, emotionality, stress responsiveness, and social abilities (Capitanio et al. 2006; Fairbanks 2001). Such a strategy could document the variation that exists along these dimensions, and could allow both colony managers and researchers to know the specific characteristics of the particular animals that are being assigned relative to the population at large. If such information is quantitative, it could be used in statistical analyses as covariates, or even as additional independent variables.

Intentional Selection

For some research projects, investigators may require animals with particular characteristics, which can include species, age and/or sex, genotype, temperament, rearing history, viral status, origin, and prior research experience. Some of the factors that might be considered in animal selection are briefly discussed below.

Species

A variety of LOWMS may be available for selection, and although they all are equally related to humans and there is considerable overlap in behavioral and biological processes, there are advantages and disadvantages of each. The rhesus monkey has been the most commonly used species in biomedical research for decades. Consequently, more is probably known about the biology of this species than about any other nonhuman primate species. This situation has resulted, for example, in a wide range of species-specific reagents for rhesus macaques. However, concerns have been raised about a shortage of rhesus monkeys (especially those of Indian origin) for biomedical research (Cohen 2000), and an effort is being made to encourage investigators to use other species. For example, cynomolgus monkeys can be productively infected with the simian immunodeficiency virus (SIV[1]), and baboons are excellent models for prenatal and neonatal research due to their large size. Vervets can be obtained for lower cost and carry fewer pathogens than the commonly used Asian macaques (Baulu et al. 2002). We refer the reader to a recent report describing alternative species for consideration and recommendations that have been made to alleviate the shortage of Indian-origin rhesus macaques (ILAR Workshop 2003). We note, however, that species differences, even within the genus *Macaca*, have been found for behavioral and physiological measures (Clarke et al. 1988). Investigators should be aware of these differences because selection of a particular species or a change to a different species could affect research results.

Age

The delayed development and long life span of LOWMS make age an important characteristic for subject selection. Some research is targeted to a specific stage of development such as the neonatal period, adolescence, or old age, and subjects are selected accordingly. More often, the research topic is not age specific, but the stage of development of the research subjects is still an important consideration. Subjects entering a research protocol at 2 yr of age will go through puberty and adolescence during the course of a 24-mo study and will undergo marked changes in behavioral propensities and in hormonal and neurochemical profiles. Physiological changes through the course of adulthood are more gradual, but the difference between young (5- to 6-yr-old) adult and old (≥20-yr-old) adult can be substantial. One of the advantages of using animals from domestic sources is that exact age is known and can be taken into account in experimental designs.

Sex

Although some research questions obviously require animals of one sex or the other, sex should be an intentional consideration in animal selection. As the reader may know, the National Institutes of Health, concerned about the lack of inclusion of women and minorities in human clinical research, and concerned that treatments under study may affect women and members of minority groups differently than males of European ancestry, developed a policy ensuring the inclusion of women and minorities as subjects in clinical research (NIH 2001). Although we are aware of no similar policy being proposed for animal research, the issues of whether data generated on nonhuman primate males apply equally to females should be a relevant consideration in subject selection (e.g., Kaplan et al. 1991).

Genotype

Advances in our understanding of genetics have led many to argue that no laboratory study should be conducted with animals of unknown genetic background (Rogers 2005). These advances, coupled with the easy availability of genetic assays, have resulted in many researchers finding it desirable to select animals that possess specific genetic characteristics. Examples include polymorphisms in the serotonin transporter (Bennett et al. 2002; Lesch et al. 1997) and the dopamine D4 receptor gene (Bailey et al., under review), which are useful in biobehavioral research. Another example is the major histocompatibility complex Class I allele MAMU-A*01 (Allen et al. 1998), which is valuable in SIV-related research.

Temperament

Although few breeding facilities have screening programs that can identify animals with particular characteristics, investigators may perform their own screening to select specific types of animals, such as those high or low in some personality trait such as sociability (Maninger et al. 2003) or impulsivity (Fairbanks 2001). Investigators may also select animals for practical reasons, such as the likelihood that they will adapt to a particular apparatus (e.g., a primate chair or a testing apparatus) (Coleman et al. 2005). Characteristics that may be selected include activity level, attention, and tractability.

Rearing History

Animals may be selected that have had specific types of early rearing; often, this history includes rearing in a nursery rather than in a social setting with mother and other conspecifics. Nursery rearing provides the investigator with unparalleled access to the animals for sample collection and administration of treatments, as well as experimental control of factors that might compromise the research. It is important to note, however, that there is a large body of data showing that nursery rearing can be associated with alterations in behavioral, neuroendocrine, and immunological functioning that can be remarkably long-lasting (Capitanio 1986; Capitanio et al. 2005; Coe et al. 1989; Lubach et al. 1995; Sackett et al. 2006; Shannon et al. 1998). It is also worth noting that there are probably as many definitions of "nursery rearing" as there are facilities that engage in this practice. Factors that may differ from colony to colony include the age at which the infant is removed from its mother, the feeding regimen the animal is put on in the nursery, the degree of human contact the infant receives, and the age socialization begins. Between-colony differences in the implementation of nursery rearing may limit generalizations across colonies.

Viral Status

A growing trend in breeding programs for LOWMS is the development of SPF animals (e.g., Lerche et al. 1994). While one motivation for such programs is to reduce the prevalence of pathogens that are harmful to humans, such as cercopithecine herpesvirus 1 (CHV-1, commonly known as B-virus), another goal has been to produce animals that are free of viruses, such as simian retrovirus (SRV) type D and simian T-cell lymphotropic virus (STLV), which might compromise infectious disease research. Many of the pathogens that are being "managed out" of animal colonies are transmitted through exchange of body fluids during aggressive and sexual activity. One major strategy for deriving SPF animals involves nursery rearing, as described in the preceding paragraph. Once such an animal itself reproduces, the offspring presumably do not need to be nursery reared. Thus, while nursery rearing of the progenitors of a derived colony may exhibit biobehavioral differences compared with more normally raised monkeys, their offspring may not exhibit the same characteristics (although see Francis et al. 1999 for evidence of nongenomic transmission of characteristics across generations in rodents). Thus, even within the category of "SPF animals," there may be variation depending on the derivation strategy used, the generation (progenitor vs. F1) of the animals, and other factors, which could complicate comparisons.

Origin

OWMs residing and being bred in domestic colonies for experimental research can originate from multiple geographic locations. Rhesus monkeys can originate from India, China, or elsewhere; cynomolgus monkeys from the Phillipines, Viet Nam, Indonesia, and Mauritius; and vervets from Barbados, St. Kitts, or East Africa. In all of these cases, populations may have been reproductively isolated for hundreds if not thousands of years, may have originated from a small set of founder animals (thereby introducing a restricted gene pool), and may show differences in behavioral and physiological functioning. Best studied are rhesus macaques. Genetic differences have been found between Chinese- and Indian-origin rhesus (Kanthaswamy and Smith 2004), as well as between those of Chinese and Nepali origin (Kyes et al. 2006). Researchers have also noted differences in temperament, concentrations of serotonin metabolites in cerebrospinal fluid, and hematological measures between Indian- and Chinese-origin rhesus (Champoux, et al. 1994, 1996, 1997). SIV research has also revealed significant differences between rhesus of Chinese versus Indian origin in disease-related measures (Ling et al. 2002; Marthas et al. 2001; Trichel, et al. 2002). This information has resulted in recognition of geographic origin of rhesus macaques as an important selection criterion for particular kinds of research.

Prior Research Experience

Many research studies involve procedures such as training, drug administration, or sample collection, which may have only short-term effects on behavior and physiology. We advise investigators to be aware of prior experimental history, and to consider the re-use of such animals wherever possible. The advantages and disadvantages of re-use will vary according to the kind of past and present use, but there are many circumstances in which re-use of animals can save investigators time and money and can make more effective use of valuable nonhuman primate subjects without compromising research goals or animal welfare.

Some facilities that maintain pedigreed, genotyped primate colonies (e.g., the Southwest National Primate Research Center) encourage multiple investigators to share a common resource (Rogers and Hixson 1997). Animals that have been habituated and trained to perform operant tasks in one protocol could be re-used in a second protocol, thereby saving many days of training and the associated expense of getting naive animals to work in an apparatus. Continued longitudinal assessment of individuals that takes advantage of differential treatment from a prior protocol would also reduce the total number of research animals and save the investigator time and money (e.g., Clarke and Schneider 1997).

Of course, there are also circumstances in which an investigator may require a research-naive subject, and some aspects of re-use of animals (e.g., the allowable number of survival surgeries) are regulated by law in the United States. Nevertheless, prior research experience should be considered when selecting subjects; savings in time and money could be considerable, and such re-use of animals can conserve increasingly scarce primate resources.

Conclusion

Because breeding colonies of LOWMS have been set up intentionally to provide animals for research, investigators often have some choice regarding which animals they select for their studies. Even when investigators do not explicitly state particular selection criteria, they should still be aware that selection criteria are almost certainly being used. In either case, it is unlikely that researchers will have a completely random sample of the available population. Depending on the goal of the research, this fact could have consequences that range from the trivial to the dramatic in terms of the ability of the investigators to generalize their results broadly. We note, however, that this situation does not necessarily invalidate research that is being conducted with nonhuman primates, just as the similar problem of subject selection in human research, described above, has not invalidated that research. Rather, as with humans, the issue is broad generalizability of results. We believe that an understanding of the relevant characteristics of the selected animals, particularly in relation to the population from which they were drawn, should be an important component of animal assignment.

Intentional selection of animals with particular characteristics can have the added benefit of contributing to a reduction in the number of animals needed and thereby the expense of the research. This benefit is due to the fact that the sample size required to achieve a particular statistical effect depends on the variation within the group. More uniformity in the composition of experimental groups through intentional selection reduces the variation and, all other things being equal, the size of the sample needed to demonstrate the desired result.

Animal Conditioning

Once animals have been selected for a project, the investigator must consider the numerous changes that the animals typically experience. For example, in a domestic breeding colony, animals might be relocated from a large breeding cage outdoors to individual housing indoors. We believe that an important but often overlooked question is, How much time should elapse after relocation before the research begins? Unfortunately, we are aware of very little scientific literature that addresses this issue directly. There are, however, published studies, conducted for other reasons, that suggest some answers to this question. In the text below, we describe some of the experiences that animals undergo in the transition from living in a breeding colony to being enrolled in a project, and we cite some of the relevant literature that can help answer the question posed above. Because behavioral and biomedical research protocols vary considerably, we do not consider aspects of conditioning that are more project specific, nor do we address the growing literature demonstrating the beneficial effects of training the animals to cooperate on research-related tasks such as phlebotomy, receipt of injections, and other tasks (Bentson et al. 2003; Reinhardt 2003; Schapiro et al. 2003, 2005).

It is important to note that the studies we review below often utilize different outcome measures, making comparisons difficult. For example, in a recently published study of adult male rhesus macaques (Ruys et al. 2004), animals were chaired for 2-hr periods on several consecutive days. The behavioral response to placement in the chair disappeared within a few days, suggesting the animals had habituated to the procedure. Examination of plasma cortisol concentrations, however, suggested that physiological habituation did not take place; rather, the reduction in cortisol concentrations that were observed appeared to reflect not habituation (i.e., learning that the procedure was not stressful) but instead, physiological adaptation to repeated stimulation of the hypothalamic-pituitary-adrenal axis. In fact, when animals were chaired again 6 mo after the initial series, the behavioral response was still minimal but cortisol concentrations were again elevated. This study reminds us that different outcome measures can be affected differently by particular procedures, and that a dissociation can exist between measures. It is important to keep in mind while reading the review below that normalization of one particular class of responses (behavioral, hormonal, or immunological) does not necessarily imply normalization of all classes of responses.

Shipping

A substantial amount of research is conducted at sites that have their own breeding facilities; however, in many other cases, research programs rely on the purchase of animals from a domestic breeding source and the shipping of the animals to the research site. The process of shipping involves many of the experiences we describe below, but whereas transfer of animals from a breeding cage to a test cage within the same facility may take only a few minutes, shipping from one facility to another may take many hours or longer.

What is the impact of shipping? An early report by Sackett (1981) reported lower viability for pregnant female pigtailed macaques shipped in the first or second, compared with the third, trimester. This effect was marginally ($p = 0.07$) significant. After delivery, many females were shipped back to the breeding colony. Those that conceived within 90 days of their return had significantly reduced viability compared with those that conceived more than 90 days after return. This result may have been related to the fact that about half of the females that returned to the breeding facility were placed in new social groups, suggesting that social stress experienced in the first 90 days of group formation may have an impact on reproduction. Honess and coauthors (2004) found that by 3 wk after shipping, behavioral indicators of distress did not return to preshipping levels in eight juvenile cynomolgus monkeys. It is important to note, however, that the preshipping data were obtained while the animals lived in a 10-member group. Two animals were not shipped; consequently the postshipping social environment was different.

Kim and colleagues (2005) reported that while many hematological values (e.g., white blood cell count) were unchanged for five cynomolgus males upon arrival after a 15-hr transit, the neutrophil:lymphocyte ratio and serum cortisol concentrations were elevated upon arrival. These two indicators returned to levels that were within the reference ranges by 1 wk after shipping. Finally, data from the New World species—Weid's marmosets *Callithrix kuhlii* (Schaffner and Smith 2005)—may be relevant. Unlike LOWMS, these marmosets are a pair-bonded species, which involves long-term attachment relationships between adult male and adult female pairs. During this study, one established pair was shipped along with a previously unfamiliar male and female that were newly paired upon arrival at the new facility. Whereas the newly established pair had elevated urinary cortisol for the entire 8-wk postshipping period compared with preshipping levels, the established pair showed no such elevations at either the 4- or 8-wk follow-up time points. These data suggest that being shipped and housed with a pair-mate might buffer the stressful consequences of shipping and relocation.

Although sample sizes are small and many confounding factors exist, the studies cited above are consistent in suggesting that shipping combined with social change can constitute a long-term stressor that requires many weeks of adaptation. These studies also suggest that the presence of a pair-mate may buffer shipping stress. It is important to reiterate, however, that adult LOWMS do not form the same kind of adult male-female bonds that callitrichids form. Thus, it remains to be learned whether shipping of familiar pairs of LOWMS (whether heterosexual or isosexual pairs) results in a similar buffering effect. Because infants of LOWMS do form strong attachment bonds with their mothers, domestic shipping of infants and mothers together may indeed produce a buffering effect, at least for the infants.

Social Separation and Relocation

Relocation of animals from a breeding cage to an experimental cage typically involves exposure to a novel environment and separation from familiar social companions. Several decades of stress research have demonstrated that social separation and exposure to a novel environment are two of the most reliable ways of eliciting behavioral or physiological indicators of stress. While there may be a tendency to view all social separations as stressful, evidence suggests that separations are stressful principally for attachment-type relationships (i.e., social relationships that involve a strong emotional bond). Disruption of such a bond results in profound behavioral and physiological changes (Mendoza and Mason 1989). In LOWMS, the strongest bonds exist between infants and mothers, and a substantial body of data has demonstrated behavioral and physiological responses to such separations. Importantly, different patterns of infant responses to separation have been observed depending on whether the infant remains in the familiar group (with mother removed) or whether the infant is removed (and thereby also experiences a novel environment). We review below the data on infant-mother separation, as well as data that suggest there are effects of removal from group (typically also including exposure to novel environments) on members of other age/sex classes.

Infant-Mother Separation

In LOWMS, removal of an infant from its mother typically results in a phase of increased activity, vocalization, heart rate, and body temperature, which together are typically referred to as a "protest" phase. This phase is followed by a period of hypoactivity—a seemingly depressed affect—accompanied by reductions in heart rate and body temperature, increases in cardiac arrhythmias, disturbed sleep, and changes in cellular immune function, often referred to as a "despair" phase (see reviews by Mineka and Suomi 1978; Reite and Capitanio 1985). Factors that influence this pattern of response have been reviewed by Mineka and Suomi (1978) and include species differences (e.g., bonnet macaques tend to show minimal responses). Importantly, within the age range of approximately 2 to 12 mo, there appear to be few age or sex differences. In terms of conditioning animals for research, we believe it is important to consider the following two questions.

1. Following separation from mother, at what point does the infant's behavior normalize? Suomi and coworkers (1973) addressed this question by separating animals at ages 60, 90, or 120 days into either single housing or pair housing in a novel cage and room. Animals were followed until 180 days of age. Although all animals responded with protest, only those animals that were housed singly following separation showed a despair response, suggesting the important role of companionship in buffering the separation response. Importantly, the authors report that by 6 mo of age, the pair-housed animals were performing normally in terms of social behavior compared with historical data on nonseparated well-socialized animals of the same age.
2. The Suomi and coworkers' 1973 study points to the second question: What role does the postseparation environment play in the infants' responses? Suomi and colleagues conclude that the environment after separation is a critical feature of how well the animals adapt, and that pair housing can have a beneficial effect. Studies by Hinde and coworkers (reviewed by Hinde and McGinnis 1977) confirm that the postseparation social environment can be influential: Rhesus infants that remained behind in their familiar groups while mothers were removed showed brief protest and more profound despair responses, whereas animals that were removed and relocated to individual housing showed more prolonged protest. Using physiological measures, Boccia and colleagues (1997) also showed that social companionship buffered the immune consequences of maternal separation in bonnet macaque infants, although they noted that species differences in immune consequences exist (cf. Laudenslager et al. 1990). Conflicting data were presented by Coe and colleagues (1989) for rhesus, however: Regardless of whether the postseparation environment did or did not contain peers, infants separated at 6 mo of age showed comparable immune responses approximately 1 yr after separation. These responses were intermediate between those of animals that were nursery reared and those that were still living with their mothers in their natal social groups. Cortisol concentrations did not differ between the groups.

Finally, it is worth noting that studies have shown that even short-term separations from the mother can have long-lasting, though sometimes subtle, effects. Months to years after a separation that lasted only a couple of weeks, effects were found in measures of immune (Coe et al. 1989; Laudenslager et al. 1985) and behavioral (Capitanio and Reite 1984; Capitanio et al. 1986; Spencer-Booth and Hinde 1971) functioning.

Other Social Separations

Among LOWMS, social bonds also exist for other matrilineal kin and among members of the same social group. Evidence exists that separation from familiar companions and relocation to novel surroundings result in physiological changes that can be persistent. Some of the most systematically collected data have been reported for rhesus monkeys by Gust and colleagues (see below), who generally used plasma cortisol concentrations and numbers of lymphocyte subsets as outcome measures. Gordon and colleagues (1992) studied 2-yr-olds that were relocated from a large outdoor cage to individual housing indoors for an 11-wk period, and the investigators compared data with matched animals that remained in the social environment. In the separated/relocated (SR[1]) animals, cortisol concentrations increased substantially 24 hr after the manipulation and remained elevated (albeit at lower levels) for 8 wk thereafter. Similarly, the SR animals had reduced lymphocyte and lymphocyte-subset numbers for periods ranging from 7 to the full 11 wk. In a parallel study, Gust and coworkers (1992) compared similarly aged animals that were removed from the social group but were relocated together to an outdoor cage, rather than to individual caging indoors as in the Gordon et al. (1992) study. The results were remarkably similar, with increased cortisol concentrations lasting for 2 wk, but reduced numbers (compared with controls) of immune cells in peripheral blood that persisted, for many cell types, for the full 11-wk period. It is worth noting in both of these studies, however, that cell numbers did return to baseline levels by 11 wk in the SR animals. It was only in comparison with the nonrelocated controls that the differences persisted, due largely to the increases observed over the 11-wk period in the control animals. Given that the SR animals were taken from the same cage in which the control animals were left behind, it is probably unwarranted to assume that the controls were living under "undisturbed" conditions.

Studies with adult rhesus monkeys reveal a somewhat more complex picture. When four adult males were removed from a social group and rehoused together in a social cage, no significant changes in cortisol or immune cell numbers were found 24 hr after the SR (Gust et al. 1993). In contrast, when adult females were removed from a group and housed either alone or in pairs (the same animals experienced both relocation conditions in counterbalanced order), elevations in cortisol concentrations were evident across the 96-hr follow-up period (Gust et al. 1994). There was no effect of postrelocation housing. When lymphocyte subset numbers were examined, numbers were found to be suppressed in the individual housing condition across the 96 hr, but the suppression was less for the females housed in pairs. Among vervets separated from their social groups and housed individually for 6 hr, cortisol response and behavioral agitation were significantly greater for juveniles than for adults (L.A.F. and M. Laudenslager, manuscript in preparation). Together, these studies suggest that adult responses are different from those of juveniles, and that adult males' and females' responses are different from each other.

Three other studies that explored separation in noninfant animals are also worth mentioning. In one study, Suomi and colleagues (1975) separated sexually mature 5-yr-old rhesus monkeys from their natal groups either into social conditions or into individual housing. The buffering effect of social housing (whether with familiar or unfamiliar companions) was evident. These animals displayed few adverse effects, whereas animals separated into individual housing showed evidence of a despair response—increased self-clasping and stereotypy and reduced self-grooming.

In another study, Capitanio and coworkers (1998a) separated and relocated 36 adult male rhesus monkeys from outdoor field cages to individual housing indoors. Plasma cortisol concentrations and leukocyte numbers were assessed under several conditions, including on three occasions while the animals lived in their new environment. For several measures, values at the first time point (approximately 1 mo after SR) were different from values at the next two time points (taken at 5 and 10.5 mo after SR). The authors suggested that adaptation to the SR might occur between 1 and 5 mo following SR.

In a third study, Capitanio and Lerche (1998) examined the independent contributions of separation and relocation to survival during SIV infection in an archival cohort of 298 rhesus monkeys of all ages. Two preinoculation time periods were examined—the entire period prior to inoculation versus the 90-day period immediately preceding inoculation. Numbers of social separations or relocations in the entire preinoculation period were not associated with survival, but the numbers experienced by the animals in the 90 days before inoculation were, and this effect was particularly true for number of separations, which ranged from none to five: Animals that experienced more social separations in the 90 days before SIV inoculation had shorter survival. Moreover, when the number of separations in the first 30 days after inoculation (0 vs. ≥ 1) was examined, it was found that separation was significantly associated with shorter survival.

Together these data suggest that biobehavioral changes are commonly seen in response to separation and relocation, although it is unclear whether the effects are due primarily to the separation or to the relocation. It appears that differences exist in the persistence of the effects of SR between adult males and females as well as between adults and immature animals, and it appears that the presence of companions (especially familiar companions) can buffer the effects of SR to some extent. Unfortunately, systematic assessments with large numbers of animals, as well as long-term follow-up data, are not available to provide a complete picture of the effects of these manipulations on behavior and physiology.

Relocation to Novel Cage or Room

After animals have been habituated to individual housing, research or animal husbandry needs may require further relocation to different cages or new rooms. There are surprisingly few published studies that have examined the time course of adaptation to *only* relocation to a novel room or cage (i.e., relocations that do not also involve separations or other factors described below). The principal exception is a research program of Crockett and colleagues, who studied responses to a variety of common laboratory procedures in cynomolgus (Crockett et al. 1993, 1995) and pigtailed (Crockett et al. 2000) macaques, including responses of individually housed animals to relocation to individual housing in a new cage room. In general, relocation of animals from a standard-sized individual cage to an identical cage in a new room was moderately distressing for long-tailed monkeys, as indexed by behavior and food intake (Crockett et al. 1995) and urinary cortisol excretion (Crockett et al. 1993). This effect lasted, however, for only the first night and day following relocation, and it was not as severe when animals were relocated to different cages within the same room. Pigtailed macaques exposed to identical conditions showed milder disturbance (Crockett et al. 2000). The authors note, however, that the pigtailed macaques in their studies had been housed in single cages substantially longer (18-35 mo) than had the long-tailed monkeys (6-9 mo), and so it is unclear whether the differences between the species reflect basic differences in responsiveness or amount of previous exposure to individual laboratory housing.

Finally, it is worth mentioning that animals within a colony room do develop rudimentary relationships with each other. Although in this article we have focused on the animal that is moved from one room to another, we caution that such a disruption may also have an impact on the animals housed in the old room (from which the animal was relocated) as well as the animals in the new room, as new relationships are formed. Although we are aware of no data directly addressing this issue, we expect that the disruption for existing residents is likely of a transient nature inasmuch as the data of Crockett and coworkers show only a short-term disruption for the relocated animal itself.

Restriction of Space and Activity

Removal of animals from a larger breeding enclosure to project-related housing also involves a restriction of space. Although there are data describing the effects of cage size on behavior, we are unaware of longitudinal data showing the time course of changes in behavior over periods of time that exceed a few days. Exceptions include retrospective studies showing that duration of individual housing is associated with display of abnormal behavior (Bellanca and Crockett 2002; Lutz et al. 2003). One of the most relevant experimental studies in this context was that of Draper and Bernstein (1963), who studied 12 wild-born adolescent rhesus monkeys that had been living either individually or in small groups in outdoor enclosures. Animals were relocated to cages of 9 ft^2, 12 ft^2, or 1152 ft^2 on 10 occasions. A variety of behavioral differences were found based on cage size, and the most notable finding was the tendency for stereotyped behavior and cage-directed behavior to increase as cage size decreased. Paulk and colleagues (1977) found similar results with rhesus monkeys of various ages, despite the animals having been allowed to explore the test cages for at least 20 hr. Of course, relocation to smaller or larger cages also typically involves novelty and separation, which can make it difficult to disentangle those influences. One research program (C.M. Crockett and colleagues) that did so, however, found no changes in behavior or urinary cortisol concentrations when individually housed pigtailed or long-tailed macaques were rehoused from standard-sized individual cages to those either 77% or 148% of standard size for 2-wk periods (Crockett et al. 1995, 2000). These data suggest that minor changes in housing have minimal impact, but that larger changes result in an ongoing adaptation by the animals that can lead to abnormal behavior if sufficient time elapses.

Alteration in Photoperiod and Illumination

Relocation may involve alteration in light/dark cycles (e.g., moving from an outdoor cage where seasonal changes are the norm to indoor cages where light/dark cycles tend to be fixed throughout the year) and/or changes in illumination. Few published studies have explored the consequences of such changes. An experimental manipulation of photoperiod, however, did show changes in reproductive function in adult male rhesus monkeys (Chik et al. 1992). Specifically, rapid growth in testicular size and elevations in testosterone levels were observed during the switch to a “short day” (8L:16D), with testicular regression and reduction in testosterone observed upon reversion to a “long day” (16L:8D). These effects were seen only in pubertal animals. Prolactin also was sensitive to day length, with changes reciprocal to those seen for testosterone (i.e., lower prolactin concentrations during short days, and higher concentrations during long days), but the prolactin changes were observed in both pubertal and prepubertal males.

Takasu and colleagues (2002) compared Japanese macaques under two conditions of illumination but with the L/D cycle held constant. The investigators observed that onset of activity was earlier and core night-time body temperature was lower in a “bright,” compared with a “dim,” illumination condition (Takasu et al. 2002). Some (e.g., Reinhardt and Reinhardt 1999) believe that illumination differences as experienced by animals housed in upper versus lower tiers of indoor caging might result in behavioral or physiological differences. Others (Crockett et al. 2000; Schapiro and Bloomsmith 2001) have found no such differences, and the difference found by Capitanio and colleagues (1996) for plasma cortisol concentrations was found to be due not to tier per se, but rather to differential access to a viewing window in the door separating the housing room from the anteroom. Once this window was covered, no differences in cortisol or other measures were found based on housing location.

Diet

Relocation may involve changes in diet. In many cases, the food itself, which is usually commercially available monkey chow often supplemented with fruit and vegetables, may not change; however, the timing of feedings may well change, and the animal may need time to adapt to the change. In fact, timing of feedings is an often-overlooked factor in research. For example, Cameron (1996) has reported that reproductive hormone secretion can change in response to missing a single meal, and Mattern and colleagues (1993) found that shifting the daily feed by 6 hr resulted in a concomitant shift in luteinizing hormone and testosterone secretion in males 6 to 8 wk after the change in feeding schedule.

In other cases, diet may be manipulated intentionally, either for weight control or for experimental reasons. For example, solid food or water may be restricted to motivate animals to engage in desired behaviors such as performance on learning tasks. It is important to note that not all animals adapt to such restriction. At the California National Primate Research Center, approximately one third of animals do not tolerate water restriction as implemented, another third tolerate it, and the remaining third adjust quite well (K. Christe, California National Primate Research Center, personal communication, 2006). Thus, there may be animal selection issues associated with experimentally manipulated changes in diet. Care must also be taken to prevent animals from overeating following a period of food restriction, as gastric dilation may result (Pond et al. 1982). Finally, long-term controlled caloric restriction was shown to have beneficial effects on morbidity, insulin sensitivity, and mortality in rhesus monkeys (Bodkin et al. 2003). Together, these results underscore the importance of considering the potential inadvertent effects of diet on research outcomes.

Resocialization

To enhance psychological well-being, animals relocated for research protocols may be paired, either permanently or intermittently (e.g., during caretakers' working hours). The studies reviewed above suggest that relocating an animal with a familiar companion might buffer some of the adverse effects of stress. However, once animals are relocated, they often experience a period of individual housing (e.g., for health screening) before a pairing procedure is begun in which previously unfamiliar animals are introduced to each other. Both the duration of individual housing and the social complexity of the new group (pair vs. group housing; e.g., Reinhardt 1991) could be a factor in the success of the resocialization effort. For example, Taylor and coworkers (1998) compared adult male bonnet macaques that had been individually housed for 24 mo with comparably aged males that had been individually housed for only 1 mo. When the 24-mo animals were placed together in a group of three, they spent significantly more time alone and less time grooming than the 1-mo males when they were placed in a three-member group.

When animals are paired, prior familiarity can result in more harmonious interactions. When previously unfamiliar animals are paired, success can depend on the establishment of clear-cut dominance relations. In fact, Schino and colleagues (1990) reported that long-tailed macaques that were unfamiliar with each other but that quickly established dominance relationships were similar to familiar pairs in their display of affiliation. Unfamiliar pairs that did not establish clear-cut dominance relations, however, continued to display conflict, tension, and less affiliation (Schino et al. 1990). So important is the establishment of dominance relationships to the success of pairing efforts that exposure of previously unfamiliar animals to each other in order to facilitate resolution of dominance conflicts prior to physical pairing has become a commonplace method of pairing (e.g., Eaton et al. 1994; Lynch 1998; Reinhardt 1994).

Two studies are relevant in the context of addressing the question of how long it may take animals to achieve biobehavioral stability following resocialization. In one study, Coe and colleagues (1992) found that aged females that were paired with juveniles showed a decrement in immunity that persisted for the 3-mo period of the study. Allowing the aged females to control access of the juveniles to their cages eliminated that decrement. In another study, Capitanio and coworkers (1998b) placed previously unfamiliar adult male rhesus monkeys together for 100 min per day for social interaction in social groups that were either stable (same three-member groups per day) or unstable (number and identity of partners changed daily). Concentrations of basal plasma cortisol decreased significantly for members of both groups through week 8 after socialization began, suggesting that the animals were stressed. (During chronic stress, regulation of the hypothalamic-pituitary-adrenal axis is altered, so basal cortisol concentrations [i.e., concentrations when the animal is not acutely stressed] decrease; Mendoza et al. 2000.) For animals in the unstable condition, levels continued to decrease, but for animals in the stable social condition, concentrations then increased to presocialization levels after another 4 to 8 wk. This increase in basal cortisol to presocialization levels suggests that stress associated with resocialization may persist through 8 wk, and may normalize by 12 to 16 wk.

Overall Recommendations

The preceding section (Animal Conditioning) describes current knowledge about behavioral and physiological responses of LOWMS to manipulations that are likely to be encountered as they rotate onto research projects. Regardless of whether the animals are shipped from one facility to another, they are likely to have a variety of new experiences that might affect the research, including separations from familiar companions, relocation to new housing, restriction of space, alteration of photoperiod and diet, and introduction to new companions. How much time should elapse between the time such substantial changes occur and the time the animals' behavior and physiology have stabilized sufficiently so that the research protocol is not affected by the animals' attempts to adapt? Unfortunately, there is no clear answer, given the number and complexity of the factors involved and the lack of systematic research.

We believe, however, that the data reviewed in this article suggest that when housing changes involve most or all of the experiences described above, that a period of up to 3 mo should elapse before protocol-related data collection begins. This recommendation is based on several published results, which are described above and may be summarized as follows:

1. Gust and colleagues (Gordon et al. 1992) showed normalization of immune measures by 11 wk;
2. Capitanio and Lerche (1998) found effects on survival for SIV-infected monkeys of separations and relocations occurring in the 90-day period before inoculation but not in a longer time frame;
3. Coe and coworkers (1992) showed that immune measures for aged females were suppressed for 3 mo after resocialization;
4. Capitanio and colleagues (1998b) showed that cortisol values normalized by 12 to 16 wk after adult male rhesus were placed into three-member groups daily;
5. Capitanio and coworkers (1998a) suggested normalization of basal cortisol concentrations and leukocyte numbers between 1 and 5 mo after separation and relocation;
6. Sackett (1981) reported that reproduction was impaired

when females conceived within 90 days of air transport and subsequent placement in social groups; and

7. Suomi and colleagues (1973) reported normalized social behavior in paired infant monkeys 2 to 4 mo after permanent maternal separation.

These reports generally suggest that within a period of approximately 3 mo, biobehavioral changes might still be occurring that could have an impact on experimental data. When fewer of the aforementioned aspects of the separation/relocation experience occur, or if animals are relocated with familiar companions, this time period will likely be shorter. In addition, for some experiences (e.g., moving to a different cage within the same room), the recommended delay may be on the order of 1 or 2 days. We recognize that a delay of up to 3 mo may seem like an expensive length of unproductive time. However, this period may be useful for training animals to cooperate during venipuncture, for identifying compatible social partners, and for habituating the animals to personnel, research-related apparatus and procedures, and indoor cleaning and husbandry procedures.

We believe that several additional considerations are relevant in the context of preparing animals for research projects.

- Note that there are significant individual differences in behavioral and physiological responsiveness to the kinds of manipulations discussed above. In other words, the period of adjustment to new housing may vary depending on the animals' temperament, early history, and/or genotype.
- Carefully consider the measures used to show adjustment to the new housing situation. As described above, Ruys and coworkers (2004) found dissociation between the behavioral and the adrenocortical response to repeated chairing in adult male rhesus monkeys. Different measures may give different answers to the question of whether the animals have "habituated" to the housing change. Obviously, the best measure that can be used to answer this question for any particular research study is the measure that the study is investigating, whether it is behavior, cellular immune function, liver enzymes, or cardiovascular responsiveness.
- Pay close attention to counterbalancing in experimental design. As described above, not all animals respond in the same way to the stressor of relocation. In fact, experiences that animals may have had, such as early separation, may lead to long-lasting alterations in behavioral or physiological function, as described above. Individual differences should be stratified across all treatment conditions whenever possible.

Concluding Thoughts

In colonies of LOWMS, animals are selected (whether intentionally or inadvertently) for enrollment into research protocols, and that selection typically involves moving the animal from one environment to another that is more suitable for the research project. It is important for the researcher to allow sufficient time for the animal to adapt to the changed circumstances. We have reviewed research pertinent to this adaptation, but we acknowledge that more systematic investigation of this adjustment process is warranted. As we await additional science-based information, we believe the existing data suggest that a period of up to 3 mo may be necessary for full adaptation of individuals experiencing major changes in social and housing circumstances. Colony managers and researchers alike must attend to issues of animal selection and preparation to promote the highest quality science with the fewest animals possible.

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