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Frequency of Feeding Enrichment and Response of Laboratory Nonhuman Primates to Unfamiliar People

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Although environmental enhancement plans for nonhuman primates vary between facilities, feeding enrichment represents a component of most programs. As part of a facility's feeding enrichment program, offering hand-fed food items by trained staff provides an opportunity for positive human interaction. We hypothesized that increased implementation of such enrichment would be associated with increased likelihood of a monkey accepting a hand-fed treat from a stranger. Several species of monkeys were tested at the Tulane National Primate Research Center. In 2002 and 2005, we recorded the number of caged monkeys that accepted a treat tablet from an unfamiliar person within 10 s. We compared the frequency of caretaker-implemented feeding enrichment documented for each animal room during the month proceeding data collection with the proportion of animals within the room that accepted the treat from the stranger. In 2002, 29.8% of the 500 subjects accepted the treat from the unfamiliar person. The proportion of animals that accepted the treat was significantly correlated with the number of days during which feeding enrichment had been implemented. In 2005, feeding enrichment frequency had increased by 76%, and 53.4% of the 676 subjects accepted the treat. These findings suggest that this simple form of enrichment may improve monkeys' responses to unfamiliar people, and that it holds promise as a method for mediating the stress imposed by human activity in animal rooms. In addition, a stranger's treat-feeding attempts may be a useful indicator of an institution's implementation of their environmental enrichment program.

The entry of an unfamiliar person into a housing room containing caged nonhuman primates can elicit a wide range of responses from the animals, including fear, aggression, affiliation, or calm watchfulness. Staff and visitors often take note of these reactions and intuitively use these observations to form judgments about the animals' psychological well-being and the quality of behavioral management these animals receive. Many variables doubtless influence response to room entry, including species, sex, rearing background, research use, and duration that individuals have been housed in indoor cages. In the face of this variability, the validity of the idea that response to room entry can reflect the care of the animals merits evaluation.

One aspect of behavioral management that may influence the way primates react to room entry is a facility's feeding enrichment program. The provision of food treats is a component of most, if not all, laboratory facilities housing nonhuman primates.[4] Facilities tend to rely most heavily on husbandry staff to distribute this feeding enrichment, and many facilities also stress the role of positive human interaction during treat feeding.[4] Human interaction has been promoted as a valid means to enhance captive environments,[6,13,16-20] and there is experimental support for the idea that human interaction contributes to the psychological well-being of nonhuman primates. Direct human interaction (positive reinforcement training and unstructured interaction) has resulted in decreased abnormal behavior,[2,5,8] decreased anxiety-related behavior,[3,8] increased affiliation,[2,8] decreased aggression,[7,8] and decreased inactivity.[2] However, routine monitoring or observation of macaques by familiar personnel can result in persistent stress responses,[12,14,15] and other species show patterns of wounding[11] and timing of births during the week[1] that suggests a relationship between level of stress and level of human activity in the laboratory environment. The degree to which this effect can be moderated has not been explored.

Although the feeding of treats to nonhuman primates has been a component of the environmental enhancement program at the Tulane National Primate Research Center for many years, implementation levels have always varied somewhat between animal rooms. This variation affords an opportunity to evaluate the relationship between the frequency of feeding enrichment and the behavior of the animals in different rooms. In addition, implementation levels have risen markedly over the past several years. This article focuses on the reaction of nonhuman primates at 2 time points, 3 y apart, to attempted hand-feeding of edible treats by facility employees that do not regularly interact with the animals. By quantifying this response to unfamiliar people, we tested whether it was associated with recent changes in behavioral management. These findings may have implications for moderating the effect of human activity around caged primates; our results also bear on the possible use of this measure as a valid piece of evidence regarding the care of captive primates.

Materials and Methods

Animal housing and husbandry. All subjects derived from the research colony at the Tulane National Primate Research Center (Covington, LA), which is accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care, International. Study subjects were housed indoors in stainless steel cages with a height of 36 in. and either 4.3 or 8.6 ft^2 of floor space, depending on body weight and in accordance with federal animal welfare regulations. Animal rooms were maintained on a 12:12-h light:dark cycle, and the ambient temperature of

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animal rooms was maintained between 64 to 72 °F (17 to 22 °C) with a relative humidity of 30% to 70%. Animal care staff provided food biscuits (Teklad NIB Primate Diet, Harlan Teklad, Madison, WI) twice daily; monkeys had access to fresh clean water ad libitum. Each cage included a manipulable object such as a toy, polyvinylchloride elbow joint, or hardwood segment, and most cages contained perches. Animal rooms contained 3 to 28 individuals. Subjects were assigned to various research or animal holding protocols approved by Tulane's animal care and use committee. Rooms comprising the monkey nursery, infirmary, and quarantine were not sampled. We also excluded rooms housing monkeys being trained or used in projects requiring monkeys to perform behavioral tests in order to receive a portion of their daily diet. All aspects of management and research use conformed to applicable federal regulations and the guidelines described in the *Guide for the Care and Use of Laboratory Animals*.[17]

Subjects. Most subjects were rhesus macaques (*Macaca mulatta*); the sample also included pigtailed macaques (*M. nemestrina*), savannah baboons (*Papio* spp.), African green monkeys (*Chlorocebus aethiops*), mangabeys (*Cercocebus* spp.), patas monkeys (*Erythrocebus patas*), and squirrel monkeys (*Saimiri scuireus*). Table 1 shows the number and proportion of individuals of different species included in the 2002 and 2005 samples. Room composition varied considerably within both phases of the study, with respect to whether rooms contained both sexes, mixed ages, or multiple species.

In 2002, subjects included 500 singly caged monkeys (308 females and 192 males) housed among 34 animal rooms. In 2005, subjects consisted of 676 caged monkeys, 585 singly caged and 91 caged in pairs or trios; 507 subjects were female, and 169 were male. Subjects in 51 rooms were included in this phase of the study.

Feeding enrichment. Monkeys at the Tulane National Primate Research Center have always received a variety of foods in addition to nutritionally complete biscuits. These foods have been given for a variety of purposes; for example, in association with interventions for abnormal behaviors, as a vehicle for delivering clinical treatments, to facilitate observation, and in order to foster cooperation with management or clinical procedures. In addition, a colony-wide feeding enrichment program, implemented by husbandry staff, has long been in place to systematically deliver items to all animals; we refer to this program as "feeding enrichment" hereafter.

Implementation of feeding enrichment varied between the 2 data collection periods. In 2002, documentation of feeding enrichment in the month preceding data collection averaged 8.2 (range, 1 to 29) feedings per month over all rooms. In 2005, feeding enrichment was documented at an average implementation level of 14.4 (range, 6 to 29) times per month. The degree of variety also differed between the 2 study periods. In 2002, subjects were fed a quarter or half of an apple or orange. By 2005, subjects were fed similar portions but a larger variety of fruits and vegetables. Each month, approximately 5 types of produce from the following list were distributed: apples, asparagus, bananas, broccoli, cabbage, carrots, cauliflower, celery, cilantro, collard greens, cucumbers, eggplant, grapefruits, grapes, green beans, lemons, lettuce, limes, mint, mustard greens, onions, oranges, parsley, pears, peppers, satsumas, squash, sprouts, sweet potatoes, tomatoes, and turnips (roots and greens). In comparison to familiar foods, novel food items were reported by husbandry staff to be eaten by a smaller proportion of animals upon the first several presentations, as would be expected given the food neophobia demonstrated by nonhuman primates.[10] The proportion of animals that consumed particular types of feeding enrichment was not quantified in either 2002 or 2005, but any novel produce item that was not eaten by a considerable proportion of the animals after several feedings was discontinued and is not included in the above list.

Table 1. Species composition of study population

Species	No. of subjects (% of sample)	
	2002	2005
Rhesus macaque	437 (87.4%)	587 (86.8%)
Pigtailed macaque	27 (5.4%)	47 (7.0%)
Savannah baboon	12 (2.4%)	12 (1.8%)
African green monkey	9 (1.8%)	10 (1.5%)
Mangabey	8 (1.6%)	13 (1.9%)
Patas monkey	4 (0.8%)	4 (0.6%)
Squirrel monkey	3 (0.6%)	3 (0.4%)

Data collection and analysis. Data were collected over a 3-wk period in August 2002 and again in March 2005. The identity of the experimenters varied between the 2002 and 2005 data collection period (though both were women). Both experimenters were unfamiliar to the study subjects, but both were knowledgeable about the social signals of the species included in the study and the mechanisms by which people can avoid antagonizing or frightening caged monkeys.

In both 2002 and 2005, each subject was offered one of a variety of Supplemate fruit-flavored treat tablets (P. J. Noyes, New Brunswick, NJ). These tablets were neither entirely novel nor entirely routine to the colony. For at least a decade, they have been included in the foods distributed to subsets of animals, as outlined earlier, or as occasional substitutes for fresh produce. The tablets were offered in an open palm held in front of the biscuit opening of the monkey's cage. At all times, the experimenter wore appropriate personal protective equipment, including cut-resistant gloves and gauntlets. The experimenter avoided eye contact with monkeys and stood at an angle relative to the cage front while offering treats. Each offer lasted 10 s. All animals in a particular room were offered treats during the same visit.

Data were collected between the hours of 10:00 and 15:00. The experimenter recorded the room number, total number of animals in the room, and number of animals that took the treat within 10 s. The feeding enrichment implemented in a particular room was recorded by husbandry staff on a daily basis. All statistical tests were performed using Statistica (Tulsa, OK); all analyses were conducted with a significance level set at $P < 0.05$. For each room, the proportion of monkeys that took treats was compared with the number of days during which feeding enrichment was distributed in that room in the month preceding data collection, and these data were analyzed using simple regression. The proportion of animals taking treats within each room was compared between 2002 and 2005 by using one-way analysis of variance. All analyses were run twice, both excluding and including the data derived from the rooms containing socially housed animals. Exclusion of these data did not alter the direction or significance of any results; therefore findings involving all subjects are reported.

Results

In 2002, during which feeding enrichment was implemented an average of 8.2 times during the month preceding data collection, 149 of the 500 subjects accepted the hand-fed tablet from the experimenter within 10 s. The proportion of animals in a room that accepted treats was significantly correlated ($r^2 = 0.35$, $P < 0.0005$) with the number of days during which feeding

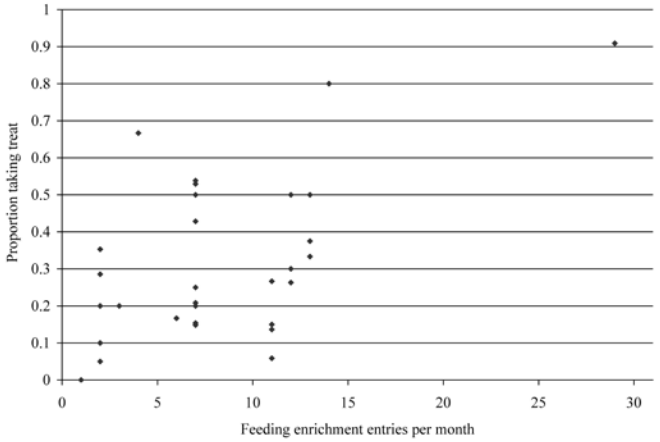

Figure 1. Relationship between feeding enrichment levels and proportion of monkeys in a room that accepted a treat from an unfamiliar person.

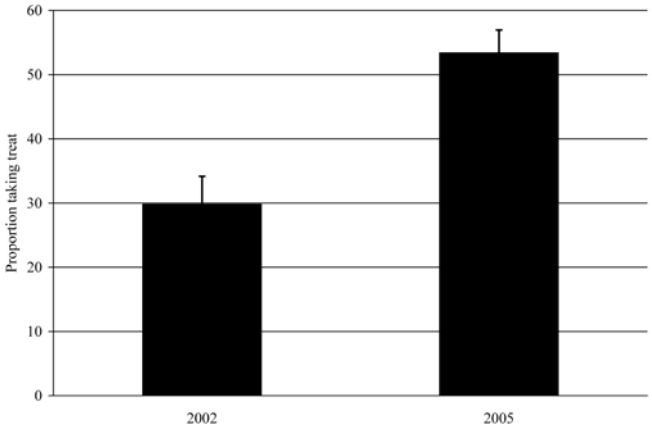

Figure 2. Proportion (mean ± standard error) of monkeys accepting treats in 2002 and 2005.

enrichment was distributed (Figure 1).

In 2005, during which feeding enrichment was implemented 14.4 times per month, 361 of the 676 subjects hand-fed from the experimenter. The proportion of animals taking treats in 2005 was not significantly correlated ($r^2 = 0.04$) with the number of days during which feeding enrichment was distributed. Feeding enrichment documentation was significantly ($F[1,83] = 9.9$; $P < 0.005$) higher in 2005 than 2002. The proportion of animals taking treats was also significantly ($F[1,83] = 12.6$; $P < 0.001$) higher in 2005 than 2002 (Figure 2).

Discussion

The data presented here support the hypothesis that levels of treat feeding influence monkeys' receptivity to unfamiliar people and that changes in the frequency of receiving feeding enrichment influences monkeys' likelihood of accepting a food treat from such a person. By 2005, the primates at the Tulane National Primate Research Center had experienced a significant increase in the number of times they were provided with feeding enrichment on a monthly basis. Their receptivity to an unfamiliar person, as measured by acceptance of a hand-fed treat from a stranger, nearly doubled over levels in 2002, from 29.8% to 53.4%.

Because the individual identity of study subjects was not recorded along with whether the treat was accepted and because the vast majority of rooms containing species other than rhesus macaques contained more than one species, we cannot report on species differences in receptivity to hand-fed treats in general nor on how the increase in feeding enrichment might have affected species differently. However, it is interesting to note that in the 2 rooms studied in 2002 that contained no rhesus macaques (only baboons and mangabeys), treats were accepted by 80% of the animals, and in 2005, among the 4 rooms containing only species other than rhesus macaques (including baboons, mangabeys, African green monkeys, and pigtailed macaques), 89% of subjects accepted the treat. Although the sample size is small (20 individuals in 2002 and 45 in 2005), these data suggest that the other species of laboratory primates included in this study may be more receptive to treat feeding from unfamiliar people than are rhesus macaques. However, an evaluation of this suggestion would need to control for many variables that this study cannot, including age, sex, current and past research use, rearing, and tenure in single housing.

Although the differences in representation of the species in the 2 sampling periods were so small as to be unlikely to represent a significant confound, several other caveats warrant consideration. First, although the data were analyzed as unpaired, a proportion of study subjects may have been sampled in both time periods. This situation introduces a methodological irregularity that cannot be controlled for because the individual identity of study subjects was not recorded along with whether the treat was accepted. Although that fact is unlikely to have influenced our findings, a paired assessment would have been preferable in order to control for individual differences and potentially confounding variables. Second, sex differences could not be assessed directly because individual identity was not recorded. This situation is a potential confound because a smaller proportion (25%) of the subjects were male in 2005 than in 2002 (38%). If male monkeys are less likely to take treats, our findings could be attributed to this difference. However, in 2005, several rooms housed 1 sex of rhesus macaques only. Comparing the 7 all-female rooms (treat offered a mean of 12 times per month) with the 4 all-male rooms (treat offered a mean of 10 times per month) revealed no tendency for male monkeys to show lower receptivity (mean, 69%) in comparison with female animals (mean, 42%). This finding suggests that the varying sex ratio between the cohorts sampled in 2002 and 2005 does not account for the difference in receptivity between years.

In addition, this study could not control for rearing background. We predict that nursery-reared animals would be more likely to take treats from unfamiliar people. The data also do not permit assessment of the duration of housing in cages (as opposed to outdoor enclosures), and increased duration in cages may increase receptivity to treats over time. Although we are unable to quantify mean tenure in caging among our samples, we have no reason to suspect that there was a higher mean tenure in the 2005 sample. It is more likely, in fact, that the mean tenure was lower because the overall size of the colony grew between the 2 sampling periods, primarily through the transfer of animals from our outdoor-housed breeding colony corrals.

This study also cannot control for research use (with the exception that monkeys receiving behavioral testing and training were excluded). The frequency of invasive access and degree to which protocols influence animal health can be expected to influence their response to treat feeding. For example, it is likely that during both sampling periods there were subjects whose response to the treat offer was influenced by anorexia due to clinical condition or recent sedation.[9] Although the same general categories of research were being undertaken in both

years and although there was continuity in terms of standards of veterinary care and clinical treatment, we cannot demonstrate that a similar proportion of animals in both time periods refused treats for reasons unrelated to their response to people. Further study involving individuals as their own controls could address these potential confounds.

Further, the identity of the tester varied between the 2 periods. However, treats were offered in a prescribed manner (involving a predetermined duration and demeanor during the offer) during both periods. In addition, the experimenter in 2005 had fewer years of experience and expertise working around nonhuman primates, making it more likely that receptivity to treat feeding would have been reduced rather than enhanced, as was found in this study.

Finally, Tulane's environmental enhancement program changed in several other ways between 2002 and 2005, due to the expansion of its enrichment department. For example, this span of time saw an increase in the variety of toy and food types, consistency in the provisioning of perching, enrichment staffing levels, and enrichment intervention for an increased proportion of monkeys.

During both phases of data collection, enrichment staff activities involved treat feeding that was not recorded on caretaker records used to provide data for this study. When enrichment staff personnel enter an animal room, it is a standard practice for them to treat-feed the entire room before proceeding with the room visit's main objective (for example, social introduction, rotation of enrichment devices). In 2005, because of the increase in the number of enrichment staff, monkeys received food treats from a larger number of different individuals, and the documentation of feeding enrichment underreported actual implementation among all staff types to a much larger degree in 2005 than 2002. The magnitude of change in feeding enrichment frequency is likely to be larger, on average, than the 76% increase reflected in caretaker records.

In addition, because the levels of enrichment staff activities varied between rooms, levels of feeding enrichment implemented by enrichment staff varied as well. This variation was more pronounced in 2005, because of the increase in enrichment staff activities, and this difference may explain why no relationship between treat-feeding levels and proportion of animals taking a treat was detected in 2005 as it was in 2002.

By 2005, training of husbandry staff had involved increased emphasis on hand-feeding monkeys (while wearing appropriate personal protective equipment) rather than on placing the food treats directly into cages without interaction. The manner rather than just the frequency of providing feeding enrichment may have contributed to our findings. However, the tight correspondence in the 2002 data between feeding enrichment levels and monkeys' response to attempted hand-feeding suggests that feeding enrichment frequency is likely to be the cause of the increased willingness to accept treats from strangers. In the face of the potential confounds and variation between sampling periods, dramatic differences between monkeys' responses to attempted hand-feeding emerged. This finding suggests that a change in the implementation level of one form of feeding enrichment has a significant impact on the animals' overall receptivity to humans.

Decreased fear of humans is likely to positively influence the well-being of captive primates. The results of this study suggest that the stress response among caged macaques can be ameliorated through these simple steps, a suggestion that may merit further examination. An assessment using a paired design of the response to unfamiliar people could be conducted, not only involving behavioral measures but also cortisol levels, heart rate, or blood pressure. Whether there is corresponding improvement in behavioral measures of well-being accompanying receptivity to treat feeding was not explored in this study, but building upon the work of Bayne and colleagues,[5] we currently are evaluating different levels and styles of human interaction on the well-being of rhesus macaques.[3,7]

Our results suggest that monkeys' responses to visitors, as measured by willingness to hand-feed, may be a valid reflection of a facility's behavioral management practices. We therefore propose that observed changes over time in this respect could be a useful tool in validating an enrichment program's changing standards or for evaluating the success of a program. These findings may be of use for internal measures of success and both internal and external inspections.

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