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Positive reinforcement training as enrichment for singly housed rhesus macaques (*Macaca mulatta*)

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Abstract

Positive reinforcement training is one component of behavioural management employed to improve psychological well-being. There has been regulatory promotion to compensate for restricted social housing in part by providing human interaction to singly caged primates, implying an efficacy standard for evaluating human interaction. The effect of positive reinforcement training on the behaviour of 61 singly housed laboratory rhesus macaques (*Macaca mulatta*) was evaluated at two large primate facilities. Training involved body part presentation and basic control behaviours. Baseline data were compared to two treatment phases presented in varying order across individuals, six minutes per week of positive reinforcement training and six minutes per week of unstructured human interaction. While a MANOVA involving behavioural categories and study conditions across study subjects was significant, univariate ANOVAs found no effect of phase within any behavioural category. Categorising subjects according to rearing, housing facility, or baseline levels of abnormal behaviour did not reveal changes in behaviour with positive reinforcement training or human interaction. This study failed to detect, to any degree, the types of behavioural changes documented in the scientific literature to result from pairing singly housed monkeys. Implementing short durations of positive reinforcement training across large numbers of singly housed animals may not be the most effective manner for incorporating positive reinforcement training in the behavioural management of laboratory macaques. Rather, directing efforts toward individuals with specific behavioural, management, clinical, research or therapeutic needs may represent a more fruitful approach to improving psychological well-being with this technique.

Keywords

animal welfare; behavioural management; human interaction; laboratory primates; positive reinforcement training; single housing

Introduction

Recent surveys concerning the social housing of laboratory non-human primates living indoors have found that approximately half are housed singly (Baker *et al* 2007; Crockett *et al* 2008). Although some progress in the use of social housing has been made over the previous decade (ie in comparison to the findings of Reinhardt [1994]), a significant proportion of laboratory primates will remain in this housing condition for the foreseeable future. It is critical to address the behavioural management of this population since single housing deprives animals of social contact and increases spatial restriction. Single housing also removes a potential source of buffering against environmental stressors and reduces opportunities for species-appropriate behaviour, cognitive challenge, physical activity, choice and control. To address the variety of environmental deficits associated with single housing requires the application of multiple techniques including the provision of manipulable objects, structural complexities, increased variety and frequency of feeding, foraging devices, and visual and auditory stimuli, as well as positive reinforcement training (PRT) and human interaction. While the majority of facilities report that social housing, objects, structures, feeding enrichment, and human interaction are elements of their behavioural management programmes, only approximately half employ PRT, and few have dedicated trainers (Baker *et al* 2007).

There are two general approaches in behavioural management to allocating training efforts to laboratory primates. The first is targeting training to individual animals exhibiting behavioural problems, causing management challenges, or undergoing stressful research and clinical procedures. For example, PRT can be an effective intervention for abnormal behaviour, as has been demonstrated in several case studies involving Great Apes (Morgan *et al* 1993; Raper *et al* 2002; Bourgeois *et al* 2007, but see Dorey *et al* 2009) and in a meta-analysis of some relevant human literature (Kahng *et al* 2002). Problems encountered with moving non-human primates between enclosures or with competition when groups are fed have been successfully addressed with PRT in chimpanzees (*Pan troglodytes*) and mangabeys (*Cercocebus atys*) (Bloomsmith *et al* 1994, 1998; Veeder *et al* 2009). PRT can also be used to reduce stress responses to common procedures in the biomedical environment (Reinhardt *et al* 1990; Koban *et al* 2005; Videan *et al* 2005; Lambeth *et al* 2006). The second approach is to apply PRT in a manner more analogous to routine environmental enrichment implementation. In other words, it is 'distributed' to a large number of animals, regardless of the nature, frequency, or context of any behaviour problems. Most studies of this 'training as enrichment' have been evaluated in Great Apes. Among chimpanzees, training for compliance with basic husbandry commands resulted in lower levels of abnormal and anxiety-related behaviour, in one study only during training sessions (Bloomsmith *et al* 1999), but in another during periods of time outside the training sessions (Pomerantz & Terkel 2009). Both studies detected generalised improvement in social dynamics (Bloomsmith *et al* 1999; Pomerantz & Terkel 2009). However, unstructured human interaction for chimpanzees can have these same effects (Bloomsmith *et al* 1999; Baker 2004). In addition, unstructured human interaction for as little as six minutes per week has reduced abnormal behaviour among singly housed rhesus macaques (*Macaca mulatta*) (Bayne *et al* 1993). It is important therefore to evaluate PRT in such a way as to

differentiate its specific effects from those resulting from the simple addition of human attention and the foods that may be associated with the training.

Quantifying the effects of either form of human interaction is of importance not only for guiding behavioural management programme decisions but also for meeting US regulatory and accreditation expectations, as well as eligibility for National Institutes of Health funding. The National Research Council *Guide for the Care and Use of Laboratory Animals* (1996) states that:

It is desirable that social animals be housed in groups; however, when they must be housed alone, other forms of enrichment should be provided to compensate for the absence of other animals such as safe and positive interaction with the carestaff and enrichment of the structural environment.

This standard logically implies that the effects of human interaction should resemble those of conspecific social housing, setting a performance standard for determining the sufficiency of a programme's human interaction component. Fortunately, the behavioural effects of pair housing have been relatively well studied. Most studies examining the effects of introducing singly housed macaques into compatible pairs have found that it can be highly effective for reducing abnormal and anxiety-related behaviour, as well as increasing activity levels (Reinhardt *et al* 1988; Line *et al* 1990; Eaton *et al* 1994; Schapiro *et al* 1996; Doyle *et al* 2008). The effects of conspecific social housing are used here as a standard for evaluating the behavioural effects of PRT provided as environmental enrichment to singly housed rhesus macaques, one of the most prevalent non-human primate species held in laboratories in the United States (Carlsson *et al* 2004; Conlee *et al* 2004). This study involved a large sample of rhesus macaques, allowing an assessment of the role of rearing history on the effects of training. This variable is relevant because rearing has long been known to affect development in a variety of respects relevant to training, such as sociality and cognition (reviewed in Novak & Sackett 2006). Since a previous study, focusing on different abnormal behaviour types among this same study group, found differential responses by individuals with varying baseline levels of abnormal behaviour (Baker *et al* 2009), this variable was also applied to the current broader assessment of behaviour.

Materials and methods

This experiment was designed as a collaborative research project between the Tulane National Primate Research Center (TNPRC) and Yerkes National Primate Research Center (YNPRC) and used identical methodology.

Study animals

The subjects consisted of 30 male and 31 female rhesus macaques between 3.3 and 16.7 years (6.9 ± 0.3) at the start of the study. Subjects were drawn from those assigned to research projects with compatible design and timeline. Approximately half of the subjects were included due to observation of relatively high levels of abnormal behaviours. Subjects' rearing backgrounds included mother rearing in a social group for at least the first 6 months of life ($n = 14$), mother rearing in mother-infant pairs for at least the first 6 months ($n = 20$), nursery rearing with peer contact for at least 2 months during the first year of life ($n = 14$),

or nursery rearing without social contact during the first 2 years of life ($n = 13$). All animals were naïve to positive reinforcement training techniques.

Housing and husbandry

Subjects were housed at the TNPRC ($n = 29$) and the YNPRC ($n = 32$). All aspects of management and research use conformed to applicable United States federal regulations and the guidelines described in the *Guide for the Care and Use of Laboratory Animals* (National Research Council 1996). Subjects were assigned to various research or animal-holding protocols approved by the facilities' Institutional Animal Care and Use Committee.

Subjects were housed indoors in individual stainless steel cages with a height of 76–91 cm, and floor space of 0.40–0.74 m² depending on bodyweight and in accordance with United States federal animal welfare regulations. Animal care staff provided food biscuits twice daily, and water was available *ad libitum*. Feeding enrichment consisted of fruit, vegetables, and other food treats, which were distributed three to five times per week. Each cage included a perch and a manipulable object such as a toy, PVC piece or hardwood segment. Many cages were also equipped with foraging or grooming devices, and the inanimate environmental enrichment conditions were held constant through the period of study for all subjects. Baseline levels of human interaction were estimated at 30 s per day in the course of routine husbandry and feeding enrichment implementation at both facilities.

Experimental procedures

This study involved three conditions, a baseline condition lasting four weeks and two experimental conditions each lasting eight weeks. The baseline condition was compared, using a within-subjects design, to two phases in which each subject received six minutes per week of PRT or six minutes per week of human interaction. Subjects were exposed to these experimental conditions in a counterbalanced order with some subjects receiving the PRT condition before the human interaction, and some the reverse. One person implemented all experimental phases for a particular animal. Types of treats and quantity given were held constant across all phases. Personnel safety was ensured through compliance with standard operating procedures for personal protective equipment (PPE), and the use of additional PPE such as cut-resistant gloves and gauntlets, which were not required for other types of animal care procedures at either facility. In addition, research technicians were familiar with macaque social behaviour and individual subjects' temperaments.

During both PRT and human interaction phases, sessions were conducted two or three times per week. PRT sessions involved teaching the animals presentation of body parts and basic control behaviours (eg sitting, standing, stationing which required the animal to touch an object clipped to the outside of its cage). Reinforcers consisted of food, praise, or tactile contact, and a small handheld clicker was employed as a secondary reinforcer or 'bridge'. The use of various reinforcers was left to the discretion of the trainer, and multiple reinforcers were sometimes applied in the same training session. The human interaction phase consisted of treat feeding (not contingent upon the subject's behaviour), play, and/or grooming (only if the animal presented for grooming against the cage bars; the interactor did not reach into the cages).

When subjects performed abnormal behaviour or were very aggressive to the person during training or interaction sessions, the person ceased interacting with the animal and resumed once the behaviour stopped. If bouts of abnormal behaviour were longer than a few seconds, or involved self-biting, the person turned her back until the behaviour ceased. These 'time-outs' occurred during fewer than 10% of sessions and lasted no more than 10 s. 'Time-outs' are commonly used by those applying PRT (eg Morgan *et al* 1993), and were intended to reduce the likelihood of inadvertently reinforcing an undesirable behaviour with human attention. However, it is possible that in some animals, the behaviour could have been reinforced by the removal of human attention.

Data collection

Data were collected and coded by four individuals after establishing inter-observer reliability among a minimum of two observers with a minimum of 85% agreement. After the collection of baseline data, each experimental phase was implemented for four weeks before the onset of data collection to characterise relatively long-term effects and not initial adjustment to the treatment conditions. Videotaping was employed to collect 60-min focal observations. All focal observations were collected outside of the times during which the person was working with the subject. Observation time was held steady over phases in recognition of the effect of time of day on behaviour. In each phase, 4–8 h of data were collected per animal, for a total of 1,001 h. Data were coded with an exhaustive and mutually exclusive ethogram of 62 behaviours, using instantaneous sampling with a 15 s intersample interval. Pre-determined decision rules were applied for priority of data entry for samples in which more than one behaviour occurred. Point samples for individual subjects were pooled across observation periods, and statistical analyses were performed using percentage of samples for each behaviour in each study phase. Behaviours were collapsed into nine categories for analysis (see Table 1 for operational definitions).

Statistical analysis

All statistics were calculated using Statistica 6.0 for Windows. Measures of skewness, kurtosis, and homogeneity of variance failed to meet required assumptions for parametric tests, so data were transformed using an arcsin square-root transformation prior to analysis. Means and standard errors are reported based upon back-transformed values. Multivariate analyses of variance (MANOVA) for repeated measures were used for the analyses of the nine behavioural categories across the three study conditions. A significant overall test (with α set at 0.05, two-tailed) was followed by univariate tests of the separate behavioural categories, with alpha adjusted to 0.01 to correct for multiple comparisons. When significant, ANOVAs were followed by *t*-tests, also with alpha adjusted to 0.01.

Subjects were classified according to early rearing history (grouped for analysis as either mother-reared in a social setting or any other settings) and the facility in which they were housed. They were also ranked by the percentage of time devoted to abnormal behaviour during the baseline period. A quartile division was performed and subjects were categorised as low-abnormal (< 8.4% of samples), moderate-abnormal (8.4 — < 25.0%), or high-abnormal (> 25.0%). These factors were used as grouping variables in a series of separate MANOVAs including to test for interaction effects between these variables and study phase.

As outlined above, a significant overall test was followed by univariate tests of the separate behavioural categories, followed by *t*-tests when appropriate.

Results

A MANOVA applied to the nine categories of behaviour showed a main effect of study phase ($F_{18,43} = 72.64$; $P < 0.001$). However, subsequent univariate ANOVAs found no effect of phase within any behavioural category (see Table 2). This unusual finding — a significant omnibus result for a MANOVA without the following ANOVA tests being statistically significant — can occur due to the magnitude of within-group intercorrelations (Bray & Maxwell 1985).

With respect to the series of between-subject ANOVAs used to test for effects of independent variables, rearing showed no interaction effect with study phase ($F_{18,42} = 0.83$; $P = 0.65$). An interaction effect between facility and study phase was detected ($F_{18,42} = 1.90$; $P < 0.05$). However, subsequent univariate ANOVAs revealed no interaction effects on any behavioural category (see Table 3).

The MANOVA assessing the interaction effect between study phase and abnormal behaviour quartile across all behavioural categories was significant ($F_{36,82} = 3.74$; $P < 0.001$), but subsequent univariate ANOVAs revealed no interaction effects on any behavioural category (see Table 4).

Discussion

This study found no evidence that the amount of PRT or unstructured human interaction evaluated in this experiment altered behaviour in singly housed rhesus macaques. Nor could any subset of animals (eg with a particular rearing background or level of abnormal behaviour) be identified which might benefit from these strategies. One might have expected this quantity of training as enrichment to be fruitful for several reasons. In a prior study of rhesus macaques (Bayne *et al* 1993) and in three studies of chimpanzees (Bloomsmith *et al* 1999; Baker 2004; Pomerantz & Terkel 2009), unstructured human interaction offered as enrichment did improve abnormal behaviour. Since training provides not only human interaction but also cognitive stimulation and opportunities for choice and control, it would be surprising that its benefits would not include those associated with human interaction. However, in the current study, there were also no positive effects of human interaction on rhesus macaques. This result contrasts with the findings of Bayne *et al* (1993), which documented a 13% reduction in abnormal behaviour associated with 6 min per week of human interaction with rhesus monkeys. There are several factors that could be involved in the disparity in findings between the Bayne *et al* (1993) study and the current study. Heritable biobehavioural differences in subjects' social reactivity (reviewed in Suomi [2005]) may present confounds in cross-facility comparisons involving small sample sizes. Another difference that could possibly account for the conflicting findings is that during the Bayne *et al* (1993) study, no enrichment devices were present. Perhaps this relatively lower level of stimulation available to the monkeys accounted for the positive effect of the intervention. Although rearing backgrounds and the levels of abnormal behaviour expressed

by individual subjects in the Bayne *et al* (1993) study were not indicated, the current study did not detect any role of these variables in our findings, making them unlikely to explain the disparity in findings. The current study is more robust in that it included a much larger sample size and many more hours of observational data. In addition, in the Bayne *et al* (1993) study, the beneficial changes in stereotypic and abnormal behaviour associated with human interaction also continued in the post-treatment phase of the study, indicating that there may have been a confounding factor influencing the results.

The paucity of evidence of an enriching effect of PRT makes it important to characterise the nature of the nonhuman primate-human interactions that occurred during this study. For example, there is some suggestion that whereas training neutral behaviours does not induce distress (O'Brien *et al* 2008) the process of training for co-operation with potentially frightening procedures may induce distress that counteracts PRT's potential benefits during the training period, at least with respect to some abnormal behaviours (Coleman & Meier 2010). In the current study, subjects chose to interact with the trainer during 96% of the sessions, accepted treats during 86% of human interaction sessions, and directed affiliation toward the human in 40% of sessions for both interaction phases (Maloney *et al* 2007). The duration of training was sufficient to obtain reliable performance for a mean of three different commands, suggesting that the animals understood the contingencies and chose to co-operate. Preliminary analyses of records kept by the trainers during all training and interaction sessions found no differences between treatment phases in the proportion of sessions in which abnormal behaviours were performed (Maloney *et al* 2007). However, fearful behaviour was shown during a larger proportion of training sessions than human interaction sessions and the frequency of fearful behaviour increased over the training phase but not over the human interaction phase (Maloney *et al* 2007). However, closer examination of the data revealed that 90% of fearful responses during training involved brief apprehension toward novel objects which were used only during the training sessions. It is difficult to imagine that this fleeting aversion could negate any benefits of cognitive stimulation and control, nor how a training programme could progress without the introduction of unfamiliar items and commands. The training technique of desensitisation was not used in the current study (Clay *et al* 2009), but could be recommended to help animals adjust to novel objects. It is likely that the brief aversion measured is vastly outweighed by the reduced stress associated with aversive clinical and research procedures when training is used for this purpose (eg Reinhardt *et al* 1990; Videan *et al* 2005).

It is notable that the majority of studies demonstrating a positive effect of training on well-being involved targeted training intervention designed to address specific stressors, management challenges or behavioural problems in individual animals (Reinhardt *et al* 1990; Morgan *et al* 1993; Raper *et al* 2002; Koban *et al* 2005; Videan *et al* 2005; Lambeth *et al* 2006; Bourgeois *et al* 2007; Dorey *et al* 2009), rather than a more general and broad application as was tested in the current study. This difference suggests that behavioural managers may want to consider implementing individualised training programmes to combat particular issues in a subset of animals rather than attempting to apply generalised training as enrichment on a broad scale, which might only allow providing the small duration of training studied here. 'Functional assessment' techniques used in human therapy,

which involve investigating the causes and contexts of an undesirable behaviour and tailoring the intervention to the specific reinforcing contingencies that maintain the behaviour, may be useful in addressing undesirable behaviours in captive primates (Bloomsmithe *et al* 2007; Martin *et al* 2007; Dorey *et al* 2009). The current study cannot address the potential for training to prevent the development of poor well-being in a caged environment. It is possible, for example, that desensitisation and PRT applied when macaques are initially moved from social groups to indoor caging could be effective for influencing the human-animal relationship and long-term changes in behaviour over the course of single housing.

While this study did not directly compare the effects of human vs conspecific social interaction in the same subjects, results from this study indicate that the level of human interaction evaluated here did not produce effects similar to the effects of conspecific social companionship. Descriptively, across subjects there was no difference in either phase in the level of abnormal behaviour (which changed by no more than 2%). Even the most severely affected subjects (those in the highest quartile of abnormal behaviour) showed only a 10% reduction in abnormal behaviour. This reduction pales in comparison to the effect of moving rhesus macaques from single housing to compatible pairs, which has reduced abnormal behaviour by 62–81% (Line *et al* 1990; Schapiro *et al* 1996). Also, whereas social introduction can reduce anxiety-related behaviours by over 25% (Doyle *et al* 2008), PRT and human interaction increased these behaviours by 7 and 11%, respectively (small numeric changes that were not statistically significant). In addition, levels of locomotion in pair housing have been observed to double over levels in single housing (Doyle *et al* 2008) and inactivity has been reduced by about 15% (Line *et al* 1990). In the current study, levels of both behaviours remained unchanged in the PRT condition and locomotion dropped 25% in the human interaction phase. The absence of changes in behaviour similar to those associated with social housing suggests that human interaction and training may not have much potential for replacing conspecific social housing in rhesus macaques, at least with regard to behavioural measures of their welfare. However, this study evaluated only six minutes per week of PRT or human interaction. More intensive schedules than those investigated in this study may result in behavioural changes, a suggestion being pursued by further study. However, intensive schedules are less likely to be feasibly applied in large primate colonies as general enrichment since it would require a full-time trainer for as few as 100 primates. As few laboratory facilities in the United States staff enrichment programmes this heavily (facilities average one enrichment technician for about 360 caged non-human primates [Baker *et al* 2007]), large amounts of training for large numbers of animals would require a significant increase in staffing. If the goal is general improvement in behaviour, the time that would be invested in generalised PRT may be more effective if instead it were devoted to increasing the use of social housing or enrichment strategies of demonstrated benefit. The findings of this study may be useful in guiding the allocation of human and financial resources toward the most effective techniques for improving the well-being of captive primates. For example, facilities considering the addition of PRT or human interaction as generalised enrichment may want to implement higher levels, or instead focus on a subset of the colony to improve their behavioural health. There are many future possibilities for related research that should be conducted, including the effect of larger

amounts of PRT and targeting PRT to intervene in the behavioural problems of singly housed macaques.

Animal welfare implications

The present study found no evidence that short durations of PRT or human interaction can compensate for a lack of conspecific companionship in rhesus macaques. At the level implemented in the current study (6 min per week), neither positive reinforcement training nor unstructured human interaction altered the behaviour of singly housed rhesus macaques. This finding contrasts with the known benefits of moving singly housed individuals into pair housing. Rather than distributing brief durations of positive reinforcement training to large numbers of singly housed animals regardless of specific behavioural, management, clinical, research or therapeutic contexts, tailoring training to individual animals may be a more fruitful application of the same training resources.

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Table 1

Behavioural categories analysed.

Behaviour	Explanation
Abnormal behaviour	Qualitatively species-inappropriate behaviours, including stereotypic, appetitive, non-injurious self-directed and self-injurious behaviours
Affiliative behaviour	Non-contact pro-social behaviour directed outside of the cage, to another monkey or human
Agonistic	Non-contact aggressive or submissive behaviour directed outside the cage, to another monkey or a human
Eat/drink	Common usage
Inactive	Passive or appearing to sleep
Locomote	Walk, climb, jump
Manipulate	Using hands, feet or mouth to explore inanimate objects or caging
Scratch	Repeated vigorous strokes of the hair
Self-groom	Picking, stroking and/or licking of one's own body hair

Table 2

No main effects of study phase on behavioural categories.

Behaviour	Baseline	PRT	Human interaction	$F_{1,120}$	P -value
Abnormal	17.9 (\pm 1.6)	17.6 (\pm 1.7)	17.5 (\pm 1.6)	0.24	0.79
Affiliative	0.3 (\pm 0.04)	0.3 (\pm 0.06)	0.3 (\pm 0.8)	2.73	0.07
Agonistic	0.8 (\pm 0.1)	0.7 (\pm 0.08)	0.8 (\pm 0.1)	0.33	0.72
Eat/drink	14.8 (\pm 1.1)	14.9 (\pm 1.0)	16.8 (\pm 1.2)	1.66	0.19
Inactive	38.9 (\pm 2.2)	39.7 (\pm 2.0)	38.2 (\pm 1.8)	0.29	0.75
Locomote	2.0 (\pm 0.3)	1.9 (\pm 0.2)	1.5 (\pm 0.2)	1.51	0.23
Manipulate	16.1 (\pm 1.6)	14.7 (\pm 1.2)	13.7 (\pm 1.2)	0.59	0.60
Scratch	1.3 (\pm 0.1)	1.4 (\pm 0.1)	1.3 (\pm 0.1)	0.49	0.61
Self-groom	7.7 (\pm 0.9)	8.6 (\pm 1.0)	8.3 (\pm 0.9)	1.27	0.29

Table 3

No interaction effects between facility and study phase.

Behaviour	$F_{2,118}$	P-value
Abnormal	1.55	0.22
Affiliative	0.39	0.68
Agonistic	0.07	0.93
Eat/drink	0.82	0.44
Inactive	0.51	0.60
Locomote	0.75	0.48
Manipulate	0.31	0.74
Scratch	0.14	0.71
Self-groom	3.01	0.05

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Table 4

No interaction effects between abnormal behaviour quartile and study phase.

Behaviour	$F_{2,58}$	P-value
Abnormal	1.65	0.17
Affiliative	0.64	0.64
Agonistic	0.83	0.51
Eat/drink	1.17	0.33
Inactive	0.64	0.64
Locomote	1.62	0.18
Manipulate	0.21	0.93
Scratch	0.52	0.72
Self-groom	0.78	0.54

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