LOWER-ROW CAGING IN A TWO-TIERED HOUSING SYSTEM DOES NOT AFFECT THE BEHAVIOUR OF YOUNG, SINGLY HOUSED RHESUS MACAQUES

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Abstract

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*It has been suggested that housing of laboratory primates in two-tiered racks adversely affects the psychological well-being of those primates housed on the lower row. Excessive darkness and its consequences are among the factors suggested to account for the supposed diminished well-being of lower-row inhabitants. Additionally, two-tiered housing has been suggested to introduce unacceptable variation into experimental designs, potentially necessitating additional subjects and/or invalidating results. Only recently have data been published to address these issues, but all studies have involved small numbers of subjects. In the present study, we compared the behaviour of 45 yearling rhesus macaques (*Macaca mulatta*) housed in upper-row cages with the behaviour of 48 yearling rhesus macaques housed in lower-row cages during a year of single housing. There were no significant differences across cage locations for time spent performing behaviours indicative of diminished psychological well-being (abnormal behaviour, inactivity, vocalisation, self-directed grooming) or for species-typical activities (feeding, playing). The difference in time spent exploring between macaques housed on the lower row and those housed on the upper row approached significance, with lower-row-housed animals spending more time exploring. Although lower-row cages are significantly darker than upper-row cages at our facility, the data from the present study demonstrate that the diminished lighting and other supposed disadvantages experienced by lower-row-housed monkeys have few behavioural consequences. Thus, there are now additional empirical data that suggest that lower-row-housed monkeys are not suffering in a "monkey cave", and that the findings of research projects using two-tiered housing systems are unlikely to be compromised.*

Keywords: *animal welfare, behaviour, captive housing, psychological well-being, rhesus macaque, single housing*

Introduction

Laboratory primates have traditionally been housed in two-tiered cages for any number of financial, convenience and space-related reasons. Reinhardt (1997) and Reinhardt and Reinhardt (1999, 2000) claim that primates housed in darker, lower-row cages suffer in comparison to primates housed in brighter, upper-row cages, and have recently referred to lower-row cages as "monkey caves". Additionally, they state that the housing of some monkeys on upper rows and of some on lower rows during experimental procedures introduces unwanted variation into the experimental design, necessitating additional subjects and potentially invalidating the research (Reinhardt 1997; Reinhardt & Reinhardt 2000). Although some of their contentions have been supported by empirical data (Schapiro *et al* 2000), others have not (Schapiro *et al* 2000; Crockett *et al* 1993, 2000).

As Reinhardt and Reinhardt (1999, 2000) suggest, relatively little attention is typically paid to the lower-row/upper-row distinction when discussing research using nonhuman primates as subjects. Much of our research on single- and pair-housed rhesus macaques has been conducted using a two-tiered housing system, yet this dimension was not examined in our earlier publications (Schapiro & Bloomsmith 1994, 1995; Schapiro *et al* 1995, 1996a,b). Only Crockett and colleagues (Crockett *et al* 1993, 2000) and Schapiro and colleagues (Schapiro *et al* 2000) have explicitly addressed cage location (upper row versus lower row) as an independent variable in their research. Contrary to Reinhardt's (1997; Reinhardt & Reinhardt 2000) predictions, no significant differences in behaviour (Schapiro *et al* 2000; Crockett *et al* 1993, 2000) or in physiological responses (Crockett *et al* 1993, 2000) were found between monkeys housed in lower-row cages and those housed in upper-row cages. However, these studies involved small sample sizes. The lack of an effect of cage location in these small studies suggests that cage location may not be as important an influence on experimental design and research findings as Reinhardt (1997; Reinhardt & Reinhardt 2000) maintains, even though Schapiro and colleagues (2000) found significant differences in light levels between lower- and upper-row cages.

Our previous studies (Schapiro & Bloomsmith 1994, 1995; Schapiro *et al* 1996a, 1998, 2000) have examined rhesus macaque behaviour and immune responses as a function of psychosocial manipulations including enrichment, dominance status and social housing condition. We have typically presented behavioural and immunological data as a means of assessing differences in psychological well-being. In our interpretive framework, increases in abnormal behaviour, self-directed grooming (particularly for socially restricted monkeys) and inactivity in response to a manipulation can be taken as indicators of diminished well-being. Increases in more species-typical activities (ie social grooming for socially housed monkeys, playing, feeding) in response to a manipulation can be taken as indicators of enhanced well-being. Similarly, decreases in $CD4^+/CD8^+$ ratios (Lubach *et al* 1995) and *in vitro* T-cell proliferation responses to mitogens and diarrhoea-inducing pathogens (Line *et al* 1996; Laudenslager & Boccia 1996; Schapiro *et al* 2000) can be taken as immunological correlates of diminished well-being. We will continue to apply this same interpretive framework to the behavioural data analysed for the present study.

This report will address the hypothesis that housing monkeys in lower-row cages or upper-row cages results in behavioural differences indicative of different levels of psychological well-being. We will provide empirical data from a large sample (n = 93) of young rhesus macaques observed during a year of single housing. These are the type of longitudinal data that are necessary to help determine whether darkness, proximity to the ground, lack of a potential safe place to escape a human threat, and/or diminished human attention (Reinhardt & Reinhardt 1999, 2000) adversely affect the behaviour and, thus, the psychological well-being and suitability as research subjects of monkeys housed on the lower row.

Methods

Subjects

Ninety-three rhesus macaques (*Macaca mulatta*) from three birth cohorts were observed while singly housed as yearlings. Slightly less than half (n = 44) of the subjects received a multiphase enrichment program (Schapiro *et al* 1995), while the remainder (n = 49) received no enrichment. There were 47 males and 46 females in the study group. All animals were part of the specific pathogen free (SPF) breeding programme at the University of Texas M D Anderson Cancer Center's Department of Veterinary Sciences (Buchl *et al* 1997; Schapiro *et al* 1994). All subjects had spent their first year of life in their natal groups. The natal groups comprised one adult male, four to seven adult females, and their most recent offspring. Subjects were singly housed during the study period to promote our dual goals: first, to establish an SPF breeding colony (Buchl *et al* 1997; Schapiro *et al* 1994); and second, to systematically study the effects of different environmental enrichment strategies on singly housed rhesus macaques (Schapiro & Bloomsmith 1995; Schapiro *et al* 1995, 1996a,b). Although housing nonhuman primates singly clearly incurs welfare costs, the prevailing wisdom at the time at which this study was conducted was that a limited period of social restriction (single housing) was necessary in order to ensure the development of a monkey colony free of the target pathogens from a colony possessing at least one of these pathogens (Buchl *et al* 1997). The potential adverse long-term behavioural consequences of such procedures were well known — hence our decision to leave subjects in their natal groups until one year of age and to systematically and extensively enrich the single cage during the period of social restriction. The development and maintenance of a viable breeding population of SPF rhesus macaques was, and is (Cohen 2000), a national research goal of the National Institutes of Health (USA) that has been deemed critical enough to warrant both the incurred welfare costs and the expense of systematically studying enrichment effects. For additional justification of our SPF derivation strategy, see Buchl *et al* (1997), and for additional descriptions and analyses of the behavioural management strategies applied to minimise the welfare costs experienced by our subjects, see Schapiro and colleagues (1994, 1995, 1996a,b).

Housing

During the period from one to two years of age, all subjects were housed singly in rack-mounted, solid-sided stainless steel cages (0.4 m^2 x 0.86 m high) with visual, auditory and olfactory access to similarly housed monkeys. Forty-eight of the subjects were randomly assigned to cages on the lower of the two rows and the other 45 subjects were randomly assigned to cages on the upper row of the rack. Across the years, there were some differences in the ways in which the cohorts of monkeys were managed (Schapiro *et al* 1995, 1996b), but there were never any differences in experimental treatment between monkeys housed on the upper row and those housed on the lower row. All monkeys were fed primate chow in the morning and the afternoon and were provided with fresh fruit three times per week. Water was available *ad libitum*.

Data collection

The frequency and duration of behaviour for each of the 93 subjects was observed for two to three 15 min focal-animal samples per week over the year of single caging (approximately 2700 hours of observations), using our standard observational techniques and macaque ethogram (Schapiro & Bloomsmith 1994). There were 41 mutually exclusive behaviours on the ethogram that were grouped into a number of composite categories for analysis (see Table 1). Observations were conducted between 0845h and 1730h and began on the day on which subjects were separated from their natal groups and housed in single cages.

Table 1 **Ethogram of behaviours recorded with composite categories for analysis (operational definitions in Schapiro & Bloomsmith 1994).**

Composite category for analysis[1]	Behaviours recorded
Abnormal behaviour	Urophagy, self-pick, suck digit, salute, pace, head toss, coprophagy, self-aggression, abnormal (other)
Inactivity	Sit, sleep
Groom	Self-groom
Feed	Eat, forage
Explore	Investigate, look, olfactory explore
Play	Self-directed play
Sexual behaviour	Masturbate
Agonistic behaviour	Threaten, submit
Other behaviour	Drink, locomote, manipulate, urinate, defecate
Vocalise[2]	Vocalise

[1] Duration data analysed
[2] Frequency data analysed

Data analysis

Behavioural data were compared using independent *t*-tests with upper-row versus lower-row as the dimension of comparison. (Preliminary analyses of variance revealed that there were no significant cage location [upper versus lower] by enrichment [enriched versus unenriched] interaction effects, so, for clarity of presentation, enriched and unenriched subjects were grouped together.) Duration data were used for analyses of all behavioural categories except vocalisation, for which frequency data were used.

Results

The difference in time spent exploring only approached statistical significance ($t_{[91]} = 1.96$, $P \leq 0.06$), with macaques housed singly on the lower row (n = 48) spending, on average, 19.5 per cent of observation time exploring and macaques housed on the upper row (n = 45) spending 17.8 per cent of observation time exploring. None of the other differences in species-typical or abnormal behaviour patterns between lower-row subjects and upper-row subjects even approached statistical significance (see Table 2). Of particular relevance are the findings that abnormal behaviour, inactivity, vocalisation and self-grooming — all potential indicators of stress and/or a lack of psychological well-being (Schapiro & Bloomsmith 1994, 1995; Crockett *et al* 1995; Novak & Suomi 1988; Bayne *et al* 1991; Rosenblum & Andrews 1995) — did not differ significantly as a function of cage location. Similarly, species-typical activities such as playing and feeding did not differ significantly with cage location.

Inferential statistics, such as the *t*-tests employed in the present study, are appropriate for attempting to prove that a difference between two groups is significant (proving the alternative hypothesis), but are not appropriate to support the claim that there is no difference between groups (proving the null hypothesis). Power analysis (Cohen 1988) is the most applicable technique to support the assertion that the behaviour of monkeys housed on the lower row does not differ from the behaviour of monkeys housed on the upper row. If the sample sizes are large enough, and if the power of the experimental design is sufficient to detect a meaningful difference (usually 0.80), then it is possible to state that any differences observed were so negligible that they can be considered trivial. As there were 45 upper-row subjects and 48 lower-row subjects, analyses revealed that the power was greater than or equal to 0.80 for all of the behaviours tested above. This confirms that the experimental design was sensitive enough to detect meaningful differences (had there been any) and that any differences in behaviour as a function of cage location were trivial.

Table 2 **Mean percentage of time spent in selected categories of behaviour by singly housed subjects. Values in parentheses represent the standard deviation.**

Location	Upper-row cages (n = 45)	Lower-row cages (n = 48)
Category of Behaviour		
Abnormal	9.8 (± 9.8)	7.2 (± 4.7)
Inactivity	4.2 (± 2.9)	4.7 (± 3.6)
Groom	9.9 (± 3.5)	10.5 (± 2.5)
Feed	28.2 (± 6.1)	28.5 (± 6.3)
*Explore**	17.8 (± 4.2)	19.5 (± 4.2)
Play	1.0 (± 1.0)	1.1 (± 1.2)
Sexual	1.0 (± 3.3)	0.3 (± 0.4)
Vocalisation[1]	14.7 (± 10.1)	11.7 (± 7.7)

[1] Mean frequency per observation session (not duration)

* $P \leq 0.06$

Discussion

This report provides the empirical data necessary to confirm or refute the following two contentions: first, that laboratory primates housed in lower-row cages 'suffer' when compared to conspecifics housed in upper-row cages; and second, that double-tiered housing introduces a potential confound to research designs. Although the assertion of Reinhardt and Reinhardt (1999) that lower-row cages are darker than upper-row cages has been empirically confirmed previously at our facility (Schapiro *et al* 2000), their claim that lower-row-housed primates suffer behaviourally was, again, not supported. Young rhesus macaques housed in lower-row cages only differed marginally in the amount of time they spent exploring their environment compared with upper-row inhabitants, and displayed no meaningful differences in a variety of species-typical and abnormal behaviours that have been related to, and are used to assess, psychological well-being (Schapiro & Bloomsmith 1994, 1995; Schapiro *et al* 1996a; Bayne *et al* 1991; Crockett *et al* 1995; Novak & Suomi 1988; Rosenblum & Andrews 1995). Similar patterns of limited, or no, behavioural differences have also been found when comparing upper-row to lower-row cage locations for pigtailed (Crockett *et al* 2000) and cynomolgus macaques (Schapiro *et al* 2000).

There are several strengths to the present data set. The sample size is quite large and represents macaques born in three different years. The three cohorts were treated slightly differently, but treatment of upper-row and lower-row subjects never differed as a function of the experiment. Some would contend that the treatment of the two subsets of subjects differed as a function of the row on which they were housed. Although this is possible, differences in treatment did not translate into relevant differences in behaviour across this sample of 93 rhesus macaques.

Additional strengths of the current data set include the timing, quantity and duration of the observations. All subjects were observed several times each week while singly caged (2700 hours in total), beginning at initial separation from the natal group and continuing longitudinally until one year later. This is a substantial period that includes intervals of considerable disturbance and intervals of potential acclimatisation. If the upper-row/lower-row dimension were an important influence on behavioural responses, then one would have expected this sample of young, socially restricted monkeys to be particularly susceptible to its effect.

The fact that monkeys housed on the lower row spent a slightly higher percentage of their time exploring their environment is difficult to explain. As indicated in Table 1, the composite category of exploration included three behaviours: looking (oriented outside the cage), investigating (oriented inside the cage), and olfactory exploring. Although some of our previous manipulations of enrichment and housing conditions have resulted in differences in the exploration category of activity (Schapiro & Bloomsmith 1994; Schapiro *et al* 1995, 1996a,b), we consider it to be of substantially less relevance for assessing subjects' psychological well-being than many of the other activities for which no differences were evident.

The goal of this study was simply to determine whether cage location (upper versus lower row) affects behavioural profiles. We were not specifically interested in the effects of light levels on behaviour, except as a subsidiary influence of cage location. In an earlier report, we found no significant relationships between any of the behaviours and levels of brightness or darkness, suggesting that, for those subjects (female cynomolgus macaques), the amount of light by itself did not influence behaviour (Schapiro *et al* 2000). However, as others have suggested (Clough 1982; Reinhardt 1997), light levels varied greatly within our rooms and within each cage.

Although lower-row cages are clearly darker than upper-row cages at our facility (Schapiro *et al* 2000), they are by no means too dark for personnel to conduct their husbandry, observational, and experimental duties. While the additional light and escape height available in upper-row cages may provide some advantages to the monkeys, these advantages do not appear to translate into behavioural differences across groups of monkeys. In our opinion, there is an increased probability of accidents when handling monkeys above human shoulder level, although we have no empirical data to support this contention.

Animal welfare implications

Overall, these data suggest that there are limited, if any, behavioural consequences of housing monkeys on the lower row in a two-tiered housing system. While lower- and upper-row cages differ in terms of light levels, height above the floor and the macaques' ability to escape threatening human stimuli by climbing above human eye level, these differences do not affect behaviour. The data do not support cage location as a significant source of variation or as a confounding influence on studies of behaviour. Therefore, some of the potentially costly solutions to the "monkey cave" problem, as delineated by Reinhardt (1997) and Reinhardt and Reinhardt (1999, 2000), do not appear to be warranted by the empirical data (Schapiro *et al* 2000; Crockett *et al* 1993, 2000) at present.

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