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Cage Size Preference in Rats in the Laboratory

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The size of an enclosure is an integral part of how well it accommodates a nonhuman animal's welfare; however, most enrichment studies concentrate on modifying the area inside the enclosure rather than enlarging it. It has been suggested that rats have little need for more cage space, but there is no empirical evidence about rats' need for space. This experiment provides preliminary evidence for the preferences of 5 male and 5 female albino rats using T-maze choices followed by 5 min dwelling times. The rats showed a moderate but significant preference for the larger of 2 cages (540 cm2 vs. 1,620 cm2; binomial $z, p < .05$). When the rats shared the chosen cage with 4 familiar cage mates, their preference for the larger cage did not become any stronger (paired $t(9) = -.820, p > .05$). The results suggest that rats should be given a somewhat larger space allowance but could share it with up to 4 other rats.

Appropriate enrichment can be based on observations of wild and feral populations (Brain, 1992; Patterson-Kane, in press). Observations of free-living rats (*Rattus norvegicus*) have indicated that they typically form large colonies in areas in which there is plentiful ground cover and they create complex burrows (Boice, 1977). Considerable research has demonstrated that these same variables can be used to enrich the caging of rats in the laboratory and substantially improve their welfare: (a) social contact (R. N. Hughes, 1969; Johnson, DeSisto, & Koenig, 1972; Morgan & Einon, 1975; Perez et al., 1997), (b) more complex environments (Anzaldo, Harrison, Riskowski, Maghirangi, & Gonyou, 1994;

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Bradshaw & Poling, 1991; Denny, 1975), and (c) shelters (Manser, Broom, Overend, & Morris,1998a, 1998b; Patterson-Kane, Harper, & Hunt, 2001).

Little attention has been paid, however, to the small amount of space a laboratory rat has access to compared with a free-living animal. Some researchers consider space relatively unimportant for laboratory rats, either because they have been bred in captivity for many generations and so presumably have adapted to existing laboratory conditions (Bantin & Sanders, 1989; Beilharz, 1994) or because rats in the wild generally occupy restricted spaces under cover or in burrows and, presumably because of the risk of predation, avoid open areas (Rose, 1990; Stricklin, 1995). Existing research has suggested that the effects of factors such as environmental complexity and security (Blom, 1993) and social contact (Lawlor, 1990) are strong enough to obscure any responses by rats to concurrent differences in cage size. However, no experiment actually has measured rats' responses to increases in cage size.

An experiment is required to establish whether rats will demonstrate a preference for the larger of two cages. It is apparent that rats prefer the more complex of two cages of equal size (Anzaldo et al., 1994; Bradshaw & Poling, 1991; Denny, 1975) and that large open areas can be stressful for rats, especially isolates (R. N. Hughes, 1969). It also seems that rats will choose small, enclosed cages over larger cages incorporating open space (Blom, 1993), but it is not known whether rats would prefer cages that are larger without incorporating significantly more open space.

Although space receives little attention in the research literature, it is mentioned extensively in legislation, codes, and guidelines relating to animal welfare. Such guides suggest a minimum amount of floor area that should be provided to each animal (whether kept individually or in a group). That size of this area is determined by species and body weight and is fairly consistent across different agencies. The National Research Council (NRC), the Council of Europe and Royal Society/Universities Federation for Animal Welfare (RS/UFAW) all recommend approximately 225 cm2 of floor space for a 300 gm rat (Hargreaves, 2000). Prescribing floor area by weight provides a greater minimum area for adults than juveniles and males than females, whereas group enclosures generally provide about as much space as an equivalent number of single-rat cages would.

One reason for the dearth of research into cage size may be the expense and inconvenience of changing to larger cages. The few commercially available large rat cages (Scanbur™; Martin™; Fern™) use wire mesh or bar construction and are out of step with the new movement to microbiological barriers such as filters and ventilation. There is some scope, however, for using cages originally designed for larger species such as rabbits and guinea pigs.

Another consideration might be that it is relatively tricky to measure spatial preferences. Gross health or productivity measures are too insensitive, and subtler physiological responses such as brain development require killing the animal, thereby increasing the number of subjects used and the level of invasiveness. A better approach might be to use animals' preferences as a guide. This allows the subjects to be reused, and generates sensitive within-subject comparisons of a number of alternatives. Preference has been used successfully with a wide range of species—including fish (Giattina & Garton, 1983), cows (Matthews & Temple, 1979), and mice (Banjanin & Mrosovsky, 2000).

Preference tests, however, are not without their difficulties. The apparent simplicity of preference as a measure relevant to animal welfare is compromised by the shortcomings of particular methods of testing. The type of preference test used has to be carefully matched with the variable and species under consideration.

The most popular method for measuring animal motivation is to join two cages together and record the animal's relative dwelling times in each (Blom, 1993; Blom, Baumans, van Vorstenbosch, van Zutphen, & Beyen, 1993). This method has been successfully used with rats to assess preferences for various objects, shelters, nesting material, and other variables with relevance to their welfare (Blom, van Tintelen, van Vorstenbosch, Baumans, & Beyen, 1996; Chmeil & Noonan, 1996; Manser et al., 1998a, 1998b; Patterson-Kane et al., 2001; Townsend, 1997).

However, dwelling time tests are difficult to use in some circumstances. For example, dwelling time could not be used to compare rats' demand for nesting material versus nest boxes as the rats moved the nesting material into the nest-box cage (Manser et al., 1998a); however, use of a demand (Manser et al., 1998b) or T-maze procedures prevented this behavior and revealed that shelters were the preferred option.

Dwelling time measures also are difficult to use when the environments are different sizes. The problem with unequally sized options is that an animal may choose between environments that differ in size or may move about randomly and thus spend more time in the larger cage without necessarily making any choice to do so. It is difficult, even post hoc, to tell which strategy is in use and to what extent, and thus to determine the correct baseline for analysis.

An alternative method requires that animals work for access to enriched cages and quantify their motivation, economically, by the effort they are willing to make. Unfortunately, rats and mice have a strong tendency to access and patrol any area that is available regardless of its quality, presumably to gather information about food sources and escape routes that might be of future use. Thus, mice (Sherwin, 1996; Sherwin & Nicol, 1995, 1997) and rats (Patterson-Kane, Hunt, & Harper, in press) tend to show high demand even for small empty cages, reducing the sensitivity of the procedure.

Another, more promising option in this case is simple T-maze preference testing that requires the rat to choose between two cages before being trapped in the chosen cages for a period of time. For animals such as hens who can see the differing sizes of the available environments before entering, dwelling times of many hours can be used (B. D. Hughes, 1975; Lindberg & Nicol, 1996). However, rats, particularly albinos, may have poor eyesight (Barnett, 1976), and therefore discrimination of cage size may depend more on shorter dwelling times and the animal's spatial memory. For the same reason, trials with rats should run in blocks rather than having the options appear randomly on the left or right throughout the session. A T-maze procedure using 5-min dwelling times proved successful for Dawkins (1978, 1982) and Patterson-Kane et al. (2001), and the same duration was used in this experiment.

The following experiment used a T maze to offer male and female rats choices between cages differing in size. They were tested once when they spent the dwelling time alone and once when they shared it with four familiar conspecifics. This allowed a rat's preference for a larger cage to be quantified as well as the effects of sex and the availability of social contact.

METHOD

Subjects

This data relate to five female and five male albino Wistar rats from the University of British Columbia's Animal Care Centre. They were age 50 days at the beginning of the experiment. The rats were housed in groups of five within Macrolon transparent polycarbonate type IV wire-topped cages (1,080 cm2 × 16 cm). The cages were in a colony room with an artificial 12-hr light–dark cycle with lights on at 19:00 hr, a temperature of 22 ± 3°C, and a relative humidity of approximately 50%. During the whole period, the subjects had access to tap water and food pellets ad libitum.

Apparatus

The test cages used were a cage with a floor area of 540 cm2 and a larger cage that had a floor area of 1,620 cm2. This represents a difference in size from approximately the size of a single-rat hanging cage to approximately the size of the largest commercially available polyethylene rat cage. The cages differed only in that the larger cage included extra cage length, accessible through a 2.6cm long tunnel—thus, it did not include a great deal of open space.

A 5.2 cm diameter PVC-T pipe was used as the T maze. Cage sizes were reduced using a metal partition and increased by joining cages together lengthwise via a tunnel. Thus, all cages had a fixed width (30 cm) and height (16 cm) but varied in length.

Procedure

A T-maze procedure was used to quantify rats' preferences. The rats always were tested in the same order so that each individual's session occurred at approximately the same time each day between 08:30 and 17:00 hr. The experiment was carried out in its entirety with the female subjects and later replicated with males.

Half the rats (three males and two females) began in the social condition, and half began in the isolated condition. Each rat began its session with a forced choice in each direction (where one arm was blocked of by a Plexiglas door). After being acquainted with the two options in this way, each rat was given 10 free choices each day. Every choice was followed by a 5-min dwelling time. For rats in the social condition, their four cage mates were put in with them during this period.

All rats began with a choice between two identical 1,080 cm2 cages (so that side bias could be excluded—thus preventing it from reducing the sensitivity of the preference test and to familiarize the rats with the procedure). After each rat had made 60 free choices (10 per daily session) this was replaced by a 540 cm2 cage on the left and a 1,620 cm2 cage on the right for a further 60 free choices. Finally, the position of the large and small cages was reversed for a final 60 free choices. This procedure was repeated with the isolated condition rats being put in the social condition and vice versa.

Matching Law Analysis

Animal welfare research normally used a simple binomial analysis of preference data. Other analyses such as the matching law (from operant psychology) are applicable (Davison & McCarthy, 1988; Matthews & Temple, 1979). The matching law offers the ability to quantify separately position bias and preference. This could eliminate the effect of bias in reducing test sensitivity and allow for future experimental designs that treat bias as an independent variable rather than a confound—determining how cage size might bias preference for contact with large social groups. This study demonstrates how the matching law might be employed as an alternate form of analysis in a simple preference experiment, with the aim of exploiting its advantages in quantifying bias in future experiments.

In the following matching law analysis, each rat's data were plotted in pairs on a graph with log10 axes and the relative amount to total space available to the left plotted on the x axis and the relative number of choices made to the left on the y axis. For example, if the cage on the left was 520 cm2 and the cage on the right was 1,620 cm2, then x = 520/1,620 or 0.33.

Thus, each animal's preference can be quantified as the slope of the regression line fitted to these data, with indifference producing a slope of zero and preference for the larger cage producing a positive slope. Measured in this manner, position biases will affect the y-intercept value but not reduce the sensitivity of the slope as a measure of preference. Experimenters currently counter balance for left and right positions; but as proportion choice does not separate bias from preference, the presence of any side bias will reduce the sensitivity of proportion choice as a measure of preference. The matching line also provides a clear method for visual presenting preference data.

RESULTS

The rats appeared to behave similarly in the differently sized cages: resting, moving around, and engaging in rough and tumble play. They made no noticeable use of the short tunnel other than to pass through it.

Binomial Analysis

Data from the social and isolated conditions were combined to produce scores representing each animal's total preference for the larger cage. The mean degree of preference for the larger cage was significant (66%, binomial, $p < .05$) as was the number of rats demonstrating a preference (see Figure 1) for the larger cage (10/10).

Matching Law Analysis

Figure 2 shows mean preference shown by all subjects. It is evident from the positive slopes of the least-squares regression lines fitted to the data that, on average, the rats, whether isolated or in a social condition, preferred the larger cage. This preference (as indicated by regression line slope) was not significantly different between males and females, $t(8) = 1.527$, $p > .05$. When the subjects shared the cage with four cage mates, the preferences were not signifi-

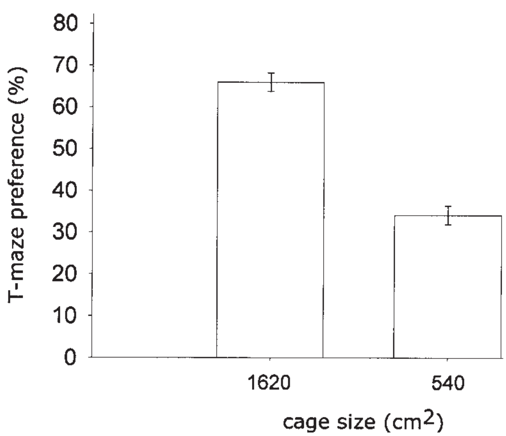

FIGURE 1 Percentage choices made for the larger and smaller cages means and standard errors; based on 240 choices each by 10 subjects.

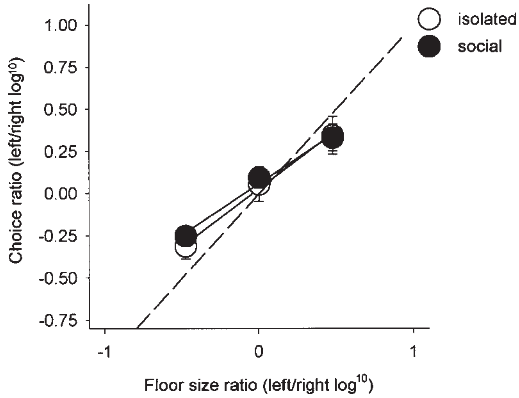

FIGURE 2 Choices (left/right log10) as a function of space (left/right log 10). Means and standard errors for 10 subjects. White circles represent the preferences for empty cage space and black circle represent preference for space shared with four familiar rats. The dotted line has a slope of +1.0 and intersects (0,0).

cantly different from when they occupied the cage alone, paired $t(9) = 0.710$, $p > .05$. On average, position bias as measured by matching line elevation was very small, with an overall left bias of 1.8% and an absolute bias (in either direction) of 2%. Figure 3 shows the individual data for each subject, demonstrating that each subject showed a preference for the larger cage under either condition (10/10, binomial probability = .00001; $p < .05$). So, percentage choice and matching law analyses are entirely consistent, both indicating significant preferences for the larger cage.

DISCUSSION

The results from this preliminary study, 10 rats of one strain and age, showed they had a preference for the larger cage that was unaffected by the presence of four familiar cage mates. The magnitude of preference for a larger cage (66% overall) was comparable with preferences for other enrichments of known importance such as a nest box (65%), and contact with cage mates (61%; Patterson-Kane et al., 2001).

This data suggests cage size is a salient feature for laboratory rats. Before specific recommendation can be made, further research is needed to determine the optimal cage size and the relative importance of cage size versus factors such as cage complexity.

Two assumptions of many weight-based space regulations are that groups of rats need as much space as an equivalent number of isolates and that males need more space than females. Neither is borne out by this data, which showed that social grouping during dwelling times did not produce stronger preferences for the larger cages and that females and males had preferences of similar strengths.

Although the individual variation in degree of preference is marked and its causes are currently unclear, every subject demonstrated a preference for the larger cage.

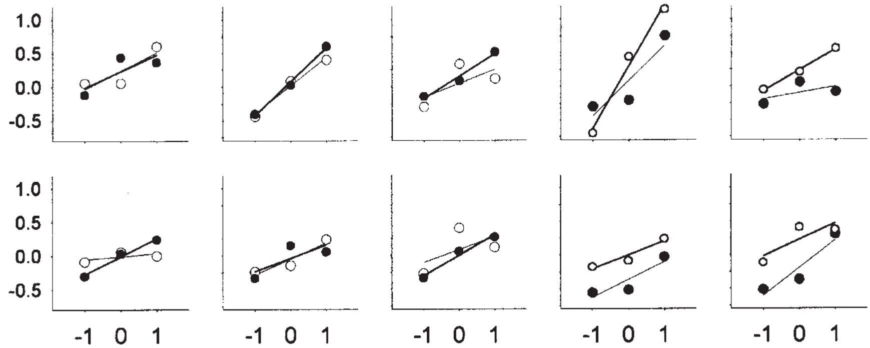

FIGURE 3 Proportion of left choices as a function of the proportion of total floor space available to the left (log10). Individual data, top five figures = female subjects, bottom five figures = male subjects.

Although sex and social group had little effect in this data, they might have an effect under other conditions as might strain, age, early experience, and home-cage experience. Also, this experiment used two specific cage sizes. Further research is needed to determine which particular cage size is sufficient and whether preferences will be shown for even larger cages or those with different dimensions.

Finally, the matching line analysis provided a clear visual method for displaying rats' preferences. In this case, however, matching line slopes were not a more sensitive measure than percentage choice as the preference was marked and position biases were negligible. The matching law might be more useful with animals who do not spontaneously alternate in a T maze (Barnett, 1976) or when the environment cannot be made so uniform across the two sides of the T maze. It also would allow the future empirical assessment of the biasing effect of variables such as cage openness or complexity on preference for cage size. In conclusion, these subjects were consistent in their preference for the larger of two cages and in their tolerance for sharing this space with familiar cage mates.

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