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Comparison of 2 Rat Breeding Schemes Using Conventional Caging

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Compared with earlier editions, the eighth edition of the *Guide for the Care and Use of Laboratory Animals* recommends more cage floor space for female rats with litters. As such, conventional rat cages often do not supply the recommended floor space to maintain 2 adult rats and a litter in the same cage. We evaluated 2 breeding schemes using traditional cages that afford 140 in.^{2} (903 cm^2) of floor space: (1) monogamous pairs housed continuously and (2) monogamous pairs cohoused intermittently with removal of the male rat after parturition. The results did not demonstrate a significant difference between breeding schemes in generation time, number of litters per breeding pair, percentage of litters weaned, number of pups born per breeding pair, and number of pups weaned per breeding pair. However, the average weaning weight of pups was significantly higher with scheme 1 compared with scheme 2. Collectively, these results indicate continuous housing of monogamous breeding pairs may be preferable to intermittent housing when conventional cages are used.

Rodents constitute the vast majority of animals used in biomedical research. Historically, mice have been the predominant mammals used in animal-based studies, even though rats are physiologically more similar to humans in many ways and make better surgical models due to their larger size.[40] The main reason mice have been the rodent of choice has been related to the ability to produce genetically engineered animals in this species.[16] Until recently, genetic engineering was not feasible in rats. Current advances involving chemical mutagenesis, transposon-mediated mutagenesis, zinc-finger nuclease technology, and modification of genes in rat embryonic stem cells have allowed researchers to create genetically engineered rat models efficiently.[1,13,18,25,28,30,41] The use of rats in biomedical research may increase dramatically in the future as a result of these technologic advances.

Recent changes to the housing standards for rats as published in the eighth edition of the *Guide for the Care and Use of Laboratory Animals* (the *Guide*)[23] likely will present operational challenges for institutions housing and breeding this species. The *Guide* recommends 124 in.^{2} (800 cm^2) floor space for an adult rat and her litter and a graduated amount of space for individual animals depending on size (17 in.^{2} [109.6 cm^2] for a rat weighing less than 100 g and at least 70 in.^{2} [451.5 cm^2] for a rat heavier than 500 g).[23] However, the standard rat cages currently used by many research institutions (including our own) provide approximately 140 in.^{2} (903 cm^2) of floor space. As such, these cages do not afford the recommended amount of floor space for a female rat with a litter to be housed with another adult animal (for example, as a monogamous breeding pair).

Studies have been conducted regarding cage size preferences in rats,[33] but the literature lacks publications regarding cage floor space and its effect on animal welfare in rats, particularly in regard to floor-space recommendations as noted in the new *Guide*. Our institution currently houses approximately 8000 rats in 3900 conventional cages that each provides approximately 140 in.^{2} (903 cm^2) of floor space; 75% of these cages are used for breeding. Replacing the conventional cages with larger cages to accommodate the space requirements for breeding according to recommendations of the new *Guide* would cost approximately $840,000. We therefore wanted to evaluate a potential alternative breeding management option using conventional cages so that objective management decisions could be made regarding rat housing at our institution. We compared breeding and pup growth by using the conventional housing method and a proposed alternative breeding option that meets the space recommendations of the new *Guide*. Two breeding schemes were evaluated: (1) a traditional breeding scheme using monogamous pairs housed continuously and (2) a scheme by which monogamous pairs are cohoused intermittently and that meets the new recommendations regarding floor space. We hypothesized that selected breeding parameter values would be statistically similar between the 2 breeding schemes.

Materials and Methods

Animal care. We performed this study in a facility that houses rodents used for research purposes. All animal use activities were approved by the Medical College of Wisconsin IACUC, and the animal care and use program is fully accredited by AAALAC. We used Dahl salt-sensitive (SS, SS/JrHsdMcwi) rats because this strain is the most common background strain for rats housed in our facilities.[15,22,29] Sentinel rats were exposed to dirty bedding from the cages housing study animals. Sentinels were negative for Sendai virus, pneumonia virus of mice, sialodacryoadenitis virus, Kilham rat virus, Toolan H1 virus, rat parvovirus, rat minute virus, reovirus 3, rat theilovirus, *Mycoplasma pulmonis*, *Pneumocystis carinii*, pinworms, and fur mites. The environment of the room in which the rats were housed was controlled (temperature, 68 to 72 °F [20.0 to 22.2 °C]; relative humidity, 30% to 70%; 14:10-h light:dark cycle). Rats were housed in individually ventilated caging (model no. RS10147U40MVSPSHR-R, Allentown Caging, Allentown, NJ). Cage changing was performed in laminar-flow cage-changing stations (model 612, Allegard Dual Access Small Animal Cage

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Changing and Transfer Stations, Nuaire, Plymouth, MN). Cages contained hardwood bedding (Sani-Chips, PJ Murphy, Montville, NJ). Nesting material, consisting of a paper towel, was added to each cage when it was changed. Cages were changed at least every 14 d and more often as necessary, according to established standard operating procedures. Criteria for cage change were: at least 10% of the cage floor space visibly wet, or more than 33% of the floor was covered with fecal material. Even though mandatory cage changes were scheduled to occur every 14 d, cages were typically changed every week because they met one or both of the cage-change criteria. The number of cage changes was not recorded. Cages and caging supplies, including bedding, were autoclaved prior to use. Food provided was a low-salt (0.4%) experimental diet (no. 113755, Dyets, Bethlehem, PA). Water provided underwent reverse osmosis filtration and hyperchlorination to 3 ppm prior to animal cage supply by an automatic watering system (Edstrom Industries, Waterford, WI). Animal care staff wore dedicated footwear and personal protective equipment that consisted of a disposable gown and gloves when performing animal husbandry tasks. Animal rooms were swept and then mopped by using a quaternary ammonium compound (Labsan 256 CPQ, Sanitation Strategies, Okemos, MI) daily except on weekends and holidays.

Experimental design. Two breeding schemes were evaluated: (1) monogamous pairs housed continuously and (2) monogamous pairs cohoused intermittently. In the second breeding scheme, the male rat remained with the female for 48 to 72 h after parturition to permit breeding during postpartum estrus. After this time, the male rat was removed from the female rat's cage but subsequently was returned to that cage immediately after weaning of each litter, again remaining until 48 to 72 h after parturition. We acknowledge that under this scheme, the space recommendations in the new *Guide* were exceeded for 48 to 72 h until the male rat was separated from the dam with litter. Within 24 h after parturition, female rats ovulate and become sexually receptive. During this period female rats may become pregnant.[40] On the basis of this fact, we concluded that any breeding scheme that failed to accommodate breeding during the postpartum estrus would be problematic because production would be cut by approximately 50%, due to time lost during the preweaning period when the dam could be pregnant with developing pups.

We evaluated several breeding parameters throughout the course of the study. As noted previously, we hypothesized that there would be no significant difference in selected breeding parameter values between the 2 breeding schemes. A significant difference would be interpreted as having a negative or positive effect regarding one or the other breeding scheme and therefore provide justification for preferential utilization of a particular scheme.

A total of 15 breeding pairs were used to assess each breeding scheme, and rats were housed in cages that provided 140 in.^{2} (903 cm^2) of floor space. Rats used in the study were 12 to 18 wk of age at the outset and had had at least one litter previously. Rats were allowed to breed for 12 wk. Several breeding criteria, based on parameters noted in the literature, were evaluated,[35] including generation time (time between litters), number of litters per breeding pair, percentage of litters weaned, number of pups born per breeding pair, number of pups weaned per breeding pair, and average weight of pups at weaning.

Statistical analysis. Statistical comparison was performed by using 2-tailed, unpaired *t* tests (GraphPad Prism version 5.04 for Windows, GraphPad Software, San Diego, CA); differences were considered to be significant at a *P* value of less than 0.05. The unpaired 2-tailed *t* tests were used to determine whether breeding parameters were significantly different between breeding schemes. In addition, for each comparison, an F test was used to compare variances between groups. The variances between groups were not significantly different for all of the comparisons made, suggesting that the populations had equal standard deviations.

Results

There were no significant differences between breeding schemes (continuous presence compared with intermittent presence of the male rat) in generation time (36.2 ± 2.6 compared with 39.4 ± 3.1 d, $P = 0.4236$, t = 0.8063, df = 55), number of litters per breeding pair (1.9 ± 0.3 compared with 1.9 ± 0.3, $P = 1.00$, t = 0.0, df = 28), percentage of litters weaned (85.9 ± 8.4 compared with 91.7 ± 5.6, $P = 0.5805$, t = 0.5605, df = 23), number of pups born per breeding pair (13.5 ± 2.1 compared with 12.9 ± 2.3, $P = 0.8467$, t = 0.1951, df = 28), and number of pups weaned per breeding pair (12.0 ± 2.1 compared with 11.3 ± 1.9, $P = 0.8165$, t = 0.2342, df = 28). The average weaning weight of pups was significantly higher (47.6 ± 1.2 compared with 41.4 ±1.7 g, $P = 0.0169$, t = 2.475, df = 48) when the male rat was continuously compared with intermittently housed with the dam and pups.

There were no significant differences in breeding parameters evaluated except that mean weaning weight was significantly ($P < 0.05$) higher for continually housed monogamous breeding pairs than for intermittently housed pairs. Published studies have indicated a positive correlation between increased weaning weight and wellbeing in rats.[4,7,14] Accordingly, we conclude that a breeding scheme involving continuous housing of the male rat with the dam and litter was preferable to intermittent presence of the sire with regard to promoting wellbeing. We also noted that the amount of husbandry time required for maintaining the intermittent-housing scheme was nearly twice that for keeping the male rat continuously with the dam and pups (data not shown).

Discussion

Social crowding is considered a stressor in rats, affecting both behavioral and physiologic responses[2,5,26] and leading to the conclusion that overcrowding has a negative effect on animal welfare. The new *Guide* recommends increased cage floor space for a female rat with a litter as compared with previous recommendations.[23] However, the amount of cage floor space required to house rats without compromising animal welfare has not been well defined empirically. To assess animal welfare, animal-centered outcomes and behavior should be evaluated.[11,21,34] One such outcome is reproductive performance. Institutions that breed rats must consider space requirements and the effects of crowding and stress in the context of reproduction.

Reproduction and pregnancy causes complex neuroendocrine, behavioral, and physiologic changes in animals.[9,36] Depending on the type and intensity of stressors, the resulting stress may have adverse or positive effects on dams and the growth of offspring.[10,17,19,27,31,32,35] However, because rats are social animals, social interaction may be more important to stress reduction than is additional floor space.[37] Consistent with this opinion, the *Guide* indicates that the social needs of animals should be addressed in regard to housing.[23] Several studies have addressed the importance of maternal and paternal influences on growth and social development and interaction in rats.[12,24,45]

One study in rats showed that maternal deprivation had negative effects on growth rates at weaning into adulthood and that deprivation differentially affects circadian clock and stress responses depending on the daily length and time of deprivation during the preweaning period.[45] Another study showed that rats undergoing maternal deprivation during the preweaning period experienced deficits in somatic growth with decreased values in body weight, nose–rump length, and tail length.[12] In addition, several studies have evaluated paternal influence on pups in California mice (*Peromyscus californicus*) and have shown that presence of the father exhibits a positive influence on pups during the suckling period by increasing the number of pups reared, increasing survivability, and promoting physical and cognitive development.[6,8,20,44] Another study in CD1 mice indicated the presence of the father increases pup care.[43] One study that examined paternal deprivation during the preweaning period involved studying litters of California mice housed with either (1) their mother or (2) their mother and father. The results of the study indicated that the presence of the father increased physical interaction between members of the litter but did not affect pup physical growth or behavioral development.[42] We did not evaluate behavior or social interactions in our study, but the significantly lower pup weaning weights noted in the breeding scheme that involved intermittent removal of the father from the breeding cage suggests that removal of the father may be more detrimental to pup development and growth than is decreased floor space under the study conditions. From the results of such studies, we conclude that removal of the father from the parental unit likely has a negative effect on animal welfare.

A limitation of the current study is that we did not compare the effects of housing rats in conventional and larger cages. The cost and availability of caging limited our ability to perform this type of study. A second limitation is that we used SS rats, and floor space may have variable effects on rat reproductive parameters depending on the strain studied. Finally, we did not record the number of cage changes performed for each breeding scheme. We assume that the number of cage changes would be higher for the traditional scheme, given that the father remained with the mother and pups throughout the study period. In the alternative scheme, the father was removed intermittently, thereby reducing the number of adult animals in each breeding cage when pups were present. During the periods when the father was absent, soiling in the breeding cages was decreased, likely resulting in a reduction in cage-changing frequency. Although cage changing is considered to cause stress in rats,[3,37-39] weaning weights were greater for the traditional breeding scheme even though the frequency of cage changes likely was higher than for the alternative scheme. In addition, use of the alternative breeding scheme required far more facility resources than did the traditional breeding scheme. The time required for daily husbandry tasks for the alternative scheme was nearly twice that for the traditional scheme and required approximately 50% more cages.

In summary, the current study did not show any advantage of removing breeder male rats to provide additional cage space to female rats with litters. Conversely, the data suggest that continuous monogamous-pair housing enhances animal welfare as evidenced by the significantly higher weaning weights of pups raised with both mother and father in the same cage. Additional studies are warranted to further investigate floor space requirements and welfare in laboratory-housed rats housed as monogamous breeding pairs in conventional and larger caging that meets the revised floor-space guidelines in the new *Guide*.

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