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Rat Breeding Parameters According to Floor Space Available in Cage

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The cage floor space recommended for a female rat with a litter is greater in the 8th edition of the *Guide for the Care and Use of Laboratory Animals* than in previous editions. As a result, research institutions using commonly available cages to house rats may not offer the recommended amount of space for a breeding pair and litter housed in the same cage. We evaluated breeding parameters in rats housed in cages with 143 in^2 (922.6 cm^2) compared with 210 in^2 (1355 cm^2) of floor space. Given the strains of rats typically used at our institution, a monogamous breeding pair and litter requires 164 in^2 (1058.1 cm^2) of floor space according to the *Guide*. Pairs of breeding animals were housed in each type of cage; and average time between litters, number of litters born, percentage of litter weaned, numbers of pups born and weaned, and average weaning weights were evaluated. None of the breeding parameters evaluated differed according to the floor space of the cage in which the rats were housed.

Rodents are well-known animal models for many types of biomedical research studies.[3,10,20,31,32] Historically, the rat was the first mammal that was domesticated for scientific research purposes.[20] All major organ systems in rats have been studied, and a wealth of knowledge is readily available regarding the physiology of the rat.[3,10,20,22,23,32] Therefore, rats are an ideal animal model for systems biology and use in translational studies.[10] Rats are larger than mice, making rats a superior surgical model, and the physiologic systems of rats are more similar to human systems in numerous ways than are those of mice.[3,10,32] Prior to the year 2000, more published scientific studies involved rats than mice.[10]

A dramatic increase in the use of research mice occurred after a 1990 publication regarding the creation of the first knockout mouse, which demonstrated that the mouse genome could be manipulated through embryonic stem cell technology.[43] After the aforementioned publication, the number of published studies involving mice increased exponentially, because the creation of genetically modified murine animal models was possible.[31,43] Until recently, genetic engineering in rats was not possible because their embryonic stem cells could not be maintained in culture; however, this is no longer the case, and transgenic and knockout rats can now be generated.[10,27] In addition, recent advances involving chemical mutagenesis, transposon-mediated mutagenesis, viral vectors, and the ZFN, TALEN, and CRISPR/Cas technologies have allowed researchers to create genetically modified rats in their own laboratories.[1,10,13,14,17,21,24,26-28,30,37,42,46] As a result, the use of rats in biomedical research may increase dramatically in the future.

In 2011, the 8th edition of the *Guide for the Care and Use of Laboratory Animals* (*Guide*) was published.[19] As is the case for mice, the recommended amount of floor space for a female rat with litter is considerably greater than that in previous versions. The *Guide* now recommends 124 in^2 (800 cm^2) of floor space for an adult rat and her litter and additional space for other animals in the cage, depending on their size. For example, an additional rat that weighs less than 100 g requires another 17 in^2 (109.6 cm^2) of floor space, and a rat that weighs between 300 and 400 g needs a minimum of 40 in^2 (258.0 cm^2) more.[19] Adult rats housed at our institution typically weigh between 300 and 400 g, and the commonly available rat cages currently used by many research institutions provide approximately 140 in^2 (903 cm^2) of floor space.[2] Cages of this size often do not provide the recommended floor space when a female rat with a litter and an additional adult are housed together in the same cage, as is typical when housing a monogamous breeding pair. For example, given the strains of rats typically used at our institution, a monogamous breeding pair and litter requires 164 in^2 (1058.1 cm^2) of floor space.

A recently published study, using rats, showed that continuous housing of monogamous breeding pairs may be preferable to intermittent housing of animals to accommodate the current floor space recommendations of the *Guide*.[2] Commonly available cages were used in the aforementioned study, which do not allow a breeding pair and litter to be housed in the same cage continuously under the current floor space recommendations in the *Guide*.[19] In that study, larger cages that could accommodate a breeding pair and litter, adhering to the *Guide* recommendations, were not evaluated. We performed the current study to evaluate the breeding efficacy of rats housed in commonly available compared with larger cages, to determine whether increased floor space, as recommended by the *Guide*, affects selected breeding parameters.

Materials and Methods

Animal care. This study was performed in an animal research facility that houses mice and rats exclusively. The animal care and use program is AAALAC-accredited, and all activities involving animals were approved by the Medical College of Wisconsin IACUC prior to initiation. Salt-sensitive rats were used for this study because these strains are commonly used for research purposes and the Dahl salt-sensitive (SS/JrHsdMcwi) rat is the most common background rat strain currently housed at our

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institution.[12,18,29] A disease-monitoring program is in place for rodents being used for research purposes. This program involves exposure of sentinel animals to dirty bedding from cages housing study animals at every cage change. Sentinels were negative for rat coronavirus/sialodacryoadenitis virus, rat parvovirus, rat minute virus, Kilham rat virus, Toolan H1 virus, rat theilovirus, Sendai virus, pneumonia virus of mice, *Mycoplasma pulmonis*, reovirus 3, lymphocytic choriomeningitis virus, cilia-associated respiratory bacillus, Hantaan virus, *Clostridium piliforme*, mouse adenovirus 1, pinworms, and fur mites. The animals were housed in a room with controlled temperature (68 to 72 °F [20.0 to 22.2 °C]) and a relative humidity of 30% to 70% with a 14:10-h light:dark cycle and 14 to 17 air changes hourly. Two sizes of rat cage were used in this study: those with 143 in^2 (922.6 cm^2) of floor space (model PC10147HT, Allentown Caging, Allentown, NJ) and those with 210 in^2 (1355 cm^2) of floor space; (model PCT4SRT, Allentown Caging). Cages were changed in a laminar flow cage changing station (model 612, Allegard Dual Access Small Animal Cage Changing and Transfer Station, Nuaire, Plymouth, MN). The cage bedding was hardwood chips (Sani-Chips, PJ Murphy, Montville, NJ), and nesting material consisting of 2 paper towels was added to each cage during each cage-changing procedure. Water provided to rats underwent reverse-osmosis purification and hyperchlorination to 3 ppm prior to animal consumption. Rats were provided a low-salt (0.4%) diet (experimental diet number 11375, Dyets, Bethlehem, PA). All caging and cage accessories were autoclaved prior to use. Cages were changed twice weekly on a defined schedule. Animal care staff wore dedicated footwear and a disposable gown and gloves while performing animal care and husbandry activities. The animal room was swept and mopped daily. The floor was cleaned by using a quaternary ammonium compound (Labsan 256 CPQ, Sanitation Strategies, Okemos, MI).

Experimental design. Two housing schemes were evaluated. One group comprised 15 monogamous breeding pairs, each of which was housed continuously in a cage with 143 in^2 (922.6 cm^2) of floor space (small-cage group); the other group consisted of 15 monogamous breeding pairs, each of which was housed continuously in a cage with 210 in^2 (1355 cm^2) of floor space (large-cage group). Each member of the breeding pairs was 12 to 18 wk old and had had at least one litter prior to inclusion in the study. Pups were weaned and removed from the cage at 21 d and transferred to other animal use protocols as appropriate. Breeding criteria evaluated were based on parameters noted previously in published studies.[2,35] The criteria evaluated included average time between litters, number of litters born and percentage of litters weaned per breeding pair, numbers of pups born and weaned per breeding pair, and average pup weight (g) at weaning.[2] The study lasted 12 wk.

Statistical analysis. Statistical comparison of the groups was performed by using statistical software (GraphPad Prism version 5.04 for Windows, GraphPad Software, San Diego, CA). Unpaired, 2-tailed, t tests were performed for each parameter, and differences between groups were considered significant at a P value of less than 0.05. In addition, an F test was used for variance comparison between groups. Variances between groups were not significantly different for any parameter, suggesting equal standard deviations for the populations evaluated.

Results

There was no significant intergroup difference in average time between litters (small cage, 34.6 ± 2.4 d; large cage, 40.5 ± 3.9 d, $P = 0.1873$, $t = 1.341$, $df = 42$), number of litters per breeding pair (small cage, 2.4 ± 0.27 litters; large cage, 2.0 ± 0.21, $P = 0.2546$, $t = 1.177$, $df = 18$), percentage of litters weaned per breeding pair (small cage, 91.7 ± 2.7; large cage, 91.7 ± 5.7, $P = 1.0$, $t = 0.0$, $df = 18$), number of pups born per breeding pair (small cage, 14.0 ± 1.4 pups; large cage, 12.6 ± 1.7 pups, $P = 0.5348$, $t = 0.6329$, $df = 18$), number of pups weaned per breeding pair (small cage, 12.8 ± 1.3 pups; large cage, 12.1 ± 1.8 pups, $P = 0.7630$, $t = 0.3062$, $df = 18$), or mean weaning weight of pups (small cage, 45.96 ± 2.3 g; large cage, 44.65 ± 2.6 g, $P = 0.7072$, $t = 0.3792$, $df = 30$).

Discussion

The 8th edition of the *Guide for the Care and Use of Laboratory Animals (Guide)* recommends a defined amount (124 in^2 [800 cm^2]) of cage floor space for a female rat with a litter.[19] The rat cages used at our institution afford approximately 140 in^2 (903 cm^2) of cage floor space in an individual rat cage. Therefore, if we strictly adhered to the *Guide* recommendations, only 16 in^2 (103 cm^2) of space would be left to accommodate any additional animals. This is insufficient to accommodate an adult male rat in the same cage as an adult female and her litter. Some research regarding cage size preference and cage density in rats has been published;[4,6,7,25,33,38-41] however, the cage space recommendations in the *Guide* do not appear to be based on any specific studies. Because the *Guide* indicates that performance indices should be considered when determining the amount of space required for housing animals,[19] we performed the current study to determine whether housing rats in larger cages was warranted as determined by measurement of selected breeding parameters. Collectively, the results of the study showed no significant influence of cage size on any of the breeding parameters evaluated.

Many factors need to be considered in addition to cage floor space when housing animals. For example, the *Guide* indicates that an animal's social needs should be considered when determining how it will be housed.[19] Housing a rat independently may induce more stress in the animal than does housing it with conspecifics, and meeting the need for social housing may be more important, in terms of animal wellbeing, than is the recommendation for increased cage floor space.[38-41] Furthermore, the number of rats that can be housed in a defined space needs to be considered carefully, given that overcrowding can result in negative effects on animals housed together and may affect experimental outcomes.[4,7,25] In addition to floor space and housing density, outcomes and behavior should be taken into consideration when determining the cage environment.[9,16,34]

Traditionally, reproductive performance has been associated with animal wellbeing, particularly in production agriculture, where housing animals in settings conducive to their wellbeing is important to maximize reproduction. That being said, it is inappropriate to consider reproductive performance as the sole measure of animal wellbeing. The 3rd edition of the *Guide for the Care and Use of Agricultural Animals in Research and Testing* notes that multiple indicators, particularly in 4 categories, provide the best means to assess animal wellbeing.[11] These indicators include behavior patterns, pathologic and immunologic traits, physiologic and biochemical properties, and reproductive and productive performance of the individual animal.[11] Overall it can be concluded that reproductive performance is only one of multiple indicators of animal wellbeing; however, reproductive data should be considered along with other measures when making decisions on animal wellbeing. Therefore, a comprehensive approach should be used to collect data that can be used to assess animal wellbeing when housing is evaluated. For example, in addition to the evaluation of reproductive indicators, evaluating fecal corticosterone as a measure of animal

stress and using behavior tests to measure animal anxiety, fear, and so forth in rats as a result of housing may yield additional data to help determine whether housing rats in larger cages is superior to housing them in smaller cages.[8,36,44,45]

A limitation to this study is the fact only Dahl salt-sensitive rats were evaluated. Different rat strains may react differently to the amount of floor space afforded by different cages under different housing density conditions. In addition, the commonly available cages may need to be changed more frequently than do the large cages we used. We changed the cages in both breeding schemes twice weekly on a defined schedule to reduce experimental variability; however, the large cages could have been changed less frequently in many instances. Common husbandry procedures are well-known to induce stress in rats.[5,15,38-41] Using larger cages could be beneficial in terms of decreasing stress levels in rats, given that the frequency of cage changing might be reduced. The use of different strains of rats and the effects of various husbandry procedures and schedules on rats housed in both cage types warrant further study.

In summary, this study did not reveal a significant difference between breeding schemes associated with 2 cage sizes according to the breeding parameters evaluated. According to this information, using the smaller, commonly available caging may be acceptable for continuous housing of rats in monogamous breeding pairs, even though doing so would be considered overcrowding, according to the space recommendations of the *Guide*, if a litter was included in a cage housing an adult breeding pair. Additional research is needed to determine the ideal cage environment for rats. In addition, such studies are needed for future editions of the *Guide*, so that housing recommendations for rats can be defined clearly and justified scientifically.

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