Effects of housing density on Long Evans and Fischer 344 rats

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At many breeding facilities, rats are housed at relatively high densities until they are 5 weeks old, at which point they are either shipped for research or rehoused at standard cage densities according to weight. The authors carried out a pilot study in Long Evans and in Fischer 344 rats to investigate whether continuing to house rats at high densities (24 in² floor space per rat) past the age of 5 weeks, through puberty and into adulthood would alter behavioral or physiological parameters compared with raising rats at standard densities (about 72 in² floor space per rat). After rats reached puberty, the authors rehoused them with unfamiliar cagemates. The researchers evaluated clinical and behavioral signs of stress, weight, blood glucose concentration, white blood cell count and serum corticosterone concentration. Overall, cage density had little effect on the parameters measured, though gender seemed to affect stress in Long Evans rats. The results suggest that rats of these strains can be raised at the higher densities tested until any age and regrouped with unfamiliar cagemates without compromising rats’ welfare or subsequent experimental data.
blood cell (WBC) count and serum corticosterone concentration. Changes to these parameters might indicate distress that is not evident by observation and that could affect research data collected from rats.

Previous studies have evaluated the effects of housing density on young mice or rats but have not continued to investigate whether these animals would be useful for research purposes if regrouped with unfamiliar animals at an older age. One group of researchers reported that housing post-pubertal Sprague Dawley rats at high densities resulted in decreased muscle mass in males but not in females. In another study, 56-d-old Sprague Dawley rats that were arbitrarily grouped at high densities (3 rats per cage) showed no significant differences in fighting frequency or weight gain compared with rats that were housed singly; in addition, group-housed rats had lower blood pressure at night. In several studies of adult rats that were housed at high densities with unfamiliar rats, researchers observed behavioral changes associated with fighting and dominance in males, though not in females. Studies that evaluated social behaviors related to fighting, dominance and territorial aggression reported that male rats older than 100 d showed territorial aggression and social dominance, whereas juvenile rats only showed play-fighting behavior. Another researcher showed that in male Wistar rats, the behavioral and physiological effects of high-density housing were dependent on the developmental stage of the rats. These reports indicate that both gender and age may affect behavioral and physiological parameters in rats housed at high densities.

In this study, we examined male and female rats from two strains that are commonly used in research, the outbred Long Evans (LE) strain and the inbred Fischer 344 (F344) strain. The results of this study might be helpful in defining space recommendations for LE and F344 rats.

METHODS

Rats and initial housing conditions

The protocol in this experiment was reviewed and approved by the IACUC of Walter Reed Army Institute of Research/Naval Medical Research Center (WRAIR/NMRC). All procedures were carried out in a facility accredited by AAALAC, International.

We obtained LE (n = 28; 14 males and 14 females) and F344 rats (n = 32; 16 males and 16 females) from Charles River Laboratories (Wilmington, MA). At the breeding facility, LE rats were housed in groups of 40 in cages with total floor space of 24 in × 24 in (14.4 in² per rat). F344 rats were housed in groups of 30 in cages with total floor space of 13.88 in × 22.63 in (10.47 in² per rat). Rats were housed in these conditions from weaning until they were 5 weeks old (Technical Services Department, Charles River Laboratories, Wilmington, MA; personal communication). These floor space allocations are slightly smaller than those recommended in the Guide for rats weighing <100 g (17 in² per rat). At Charles River Laboratories, rats younger than 5 weeks are commonly housed at higher densities without high frequency of wounds, failure to thrive or other visible stress reactions. At 5 weeks of age, rats are either shipped to research facilities or housed according to the Guide’s current space recommendations.

When they arrived at our facility, the F344 rats were 35–42 d old and the LE rats were 30–36 d old. Rats were free of Sendai virus, pneumonia virus of mice, coronavirus, Kilham’s rat virus, Toolan’s H-1 virus, rat parvovirus, rat minute virus, reovirus, Mycoplasma pulmonis, lymphocytic choriomeningitis virus, hantavirus, cilia-associated respiratory bacillus, Salmonella spp., Shigella spp., Spirillum minus, Helicobacter spp., and ectoparasites. We continued to monitor rats with sentinel testing throughout all stages of research.

First stage: housing density study

Rats arrived at our facility weighing 75–110 g each. Rats were ear-tagged for identification and then used in a separate study in which they were housed for 8 weeks at either a high density (5–7 rats per cage; 20.5–28 in² floor space per rat) or a low density (2 rats per cage; 72 in² per rat). Rats were separated by gender and strain. For high-density housing, LE and F344 rats were housed, respectively, at 2–3 times and 1.1–1.4 times less space per rat than what is recommended in the Guide. For low-density housing, the amounts of floor space per rat for both strains were equal to or greater than the Guide’s standard allowances.

During this stage of the study, all rats were subjected to similar manipulations and data collection procedures. None of the rats showed evidence of dominant or submissive behavior such as biting or barbering (excessive grooming). Each rat was within the expected weight range for its age, strain and gender. No other signs of excessive stress or aggression, including inappetence, hunched posture or lethargy, were observed in any of the rats. Chromodacryorrhea was noted in some of the rats during this stage of the study.

Second stage: regrouping with unfamiliar rats

We began the second stage of research when rats were 12–13 weeks old. The average age of puberty for rats is

| TABLE 1 | The Guide’s minimum floor space recommendations for rats, according to weight |
| Rat weight (g) | Floor space per rat |
| <100 | 17 in² |
| 100–200 | 23 in² |
| 200–300 | 29 in² |
| 300–400 | 40 in² |
| 400–500 | 60 in² |
| >500 | ≥70 in² |
50 ± 10 d (7–9 weeks; ref. 29). At this stage of the study, no rats were actively showing any unusual clinical signs.

We redistributed the groups of rats and rehoused them such that no rats that were previously housed together were assigned to the same cage. Rats that had been housed at a high density during the first stage of the study continued to be housed at a higher density than recommended (these rats are hereafter referred to as ‘HDH’), and rats that had been housed at a low density continued to be housed in an amount of space that was equal to or greater than that recommended in the Guide (hereafter referred to as ‘LDH’). We separated rats by gender and strain; otherwise, the selection of groups was arbitrary. All rats were subjected to the same manipulations and data collection procedures.

We housed LE rats in groups of six for HDH (24 in² floor space per rat) and in groups of two for LDH (72 in² floor space per rat). At the beginning of this stage of the study, LE males and females weighed 360–440 g and 230–300 g, respectively, and at the end of the study they weighed 395–495 g and 255–330 g, respectively. The HDH groups of LE rats were housed in 1.2–2.5 times less than the recommended floor space for rats of these weights.

We housed F344 rats in groups of seven for HDH (21 in² floor space per rat) and in groups of two for LDH (72 in² floor space per rat). At the beginning of this stage of the study, F344 males and females weighed 245–280 g and 155–180 g, respectively, and at the end of the study they weighed 260–315 g and 165–195 g, respectively. The HDH groups of F344 rats were housed in 1.1–1.9 times less than the recommended floor space for rats of these weights.

For each strain, there were two cages of HDH males, two cages of HDH females, one cage of LDH males and one cage of LDH females. Most experiments at WRAIR/NMRC use rats housed at low densities as described above; therefore, we considered LDH rats to be a control group for comparison with HDH rats.

**Husbandry and management (second stage)**

We housed rats in standard filter-top covered polycarbonate cages (144 in² of floor space, 8 in high; Lab Products, Inc., Seaford, DE) on woodchip bedding (Beta Chip, Northeastern Products Corp, Warrensburg, NY). All rats received rodent chow *ad libitum* (LabDiet RMH3000, PMI Nutrition International, LLC, Brentwood, MO) and drank municipal water, which was filtered by reverse osmosis, through an automatic watering system. For environmental enrichment, we put two plastic pipes (6 in long, 3 in in diameter) in each HDH cage and one plastic pipe in each LDH cage. We maintained cages in a climate-controlled room with temperature set at 18–26 °C and humidity at 30–70%, with a light:dark cycle of 12 h:12 h.

We maintained rats under these conditions for 4 weeks. We changed cages two times a week for the LDH groups and an average of three times a week for the HDH groups, as needed. We carried out cage changes after completing data collection for the day.

**Rat manipulations (second stage)**

We handled and examined all rats for at least 20 s twice daily. We examined rats for clinical and behavioral signs of stress, including chromodacryorrhea, hair loss, wounds, inappetence, lethargy, ruffled haircoat and hunched appearance. We scored each rat’s condition once daily, using a subjective system in which 0 indicated no signs of stress, and 1 indicated that at least one sign was observed. At the end of the study period (29 d), we totaled these scores for each rat. We also noted whether any rats required medical treatment, which would result in removal from the study. We weighed each rat once per week (SK-LOK scale, A&D Co., Ltd, Seoul, Korea).

We collected blood once every 2 weeks at the same time of day (three samplings total; the first sampling was on the last day of the first stage of the study (day 0), before rats were regrouped, to evaluate WBC count, blood glucose concentration and serum corticosterone concentration). For blood sampling, we anesthetized rats with 2% isoflurane using an IMPAC6 anesthesia system (VetEquip, Pleasanton, CA). We collected blood samples (0.5–1.0 ml) from the orbital venous plexus using heparinized hematocrit tubes. For WBC counts and measurement of glucose concentration, we collected blood samples (0.25–0.5 ml) from all rats and stored them in anticoagulant (K₂-EDTA) microcontainers. We measured glucose concentration immediately after blood collection using a commercial glucometer (ACCU-Check Advantage, Roche, Mannheim, Germany). We obtained the WBC count within 2 h of blood sampling using a Beckman Coulter AcT Diff hematology analyzer (Beckman Coulter, Miami, FL).

For serum corticosterone analysis, we collected additional blood (0.25–0.5 ml) from at least one rat in each cage (n = 10 for each strain) and stored the samples in serum separator microcontainers. We selected this subgroup of rats arbitrarily at the beginning of the study, and we collected blood for corticosterone analysis from the same rats at each sampling session. We froze serum at –29 °C and sent it to the Michigan State College of Veterinary Medicine Endocrinology Laboratory for corticosterone analysis.

We considered the measurements recorded on day 0 to be baseline parameters.

**Statistics**

We analyzed data separately for each strain, comparing cage groups according to gender and housing density. We analyzed clinical and behavioral signs of stress
signs in LDH males was slightly higher than that in HDH males, though results were not statistically significant. HDH females showed a significantly higher incidence of stress signs compared with LDH females ($P < 0.0001$). Over the course of the 29-d study, we noted signs of stress in HDH females about 1.3 times on average for each rat, whereas in LDH females, we observed no signs of stress (Fig. 1b).

**Weight gain**

On the first day of the second stage of the study, the weights of all subjects were within the expected range for rats aged 80–90 d (ref. 28). In LE rats, initial weight was significantly influenced by gender ($P < 0.0001$; males were significantly heavier than females) but not by cage density ($P = 0.3354$). At the end of the study, all rats gained weight, but males gained more weight than females did. Weight gain (expressed as a percentage of rats’ initial weight) was significantly influenced by both gender ($P < 0.0165$) and cage density ($P = 0.0245$). By the end of the study, LDH males were heavier than HDH males, whereas HDH females were heavier than LDH females (Fig. 2a); this interaction effect was not statistically significant, however ($P = 0.87$).

![Figure 1](image1.png)

**FIGURE 1** Average number of occurrences per rat of clinical or behavioral signs of stress over a period of 29 d. Rats are separated by gender and by housing density. (a) LE rats. (b) F344 rats.

![Figure 2](image2.png)

**FIGURE 2** Average weight in male and female rats in LDH and HDH groups. (a) LE rats. (b) F344 rats.
Physiologic parameters
We analyzed WBC count, blood glucose concentration and serum corticosterone concentration from blood samples taken at 2-week intervals (three samples per rat). We also compared data from the third blood sample (taken on day 28) with data from the first sample (taken on day 0).

In LE rats, at least one parameter for each sampling period was significantly influenced by gender. Gender did not significantly affect the difference between data obtained from the first blood sample and the last. Average blood glucose concentration and WBC were higher in males than in females, whereas corticosterone concentration was lower in males than in females (Tables 2 and 3). Cage density significantly affected glucose concentration in the third blood sample, though in the first two samples glucose concentration was similar in HDH and LDH groups (Fig. 3). This effect can be attributed to a single LDH rat, in which glucose concentration in the third blood sample was 204 mg/dl, 50 mg/dl more than that in any other rat. If this data point is considered an outlier, the difference between the two cage densities is no longer statistically significant ($P = 0.25$).

In F344 rats, initial weight was also significantly influenced by gender ($P < 0.0001$; males were significantly heavier than females) but not by cage density ($P = 0.3189$). Weight gain was significantly influenced by gender ($P < 0.0011$), with males weighing more than females at the end of the study. We observed a significant interaction effect between cage density and gender for weight gain in this strain, suggesting that LDH males gained slightly more weight than did HDH males ($P = 0.0043$). Despite this observation, housing density had no significant effect on rats’ overall weight at the end of the study ($P = 0.1683$; Fig. 2b).

### Table 2

For LE rats, statistical significance of differences in average blood glucose concentration, serum corticosterone concentration and WBC count when comparing rats according to gender or housing density (low or high)

| Parameter          | Sampling day | Gender ($P$) | Cage density ($P$) |
|--------------------|--------------|--------------|--------------------|
| Glucose concentration | 0            | 0.4075       | 0.9055             |
|                   | 14           | 0.2475       | 0.7074             |
|                   | 28           | 0.0317*      | 0.0054*            |
| Glucose$_{28}$ – Glucose$_{0}$ | 0          | 0.2516       | 0.0110*            |
| Corticosterone concentration | 0            | 0.0813       | 0.0896             |
|                   | 14           | 0.0272*      | 0.7083             |
|                   | 28           | 0.0109*      | 0.6450             |
| Corticosterone$_{28}$ – Corticosterone$_{0}$ | 0          | 0.7774       | 0.4138             |
| WBC count | 0           | 0.1828       | 0.3388             |
|                   | 14           | 0.0007*      | 0.8469             |
|                   | 28           | <0.0001*     | 0.5454             |
| WBC$_{28}$ – WBC$_{0}$ | 0          | 0.2785       | 0.5123             |

Day 0 refers to the last day of the first stage of the study, before rats were regrouped. *$P < 0.05$

### Table 3

For LE rats, means and s.d. for physiological parameters with significant differences by gender

| Parameter          | Sampling day | Female Mean | s.d. | Male Mean | s.d. |
|--------------------|--------------|-------------|------|-----------|------|
| Glucose (mg/dl)    | 28           | 121.1       | 17.5 | 135.8     | 22.1 |
| Corticosterone (μg/dl) | 14       | 510.0       | 141.0 | 283.8     | 73.8 |
| Corticosterone (μg/dl) | 28       | 648.4       | 163.6 | 367.2     | 72.4 |
| WBC count ($10^{-3} \times$ cells/μl) | 14     | 5.9         | 1.0  | 8.7       | 2.3  |
| WBC count ($10^{-3} \times$ cells/μl) | 28     | 4.5         | 1.1  | 8.8       | 2.7  |
behavioral or physiologic parameters, compared with rats that were housed at low densities and were also rehoused with unfamiliar rats after puberty. The Guide currently recommends housing rats of the weights of those used in this study in 23–60 in² of floor space. This recommendation is aimed at preventing stress and aggression, which may occur when rats are housed at higher densities.

To determine whether rats showed signs of stress, aggression or unthriftiness when housed at higher densities, we used a subjective scoring system to evaluate the incidence of common clinical and behavioral signs of stress. Although we observed some signs of stress, neither strain showed a frequency or severity of abnormalities that would indicate that a decreased amount of floor space caused high stress in either gender. We evaluated all clinical signs equally on a 'present or absent' basis but noted that chromodacryorrhea was the most prevalent abnormality (data not shown).

LE males in both housing densities showed more clinical and behavioral signs of stress than did females of this strain. This may indicate that male LE rats are not as compatible as females in group housing situations. Cage density did not have statistically significant effects on the incidence of signs of stress in either gender. In F344 rats, LDH males showed more signs of stress than did HDH males. LDH and HDH males all showed fewer signs of stress than did HDH females. We did not observe signs of stress in F344 LDH females. This may indicate that the females of this strain would not be as compatible as males in high-density group housing situations. None of the observed abnormalities were severe enough to require medical attention or removal from the study.

We observed no overt signs of inappetence in any of the rats. In LE rats, gender and cage density each had significant effects on weight. As noted above, LDH males weighed more on average than did HDH males, whereas HDH females weighed more than LDH females. In F344 rats, cage density did not affect over-all body weight, though it did affect weight gain; LDH males gained more weight than did HDH males. All rats in both strains gained weight during the study, and their weights were in the accepted range for their ages. We did not submit rats for necropsy and did not measure organ weights. There may have been alterations in the weights of some of the organs, similar to the alterations observed in another group-housing study.

We obtained mixed results when we measured physiological parameters. In LE rats, cage density did not affect WBC or corticosterone concentration, though it did affect glucose concentration. Glucose concentration in LDH males seemed to be higher than that in HDH males. We note, however, that this observation was based on one reading for a single rat, and this reading was not statistically significant.

In F344 rats, LDH males showed more signs of stress than did HDH males. LDH and HDH males all showed fewer signs of stress than did HDH females. We did not observe signs of stress in F344 LDH females. This may indicate that the females of this strain would not be as compatible as males in high-density group housing situations. None of the observed abnormalities were severe enough to require medical attention or removal from the study.

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| Parameter                                      | Sampling day | Gender (P) | Cage density (P) |
|------------------------------------------------|--------------|------------|-----------------|
| Glucose concentration                          |              |            |                 |
| 0                                              | 0.1103       | 0.3906     |
| 14                                             | 0.0885       | 0.4924     |
| 28                                             | 0.0320*      | 0.2125     |
| Glucose(day 28) – Glucose(day 0)                |              |            |                 |
| 0                                              | 0.2900       | 0.5284     |
| 14                                             | 0.0320*      | 0.2125     |
| 28                                             | 0.2900       | 0.5284     |
| Corticosterone concentration                   |              |            |                 |
| 0                                              | 0.0840       | 0.1381     |
| 14                                             | 0.2765       | 0.0239*    |
| 28                                             | 0.0320*      | 0.2125     |
| Corticosterone(day 28) – Corticosterone(day 0) |              |            |                 |
| 0                                              | 0.0964       | 0.8824     |
| 14                                             | 0.2765       | 0.0239*    |
| 28                                             | 0.0320*      | 0.2125     |
| WBC count                                      |              |            |                 |
| 0                                              | 0.127        | 0.6216     |
| 14                                             | 0.127        | 0.6216     |
| 28                                             | 0.127        | 0.6216     |

Day 0 refers to the last day of the first stage of the study, before rats were regrouped. *P < 0.05.
collected a representative serum sample from each cage in order to determine whether any trends could be extrapolated. The differences noted suggest that corticosterone levels should be measured for all rats in future expanded studies.

Because normal husbandry activities can be stressful for animals, the different frequency of cage changes for LDH and HDH rats might have been a confounding variable. To minimize this potential effect, we changed all cages after the collection of data. Another variable that might confound comparison between strains is the difference in group size between the HDH groups of each strain. The difference in the number of rats per cage in each strain was based on the differences in the average body weight of rats in these strains. To make comparisons between the strains, it would be beneficial to standardize the group sizes and available floor space per rat. Furthermore, group size may affect rats’ behavior regardless of the amount of floor space available. In larger groups of rats, the social hierarchy is more complex than that in smaller groups, which may lead to increased incidence of aggression or stress responses. This variable should be considered when evaluating some of the differences seen between the HDH and LDH groups within each strain.

CONCLUSIONS

Our findings suggest that LE and F344 rats that have been housed at high densities from weaning through puberty may be arbitrarily regrouped and housed for at least 4 weeks at densities that are higher than the Guide’s current recommendations, without adverse effects on behavioral or physiological parameters. Housing density had little effect on the common indicators of stress that we evaluated (clinical and behavioral signs, weight and physiologic parameters). We did note that in F344 rats, corticosterone concentration was higher in HDH rats compared with LDH rats, which may indicate that high-density housing is not optimal for rats of this strain. In addition, gender affected stress in LE rats, whereas in F344 rats it did not. All rats showed normal growth with no clinical signs of severe stress or serious behavioral abnormalities that would result in exclusion from a study. More studies are needed to further investigate potential consequences of transferring post-pubertal group-housed rats into new cages.

**TABLE 5 |** For F344 rats, means and s.d. for physiological parameters with significant differences by gender

| Sampling day | Female | s.d. | Male | s.d. |
|--------------|--------|------|------|------|
| Glucose (mg/dl) | 28     | 98.5 | 9.66 | 110.56 | 19.37 |
| WBC count (10^{-3} \times cells/μl) | 0      | 6.41 | 1.45 | 7.82 | 1.87 |
| WBC count (10^{-3} \times cells/μl) | 14     | 6.75 | 1.95 | 8.40 | 2.06 |
| WBC count (10^{-3} \times cells/μl) | 28     | 5.03 | 0.64 | 6.79 | 1.64 |

was much higher than the rat’s other two measurements. Therefore, this data point is probably an outlier caused by a lab error or an unrecorded stress event at the time of sampling. If this one value is discounted, none of the parameters indicate a difference in stress level between the two cage densities. In F344 rats, cage density did not affect glucose concentration or WBC. The average corticosterone concentration in LDH rats was lower than that in HDH rats.

According to these results, increasing cage density did not seem to cause adverse physiologic effects in LE rats. F344 rats housed at high densities may have higher short-term stress compared with rats housed at low densities. Although we did not find strain-specific published reference values for all of the physiological parameters we measured, most of the glucose and WBC measurements we obtained were within the accepted normal range for rats. Corticosterone concentrations were also mostly within the published normal ranges, so the increase we observed in HDH F344 rats, which was outside of the normal range, seems to be a true elevation indicating a physiologic stress response. Handling and restraint are known to cause an increase of corticosterone concentration in rats, but as all rats were handled in the same manner, this effect should have been normalized for all groups. Baseline values of physiological parameters may differ for males and females.

We did not collect corticosterone samples from all rats because of processing and logistical constraints. Because this work was carried out as a pilot study, we collected a representative serum sample from each cage in order to determine whether any trends could be extrapolated. The differences noted suggest that corticosterone levels should be measured for all rats in future expanded studies.

Because normal husbandry activities can be stressful for animals, the different frequency of cage changes for LDH and HDH rats might have been a confounding variable. To minimize this potential effect, we changed all cages after the collection of data. Another variable that might confound comparison between strains is the difference in group size between the HDH groups of each strain. The difference in the number of rats per cage in each strain was based on the differences in the average body weight of rats in these strains. To make comparisons between the strains, it would be beneficial to standardize the group sizes and available floor space per rat. Furthermore, group size may affect rats’ behavior regardless of the amount of floor space available. In larger groups of rats, the social hierarchy is more complex than that in smaller groups, which may lead to increased incidence of aggression or stress responses. This variable should be considered when evaluating some of the differences seen between the HDH and LDH groups within each strain.

**CONCLUSIONS**

Our findings suggest that LE and F344 rats that have been housed at high densities from weaning through puberty may be arbitrarily regrouped and housed for at least 4 weeks at densities that are higher than the Guide’s current recommendations, without adverse effects on behavioral or physiological parameters. Housing density had little effect on the common indicators of stress that we evaluated (clinical and behavioral signs, weight and physiologic parameters). We did note that in F344 rats, corticosterone concentration was higher in HDH rats compared with LDH rats, which may indicate that high-density housing is not optimal for rats of this strain. In addition, gender affected stress in LE rats, whereas in F344 rats it did not. All rats showed normal growth with no clinical signs of severe stress or serious behavioral abnormalities that would result in exclusion from a study. More studies are needed to further investigate potential consequences of transferring post-pubertal group-housed rats into new cages.
groups, at less floor space per rat than what is recommended in the Guide. Another point for investigation is the effect of housing regrouped rats at high densities for long periods of time.

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COMPETING INTERESTS STATEMENT
The authors declare no competing financial interests.

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