

Letters to the Editor

The Response of C57BL/6J and BALB/cJ Mice to Increased Housing Density

Dear Editor,

We are writing in regard to the article by Nicholson A and colleagues.³ We believe that the data presented do not support the primary conclusion drawn by the authors, namely, that housing density can be increased significantly over current *Guide* standards² with few if any adverse effects. We also note that the conclusions stated in the abstract do not coincide with those in the text. The abstract states that the findings support a 50% to 100% increase over current *Guide* standards,² whereas the discussion mentions a 30% to 50% increase. Although this may stem from confusion about the number of mice per cage versus the amount of floor space per mouse, it is an important distinction that requires clarification by the authors.

Rather than supporting an increase in housing density, the data presented suggest that significant increases in density have adverse effects. A 100% increase above the current *Guide* standards² appeared detrimental to the mice, based on criteria that the authors determined were most significant for determining wellbeing. These criteria included weight gain, adrenal gland size and percentage cortex, fecal corticosterone, in-cage telemetry of activity and heart rate, and behavioral aspects such as incidence of barbering and whisker-picking, fighting, and formal tests of anxiety. Many of these parameters changed with increasing density and were interpreted by the authors to be indicative of chronic stress or anxiety. Similar findings were reported by many of the papers cited in Figure 1 of the article.³ Thus, it is unclear why the authors would claim that housing density could be increased as much as 100% without affecting overall wellbeing.

The validity of this conclusion is also called into question by the fact that the stated goal of the study was to serve as a preliminary experiment that would enable the testing of specific hypotheses in subsequent larger studies. The study was not designed to determine whether housing density could be increased; rather, the primary goal was to determine what parameters should be incorporated into future experiments. This tends to weaken any conclusions about housing density per se.

The authors emphasize in the discussion that more extensive behavioral measurements are important to determining appropriate housing density. We strongly concur and would argue that simple strategies, such as monitoring for gross evidence of negative behavior (fighting, barbering, and whisker pulling), are necessary but not sufficient for assessing wellbeing. More refined behavioral testing would add substantially to understanding the optimal housing requirements for mice. Assessments of time budget, preference testing, and demand (how hard the animal will work to obtain the preference) would shed light on how animals use the space available to them. Behavioral measurements of anxiety in the presence of a strange setting or unknown animals would help clarify the extent to which high density housing is associated with stress responses.

This topic was discussed in depth at the 2006 National AA-LAS meeting and later published in the November 2007 issue of *Lab Animal*.¹ We believe for fairness to the reader and the laboratory animal community the author should reference this publication since in that forum similar studies from the same

laboratory were criticized for weaknesses in experimental design and data interpretation.

We would also point out that the issues at hand are not strictly scientific in their reach. There are ethical concerns and public perceptions that should influence the discussion. Providing sufficient space to permit species-typical activities and social interactions is an ethical imperative. Even if studies suggest that increased housing density is devoid of negative effects, implementation of high-density housing may lead to the public perception that animals are crowded into an unacceptably small space. This perception may well diminish public confidence in the proposition that the welfare of research animals is properly assured.

Sincerely,
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References

1. Foltz C, Carbone L, DeLong D, Rollin B, Van Loo P, Whitaker J, Wolff A. 2007. Considerations for determining optimal mouse caging density. *Lab Anim* 36:40–48.
2. Institute for Laboratory Animal Research. 1996. *Guide for the care and use of laboratory animals*. Washington (DC): National Academies Press.
3. Nicholson A, Malcolm RD, Russ PL, Cough K, Touma C, Palme R, Wiles MV. 2009. The response of C57BL/6J and BALB/cJ mice to increased housing density. *J Am Assoc Lab Anim Sci* 48:740–753.

Response to Drs Foltz and DeLong's Letter to the Editor:

We thank Drs Foltz and DeLong for their comments bringing to our attention the discrepancy between the data presented in the abstract and our conclusions,³ and we regret any confusion this may have caused in the minds of readers. To clarify the ambiguity, we include here a table explicitly relating the housing densities used in our study to those recommended in the *Guide*.²

Body weight	Recommended no. of mice per pen*	Relative increase in housing density for 4, 6 or 8 mice per pen ³		
		4	6	8
> 25 g	3	33%	100%	166%
15-25 g	4	0%	50%	100%
10-15 g	6		0%	33%
< 10 g	8			0%

*Number of mice of different weight categories per pen in duplex cages of 51.7 in.² in accordance with the recommendations of the *Guide*.

The data for the largest animals (that is, those > 25 g) showed that mice housed at 4 or 6 per pen demonstrated no significant differences for most of the parameters measured. In fact, none of the female C57BL/6J mice attained a weight > 25 g during the study, so they remained within the *Guide* recommendations throughout. No significant differences were noted between these female C57BL/6J mice and other mice housed at 4 or 6 per pen. Therefore, we concluded that the housing density of mice could be increased by up to 100%, to 4 or 6 per pen, with no apparent ill effect on wellbeing as reflected in the wide range of tests employed in our study. This did not appear to be the

case for animals housed at 8 per pen.

Although we suggested, as part of our conclusions, that the housing density could be increased beyond that recommended in the *Guide*, at no point did we advocate for such an increase. In fact, on p 751 in the article, we cautioned against ascribing too much biologic significance to those parameters manifesting statistical significance.

This caveat is reinforced by the fact that very few parameters displayed a linear response to altered housing density. Such variability makes it difficult to suggest a unifying response to increased housing density; however, certain parameters are highly suggestive of significant responses to increased housing density and are worthy of inclusion in future confirmatory experiments.³

Furthermore, the most detrimental response to increased housing density was in those mice housed at 8 per pen. Some negative responses were seen relatively early on in the study before the mice exceeded 25 g in weight. For example, the growth curves presented in Figure 2 on p 746 suggest that group size may have had a greater role than floor area by itself.³ The question of group size versus cage size has been raised by several other studies referred to in our paper but none seems to have clearly distinguished between these two environmental factors.

Our study was preliminary, as our primary aim was to assess a range of test parameters to determine which were most helpful in detecting differences in the response of mice to altered housing densities. Indeed, we were able to conclude that some tests were useful and others were not. In addition, our data did not indicate that housing density increases were uniformly detrimental to the animals, and we stand by this conclusion. At the same time we strongly agree with the correspondents' calls for more extensive behavioral testing in future studies and are fully cognizant of the ethical obligations and emotional (public perception) aspects of the welfare of animals used for research. These considerations, together with the study limitations that we pointed out (that is, sample size, lack of assessment of the impact of environmental enrichment, and testing of only animals housed in single-sex groups) further induced us to recommend caution in interpretation of our results. However, we consider the data interesting and believe that our cautiously drawn conclusions are reasonable and provide food for thought about mouse wellbeing and how to assess it.

Finally we take issue with the correspondents' reference to Foltz and colleagues,¹ in which previous studies from The Jackson Laboratory (TJL) were criticized for some of the assumptions made and resulting conclusions reached. First, none of the authors in the current study were involved in the previous studies although most of us have worked at TJL. Second, we did not feel that overall the comments¹ were directly relevant to nor contributed to the assessment of our data because we included many more physiological and behavioral tests than the earlier TJL studies. We did not include all the behavioral tests suggested¹ because as with many researchers, we had to make decisions about, and restrict ourselves to, those tests we felt we could best perform to gain reliable data within our limited budget. Comprehensive housing studies that incorporate factors such as strain differences, effect of environmental enrichment, more extensive in- and out-of-cage behavioral testing, as suggested by the correspondents, are necessary, highly desirable, and expensive to conduct. Securing funding for such complex, large-scale studies is extremely difficult, because despite the importance of this area of research, with its potential for wide-

-ranging impact on much other research, those funding agencies with adequate funds appear to focus their support elsewhere.

In conclusion, we agree in large part with the suggestions of Foltz and DeLong but contend that the differences in our interpretation of the data stem from differences in emphasis. Our emphasis was to identify reliable and valid parameters for evaluation of responses to changes in housing density for use in future studies. Despite our findings suggesting that increased housing density may have no or limited apparent detrimental impact on the 2 strains of mice studied, we did not advocate implementing such an increase. We are well aware of the need for further studies before concluding that one way or the other is preferable or even adequate. Our intentions were to contribute to this important and ongoing debate concerning laboratory mouse welfare.

Respectfully yours,

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1. Foltz C, Carbone L, DeLong D, Rollin B, Van Loo P, Whitaker J, Wolff A. 2007. Considerations for determining optimal mouse caging density. *Lab Anim* 36:40–48.
2. Institute for Laboratory Animal Research. 1996. Guide for the care and use of laboratory animals. Washington (DC): National Academies Press.
3. Nicholson A, Malcolm RD, Russ PL, Cough K, Touma C, Palme R, Wiles MV. 2009. The response of C57BL/6J and BALB/c mice to increased housing density. *J Am Assoc Lab Anim Sci* 48:740–753.

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