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Mice are sensitive to minor changes in food, bedding and light exposure.

REPRODUCIBILITY

A mouse's house may ruin studies

Environmental factors lie behind many irreproducible rodent experiments.

BY SARA REARDON

It's no secret that therapies that look promising in mice rarely work in people. But, too often, experimental treatments that succeed in one mouse population do not even work in other mice, suggesting that many rodent studies are flawed from the start.

"We say mice are simpler, but I think the problem is deeper than that," says Caroline Zeiss, a veterinary neuropathologist at Yale University in New Haven, Connecticut. Researchers rarely report on subtle environmental factors such as their rodents' food, bedding or exposure to light; as a result, conditions vary widely across labs despite research showing that these factors can significantly affect the animals' biology.

"It's sort of surprising how many people are surprised by the extent of the variation" between mice that receive different care, says Cory Brayton, a pathologist at Johns Hopkins University in Baltimore, Maryland. At a meeting on mouse models at the Wellcome Genome Campus in Hinxton, UK, on 9–11 February, she and others explored the many biological factors that prevent mouse studies from being reproduced.

Christopher Colwell, a neuroscientist at the University of California, Los Angeles, has first-hand experience with these issues. He and a colleague studied autism in the same genetically modified mouse line, but got different results on the same behavioural tests. Eventually, they worked out why: Colwell, who studies circadian rhythms, keeps his mice dark in the daytime to trick their body clocks into thinking day is night, so that the nocturnal animals are alert when tested in the day. His colleague does not.

Nutrition can also determine whether a mouse study succeeds or fails. Some mouse foods contain oestrogens and endocrine-disrupting chemicals that can affect research on cancer, among other diseases (M. Nygaard Jensen and M. Ritskes-Hoitinga *Lab. Anim.* **41,** 1–18; 2007). And the high-fat, high-sugar food used in obesity studies goes rancid quickly; when it does, mice may stop eating and lose weight without researchers realizing why.

Food choices can also alter a mouse's gut microbiome. Catherine Hagan Gillespie, a veterinary pathologist at the Jackson Laboratory in Sacramento, California, has found that species of bacteria in the gut vary widely between mice from different vendors (A. C. Ericsson *et al. PLoS ONE* **10,** e0116704; 2015). In an unpublished study, she also found that mice with different gut bacteria showed different anxiety levels in behavioural tests.

But few behavioural scientists think about microbiology assessments, says Hagan Gillespie. Even when they do, the extra work can increase the complexity and cost of the study. Yet the mouse microbiome is sensitive to many factors, such as air quality and maternal stress.

Differences in the gut microbiome may explain why mice with the same genes can have different characteristics, or phenotypes, says George Weinstock, associate director for microbial genomics at the Jackson Lab's site in Farmington, Connecticut. The lab, which breeds and supplies mice for use in studies around the world, tightly controls factors such as the type and quantity of food and the pH of water that animals receive. Even so, it finds differences between mice at its three sites. Weinstock says that the company has begun looking into standardizing its customers' experiments by providing food and care instructions for its mice.

But even when improved mice and food are available, some researchers resist using them in case it affects their results, says Graham Tobin, former technical director of mouse-diet vendor Teklad in Alconbury, UK. He argues that standardizing results is worth any inconvenience, and notes that researchers rarely resist adopting other new technologies that can throw older data into question.

Zeiss says that the competitive nature of science might increase researchers' resistance to changing how they consider animals in research design. If scientists have to treat their animals at the right point in the experiment, analyse both clinical and biomarker changes, include old mice and both sexes to ensure that results are representative of broad populations, and control environmental variables, each experiment will take much longer and the scientists are probably not going to be able to publish as much, she says.

The US National Institutes of Health (NIH) has taken steps to address some of these problems, although some people say it is not enough. Some NIH institutes require certain animal trials to be replicated before a therapy can move into clinical trials, but the agency says that it has no plans to require this agency-wide. And in 2014, the NIH began requiring researchers to include female animals in studies, and giving out supplementary grants to those who complained about the cost. But the agency has not issued any specific grants or supplements to study other confounding factors.

That is disappointing to those who would like to see researchers control — or at least report — factors such as the strain of mice used and the type of environment they are raised in. This would allow meta-analyses of published literature that could identify any confounding factors. "The information and the wisdom is out there," says Zeiss, "but studies get funded without necessarily a lot of attention to that." ■ SEE EDITORIAL P.254