Experimental Neurology 270 (2015) 72–77

Contents lists available at ScienceDirect

Experimental Neurology

journal homepage: www.elsevier.com/locate/yexnr

The influence of the cage environment on rodent physiology and behavior: Implications for reproducibility of pre-clinical rodent research

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ARTICLE INFO

Article history:
Received 12 January 2015
Revised 13 April 2015
Accepted 16 April 2015
Available online 22 April 2015

Keywords:
Mice
Rats
Housing
Environmental enrichment

ABSTRACT

The reproducibility of pre-clinical research is an important concern that is now being voiced by constituencies that include the National Institutes of Health, the pharmaceutical industry, Congress, the public and the scientific community. An important facet of performing and publishing well-controlled reproducible pre-clinical research is to stabilize and more completely define the environment of the animal subjects. Scientists who use rodents in research generally recognize the importance of maintaining a stable animal environment. However, despite a theoretical and general awareness of these issues, many may lack a true appreciation of how significantly even seemingly minor variations in the environment can affect research outcomes. The purpose of this article is to help investigators gain a more comprehensive and substantiated understanding of the potentially significant impact of even seemingly minor environmental changes on the animals and the data. An important caveat to this article is that the examples presented were selected from a very large literature, admittedly in order to illustrate certain points. The goal of this article is not to provide an overview of the entire literature on how the environment affects rodents but rather to make preclinical scientists more aware of how these factors can potentially influence the experimental data and contribute to poor reproducibility of research.

The reproducibility of pre-clinical research is an important concern that is now being voiced by constituencies that include the National Institutes of Health, the pharmaceutical industry, Congress, the public and the scientific community. Maintaining credibility as scientists who perform meaningful research will require strengthening efforts to generate and publish well-controlled reproducible research. One important facet of furthering this goal is to stabilize and define the animal environments used in preclinical research. Scientists who use rodents in research generally recognize that maintaining a stable animal environment is an important element of rodent pre-clinical experimentation. However, despite a theoretical and general awareness of these issues, many may lack a true appreciation of how significantly even seemingly minor variations in the environment can affect research outcomes. As animal models and measurement techniques become increasingly precise and sophisticated, environmental influences become even more critical; we are now able to detect subtle effects that may have previously been part of the experimental noise. A clearly defined stable environment is essential to generating consistent experimental outcomes that support both replication and valid interpretations of the data.

To provide an example of these issues, Table 1 shows a sample scenario listing elements of the rodent housing environment that differ either within or among laboratories studying the same gene and its effect on a variety of phenotypes. Whether in the same lab or multiple labs, mice used to study different aspects of the gene's function may experience different environmental conditions. For studies of sleep, for example, mice are generally housed one per cage, whereas for other studies they are likely housed in groups. For sleep studies that use tethering, cages generally have open tops, whereas for telemetric monitoring and other types of studies, closed-top cages are probably used. The choice of bedding and the environmental temperatures may differ in static and ventilated caging. Rodents housed under this wide variety of environmental conditions may not be physiologically equivalent, particularly with regard to relating results from one segment of the study to results of other segments.

Critical aspects of the rodent environment that may vary from lab to lab and even from study to study within the same lab include general cage and environmental conditions, the presence of cage mates, manipulations associated with husbandry and testing, and the use of environmental enrichment. The purpose of this article is to help investigators gain a more comprehensive and substantiated understanding of the potential significant impact of even seemingly minor environmental changes on the animals and the data. An important caveat to this article is that the examples presented were selected from a very large literature, admittedly in order to illustrate certain points. Some of this literature is contradictory, potentially for the very reasons that will be discussed. The goal of this article is not to provide an overview of the entire literature on how the environment affects rodents but rather to make pre-clinical scientists more aware of how these factors can potentially influence the experimental data and contribute to poor reproducibility of the research.

http://dx.doi.org/10.1016/j.expneurol.2015.04.010
0014-4886/

Table 1
Sample scenario of environmental conditions experienced by mice used to characterize the effects of a gene on various phenotypes.

Phenotype	Ambient temperature	Housing density	Caging system	Bedding
Sleep	26 °C	1 per cage	Static, open top	Wood chip
Immune response	22 °C	5 per cage	Ventilated	Corn cob
Tumor growth	22 °C	5 per cage, with periodic euthanasia or death	Ventilated	Corn cob
Inflammatory pain	22 °C	I per cage	Ventilated with shelter	Corn cob
Viral infection	26 °C	5 per cage, with periodic euthanasia or death	Static, closed top	Wood chip

Caging

The rodent cage, also called the primary enclosure, comes in many varieties (e.g., open top and closed top, ventilated or non-ventilated) and can vary in terms of size, bedding, enrichment devices, and other attributes. Even the position of the cage on the rack can influence the outcome of behavioral tests (Izidio et al., 2005). Another attribute that is rarely considered is the color of the cage. This fundamental feature of the environment was recently shown to significantly influence the circadian metabolic measures in rats (Dauchy et al., 2013; Wren et al., 2014). The tint of the cage (clear, amber, blue, or red) causes significant variation in peak levels and peak durations of melatonin during the dark phase and significant alterations in the circadian timing of insulin peaks (Wren et al., 2014). The practice of exposing rats to red light during the dark phase to allow observation and manipulation was also recently shown to have significant effects on the circadian rhythms of melatonin, leptin, insulin, and other analytes in rats (Dauchy et al., in press).

A fundamental feature of the rodent cage is the bedding used. The properties of different types of rodent bedding may differentially influence the cage environment, rodent physiology and behavior, and even animal health (e.g., Horn et al., 2012; Leys et al., 2012; Royals et al., 1999; Smith et al., 2004; Whiteside et al., 2010). For example, corn cob bedding is often used because it is highly absorbent and therefore may require less frequent cage changes, thereby reducing both labor and bedding costs as well as the necessary frequency of disruption of the cage environment (Burn and Mason, 2005; Ferrecchia et al., 2014). However, corn cob bedding may not be optimal for animal comfort (Ras et al., 2002), rodents may consume it, which may confound some types of studies (Ambery et al., 2014), and it may modify some behaviors (Leys et al., 2012).

Ambient temperature is another critical feature of the rodent cage environment that is likely influenced by the type of caging system used. Two recent studies illustrate how ambient room temperature, interacting with the caging system and perhaps the tumor model, can have complicated effects on experimental outcomes and conclusions and potentially contribute to inability to reproduce preclinical data across labs (Table 2) (David et al., 2013; Kokolus et al., 2013). For example, one recent study evaluated brown fat thermogenesis in nude and SCID mice that were housed individually at an ambient temperature of 21 °C in ventilated cages with or without a shelter or in a static (non-ventilated) cage (David et al., 2013). The data showed that regardless of strain, mice housed individually in ventilated caging without a shelter had significantly greater brown adipose tissue thermogenesis and greater adrenal weights than did mice housed in either static cages or in ventilated cages with a shelter. Furthermore, when implanted with tumor cells, mice housed in static cages had greater tumor growth than did mice in the other two conditions. The authors concluded that mice housed in ventilated caging without a shelter were experiencing cold stress, which in turn interfered with tumor growth. However, another study reported that BALB/c and C57BL/6 mice housed 5 per cage at an ambient temperature of 22 °C had greater tumor growth than those maintained at 30 °C, yet did not detect a temperature effect on tumor growth when using immune-impaired nude and SCID mice (Kokolus et al., 2013). The study further determined that the anti-tumor immune response was attenuated in immune-competent mice maintained at 21 °C as compared with those housed at 30 °C.

Ambient temperature can also influence host responses to microbial challenges. For example, the thermoregulatory response of mice after injection of lipopolysaccharide (LPS) depends on whether they are maintained at a thermoneutral temperature (31 °C) or a cooler temperature (26 °C); mice housed at 31 °C show a prolonged fever, whereas the mice housed at 26 °C show a transient fever and prolonged hypothermia (Rudaya et al., 2005). Similarly, in a study of rats maintained at 22 °C or 28 °C, injection of LPS or *Escherichia coli* was associated with a hypothermic response at the cooler temperature and hyperthermia at the higher temperature (Liu et al., 2012). In mice inoculated intranasally with influenza virus, housing at temperatures of 22 °C and 26 °C was associated with both hypothermia and reductions in locomotor activity, whereas in mice housed at 30 °C, these responses were essentially absent (Jhaveri et al., 2007). Furthermore, at 72 h after infection, the inflammatory response, as indicated by concentrations of inflammatory cytokines in lung, was significantly reduced at the higher temperature, despite identical pulmonary viral titers at all three temperatures (Jhaveri et al., 2007).

Table 2
Summary of housing conditions and research outcomes in two studies.

	David et al. (2013)	Kokolus et al. (2013)
Mouse strains tested	Nude, SCID	C57BL/6, BALB/c, nude, SCID
Temperature effect on tumor growth	Yes, nude & SCID	Yes, C57BL/6 & BALB/c; no, nude & SCID
Housing systems	Ventilated (with or without shelter), static	Caging system was not identified in methods section
Housing density	1 per cage	5 per cage
Ambient temperature	21 °C; assessed cold exposure based on brown adipose tissue thermogenesis	22 to 23 °C, 30 to 31 °C
Tumor cell lines	Human epidermoid carcinoma cell line A431	4 syngeneic cell lines, 3-methylcholantrene

Noise

Noise is another environmental variable that can have a significant uncontrolled impact on laboratory animals and potentially reduce replicability both within and among laboratories. Exposure to various environmental sounds can lead to changes in multiple organ systems, making what laboratory animals hear of consequence for researchers beyond those solely interested in hearing (Turner et al., 2005). Strain and species differences in hearing can potentially contribute to non-reproducible findings and erroneous data interpretation (Turner et al., 2005).

Common sources of noise in animal research include infrastructure noises (e.g., room ventilation), technical equipment, activities associated with animal maintenance and use, and activity of the animals themselves (Turner et al., 2005). However, evaluating noise exposure in animals is complicated by differences in hearing among animal species and strains, including humans (Voipio et al., 2006). Common sounds in an animal facility, such as ventilated racks, animal transfer stations, and construction equipment, can produce sound pressure levels that are