Stressed out: providing laboratory animals with behavioral control to reduce the physiological effects of stress

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Laboratory animals experience a large amount of environmental stress. An animal's environment can include both physiological and social stressors that may require an animal to adapt to maintain allostatic balance. For example, thermal stress can lead to changes in behavior, reproduction and immune function, which has been detrimental to cancer modeling in mice. Chronic uncontrollable stress is widely acknowledged for its negative alterations to physiology. However, there is a lack in the understanding of how the laboratory environment affects animal physiology and behavior, particularly as it relates to characteristics of the human disease being modeled. Given the evidence on how stressors affect physiology, it is clear that efforts to model human physiology in animal models must consider animal stress as a confounding factor. We present evidence illustrating that providing captive animals with control or predictability is the best way to reduce the negative physiological effects of these difficult-to-manage stressors.

Hans Selye would have made a horrible laboratory animal technician. This budding young 1930s scientist accidently discovered stress physiology and the negative effect chronic exposure has on the body because of his extremely poor rat handling skills[1]. As a result, we know for a fact that husbandry affects the quality of animal models. Despite this knowledge, most labs continue to ignore stressful experiences in rodent models, often because of time or financial costs. Today's researchers and animal technicians of course receive more training and animal welfare oversight than Dr. Selye, but the slow rate of progress in minimizing general stress in the laboratory is disheartening. In addition to the obvious animal welfare ramifications of this lack of advancement, animal models in general show poor predictive validity in terms of translational outcomes in human clinical trials. Roughly 90% of compounds (for example, drugs or other therapeutics) that are 'successful' in animal trials fail in FDA human trials; the majority of these failures are a result of a lack of efficacy[2]. There are certainly many variables that contribute to this failure to translate, including reliance on measures that lack biological or psychological homology, translation of human clinical measures, or simple environmental effects[3]. In addition, we widely assume that strain differences and genetics are a large, if not the largest, influence on mouse behavior. However, in a retrospective study of influences on thermal nociception, strain only accounted for 27% of data variability[4]. On the other hand, environmental factors alone accounted for as much as 42% of data variability in the same study, and the identity of the experimenter actually had more effect on behavior than the genetics of the animal. Other examples highlight even more marked ratios, with enormous effects on experimental power and false discovery rates if not properly accounted for in data analysis[3,5]. Yet there is a substantial lack of research and funding available to determine how the laboratory environment affects animal physiology and behavior, particularly as it relates to characteristics of the human disease being modeled.

Temperature as an example of environmental stress

Many aspects of the laboratory environment are stressful to rodents and do not accurately reflect human physiology. An animal's environment can include both physiological and social stressors that require an animal to adapt to maintain allostatic balance. Thermal stress, for example, is a known stressor in the laboratory that affects temperature regulation and metabolism and results in poor modeling of human conditions in mouse models. Laboratory mice experience some degree of thermal stress under all recommended housing temperatures[6,7] (typically between 20–26 °C), but are prevented from adequately using many of their behavioral adaptations for thermoregulation and must therefore rely on thermogenic processes. At 23 °C, a mouse's metabolic rate is 60% higher than its metabolic rate at thermoneutrality, but the mouse

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uses its brown adipose tissue to produce enough heat to maintain homeothermy[6]. Housing mice at, or just below, standard housing temperatures has been shown to decrease reproduction[8,9], growth[10,11], organ weight[10], immune function[10] and increase metabolic rate[12–14]. Increasing laboratory ambient temperatures is not a solution because mice prefer different temperatures for different behaviors, times of day and between genders[15–18]. A mouse breeding cage is the best example of the disparity of thermal needs that can occur, even in one mouse cage. Day-old mice will relocate themselves along a thermal gradient by rolling and twisting until they reach their preferred temperature of 36–38 °C[19], a temperature range similar to the body temperature of lactating females[20]. This selected temperature remains relatively constant until 22 d of age, at which point it drops to approximately 34.5 °C[21,22]. Not only do young mice prefer warmer temperatures, but so do sexually mature virgin adults. Adult mice actively prefer 30–32 °C[16,17], especially when inactive and performing maintenance behaviors, such as grooming and eating. Thus, thermal environment appears to be extremely important during a mouse's inactive phase. Based on this and the large amount of historical data that indicates that mice need warmer temperatures, these thermal needs will vary based on age and behavior. Although the solution to this problem appears to be simply turning up the thermostat, warmer ambient temperatures increase aggressive interactions between male mice[23], exacerbating an already troublesome behavior in mouse colonies. Thus, a one-size-fits-all engineering solution is unobtainable.

Thermal stress and its effect on mouse models

The influence of mouse metabolism on experimental results has only recently been questioned[24–29], even though we have known that mice in normal laboratory temperatures must raise their metabolic rate to counter heat loss to their environment. Some scientists, aware of how housing temperature may influence results, approach this problem by housing mice in temperatures near thermoneutrality[30–32]. Others believe that temperatures around 20–22 °C appropriately mimic normal human metabolism[28]. The problem that neither group seems to recognize, as mentioned earlier, is that mouse thermal preferences fluctuate in and between mice, making thermoneutrality an ever moving target.

The elevated metabolic rate of laboratory mice at the high end of *The Guide's* temperature recommendations (26 °C) more accurately models the metabolic rate of a naked human exposed to ≈18 °C[26]. Notably, 15 °C is used to model a mild cold stress in lightly clothed humans. Thus, even with the beneficial insulation from clothing, metabolic increases are still necessary to counter heat loss[33]. This chronic cold stress can be expected to inherently confound the modeling of human physiological processes and potentially provide misleading measures of metabolic rate-dependent processes (for example, pharmacokinetic work). Furthermore, a 30–60% increase in metabolic rate inherently means a 30–60% increase in free radical production and oxidative stress, with the potential to affect everything from cancer to metabolic syndrome to aging to behavior (for example, ref. 34). Beyond the central effect of oxidative stress, the partitioning of resources to thermoregulation potentially affects many other systems and processes. For instance, as metabolic rate increases, limited sulfur amino acid resources must be allocated to glutathione production, rather than to homocysteine and s-adenyl-methionine production, thereby reducing methyl donor availability for gene regulation[35]. These alterations to normal function have the potential to adversely affect outcomes in vast areas of scientific research.

Oncology drug discovery has an abysmal success rate of approximately 5%[2]. Although unlikely to be the only reason for the lack of translation from animal models to humans, temperature has recently been in the spotlight as an environmental factor that is extremely influential on mouse cancer models[36]. Mice housed at thermoneutrality (30 °C) are better able to fight tumor cells with improved adaptive immunity, resulting in reduced tumor growth relative to mice housed in typical temperatures (22 °C)[27,31,37]. Specifically, $CD8^+$ T cell count and activation is improved in warmer temperatures[31]. Improving immune function simply by reducing thermal stress is likely to improve the external validity of mouse models, aiding the drug discovery process. In addition, the increased ability to fight off tumor cells suggests potential ramifications for many other fields of research in which adaptive immune function is central to the disease process (for example, Type I diabetes, vaccine studies or infectious disease studies).

Skeletal biology can also be affected by thermal environment[32]. Humans and mice both experience age-related bone loss, but it's the how and when that differs between these two species. At the point at which mice reach skeletal maturity, occurring around 4–6 months of age when bones stop elongating, they have lost the majority of their cancellous bone[38]. This early bone loss makes it difficult to model human disease associated with postmenopausal osteoporosis or bone disuse, which typically occur a substantial amount of time past skeletal maturity. A recent study in which mice were housed at 32 °C found that cortical bone mass either remained the same or increased up to 4.5 months of age[32], increasing the external validity to humans. Additional reported benefits of this warmer housing temperature were increased bone marrow adiposity, higher rates of bone formation, higher expression of osteogenic genes and decreased bone resorption.

The effect of temperature on food consumption is a well-documented phenomenon and is actually a tactic used by restaurants to influence food consumption volume[39]. Regardless, it has not been considered a confound in mouse models of obesity and other metabolic disorders until recent years[25,40]. In fact, Martin *et al.*[41] believe 'control mice' are an inherent confound, as they are sedentary, obese and glucose intolerant and are therefore likely to affect translation of mouse-model data to human health. Cold stress increases food consumption and metabolism of these control animals, which can influence a variety of diseases through increased oxidative stress. Thus, this may also be adding to the poor drug translation of metabolic disease[2].

Behavioral control over temperature

Stress in life and in the laboratory is inevitable; there is no way that it can be completely eliminated. How then do wild animals appear to cope with these inevitable stressors better than the laboratory animals under our care? Animals adapt to environmental stressors physiologically or behaviorally, thereby returning to an allostatic balance and allowing the organism to survive[42]. Depending on the degree of adaptation or biological cost required, stress may

negatively affect biological functioning and put animals in a metabolically or physiologically vulnerable state. This stress-induced shift makes the animals susceptible to disease, abnormal behavior or suppressed functioning of biological systems that are less essential for survival, such as reproduction[43,44]. The difference between wild and captive animals is that wild animals can choose less stressful habitats, such as finding shelter from the cold, or modify their behavior or environment to exert some control over the stressor. Barnett[45] documented wild house mice living and breeding in food storage freezers, which were kept at −10 °C. However, studies of hypothermia show that mice can no longer right themselves after 30–99-min exposure at a similar temperature[14]. The difference between the two scenarios is that wild mice in the freezer were able to build an insulating nest, which likely reduced heat loss and created a more favorable thermal microclimate[45].

Control, or the perception of control, and predictability in captive animals are the most effective ways of reducing the effect of negative stressors[46]. Jay Weiss in the 1970s cleverly illustrated the importance of psychological factors, such as predictability and outlets for frustration, on the overall effect of a stressor[47]. When negative stimuli are predictable, animals know when to worry and when to relax[48–50]. Control, or the perception of control, over a stressor also has a big part on the physiological effect. Allowing rodents to escape or control when a negative stressor, such as an electric shock, ends increases tumor rejection, reduces tumor growth and increases lymphocyte stimulation[51,52]. Furthermore, even the perception of control is sufficient to elicit the same psychophysiological buffering to a stressor as actual control[53]. Glass and Singer[54] illustrated this by exposing human participants to loud noise. One group was told that a button stopped the noise, but that they shouldn't push it. Although it was never pushed or proven to actually control the noise, the participants believed it would, reducing hypertension.

Providing mice with control over their microclimate may be the best solution to this thermal controversy, especially given that this more closely models human thermal behavior. In addition, it would allow us to maneuver around the problem of identifying each animal's individual thermal needs. Behavioral thermoregulation is the most cost effective, in terms of energy, and most rapid strategy to reduce heat loss[14]. Huddling and nest building are two common behaviors that mice use to control heat loss to the environment. Huddling increases insulation and reduces surface area-to-volume ratios, thereby reducing convective and radiant heat loss relative to internal heat production[55,56]. This reduction in heat loss, and subsequent reduction in metabolism, results in substantial energetic savings. Nest building provides better insulation and reduces heat loss to the environment[11], resulting in improved food conversion[11,57], better reproductive performance[8,9] and increased pup survival[8]. Although we and colleagues have shown that 8 g of nesting material is effective at reducing thermal stress in groups of three mice in static cages with aspen bedding, it is unknown how much material is needed for different group sizes, in ventilated versus static caging, or with different types of bedding material.

Conclusion

Stress is an inevitable part of life for both humans and research animals. However, research animals do not always have the means to control stressors in the ways that humans can. Providing animals with increased control or predictability may help to remove the burden from our shoulders of finding specific solutions to some of these stressors. In the case of mice, the most obvious stressor in terms of welfare, physiological, scientific and translational effects is temperature. A substantial body of literature now shows that providing control via nesting material reduces the effect of this stressor and has wide ranging effects. Thus, nesting material is a wonderful example of how good welfare is good science.

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