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The Impact of Stress on Laboratory Animals and Variability in Research Outcome

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Abstract:
The laboratory environment offers an enormous amount of chronic and/or acute stress, which can be both social and physiological and require the animal to adapt to allostatic balance. Several aspects of the laboratory environment, such as confinement, cause significant and recurrent stress in laboratory animals, which is inescapable. Several factors such as transportation, handling, noise, restrictions, experimental procedures, and may cause stress which is difficult to manage. It can be even more challenging in the absence of adequate habituation/ desensitization. These may result in several physiological as well as psychological challenges, triggered by the activation of several neuroendocrine pathways, with a variety of complications such as physiological and/or psychological damage. This type of damage may result in stereotypic behaviours like pacing and circling, self-harm, and physiological consequences such as inflammatory reactions, immune dysfunction, susceptibility to diseases, and metabolic disorders. Moreover, some of the stress-mediated outcomes are epigenetic which makes the consequences transgenerational, that is the biology of animals whose immediate generations have been captured in the wild and/or have endured stress in laboratories could be epigenetically transformed compared to their wild counterparts. It is thought that lab animals have different physiological, epigenetic, and psychological differences that make it hard to extrapolate findings from animal studies to humans. These stress factors and their consequences need to be recognized sufficiently by scientists while using animal models for experiments. We have described the physiological, behavioural, and epigenetic consequences of laboratory-induced stress among animals in this review.

INTRODUCTION

While discussing stress and distress among laboratory animals, various definitions, terminologies, and points of view are utilized. The scientific community defines stress as an actual or perceived disturbance of an organism's physiological homeostasis and/or psychological well-being. To respond to such perturbations in homeostasis, the body uses several physiological and behavioural strategies to offset the disruption (Pekow, 2005). Events that lead to *stress* (called *stressors*) can stimulate numerous adaptive changes and coping mechanisms comprising behavioural reactions, stimulation of the sympathetic nervous system and adrenal medulla, and secretion of stress hormones (e.g., glucocorticoids and prolactin), and deployment of the immune system. While the physiological reactions to stress are well known, the scientific, regulatory, and animal welfare segments dispute what constitutes distress. Although most definitions of distress describe it as a negative and aversive state in which an organism's coping strategy and adaptative response to stress fail to restore psychological and/or physiological homeostasis (National Research Council, 2008). However, philosophical differences focus on whether feelings and emotions are affected by this state of being. A state of distress usually develops over an extensive period, although short, strong stressors can also affect animal well-being and cause severe distress (National Research Council, 2008). As a result, an animal may be distressed even if, it seems to recover quickly once the stressor is removed or the experimental procedure is completed. In addition, the stress response may result in inadequate or incorrect alterations in the physiological and behavioural regulatory systems or induce insufficient or unwanted responses as their output signals. Chronic social subordination, for instance, has been demonstrated to cause behavioural withdrawal symptoms, long-term changes in the hypothalamic-pituitary-adrenal (HPA) axis response, and eventual immunosuppression, all of which make it difficult to cope and adapt (Kyrou and Tsigos, 2009; Gaskill and Garner, 2017). Research has revealed that the glucocorticoid feedback mechanisms fail in persistent distress states like depression. As a result, if stress coping strategies used by laboratory animals fail to adequately cope or induce fruitful adaptation, this may not just be unproductive but also harmful. In such conditions, corticosteroid responses are necessary for adaptation, but the excessive or sustained release can result in significant metabolic and immunological dysfunction. Most of the time, perturbation and associated stress response are usually transient, and homeostasis is quickly restored. In other cases, the stressor is chronic, and homeostasis is not reinstated, resulting in dysregulation of physiological functions in response to stress responses. A sustained challenge to homeostasis, on the other hand, may eventually lead to allostasis, which involves physiological readjustments where the homeostatic baseline is shifted to accommodate for altering conditions (e.g., elevated basal cortisol level in the absence of stress) (Logan and Barksdale, 2008). However, allostasis may come at a cost to the body in terms of higher energy needs. Here allostatic load refers to this augmented cost or demand on the body (often referred to as "wear and tear") (McEwen, 1998). Thus, stress and distress can have a negative influence on the quality of scientific data, resulting in compromised output, which demands the use of additional animals for research. The present study reports the physiological, behavioural, and epigenetic consequences of stress among laboratory animals. We have tried to elucidate the possible relationships between stress and well-being of laboratory animals as it impacts the animal welfare as well as lead to the variability in the outcome of research; as a result, extrapolation of such animal model studies on human become difficult.

Laboratory conditions

It has been found that, the life of laboratory animals is intrinsically and excessively stressful compared to their natural habitat, from which the laboratory environment differs substantially, even after the efforts of enrichment and reducing the stressors. Natural behaviours of laboratory animals are prohibited or limited in laboratory settings, housing is often considerably smaller than the animals' natural habitat, and the animals are exposed to recurrent handling and manipulations, as well as other unfamiliar influences that they attempt to escape (Balcombe *et al.,* 2004; Balcombe, 2006). Stressful events and environmental aspects in the laboratory are abundant and varied. These involve but are not restricted to procedures such weighing, general handling, saline injections restraint, anaesthesia, gavage, food restrictions, blood sampling; artificial environment and allied factors, such as temperature variation, noise, and light; solation or crowding, cage size, transportation, experimental procedures, the slaughter of fellow animals, and even environmental enrichment itself (Bailey, 2018).It has been proposed that captive-bred animals are probably unaware that laboratory stresses are not the same as those encountered in the wild. Though it can be sensed that they are not comparable to 'natural' stressors such as poor shelter, scarcity of food, and predation. However, the fact is that they are distinct, but they are also common, consistent, and unavoidable. Stress/Distress is recognized as a source of "unexplained variation within and between animal studies," as it affects "both the physiology and behaviour of animals (Bailey, 2018).

Physiological implications of stress in laboratory animals

Though different animal species have distinct stressors, variable stress ranges for adaption, varying tolerance levels, and different symptoms and consequences of excessive stress, they all have the same molecular pathways and processes that are impacted by stress. Stressors activate the HPA axis as well as the sympathetic adrenal medullary axis which are major pathways stimulated in response to stress (Boere, 2001; Kyrou, and Tsigos, 2009). Furthermore, sympathetic and parasympathetic nerves are activated when animals are stressed, and these nerves directly affect secondary lymphoid organs (Glaser and Kiecolt-Glaser, 2005; Sternberg, 2006; Tracey, 2009). Because of the activation of these pathways, the stress hormone cortisol and corticosterone rise in the body. Increased glucocorticoid (GC) levels, coupled with considerable increases in blood pressure, heart rate, and another hormone, are established indicators of anxiety, stress, and distress, and are employed in laboratories as stress biomarkers for invertebrate species (Altholtz *et al.,* 2006; Balcombe, 2006; Meijer *et al.,* 2007). These neuroendocrine alterations have direct negative impacts on innate and adaptive immunity, central nervous system, and reproductive and cardiovascular function (Obernier and Baldwin, 2006; Gurfein *et al.,* 2012), resulting in a wide range of adverse health outcomes. Psychological trauma because of a stressful environment may lead to changes in health and abnormal behaviour patterns (Tatemoto *et al.,* 2019). It is widely recognized that a variety of animals experience anxiety, pain, discontent, and depression in a laboratory environment. Studies on fish reported aggressive behaviour, increased anxiety, and reduced weight gain are related to alterations in dopamine levels due to stress (Rambo *et al.,* 2017).

Behavioural implications of stress in laboratory animals

Stereotypies are unusual, repetitive behaviour patterns found in laboratory animals that reflect poor wellbeing (Lutz, 2014; Sevillano and Fiske, 2019; Tatemoto *et al.,* 2019). Animals kept in artificial situations with little stimulation, fear, and/or frustration, physical restraint(s) are more likely to exhibit such behaviour (Tatemoto *et al.,* 2019). Little examples of stereotypic behaviour among laboratory animals include sham-chewing in sows, back flipping, circuit running, wire gnawing, and leaping in mice (Philbin, 1998; Gross *et al.,* 2011). Behavioural abnormalities in laboratory animals come in a variety of forms. Deviant behaviours, such as excessive aggression or inefficient/ inappropriate maternal behaviour, are not stereotypic since they are not recurrent, unchanging, or ritualized. Stereotypic behaviour is assessed in terms of rate of occurrence and duration, that is how frequently does the animal involve in such behavioural bouts and how extensive are these bouts? It is consequently insufficient to merely state that an animal exhibits stereotypic behaviour; it is important to measure the degrees of behaviour displayed. This is often accomplished by looking at the animal's time spent in such behaviour. It is less alarming for an animal to spend 3% to 5% of its time in stereotypic behaviour compared to the one that devotes 75% of its time to stereotypic behaviour. However, any level of stereotyping might be construed as a reason for concern and an indicator of poor well-being.

The stereotypes of laboratory captive animals may be classified into three groups: cage stereotypes, deprivation stereotypes, and miscellaneous stereotypes (Table1).

Table 1. Categories of stereotype behaviour.

Cage Stereotype	Deprivation Stereotypes	Miscellaneous Stereotypes
Pacing, Quadrepedal Bipedal, Bouncing, Somersaulting, Twirling, Spinning, Dancing	Self-orality, Rocking, Self-Clasp,huddle, Crouch, Self-abuse, Head banging, Eye press	Overgrooming, Head weaving, Picking at nothing

Cage/Locomotors stereotype

Animals in captivity during adulthood response to low stimulus develop various locomotor stereotypes. Such stereotypes may offer some advantages and work as a sort of "do-it-yourself enrichment," thus serving to enhance the well-being in an underprivileged cage setting for animals suffering from an inadequate environment lacking the stimulus (Draper and Bernstein, 1963; Lutz, 2014). For instance, six chimps housed in a small restricted cage displayed higher levels of repetitive stereotypic behaviours (e.g., swaying, rocking, or circling) than when kept in an outdoor environment (Lutz, 2014). leaping in place, pacing, and somersaulting are some examples of tedious locomotor stereotypes. These locomotors' stereotypes are believed to be the result of an animal's existing artificial habitat, as the name denotes. Locomotor or cage stereotypes are dynamic depict active whole-body motions. Dancing is a back-and-forth quadrupedal motion that is not confused with spinning or pacing. These labels, while helpful, do not capture all types of stereotypic behaviour, nor do they eliminate ambiguity about the many forms of such behaviour. In a caged environment, self-abuse is thought to be the sole way for these animals to express their frustration. It has been found that approximately 5-10% of captive primates can develop extremely aberrant behaviour for example, self-injurious behaviour (SIB), which is a substantial problem. One of the factors of the prevalence of this stereotypic and significantly aberrant behaviour is chronic stress, and sleep deprivation caused by the captive environment (Davenport *et al.,* 2008). SIB was reported to be correlated with deregulation of the stress response system, sleep disruption, intensified aggressiveness, immune dysfunction, and degeneration of glial cells (Egaña-Huguet *et al.,* 2021).

Deprivation stereotypes

Deprivation stereotypes are also known as self-directed stereotypes as, the behaviours done on the animals' bodies. Self-orality is the futile sucking of an animal on one or more bodily parts (e.g., tail, fingers, or genitalia), is an example of maternal deprivation behaviour (Latham and Mason, 2008). Other behaviours such as abnormal postures that include rocking, huddling, and crouching, and self-abuse behaviour such as biting, head pounding, or scratching are examples of deprivation behaviour. Another unusual position is the salutation, which involves the animal putting a hand in front of its face, palm out, with one or more fingers placed against the eyeball. Deprivation stereotypes are generally observed in animals that are alienated from their mothers at the time of birth or neonatal part of life and reared with peers or in total social isolation (Lutz, 2014). These stereotypic behaviours are believed to be equivalent to the customary behaviours seen in juvenile primates. However, in captive primates, such behaviours are self-directed in the absenteeism of con-specifics (Tarou *et al.,* 2005). For example, self-orality in segregated juvenile primates, precisely self-sucking of tailor digits is believed to be equivalent to nipple sucking in new-borns raised by mothers, whereas self-clasp is linked to the mother clasping or clinging. Monkeys raised in part or total social isolation after being taken from their mothers at birth or within the first year of life exhibit deprivation stereotypes (Lutz, 2014). These bizarre behaviours are supposed to be like those observed in baby and adolescent monkeys. However, in the absence of a con-specific, they become self-directed. Self-orality in isolates, particularly self-sucking of the fingers or tail, is thought to be like breast sucking in new-borns reared with their mothers. Mother clinging or clasping is connected to self-clasping. Studies show that early weaning in laboratory animals escalates the risk of anxiety, aggression, and stereotypic behaviour (Ahola *et al.,* 2017).

Miscellaneous stereotypes

Some behaviours do not fall under any of the above categories. Stereotypes like this are classified as miscellaneous groups. For instance, head weaving is a head toss that is not associated with a pacing stereotypic behaviour. This category also includes over-grooming, which occurs when an animal eliminates all the hair from one portion of its body, generally a tiny patch on the arm or shoulder. Picking at nothing is also included in this stereotype category.

Possible environmental causes of stereotypic behaviour

Stereotypic behaviours have been confirmed to arise in poor environmental conditions (Mason, 2006; Cooper and McGreevy, 2007). Laboratory primates' captive environments have been extensively studied to find out the cause of such behaviours in them. It has been found that cage size, housing type (individual vs. pairings or groups), stress, and an absence of environmental complexity are only a few aspects that correlate with stereotypies. Past belief to essentially sterilize laboratory caging led to the custom of using tiny cages that could be washed in standard cage washing machines. Stereotypic behaviour has long been linked to the utilization of such tiny cages. Wild animals kept in such tiny cages are said to acquire abnormal stereotyped behaviour (Draper and Bernstein, 1963). The major goal of defining minimal cage sizes per animal's body weight was to encourage more species-specific behaviour (such as the vertical-flight reaction) while reducing abnormal behaviour. In laboratory primates, the type of housing is an environmental factor that has been connected to the development of stereotypic behaviour (Von Borell and Hurnik, 1991; Rushen and Passillé, 1992; Mason *et al.,* 2007). Previously, laboratory macaques were kept separately to prevent the possibility of wounds from fighting and infection transmission. In recent years, social housing patterns that are in pairs or groups have been preferred over solitary individual housing whenever possible. Separate housing, even with olfactory, auditory, and visual interactions with conspecifics, has been taken by some to be similar to private confinement (Mason *et al.,* 2007). Therefore, separate housing has been believed to add to the development of stereotypes. Stress is an additional factor of concern. It has been advocated that the stress of laboratory life might add to the development of stereotypic behaviours. Laboratory animals face an excess of stressors, including blood draws, injections, usual cage changes, physical and chemical restrictions, and involvement in scientific experiments. Individual housing used for laboratory animals has been compared to solitary confinement by some despite having olfactory, auditory, and visual interaction with con-specifics. As a result, it has been suggested that individual housing contributes to the formation of stereotyped behaviours. It's been proposed that the laboratory procedures leading to stress/distress contribute to the development of stereotypic behaviours (Mason, 2006). Several laboratory procedures such as injections, gavage, blood drawing, physical restrictions, cage changes, and participation in experimentations are all possible stressors inherent in the laboratory environment. The animal's inability to flee from such situations may lead to the development of stereotyped behaviours as a way of adaptive measures to cope with an adverse environment. Moreover, boredom in the captive life may also lead to the development of stereotypic behaviour. Primates are active, intelligent, and inquisitive creatures who require stimulating, complex, settings to thrive. Laboratory primates are thought to be especially prone to boredom. This idea is reflected in the extensive study into the improvement of enrichment devices for primates in laboratories, and the impact of using such interventions on stereotypic behaviour (Mason *et al.,* 2007). It has been found that stereotypes in many cases are an indicator of existing frustration and/or chronic stress/distress, the result of brain damage, a pleasurable method to execute a natural behaviour in an artificial setting, or an effective approach to manage with laboratory stress (Poirier and Bateson, 2017). Moreover, genetic element and personality predisposition seems to influence stereotypic behaviour (Tatemoto *et al.,* 2019).

Epigenetic consequences of Stress in laboratory animals

Research shows that the maternal environment has a significant influence on epigenetic determinants of behaviour and physiology; therefore, that it should be regarded as an important variable in stress and distress assessments in laboratory environments. Offspring are usually raised with their mothers, but they can also be raised in larger social groups with other offspring and adult males and females. The type of rearing and the maternal care is an important factor in the development of stress response in captive animals. There is ample scientific evidence that individuals who have been exposed to prenatal and/or early-life stress in previous generations may be destined to face the repercussions in maturity. In the light of the information on how stressors impact physiology, it's evident that efforts to simulate human physiology in animal models must account for animal stress. Stress throughout adulthood is found to affect the methylation of numerous genes in the brain of rats. Unpredictable chronic stressors such as confinement, cage tilt; reversed light periods, cause hypermethylation of the glial cell-related neurotrophic factor (Gdnf) gene in the nucleus accumbency, resulting in reduced expression (Uchida *et al.,* 2020). Stress-related events can also change DNA methylation in the brain, as shown in animal models of post-traumatic stress disorder (PTSD). In one such study rats were put on filthy cat litter for 10 minutes every day for 7days, to simulate trauma (Chertkow-Deutsher *et al.,* 2010). Stressed rats showed hypomethylation and increased gene expression of the hippocampal (Disks-Large Associated Protein (DIgap2) gene which is a postsynaptic density protein after 7 days and is associated with the severity of the shock following behavioural testing (Chertkow-Deutsher *et al.,* 2010). In another study, rats have given a dual shock by exposing them to a cat along with social insecurity (i.e., unpredictable cage mates every day). Rats exposed to this trauma exhibited emotional and cognitive deficits like those observed in human PTSD patients, which may be mediated by long-lasting epigenetic alterations (Zoladz *et al.,* 2008). These rats after receiving psychosocial stress showed a changed methylation profile of the brain-derived neurotrophic factor (bdnf) gene in distinct sub-regions of the hippocampus along with altered bdnf gene expression (Roth *et al.,* 2011). It has been found that stressors provided to the mother around the gestation period (21–23 days in rats), such as continual light, damp bedding, noise, repeated cage changes, and restrictions, have been shown to cause long-term alterations in her offspring's brain (Bourke *et al.,* 2013a; Bourke *et al.,* 2013b; Weinstock, 2017). Research shows that stress leads to epigenetic changes in genes crucial for stress regulation, such as hypermethylation of the hippocampal GR promoter and hypo- methylation of the Corticotropin-releasing factor (CRF) promoter of the amygdala (Mueller and Bale, 2008). HPA axis plays a role through the glucocorticoid receptor (GR)and CRF in response to stress (Mueller and Bale, 2008). Hyper methylation of 11-hydroxysteroid- dehydrogenase type- 2 which typically shields the foetus from maternal glucocorticoids by converting them to inactive metabolites, is another way through which prenatal stress may have detrimental consequences on offspring who experienced prenatal stress (Jensen *et al.,* 2012). Previously, it was considered that DNA methylation occurred exclusively during cell development and differentiation (i.e., prenatal), however, new research contradicts this notion and shows epigenetic effects of stress outside of embryonic development (Weaver *et al.,* 2004; Champagne *et al.,* 2006; Wu *et al.,* 2014). Researchers have found that rats exposed to low levels of maternal care (i.e., low licking and grooming) during the first week of life, experience hypermethylation of GR in the hippocampus, decreased GR expression, and increased corticosterone levels in response to a stressor throughout life (Weaver *et al.,* 2004). Furthermore, postnatal patterns of maternal care can be handed over to progeny, resulting in high Licking and Grooming offspring becoming high Licking and grooming dams, through epigenetic programming of the estrogenic receptor in females (Champagne *et al.,* 2006). Additional early-life stress model comprises a period when maternal care was completely absent. Offspring exposed to maternal separation (3 h/day for initial 10 postnatal days) showed a long-term reduction in methylation of pituitary proopiomelanocortin and hypothalamic arginine vasopressin two genes involved in the HPA axis pathway to stress (Murgatroyd *et al.,* 2009; Wu *et al.,* 2014). These offspring later on show increased pituitary proopiomelanocortin and hypothalamic arginine vasopressin gene expression and hypersecretion of corticosterone, which is associated with behavioural deficits in stress coping and memory (Murgatroyd *et al.,* 2009; Wu *et al.,* 2014). The CRF gene, which is associated with stress response, is also affected by maternal separation. Studies suggest that maternal separation causes hypomethylation of the paraventricular nucleus (PVN), and CRF in the hippocampus (Wang et al., 2014). These methylation patterns are associated with enhanced gene expression leading to memory impairments (Wang *et al.,* 2014), synaptic dysfunction (Wang *et al.,* 2014), depression (Franklin *et al.,* 2010), and HPA-axis hypersensitivity (Chen *et al.,* 2012), and behavioural abnormalities (Yu *et al.,* 2011). These methylation-associated behavioural deficits have been reported to carry over to subsequent generations (Boku *et al.,* 2015). Moreover, impacts of maternal separation seem to include methylation of the hippocampus retinoic acid receptor promoter, involved in neural progenitor cell differentiation (Boku *et al.,* 2015), demonstrating that early-postnatal stress-induced methylation plays a role in neurogenesis.

DISCUSSION

Laboratory Animals are complex creatures that respond to several environmental factors. While living in a tightly controlled laboratory environment animals are subjected to several changes which lead to stress/distress, thus altering their physiological as well as behavioural processes, as, they respond to stressors in their premises (National Research Council, 2008). Confounding and diverse study findings might result from even minor changes in the living and experimental setting. The impact of such environmental variations on animals may not be easily apparent to the investigator, leading to study variability and potentially incorrect conclusions. Many researchers do not bother about the extent to which environmental factors impact study animals' findings when their emphasis is on regulating direct experimental variables characteristic of their research. However, there has recently been a revived focus on reproducibility in animal research findings, whether performed between various research institutions or inside the same facility, as well as an increasing weightage on reporting of circumstances that might create variability when animals are employed in the study (Bailey, 2018). Generally, low psychological and physiological well-being among laboratory animals reflects the inherent, multi-faceted stress of laboratory life, which is sometimes overpowering as compared to the more transitory, acute, and 'natural' stressors faced in the wild. As this damage is mediated by well-known trans-species biological mechanisms such as the HPA axis, sympathetic nervous system, and is influenced by oxidative stress and epigenetic modifications that impact biological system modulating numerous pathways, experimental results may be skewed if such animals are used in the experiment (Boere, 2001; Bailey, 2018). This manipulation of multiple biochemical pathways and gene expression can result in organ damage, modulation of immunological function, cardiovascular illness, autoimmune disorders, accelerated ageing and mortality, increased tumour growth, and progression of musculoskeletal atrophy. The extent to which animal studies can be applied to people is debatable, but there's little doubt that "research using animal models is more translatable to human disease when the animals' welfare is maximized" (Bailey, 2018). However, the inherent, inescapable, significant, and generally intractable nature of these stressors present in a laboratory environment makes it hard to "maximize" wellbeing substantially. Laboratory premises comprise both the micro-environment and the microenvironment. In this context, the microenvironment refers to the immediate area around an animal, such as an aquarium, pen, cage, or stall, and is described as the environment in immediate contact with the animal. Temperature, vibrations, noise, humidity, and air are among the factors that laboratory animals are exposed to in their microenvironment. The macroenvironment refers to the physical conditions that surround the microenvironment, which includes the room, pasture, or barn. Because of the open caging system or natural housing conditions, the microenvironment is often like the macroenvironment. The microclimate, on the other hand, might be very different from the macroenvironment due to the primary enclosure's design. Thanks to the ventilated cage system, different microenvironments can coexist in the same macroenvironment. The micro-and macro environments should be appropriate for the animals' genetic composition, age, and the purpose for which they are being employed. Housing settings should allow an animal to be physiologically healthy (i.e., not impact biological functioning), live a normal life, behave properly, and be free of suffering, pain, and other negative situations. Studies show that artificial conditions that do not let animals meet their behavioural needs are seen to be the root cause of stereotypical behaviour. It has been recommended that instead of calling the behaviour as abnormal behaviour, it is to be referred to as the "behaviour suggestive of an abnormal environment. Stereotypic behaviour is uncommon in wild animals, and is it thought to be unnatural and a sign of ill health. As a result, changing the cage microenvironment, cage location, and cage size can all affect stereotyped behaviour (Lutz, 2014). Stereotypic behaviour is thought to be a sign of low psychological health and general well-being. There are several regulations, policies, and guidelines that control the use of animals in research, education, and testing. These documents often include the expectations regarding care and usage of diverse species in research and describe common environmental conditions, including sanitation, housing requirement, feed, lighting, water, and temperature. The suitability of various environmental elements, whether physical, nutritional, or social enrichment, is closely related to animal well-being. Stress, death, disease, injury, and behavioural issues should all be minimized by environmental factors. Monitoring environmental factors as well as providing suitable husbandry is critical for the proper usage and care of animals.

CONCLUSION

Laboratory animals face various stressors in the laboratory environment that impact their physiology, behaviour, and genetic makeup. Monitoring and evaluating environmental factors and adopting strategies to reduce stress are critical to reducing these changes for animal welfare reasons and reducing variability in experimental results.

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CONFLICT OF INTEREST

None declared.

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