



Laboratory animal welfare: environmental enrichment shows positive effect on animal testing

Bem-estar animal em laboratório: enriquecimento ambiental apresenta efeito positivo na experimentação animal

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ABSTRACT

Reproductive and developmental toxicology has focused on the need to approach the effects of organism exposure to various drugs during pregnancy after the mid-50's, when the thalidomide tragedy stroke humanity. In recent decades, this area of study has developed a lot due to animal testing, raising awareness on the need to improve the quality of life of such animals. Therefore, this paper aims to investigate how the science of animal welfare can improve scientific research as a whole, including the reproductive and developmental toxicology fields, by emphasizing environmental enrichment in animal facilities. To do so, we conducted an integrative literature review on several quantitative and qualitative methodological approaches that are applicable to toxicology studies. Here, we present evidence that environmental enrichment improves animal welfare and prevents or reduces the negative effects of captive housing, which must be a principle of toxicological research for ethical, legal and scientific reasons.

Keywords: Animal welfare. Animal testing. Laboratory animal. Reproductive toxicology. Environmental enrichment.

RESUMO

Na toxicologia da reprodução e do desenvolvimento, a atenção necessária foi dada aos efeitos da exposição do organismo às inúmeras drogas durante o período gestacional somente após a metade dos anos 50, quando a tragédia da talidomida atingiu a humanidade. Assim, esta área alcançou desenvolvimento científico com a contribuição da experimentação animal nas últimas décadas. O uso de animais de laboratórios para a pesquisa científica expôs a necessidade de melhorar a qualidade de vida destas espécies. Portanto, este trabalho tem como objetivo investigar como a ciência do bem-estar animal pode melhorar a pesquisa científica como um todo, incluindo na área de toxicologia da reprodução e de desenvolvimento, enfatizando o enriquecimento ambiental em biotérios. Uma revisão integrativa de literatura foi realizada, incluindo abordagens quantitativas e qualitativas, quais podem ser aplicadas para estudos de toxicologia. Aqui, são mostradas evidências de que o enriquecimento ambiental melhora o bem-estar animal e previne ou reduz os efeitos negativos do cativeiro, qual deve um princípio da pesquisa toxicológica por razões éticas, argumentos legais e garantias científicas.

Palavras-chave: Bem-estar animal. Experimentação animal. Animal de laboratório. Toxicologia da reprodução. Enriquecimento ambiental.

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Introduction

Using animals in research is important to develop science and technology in different scientific fields. Studies involving laboratory animals have come up with preventive measures and treatments for diseases that affect both humans and animals (AKHTAR, 2015). Additionally, pharmacology and toxicology advances are remarkable and

have been achieved through modern technology research methods, including *in silico* screenings through computer simulation, *in vitro* studies using different cell lines, and *in vivo* animal models (PARASURAMAN, 2011).

Reproductive and developmental toxicology research has given especial attention to the effects of drugs during pregnancy after 1956, when thalidomide stroke humanity. Up until this moment, toxicity testing, required for drug release, only comprised studies on a single generation and on only one species (usually, a rodent). Despite perinatal toxicity tests being required for drug registration, most studies only tested the effect of drugs on rats or mice, thus contributing to the thalidomide tragedy, considering researchers have ran them in rats, which, unlike mice, are not sensitive to the teratogenic effects of thalidomide (SILVA et al., 2009). After this unfortunate event, medicine regulatory authorities have recommended far more sophisticated perinatal tests, including multigenerational studies and the use of different types of animals, with at least one non-rodent species (JOAQUIM et al., 2016).

In recent decades, reproductive and developmental toxicology fields have achieved scientific breakthroughs due to animal testing. Studies on the effects of toxic agents on different stages of the reproductive and developmental process fundamentally seek to assess their impact on fertility, transport and egg implantation, embryogenesis and fetal stage, birth, newborns, lactation, weaning and maternal care, postnatal abnormalities and behavior. Reproductive toxicity evaluation requires specific types of *in vivo* toxicology tests, including developmental toxicity screening, developmental toxicity test and a two-generation reproductive toxicity study. The studies from this field are the most demanding, considering the significant number of animals required for testing. According to Rovida and Hartung (2009), reproductive toxicity is one of the areas with the highest contribution to animal use and cost, with about 90% of all animals used in research and 70% of the required costs for registration. To reduce animal use, it is essential to implement the “3Rs” principle in toxicological studies, which implies replacing the use of sentient animals in alternative methods, reducing the number of used animals and refining conditions to minimize the level of animal discomfort or suffering (RUSSELL; BURCH, 1992).

Not only do toxicology studies depend on the use of animals, but also on the environments and infrastructures that surround them (RAMSDEN, 2011). Animal welfare is closely associated with the environment based on

the concept of Five Freedoms: I) Freedom from thirst, hunger, and malnutrition; II) Freedom from discomfort; III) Freedom from pain, injury and disease; IV) Freedom to express normal behavior; and V) Freedom from fear and distress (ROLLIN, 2009). However, in rodent testing, applying the concept of five freedoms has proven to be tough as procedures can cause pain and injuries, besides also socially depriving animals after submitting them to clinical or surgical procedures.

Nonetheless, it is possible to easily meet the needs addressed by the second freedom by providing a suitable environment that includes shelter and a comfortable resting area; it is possible to meet the fourth freedom by giving animals enough space, proper facilities, and company of their own kind (when there are no veterinarians or scientific reasons for single housing) (HAU; VAN HOOSIER, 2002).

Considering the need for animal testing in science, the analysis of rodents housing conditions is crucial, as it can affect them physiologically and psychologically. Research use rats and mice as measuring tools by exposing their biological systems to internal and and external factors. Environmental conditions affect laboratory animals and influence experiment outcomes (BAUMANS, 2005).

Despite many new methods complementing animal testing currently, such as *in vitro* and *in silico* techniques, animal testing is still required in many areas of the medical sciences to develop drugs destined to the human or veterinary pharmaceutical industry. We aim at investigating how the science of animal welfare can improve scientific research as a whole, including the reproductive and developmental toxicology field by emphasizing environmental enrichment in animal facilities and laboratories.

Review method

This study is an integrative literature review that includes studies of different methodological approaches (quantitative and qualitative), by following meticulous pedagogical standards. This literature survey was carried out based on a descriptive and retrospective theoretical method, only comprising studies on rodents commonly used in laboratory, including rats and mice.

We defined the following inclusion criteria: fully and freely available articles that corroborated with the objectives of this research, regardless of their language or date of publication. As exclusion criteria, we defined: duplicates, studies whose protocols were only approved by

institutional animal care and use committees (IACUCs) (without furthering the theme), and articles not fully and freely available (with only accessible abstract). Original articles resulting from online searches were organized in a table containing the following information: animals, objective, intervention, methodology, results and reference.

Results and discussion

This study discusses original articles that approach *in vivo* tests on environment enrichment. Within this context, many authors have shown that an enriched environment leads to positive results in various aspects of animal welfare. In other words, housing with appropriate stimuli to rodents allows animals to satisfy their basic behavioral and physiological needs.

An enriched environment has been more and more recognized as a way to improve the welfare of animals, by offering them conditions to follow their natural behaviors and improving their biological functions. Summarily, enrichment is a change in the captive environment (cages, in the case of laboratory rats) to enhance the physical and psychological well-being of rodents by providing stimuli for animals to satisfy their species-specific needs (DAMY et al., 2010). Environmental enrichment ideally designates changes in the hosting environment that significantly improve animal welfare, also consequently comprising social and physical settings.

Studies on environmental improvement from the reproductive and developmental toxicology fields mainly focus on the effects among females. However, we also included some studies with males to have a wider understanding on the environmental enrichment effects within this field. Although some studies suggested a positive effect on emotional and cognitive maternal aspects, there were also effects on physiology, body weight, reproduction, hormone secretion and offspring development. Despite the many positive results regarding enrichment environment described in the literature, it is worth mentioning that we also analyzed several articles with neutral and negative results, as they deserve careful attention when it comes to intervention implementation and study furthering. The increase in standard laboratory cages complexity can stimulate positive rodent behavior. Conventional laboratory cages have been improved with objects that stimulate general activity, such as wood blocks and balls, promote behaviors that reflect good welfare, such as longer bouts of sleep, while reducing behaviors that lead

to poor welfare, such as aggression. The use of inanimate objects inside cages can also optimize mating and improve reproduction (CHISARI et al., 1995; FRANCIS; MEANEY, 1999; STARK, 2001; CHAMPAGNE et al., 2003; WELBERG et al., 2006; SALE et al., 2009; HARATI et al., 2013).

Patterson-Kane et al. (1999) showed that the use of enrichment can reverse or mitigate the adverse effects associated with single-housing conditions, such as behavioral deficits and stress. In this study, we measured the behavior of female rats kept under standard, enriched, semi-enriched or single-housed conditions by using an emergency box, and employing the open field test and Hebb-Williams maze task. Female rats living in semi-enriched and enriched cages were more active and adaptable, showing the importance of physical enrichment to improve welfare. Similarly, the addition of various physical structures such as red-tinted guinea pig huts, nylon, crawl ball, ladders and nestled stimulated rodent-specific behavior and increased the ability of rats to control the environment.

Reproductive performance

Researchers found that the use of cardboard tubes to enrich the environment may increase reproduction by reducing pre-weaning mortality, and enhancing animal welfare as it allows the manifestation of species-specific behaviors, which minimizes the stress caused by confinement (MOREIRA et al., 2015). Additionally, the enrichment provided by several inanimate objects contribute to reproductive performance of mice. According to Whitaker et al. (2009), we can associate enriched cages with higher offspring weights, higher newborn surviving rates and a higher percentages of weaning pups when compared to non-enriched cages.

The use of individually ventilated cages and forced-air-ventilated systems has been growing in research, especially to reduce cross contamination between cages. Tsai et al. (2003) investigated the effects of different rack systems coupled with environmental enrichment on the breeding performance of animals. Micro-environmental systems can improve the health of animals considering they control favorably some conditions such as light intensity, the relative humidity and temperature of cages, the concentration of ammonia and CO₂, among other factors. There was a decrease in the total number of litters per dam in enriched groups, particularly in individually ventilated cages, of high abortion rate, despite enriched

groups showing a more weaned offspring. The enrichment seems to have had a positive effect on raising pups in the first breeding phase, but authors concluded that there was no significant difference in breeding indices (pups/female/week) of enriched and standard groups.

Adding objects to cages influence the development, cognition, reproductive and the performance of maternal rats. However, the effects of increased spatial complexity do not affect behavior and development as much as traditional enrichment (rats housed in traditional (single-shelf) cages), as there were minor differences in maternal behavior, such as nursing and offspring development (LYST et al., 2012). Nevertheless, females housed in larger cages showed better performance in a hippocampus-independent task (Novel Object Recognition, NOR) when compared to those housed in basic cages, which suggests that spatial enhancement can contribute to females' learning of a non-spatial task despite not affecting the development of most behaviors in experimental animals.

Additionally, when assessing the effects of cage size and enrichment in mice breeding performance and behavior, the authors found that mice raised in significantly enriched cages weighed less and had a higher survival rate when compared to those from standard cages, suggesting better reproductive performance, despite enrichment not being associated with the improvement of a pup's performance in behavioral tests (WHITAKER et al., 2009). However, another research showed that cage space did not change standardized reproductive measures, even though it can affect behavior. In this study, it was observed that pups living in larger cages (432 cm² and 800 cm² cages) were more active and were able to build more thermally active nests when compared to others housed in smaller cages (226 cm² and 305 cm² cages) (GASKILL; PRITCHETT-CORNING, 2015a). In a related study, authors have concluded that it is better to provide more biologically relevant cage complexity than to increase floor space when it comes to animal welfare (GASKILL; PRITCHETT-CORNING, 2015b).

Early life experiences can affect development and emotional behavior of all animal species in a gender-dependent design. For instance, D'Andrea et al. (2010) found that female mice raised in a socially enriched environment had a reduced depression-like response, while male mice had an increased anxiety-like behavior. This study reared mice in a communal nest, where different mothers were kept with their pups inside the same cage, increasing interaction

between mother and offspring. Consequently, authors have concluded that adult behavior is modified by gender and early life.

Additionally, enriching cages attenuated the development of repetitive behavior in females and their non-enriched offspring, corroborating to the beneficial transgenerational effect of environmental enrichment on behavior development (BECHARD; LEWIS, 2016).

Therefore, enrichment is way more advantageous to the offspring and their mother when provided during the gestational period. Researchers studied the effect of pre-reproductive environmental enrichment on offspring in rearing female rats living in an enriched cage from weaning to sexual maturity. They found out that positive maternal experiences influence the offspring, as pups born from females exposed to pre-reproductive environmental enhancement acquired early complex motor abilities; from that, it was possible to conclude that pre-reproductive maternal experiences affect offspring behavior and biochemical levels (CAPORALI et al., 2014). In a related study, females in an enriched environment had better licking and crouching, and performed better nest building activities; in other words, they showed higher maternal care when compared to standard females. The offsprings displayed higher discriminative and spatial preferences than the controls, demonstrating that pre-reproductive maternal experiences prepare their progeny to cope with the enriched environment (CUTULI et al., 2015). Finally, females housed in environmental enriched cages spent reduced time building their nest and had lower nursing frequencies when compared to dams in standard cages. Researchers have not observe anxiety-like behavior in their pups. In the wild, dams must leave their nest to defend and hunt, which suggests that environmental enrichment mimics natural maternal care (CONNORS et al., 2015).

Maternal behavior

On the other hand, research assessing the effects of physical enrichment on stress levels and maternal behavior sensitization latencies showed an increased anxiety-like behavior, which inhibited the onset of maternal behavior. It is possible to explain this apparently contradiction by how objects may distract female and prevent her from interacting with her foster pup (MANN; GERVAIS, 2011). It is known that environmental enrichment leads to more interactions between the animal and its environment, causing changes in its physiology,

health, and disease prognosis. However, environmental enrichment effects on sensory function are more complex than previously thought.

Nevertheless, male mice reared in the communal nest were more resilient to social stress and had lower corticosterone levels when compared to standard cage mice, showing that adult vulnerability to stress is associated with early experiences and characteristics of stress (BRANCHI et al., 2013).

Postnatal development

One can assess the effects of physical and social environmental enrichment on behavior, performance, and welfare of male rats by comparing three types of housing: *a*) standard cages with 48.5 cm length × 33 cm width, with the upper side delimited by metal bar cage bars of 21 cm from the cage floor for the housing of single rats; *b*) physically enriched cages as big as a standard cage, but with more elevated cage lids and many objects such as treats, nylabone, crawl ball and ladders for the housing of single rats; *c*) socially enriched cages that also have the size of a standard cage with elevated cage lids and that despite not presenting physical structures, it houses three rats. Results revealed that physically enriching the environment may improve animals welfare, considering there were significant effects on behavior and growth, such as high levels of sleep, grooming, exploration and body weight (ABOU-ISMAIL et al., 2014).

Elliott and Grunberg (2005) examined the single and combined effects of social and physical enrichment by a locomotor activity of male and female mice submitted to open field test. This test measures the information-processing or learning of animals, in which high activity rate suggests that the animal is not adapting to the environment or is not processing the new information while a low activity rate suggests fast adaptation and learning (VARTY et al., 2000).

Researchers found that social enrichment is better than physical enrichment for information-processing in females considering it was possible to note reduced activity in the open field, suggesting greater adaptation to a new environment (ELLIOTT; GRUNBERG, 2005). In addition to that, other studies showed that physical enrichment is also necessary to improve rodents' cognition, showing that group-housing and nesting material affect the behavior pattern of female mice, contributing to their emotional behavior, learning and memory. Social isolation and the

lack of nesting material have a negative impact on the learning and memory of females, leading to the conclusion that the lack of environmental stimulation impairs mouse behavior (KULESSKAYA et al., 2016).

Enriched cages made of standard polypropylene cages of 48.5 cm length × 33 cm width × 21 cm height supplied with treats, nylabone, crawl ball, ladders and nestlets have increased sleeping time, exploration, movement and feeding behavior patterns, body weights, weight gains and the relative weights of the thymus gland and spleen, reflecting a good welfare. The enriched cages have also reduced the levels of stationary behavior and the relative weight of adrenal glands, indicators of poor welfare (ABOU-ISMAIL; MAHBOUB, 2011).

A study using simultaneous *in vivo* electrophysiological recordings and optical imaging revealed that environmental enrichment modifies specific components of sensory-evoked activity in the barrel cortex of rats (DEVONSHIRE et al., 2010).

Similarly, another study showed increased level of physical activity in rats living in largely enriched cages when compared to those housed in standard cages, which may interfere in animal welfare, considering conventional cages lead to sedentary behavior patterns and overweight because of limited opportunities to do physical activity and *ad libitum* feeding. Thus, the use of largely enriched cages resulted in lower body weight gain and increased motor function and muscle strength although the food and water intake were the same when compared to standard cage animals, showing that large pen houses stimulate physical activity and are positive for animal welfare (SPANGENBERG et al., 2005).

Standard housing systems for laboratory animals disturb their emotional state causing stress due to natural behavior deprivation. A widely used method to evaluate the affective state of animals is looking at their anticipatory behavior by of measuring the behavior between a signal indicating the arrival of a reward and the arrival of the reward (MAKOWSKA; WEARY, 2016).

Researchers have shown that male rats housed in standard houses have a stronger anticipatory response for the sucrose reward, an indicative of higher sensitivity to the reward when compared to mice housed in cages enriched with objects. With this in mind, they have concluded that rats standardly housed suffer more from deprivation to behavioral needs, which leads to disturbed emotional states (VAN DER HARST et al., 2003).

Another study assessed the affective state of female rats reared and housed in standard or semi-naturalistic cages for a long period, which described and compared their anticipatory behavior for sugar. Standardly-housed rats were more active while anticipating the reward than enriched-housed females, an indicative of higher sensitivity to rewards that corroborates with the idea that standard laboratory housing compromises rodent's welfare (MAKOWSKA; WEARY, 2016).

Agonistic behavior and dominance are common when housing rodents in captivity conditions and can lead to social stress and poor welfare. Although these behaviors occur in natural habitats as well, they are way more evident in an animal testing environment. The study of the effects of enriching laboratory cages on agonistic interaction and dominance of rats revealed that animals housed in enriched cages had lower levels of aggressive and defensive bouts when compared to rats housed in standard cages, whereas enrichment did not have any effect on the social order of animals in the cage. In conclusion, adding objects in laboratory cages is enough to increase the complexity of laboratory rats' cages and results in an improvement of welfare in these animals (ABOU-ISMAIL; MAHBOUB, 2011). Early life stress impacts the social behaviors of adult mice. Benner et al. (2014) showed that neonatal social isolated rats were subordinate to group-housed mice when competing for reward access, hindering their reversal learning.

One way to reduce the effects of stress on anxiety-like behavior induced by isolation is through handling during neonatal period or reward-based eating. In this study, we divided pups into handled and non-handled groups. After gently removed from the nest, we placed the pups of the handled group in an incubator at 32 °C. We returned them to their home cages where their mothers remained after 10 min. The researcher changed gloves after dealing with each litter to avoid the spread of different odor from nest to nest. In the other group, we kept pups of non-handled sets with their mothers without any interventions until weaning. We also decided to feed them with a highly palatable diet enriched with simple carbohydrates, and prepared with condensed milk, sucrose, vitamins and minerals, powdered standard lab chow, purified soy protein, soy oil, water, methionine and lysine. The nutritional content of this diet is similar to that of a standard lab chow, despite most carbohydrates in the palatable diet being sucrose. In contrast, the standard lab chow mainly consisted of carbohydrates from starch. We offered the palatable

diet after 20 days of birth and switched the pellets daily. According to Marcolin et al. (2012), the handling reversed the animals from stress.

Early handling of female rats resulted in higher palatable food intake when compared to non-handled counterparts. Neonatal handling affects the response to palatable food in adult life, suggesting that neonatal history has important implications in altered feeding behavior and related morbidities, such as obesity in adulthood (COLMAN et al., 2015).

Considering the wide range of materials used for bedding and nesting, a study aimed at identifying the preference of young rats aged between 8 and 10 weeks for corn versus wood-based materials. This study observed that male and female rats preferred aspen chip bedding instead of corn cob, and paper strips are preferred over cornhusk as a nesting material, concluding that corn-cob products are not recommended except when air quality and or/flooding become a serious issue (RAS et al., 2002).

On the other hand, a study on the enduring effects of enriched environment in male and female rats in tasks that measured emotional reactivity, social exploratory and memory, sensorimotor gating and learning showed relevant and lasting consequences on physiological and behavioral aspects. Rats housed in largely enriched cages gained less weight, had more exploratory behavior besides having learned more than standard rats (PEÑA et al., 2009).

Sexual behavior

Concerning the effects of enriched environment on emotional and sexual behavior, some authors have observed that rearing rats mostly living in enriched cages showed both male copulatory behavior and ejaculation reduction, besides extended ejaculation latency and postejaculatory intervals (URAKAWA et al., 2014).

Biochemical patterns and others

Rats and mice are social animals and must be housed in groups to ensure normal the development of their physiological behavior. It is possible to consider social interaction as enrichment. The social isolation of laboratory rodents is a frequent practice when researchers need to obtain data from individual animals (BOGGIANO et al., 2008). However, isolation is an abnormal condition for rats and mice and causes numerous adverse effects that may threaten the validation of experimental results and the translatability to humans. One alternative to minimize the

negative effects of isolation is using a device, such as a cage divider, which ensures social stimulation in rodents while allowing data collection in individual housing studies. This device, named “buddy barrier”, enhances animals’ welfare by providing social enrichment without compromising data integrity (BOGGIANO et al., 2008). Another study established rodents’ preference to maintain limited contact through a perforated plexiglass wall separating animals instead of being individually housed. The authors observed positive effects of non-tactile contact with a social partner. Females spent more time near other animals and also demonstrated more activity when compared to males, confirming the importance of social contact to enhance female welfare (SØRENSEN et al., 2010).

Laboratory animals are widely used in energy homeostasis experiments. To record food intake or energy expenditure accurately, it is necessary to house rats separately, which is a stressful condition for social animals like them. Stress can affect different aspects of energy homeostasis, including food intake and body composition (ELLACOTT et al., 2010), so it is important to use enrichment to overcome the negative effects of single housing. Authors observed reduction in hypothalamic-pituitary-adrenal axis activity in rats housed in enriched cages despite enrichment not affecting body weight and food intake were not affected, being possible to conclude that environmental enrichment improves living conditions of animals used in energy homeostasis studies without compromising experimental outcome (BEALE et al., 2011).

Proper animal handling is important to their welfare and can influence experiment outcomes experiments and adult behavior, considering human-animal interaction is part of laboratory daily routine (AUGUSTSSON et al., 2002).

The use of a system that allows minimal animal handling has been considered a powerful tool to study animal behavior although the presence of other stressors. Automated home cage systems assessed behavioral parameters for long periods of time, such as in the analysis of across-circadian phases as well as separate analysis of both novelty-induced and baseline homecage behavior. Before concluding conclusion, authors provide more significant and valid measurements (CLEMENS et al., 2014).

A study aimed at investigating the influence of housing male rats in large groups of enriched cages (pen housing) in human-animal interaction using three behavioral tests to assess human-animal interaction: a) rats’ anticipatory

reactions to handling b) mouth gag cooperation test and c) human approach test in a Y-maze. According to the results, there was no difference in anticipatory reaction when handling with large and standard cages. However, pen housing rats were more active, weightless, had lower total cholesterol values and higher urine corticosterone values than animals from conventional cages. These effects suggest higher physical activity (AUGUSTSSON et al., 2002).

Additionally, other have shown that housing rats in solid-bottom cages have no effect on clinical and pathological parameters commonly required by toxicology studies, such as complete blood count, serum chemistry profile, urinalysis, unique creatinine and corticosterone. Although toxicologists fear that modifications in experimental conditions might impair experimental results, Sauer et al. (2006) found no relevant clinical alterations between rats housed in solid-bottom polycarbonate cages and rats in standard wire-bottom cages. These results support the recommendations of Guide and suggest that the selection of cage type for animals used in toxicology studies should follow scientific and animal welfare considerations (SAUER et al., 2006; UEHARA et al., 2012).

The use of solid floor is as important as the type of bedding material to enrich the environment inside cages. The bedding and nesting material provided in cages have significant consequences for welfare, either by improving air quality or by providing a comfortable resting surface and opportunities for development of natural behavior, such as nesting, digging and chewing (BLOM et al., 1996).

In addition, Gordon (2004) observed that the type and bedding offer affected the core temperature and locomotor activity of female mice, concluding that variations in bedding material may disturb rodent health and well-being, which can affect the outcome of toxicology and pharmacologic studies reliant on body temperature. Another study investigated the use and the effects of nesting material as enrichment for behavioral and physiological parameters of male and female mice. Although mice living in enriched cages weighed more than mice living in standard conditions, there were no major differences regarding behavior and physiological factors between these groups, demonstrating that the use of nesting material does not risk the outcome of experiments and serve as environmental enrichment (VAN DE WEERD et al., 1997).

Besides the effect on adult behavior, it is important to mention the impact of maternal enrichment on behavior and physiology factors of both dams and offspring. Studies

have revealed that enrichment reduces the body weight of female rodents while it also increases food intake of pups, promoting social interactions between mother and offspring (GIRBOVAN; PLAMONDON, 2013).

Conclusion

Environmental enrichment is not restricted to an addition of objects in cages, also comprising cage space, type of flooring, bedding material, handling and social contact. There is scientific evidence that environmental enrichment has a positive effect on emotion, cognition, behavior, physiology, body weight, reproduction, hormone secretion and offspring development. It is also a necessary step to obtain results with high accuracy and high level of

reproducibility. Besides, some enrichment conditions are well known and should be implemented when it is possible to improve animal welfare. There is need to standardize appropriate cage conditions and to raise awareness on environmental enrichment planning to obtain highly accurate and reproducible outcomes.

Conflict of interest

The authors declare that there were no conflicts of interest.

Informed consent

This article does not contain any studies with human participants or animals performed by any of the authors.

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