Environmental enrichment as an ethical principle in animal research

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Abstract

Animals used in experiments have reduced levels of welfare and are thus vulnerable to pain and suffering. Considering that environmental enrichment increases the quality of life of captive animals, we aimed to assess its scientific application regarding the promotion of animal welfare by recognizing the vulnerability of biological models. Documentary analysis and experimental tests were conducted, stating that despite this technique having been applied mainly in neuroscience studies, endorsing their feasibility and the improvement in learning, the rationale for its use has been limited to the successful development of research, that is, not in recognition of animals' vulnerability, their need for welfare and quality of life. In contrast, we proposed the application of environmental enrichment for laboratory animals, within a framework of scientific contractualism and the researcher's responsibility, as a standard to be adopted for the mutual benefit of scientific development and the quality of animal life.

Keywords: Bioethics. Bioethics-Enviromental. Animal Experimentation. Scientific misconduct. Psychology Experimental.

Resumo

Enriquecimento ambiental como princípio ético nas pesquisas com animais

Animais utilizados em experimentos dispõem de reduzidos meios de bem-estar, estando vulneráveis a dor e sofrimento. Considerando que a técnica de enriquecimento ambiental aumenta a qualidade de vida de animais cativos, objetivou-se avaliar sua aplicação científica no âmbito da promoção de bem-estar por meio do reconhecimento da vulnerabilidade de modelos biológicos. Para tanto, conduziram-se análises documentais e ensaios experimentais, atestando que apesar de a técnica de enriquecimento ambiental ter sido aplicada principalmente em estudos de neurociência, endossando sua viabilidade, e da melhora no aprendizado, a justificativa para sua utilização tem se limitado ao bom desenvolvimento da pesquisa, e não em reconhecimento da vulnerabilidade e necessidade de bem-estar e qualidade de vida do animal. Em contraponto, foi proposta a aplicação do enriquecimento ambiental em animais de laboratório, num âmbito de contratualismo científico e responsabilidade do pesquisador, como norma a ser adotada para benefício mútuo do desenvolvimento científico e qualidade de vida animal.

Palavras-chave: Bioética. Bioética-Meio ambiente. Experimentação animal. Má conduta científica. Psicologia experimental.

Resumen

El enriquecimiento ambiental como un principio ético en la investigación con animales

Los animales utilizados en experimentos disponen de bienestar reducido y están expuestos al dolor y al sufrimiento. Considerando que la técnica de enriquecimiento ambiental aumenta la calidad de vida de los animales en cautiverio, el objetivo de este estudio fue evaluar su aplicación científica en el ámbito de la promoción del bienestar mediante el reconocimiento de la vulnerabilidad de los modelos biológicos. Para ello, se realizaron análisis documentales y ensayos experimentales, señalando que a pesar de que la técnica de enriquecimiento ambiental se ha aplicado principalmente en estudios de neurociencia, respaldando su viabilidad y la mejora en el aprendizaje, la justificación para su utilización se ha limitado al desarrollo exitoso de la investigación; no hay reconocimiento de la vulnerabilidad, de la necesidad de bienestar y de calidad de vida del animal. Por el contrario, se propone la aplicación de enriquecimiento ambiental en animales de laboratorio, en un marco de contractualismo científico y de responsabilidad del investigador, como un estándar a ser adoptado para el beneficio mutuo del desarrollo científico y de la calidad de vida de los animales.

Palabras clave: Bioética. Bioética-Medioambiente. Experimentación animal. Mala conducta científica. Psicología experimental.

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Although knowledge of non-human animals has increased significantly throughout history, it has increased in recent decades through the development of technology associated with the field of neuroscience. This process has added to our understanding of the biopsychosocial needs of animals, the moral value of their lives and the legal and ethical need to respect them. This is especially true with regard to captive animals and interferences without valid reasons and for which alternatives do not yet exist, such as in research and education [1].

The definition of animal welfare (AW) involves a momentary state of harmony between the body and the environment that surrounds it, in which the animal seeks to meet its physiological needs through adaptation, aided by good health and environmental opportunities [2,3]. In order to develop technologies that promote both the diagnosis of conditions of AW and alternatives to mitigate the effects of captivity, the science of AW has developed, supporting the appropriate use of animals, and recognizing the inherent guidelines of the 3Rs: *reduction*, *replacement* e *refinement* [4].

While this behavior is widely encouraged in production systems, it is endorsed especially in the area of science by the justification that the greater the AW, the more reliable the results of research involving non-human animals will be. Recent studies reveal that a space enriched with toys, tunnels and physical activities [5] significantly stimulates the neurogenesis [6] of rodents. This improves the ability to perform cognitive tasks [5], due to increased exploration activity and new sensory experiences, thus increasing AW. The field of laboratory research with rodents, targeted mainly at experiments aimed at developing biotechnology, has proven conservative in applying techniques of environmental enrichment (EE).

The standardizations of biosecurity and husbandry techniques are important for ensuring that the material introduced into the reproductive system is practical for handling and cleaning, and does not put the animals at risk [7]. Most of the diseases that affect animals in captivity come, or are maximized by laziness [8], and EE aims to increase quality of life [9]. Thus, the application of EE in rats acts to improve the results of scientific research in experiments in the areas of both health and learning [10], as this hypothesis is based on the fact that animals display more natural physiological and behavioral characteristics [11].

Lab exercises with rats are a reproduction of the effects of environmental variables, influencing the functional relationships established by animal behavior [12,13]. One way to impose natural environmental variables would be to use experimental psychology to work with the results of the manipulation of important variables in controlled conditions [12]. Thus, it is the duty of the researcher to take care of feeding and provide a hygienically suitable environment, as well as to avoid the discomfort of animals [12]. It is important to note that all animal research should adopt ethical principles. The animals must be acquired legally and their treatment must comply with local laws and regulations [14].

For Weinberg [15], a scientist may be brilliant, imaginative and intelligent, but he or she will not go beyond being a mere scientist if he is not also responsible. Therefore, responsibility is the principle which guarantees the autonomy and freedom of the researcher. This responsibility is social, as it must ensure the quality of the research, and also be ethical to animals, as vulnerable beings are being manipulated in the research process [16].

As well as the fact that vulnerability is intrinsic to life, it must also be considered that individuals may be directly affected by unfavorable circumstances. Accordingly, being or being in a state of vulnerability refers to a range of meanings that extend from a latent state to a manifested state, or in other words, from the possibility to the probability that the vulnerable being is vulnerable [17]. Not providing welfare to a non-human animal is to leave it in a manifest situation of vulnerability, thus compromising its quality of life and, as a consequence, the results of the research.

This study is justified in bibliographic data [17,18] which shows that EE is effective in promoting AW. The question, however, is why its use remains so poorly promoted. The hypothesis tested theorizes that researchers, despite knowledge of current legislation [19] and the improved quality of life of animals provided by AW – and the consequent increase in the reliability of data – do not consider the vulnerable condition of animals when making their decisions. Therefore, this study aimed to evaluate the applicability of EE through documentary and experimental data, with a view to attributing responsibility for the promotion of AW to researchers, based on the evidence of the effectiveness of EE, as well as its link with the results of their research.

Materials and method

The present article consisted of two methodological approaches: one consisted of the systematic

documentary analysis of the use of EE in scientific research, while the other is the experimental evaluation of the effect of environmental and social enrichment on the learning of animals evaluated, to support the discussion about animal vulnerability and the ethical responsibility of the researcher with regard to the evidence of the effectiveness of EE.

Documentary analysis

Documentary analysis was performed using articles recovered from the Capes Periódicos portal, a tool that brings together indexers such as Pubmed, Scopus, ASFA, SciELO, OneFile, Medline, SpringelLink, BioOne and JSTOR. The search term "environmental enrichment" was applied, subject to the presence of the term "rats". The first stage was carried out between September and October 2014, analyzing the total records per year and then categorizing them into specific topics. The first 100 articles published between the years 2013 and 2014 were retrieved, in order to verify that the use of the term "EE" in research was aimed at achieving welfare and recognized the vulnerability of laboratory rats. Review articles that met the exclusion criteria, as well as items that could not be accessed, were rejected, leaving 80 articles.

Experimental evaluation

Research animals and location

The study was performed at the Laboratório de Análise Comportamental da Escola de Saúde e Biociências (the Behavioral Analysis Laboratory of the School of Health and Biosciences) of the Pontifícia Universidade Católica do Paraná (the Pontifical Catholic University of Paraná) (PUCPR), Curitiba campus. The behavioral patterns of 40 male rats of the *Rattus norvegicus*, aged approximately 30 days, was analyzed. The animals were provided by the PUCPR bioterium and housed in standardized boxes, arranged on shelves. They were provided with daily food and water and bedding was changed twice a week.

The *R. norvegicus* rodent was used as an experimental model because of its high genetic homogeneity and because it is commonly used in experimental studies and experimental behavior analysis. The fact that they are gregarious animals with a high exploration and locomotor activity was also taken into account [11].

Experiments

The experimental design aimed to evaluate the effect of EE and social enrichment (SE) comparing the performance of learning among individuals kept in enclosures with and without EE and SE. The animals were therefore separated into four groups, each with 10 animals ¬ Group 1: isolated rats with EE; Group 2: isolated rats without EE; Group 3: rats in pairs with EE; and Group 4: rats in pairs without EE.

Environmental enrichment

The environmental enrichment for both isolated animals and those in pairs consisted of the addition of a PVC tunnel, 7.5 cm in diameter, with an angle of 180°. The tunnel was arranged in a lateral position on bedding in a 20 cm tall by 30 cm wide box.

Social enrichment

The SE consisted of keeping the animals in pairs. A mark on the animals' tails was made with an overhead projector pen and reinforced weekly, so they could be recognized. To avoid the influence of this variable, all animals were marked, even those kept in isolation. Through behavior analysis, the subordinate and dominant animals were determined.

Evaluation of learning performance

To evaluate the learning of the animals in different conditions, tests were conducted by the classic experimental analysis of behavior procedure [12], using the Skinner box. The operant conditioning chamber is one of the main elements of behavior-analytic methodology [20].

In this methodology, the assessment of learning occurs by conditioning through positive reinforcement, using the water dispenser. It should be mentioned that for water to function as an effective reinforcement in reinforcement learning trials, it is necessary that the animal is deprived of water for 24 hours before the planned exercise [12]. The first step was to determine the operant level of the animal (OL) before behavior modeling (M). This allows the evaluation of the effect of the positive reinforcement (water) by comparing with the response frequency of response before and after the introduction of the reward [12]. The exercise had an average duration of 30 minutes and behaviors were recorded at one-minute intervals.

After determining the operant level, the animal underwent the conditioning exercise in training with the water dispenser. This process includes the

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adaptation of the possible emotional responses of the subject when hearing the noise of the water dispenser. The experiment had an average duration of 15 minutes.

The next test was response to the pressure bar after continuous reinforcement (CFR), which consisted of modelling the response to the training. The modeling here is the release of stimulus, containing water, after a previously defined response. The exercise had a duration of ten minutes[12]. Another feature evaluated was the satiation level (SL), which analyzed how long and how many drops of water were needed for the animal to be satiated. The experiment had an average duration of 60 minutes.

After the animal is conditioned to the bar pressure stimulus, the exercise of extinguishing the response to the bar pressure (E) was performed. This test aims to observe the effect on the frequency of the pressing the bar action without the presence of reinforcement. The process was complete after a minimum of fifty responses had occurred and the animal went for ten minutes without pressing the bar[12].

To again stimulate again the behavior of pressing the bar to receive a reward in the rat, reconditioning of the bar pressure response was carried out. This test had an average duration of one hour, and the animal had an interval of five minutes to press the bar. If this did not occur, the response modeling process was again carried out, continuing in this way until the moment that the subject received ten CRF stimuli.

The intermittent extinction of reinforcement (IE) was also considered in this study. This is an experiment for comparing the bar press response rate with the process of the extinction of the continuous reinforcement. The experiment began with ten stimuli sessions. After the initial phase, the control switch was switched to the manual position. Thus, when the subject pressed the bar no reward was issued[12]. The test had an average duration of 1h 30 min.

Statistical analysis and legal considerations

The test results were compared between the groups based on social and environmental enrichment. The Kolmogorov-Smirnov test was used to verify the normal distribution of variables. Comparisons between means were performed by the non-parametric Kruskal-Wallis and Mann-Whitney tests due to the absence of a normal sample. For comparison between the frequency of motor pattern values displayed by the animals the chi-squared test was used. A significant of 95% was used in all tests.

All procedures, as well as those of the bioterium that provided the animals, were in accordance with Brazilian law. The selection of the animal model was due to its prevalence in the experimental protocols of the institution, as well as being traditionally used by experimental psychology[1]. The experimental design sought to minimize the use of the animals in the performance of statistical tests.

Environmental enrichment was not provided for all animals, as its absence was one of the study variables. The deprivation of water for 24 hours also constituted a procedural variable for behavior modeling. However, to alleviate this process, we attempted to provide intervals of at least one week between the tests. Habituation was carried out prior to testing, so that the animals could get used to the presence of the researcher. Their reactions during the study were also monitored, while they underwent the minimum amount of handling and stayed in the lab for as short a time as possible.

Results

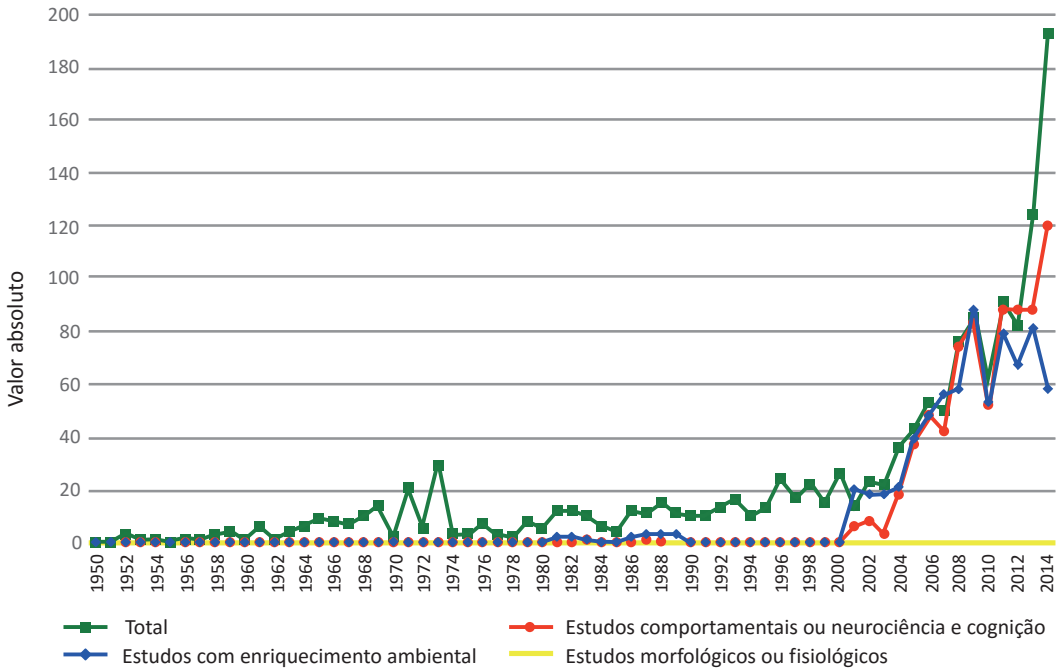
Document analysis

The scientific texts reviewed indicated a development that began with the first article found, which was from the 1950s to the peak of production in 2014. Studies that specifically use environmental enrichment and the association of the technique with neuroscience began to emerge in the scientific field in the 2000s, following the overall increase in scientific production in subsequent years (Figure 1).

Of the 80 articles retrieved, most of the tests involved cognitive or neuroanatomical-pathological procedures. The main interest of the studies was how to meet the research needs in support of the benefit sought by the study, seeking homogeneous results rather than animal welfare. Recognition of animals as vulnerable subjects in the laboratory environment was not mentioned or discussed in any article.

The studies included works on neurology, with 50% being about neurophysiology and the others dealing with neuroanatomy, neuropharmacology and neuroendocrinology. Most studies (75%) aimed to verify the effect of EE. The majority of studies (96%) found that EE had a positive effect on the results of research, while only 3.1% obtained neutral conclusions. A total of 91% of studies were neurological, followed by studies about cognition (6%), drugs (2%) and phenotypes (1%). The tests were mainly based on cognition (37%). The combination of physical and social EE (29%) was the most commonly used, followed by physical, cognitive and social (18.5%).

Figure 1. Temporal distribution of scientific texts retrieved from Capes Periódicos Portal using the terms "Environmental enrichment" and "Rats", total, approaches with environmental enrichment, behavioral or neuroscience studies based on cognition and morphological or physiological studies



Environmental enrichment

Group 1, where the animals were kept isolated and exposed to an enriched environment, performed better in learning patterns (t = -3.027; p = 0.007) when the evolution of operant level (OL) after continuous reinforcement was evaluated. The group without EE exhibited a more heterogenous mean difference in the number of responses (bar press), with double the response imprecision, as can be seen in Figure 2. The same response pattern, with greater variation and a higher average, was observed in the level of satiation in group 2 (p = 0.005) (Figure 3). There was no significant difference between the response patterns of groups 1 and 2 for the other cognitive tests. When the test performances of the individuals in pairs (groups 3 and 4) with and without enrichment were compared, the difference was not significant.

Social enrichment

There was no difference in performance in the learning process between the dominant and subordinate individuals in any of the tests, despite the stimulus of environments with and without EE. Thus, it was possible to compare the pairs, as a whole, with the isolated individuals. There was a difference in learning performance between pairs and single individuals in the intermittent extinction of reinforcement test (p = 0.043), with no influence of EE. The isolated individuals performed better and had a more homogeneous bar pressure response in the test (Figure 3). There was no significant difference between the pairs and the isolated individuals for the other tests.

Discussion

The documentary analysis and the experiments showed that the use of EE in research involving behavioral and neurological analysis is established and that its use achieves better results. However, the prioritization of neuroanatomical-pathological tests to measure the effects of EE shows that evaluations are aimed at quality of research, not quality of animal life.

The experimental data in this study indicates that EE favored the learning of rats, certified in modeling tests. These tests evaluated the bar pressure response as a learning determinant and found a significant improvement in the performance of animals kept in enriched environments. These results corroborate other studies that showed the influence of EE on cognition in rats[17,18], mice[21,22], rabbits[23] and pig[24]. Schaeffer[6] points out that enriched environments result in an increased proliferation of neural stem cells, the survival of new neurons[25] and increased brain weight[23,26,27], thus promoting improved performance in cognitive tasks, given the increase in variables in an environment is a stimulating factor.

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Figure 2. a. Mean difference between standard responses (bar pressure) of operant level for mice undergoing continuous reinforcement with EE and without EE (WEE). **b.** Level of response to bar pressure related to the satiation of isolated animals in an environment with EE and without EE (WEE)

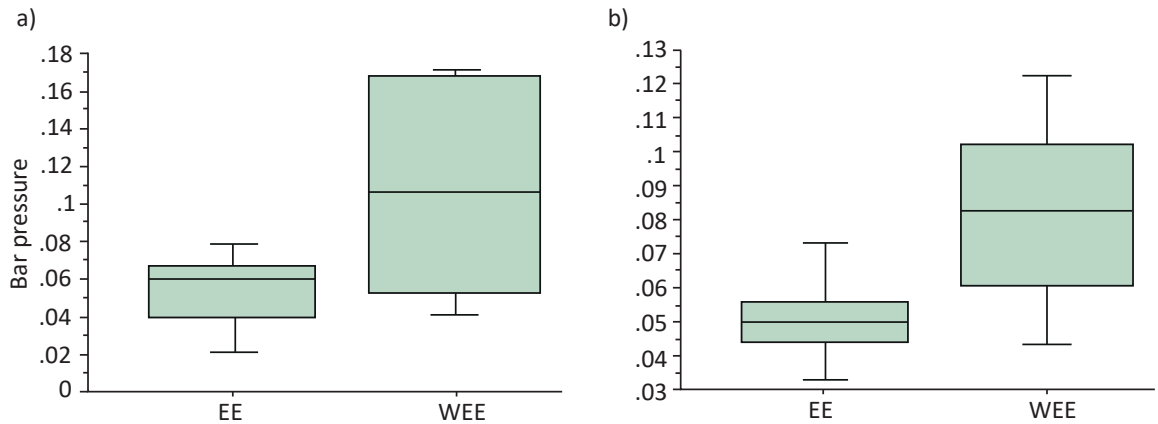
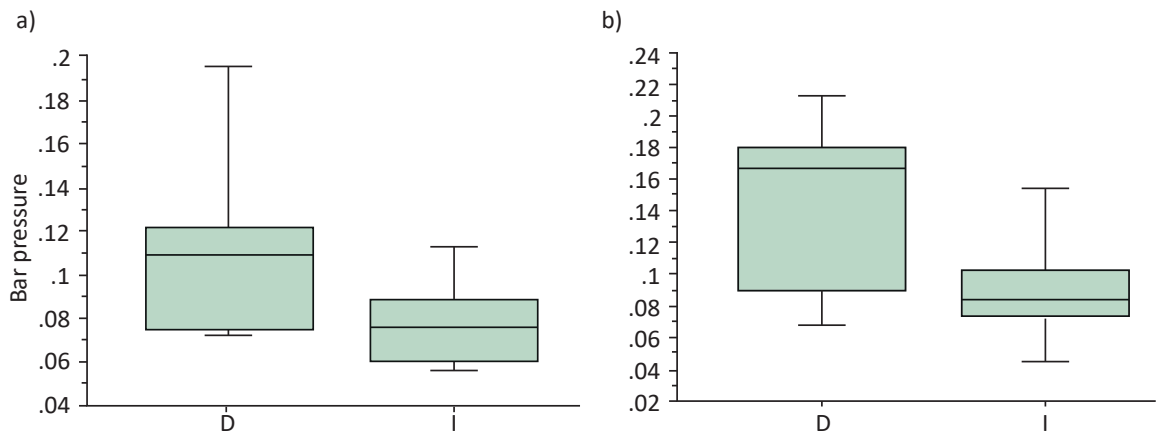


Figure 3. Response pattern (bar pressure) in the intermittent extinction of reinforcement test between individuals with social enrichment (doubles – D) and without social enrichment (isolated – I) **a.** with enrichment (EE) and **b.** without enrichment (WEE)



Many researchers have considered that the adoption or otherwise of EE is a possible compromise of experimental results, mainly due to greater variability[27,28]. However, this study found that EE contributed to cognitive ability in the responses to the initial testing, and also that the satiation level of rats was more homogeneous than for individuals without EE. Instead of the complexity of the enriched environment leading to an increased response variation among individuals due to the number of stimuli[29], what seems to occur is that the inclusion of just one PVC tube provides environmental improvement, making the individuals more able to handle the new challenges posed in testing, thus generating uniform responses, as has been observed in other studies[30-32]. Nonhuman animals in EE conditions have shown greater physiological and psychological stability, and are thus able to generate better scientific results[33].

In this study, EE consisted of the provision of an object that protected from light and aggressions, which is recognized as an increase factor for AW[34]. In the case of rodents, which are gregarious, social animals[35], it is important to consider that social isolation can stimulate behavioral disorders[36]. As for SE, the results of the present study only showed differences in the most complex phases of the learning tests, with isolated individuals exhibiting an advantage in the re-elaboration of responses to positive stimuli. This result may reflect the generation of a mild social instability generated between the pairs in cages, as literature recommends using three individuals per environment due to the reduction of aggressive behavior and better social stability[37].

The lack of a difference observed in learning performance between pairs and isolated animals in the other tests reinforces the recommendations of Van Loo and Baumans [38] who said that if there is a need to keep animals in social isolation, it is appropriate to use nests, aimed at providing rest and a sense of security, as well as suitable thermal control conditions.

The concern that the application of EE can affect the variability of results caused by environmental conditions is in fact more related to the importance of a wider discussion of the subject in the field of science. Based on the proposal of EE, several studies have been conducted aimed at identifying the variables that affect laboratory experiments. The results point to several important parameters, such as basic housing conditions [28], sex, species, the age of the animal, cage lighting conditions and temperature [39 40].

It is clear that factors that influence the results of non-human animal experiments are directly interconnected with the structure in which the individual finds itself. This fact highlights two important aspects, the first being the social responsibility of researchers regarding the quality of their experiments and the importance of ensuring reliable results. The second aspect is the duty to respect the lives of animals exposed to experimental situations in which their existential vulnerability is increased [16] due to pain, discomfort and suffering.

Social responsibility in research with non-human animals

According to Jonas [41], the human being, due to possessing the capability to understand, experiences responsibility as an act of liberty, and such responsibility as essentially ethical. Every researcher has a social responsibility to the community in general [42], and the primary social responsibility is to prevent the corruption of research data, thus ensuring the integrity of the study. The literature review [43] explained that basic conditions such as the brightness of the environment of the animals can interfere with the response of individuals. Therefore, the non-standardization of the appropriate environmental conditions for the animals, as well as an ethical failing, represents scientific misconduct with regard to research data [43].

From the principle of ethical and social responsibility linked to research, EE is proposed as a guideline and standard of ethical conduct in research with non-human animals. Giorgini et al. [44] pointed out that the role of the code of ethics in the conduct of researchers in the laboratory practice has been restrained. According to Habermas [45], in a plural society there is no traditional *ethos* or a substantial consensus of standards, values or principles to justify answers to practical questions. However, in the absence of such a consensus, there is a form of "rational consent" by independent, free and equal individuals, who identify, through deliberative communication practices, a rational justification for adopting a certain standard. This defines modern contractualism, which is rationally justified through dialogic procedures based on communicative rationality [46].

In this sense, the best rational argument among the scientific community is the establishment of strategies to promote AW, thus establishing EE in the scientific contract as a standard to be adopted for the mutual benefit of scientific development and quality of animal life.

Another important point is the vulnerability of the animal in experimental conditions. For Hossne [16], all living beings are subject to vulnerability, which is the result of the inability to an individual to protect his, her or its own interests. The vulnerable individual that suffers unmet needs becomes more prone to being easily affected and victimized [47]. Animals used in laboratory experiments suffer a reduction in the range of possibilities to achieve greater degrees of AW, even taking into account the obligation that their suffering is avoided and their survival ensured, as they are unable to protect their own interests and are thus vulnerable. This takes us back to the principle of social responsibility and the ethics of the researcher, who when handling a vulnerable life incurs the risk of compromising the search results. In addition, there is an ethical responsibility for the lives of the laboratory animals and to provide essential conditions of AW and quality of life.

Given these circumstances, the fact that research has already attested to the benefits of AW cannot be overlooked, leading us to consider whether the omission of this variable in the analysis and placing of results can be considered fraud [48]. However, it is also worth considering the vulnerability of the researcher in the face of the academic pressure to publish, the encouragement and incentives offered for positive results in high impact journals [49] and the necessity of meeting deadlines, as well as the desire for studies that are not properly justified by the results. These factors can lead the professional to only consider the immediate usefulness of

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the animal rather than the effectiveness of their research and the ethics of their behavior.

However, even considering the pressure that researchers may be under, the reliability of the research can be measured by the interpretation of the results. Therefore, it is necessary to improve the quality of animal experiments, especially considering that irresponsible practice includes the ill-treatment of laboratory animals. Studies should establish experimental protocols that comply with the justifications for the experiment, with an appropriate statistical design and detailed methodology, following the guidelines of Concea [50]. This resolution also emphasizes the importance of minimizing the pain and distress of animals so that physiological and behavioral changes are avoided, and do not lead to the misinterpretation of the data [50].

Final considerations

It was not expected that identifying the threshold between the vulnerable being and being vulnerable is a bioethical issue that would be discussed or addressed directly in a search of scientific articles involving EE, although it was hoped that such a search would find recognition and assessment of EE as an ally of quality of life and AW. The fact that EE is being tested for the best search result is a positive sign for the improvement of the conditions of captive animals. But it is important to note that so that EE can effectively protect the vulnerable, from an ethical point of view, it can only be considered adequate in a systematic assessment of the context of vulnerability.

In this sense, the concern with neuroanatomical patterns or cognitive performance in tests restricts the focus of research and makes EE an ethical procedure that should be associated with standardized protocols. Promoting AW, as corroborated by the experimental results of this study, in addition to providing appropriate environmental conditions for the animals, will lead the researcher to obtain more reliable and reproducible results. However, for this to happen it is necessary to understand the biological and behavioral needs of the animal and EE in a broad and integrated manner. This is ethical and social responsibility linked to research and to all involved, involving the creation, maintenance, handling and transport of animals.

In summary, it is proposed that EE is one of the guidelines and standards of ethical conduct in research with animals in the context of scientific contractualism. In this context, the researcher adopts the standard as a matter of social responsibility, both for the benefit of scientific development and for vulnerability.

Referências

1. Fischer ML, Oliveira GMD. Ética no uso de animais: a experiência do comitê de ética no uso de animais da Pontifícia Universidade Católica do Paraná. Estud Biol, Ambiente Divers. 2012;34(83):247-60.
2. Hughes BO. Behaviour as index of welfare. Proceedings of the Vth European poultry Conference; 1976. Malta: Poultry Science Association; 1976. p. 1005-18.
3. Broom DM, Fraser AF. Comportamento e bem-estar de animais domésticos. 4ª ed. Barueri: Manole; 2010.
4. Russel WMS, Burch RL. The principles of humane experimental techniques. Londres: Universities Federation for Animal Welfare; 1992.
5. Silva IS. Neurogênese no sistema nervoso adulto de mamíferos. Revista da Biologia. 2009 dez [acesso 15 out 2015];3:1-14. Disponível: http://bit.ly/2bWhLi3
6. Schaeffer EL. Enriquecimento ambiental como estratégia para promover a neurogênese na doença de Alzheimer: possível participação da fosfolipase A_2. [Internet]. Rev Psiq Clín. 2010 jul [acesso 3 out 2015];37(2):73-80. Disponível: http://bit.ly/2bgTwKb
7. Neves SMP, Mancini J Filho, Menezes EW, editores. Manual de cuidados e procedimentos com animais de laboratório do Biotério de Produção e Experimentação da FCF-IQ/USP. São Paulo: FCF-IQ/USP; 2013.
8. Grandin T, Johnson C. O bem-estar dos animais: proposta de uma vida melhor para todos os bichos. Rio de Janeiro: Rocco; 2010.
9. Alcock J. Comportamento animal. 9ª ed. Porto Alegre: Artmed; 2011.
10. Ratajczak P, Nowakowska E, Kus K, Danielewicz R, Herman S, Woźniak A. Neuroleptics and enrichment environment treatment in memory disorders and other central nervous system function observed in prenatally stressed rats. Hum Exp Toxicol. 2015 maio;34(5):526-37.
11. Ghidini VK. Influência do enriquecimento e do estresse imprevisível em camundongos pré-selecionados pelo perfil exploratório [tese]. Porto Alegre: Universidade Federal do Rio Grande do Sul; 2010.

12. Gomide PIC, Weber LND. Análise experimental do comportamento: manual de laboratório. Curitiba: EDUFPR; 2003.
13. Frajblat M, Amaral VLL, Rivera EAB. Ciência em animais de laboratório. Ciênc Cult. 2008;60(2):44-6.
14. Schnaider TB, Souza C. Aspectos éticos da experimentação animal. Rev Bras Anestesiol. 2003;53(2):278-85.
15. Weinberg AM. The obligations of citizenship in the republic of science. Minerva. 1978;16(1):1-3.
16. Hossne WS. Dos referenciais da bioética: a vulnerabilidade. Bioethikos. 2009;3(1):41-51.
17. Tomchesson JL. The behavioral effects of environmental enrichment in rats [tese]. Bethesda: Uniformed Services University of the Health Sciences; 2004.
18. Van Praag H, Shubert T, Zhao C, Gage FH. Exercise enhances learning and hippocampal neurogenesis in aged mice. J Neurosci. 2005;25(38):8680-5.
19. Brasil. Lei nº 11.794, de 8 de outubro de 2008. Procedimentos para uso científico dos animais. [Internet]. Diário Oficial da União. Brasília, 9 out 2008 [acesso 24 jun 2014]. Disponível: http://bit.ly/1WV52wP
20. Lattal KA. Steps and pips in the history of the cumulative recorder. J Exp Anal Behav. 2004;82(3):329-55.
21. Dong S, Li C, Wu P, Tsien JZ, Hu Y. Environment enrichment rescues the neurodegenerative phenotypes in presenilins-deficient mice. Eur J Neurosci. 2007;26(1):101-12.
22. Frick KM, Benoit JD. Use it or lose it: environmental enrichment as a means to promote successful cognitive aging. ScientificWorld Journal. 2010;10:1129-41.
23. Moura ASAMT, Barros TFM, Fernandes S, Siqueira ER. Composição do grupo social e enriquecimento ambiental modificam o comportamento de coelhos em crescimento. Botucatu: Unesp; 2012.
24. Douglas C, Bateson M, Walsh C, Bédué A, Edwards SA. Environmental enrichment induces optimistic cognitive biases in pigs. Appl Anim Behav Sci. 2012;139:65-73.
25. Van Praag H, Kempermann G, Gage FH. Neural consequences of environmental enrichment. Nat Rev Neurosci. 2000;1(3):191-8.
26. Pereira LB, Almeida ARV, Soares AF. Enriquecimento ambiental para animais que vivem em cativeiro. Anais da IX Jornada de Ensino, Pesquisa e Extensão. Recife: UFRPE; 2009 [acesso 7 set 2015]. Disponível: http://bit.ly/2bOptaG
27. Benefiel AC, Dong WK, Greenough WT. Mandatory "enriched" housing of laboratory animals: the need for evidence-based evaluation. ILAR J. 2005 [acesso 20 set 2015];46(2):95-105. Disponível: http://bit.ly/2biMndM
28. Baumans V, Van Loo PLP, Pham TM. Standardization of environmental enrichment for laboratory mice and rats: utilisation, practicality and variation in experimental results. Scand J Lab Anim Sci. 2010;37(2):101-14.
29. Eskola S, Lauhikari M, Voipio HM, Laitinen M, Nevalainen T. Environmental enrichment may alter the number of rats needed to achieve statistical significance. Scand J Lab Anim Sci. 1999;26(3):134-44.
30. Baumans V. Environmental enrichment: practical applications. In: Van Zutphen LFM, Balls M, editores. Animal alternatives, welfare and ethics. Amsterdam: Elsevier; 1997. p. 187-91.
31. Van de Weerd HA, Van Loo PLP, Van Zutphen LFM, Koolhaas JM, Baumans V. Nesting material as environmental enrichment has no adverse effects on behavior and physiology of laboratory mice. Physiol Behav. 1997;62(5):1019-28.
32. Wemelsfelder F. Animal boredom: a model of chronic suffering in captive animals and its consequences for environmental enrichment. Humane Innovations and Alternatives. 1994;8:587-91.
33. Van de Weerd HA, Aarsen EL, Mulder A, Kruitwagen CLJJ, Hendriksen CFM, Baumans V. Effects of environmental enrichment for mice: variation in experimental results. J Appl Anim Welf Sci. 2002;5(2):87-109.
34. Dawkins MS. From an animal's point of view: motivation, fitness, and animal welfare. Behav Brain Sci. 1990;13:1-61.
35. Lacey EA, Sherman PW. The ecology of sociality in rodents. In: Wolff JO, Sherman PW, editors. Rodents Societies: an ecological and evolutionary perspective. Chicago: University of Chicago; 2007. p. 243-54.
36. Arakawa H. Interaction between isolation rearing and social development on exploratory behavior in male rats. Behav Processes. 2005;70(3):223-34.
37. Baumans V. Science-based assessment of animal welfare: laboratory animals. Rev Sci Tech. 2005;24(2):503-13.
38. Van Loo PLP, Baumans V. The importance of learning young: the use of nesting material in laboratory rats. Lab Anim. 2004;38(1):17-24.
39. Galef BG Jr, Sorge RE. Use of PVC conduits by rats of various strains and ages housed singly and in pairs. J Appl Anim Welf Sci. 2000;3(4):279-92.
40. Garner JP. Stereotypies and other abnormal repetitive behaviors: potential impact on validity, reliability, and replicability of scientific outcomes. [Internet]. ILAR J. 2005 [acesso 7 set 2015];46(2):106-17. Disponível: http://bit.ly/2bPSdB2
41. Jonas H. Memórias. Madrid: Losada; 2005.
42. Frankel GEC, Austin Z. Responsibility and confidence: identifying barriers to advanced pharmacy practice. Can Pharm J. 2013;146(3):155-61.
43. Braga LMGM. Animal como um modelo experimental: noções básicas de genética, sanidade, alojamento e manutenção de animais de laboratório. In: Feijó AGS, Braga LMGM, Pitrez PMC,

organizadoras. Animais na pesquisa e no ensino: aspectos éticos e técnicos. Porto Alegre: EDIPUCRS; 2010. p. 171-86.
44. Giorgini V, Mecca JT, Gibson C, Medeiros K, Mumford MD, Connelly S *et al*. Researcher perceptions of ethical guidelines and codes of conduct. Account Res. 2015;22(3):123-38.
45. Habermas J. Direito e democracia: entre facticidade e validade. Rio de Janeiro: Tempo Brasileiro; 1997. v. 1 e 2.
46. Werle DL. Razão e democracia: uso público da razão e política deliberativa em Habermas. Trans/Form/Ação. 2013;36(1):149-76.
47. Levine C, Faden R, Grady C, Hammerschmidt D, Eckenwiler L, Sugarman J. The limitations of "vulnerability" as a protection for human research participants. Am J Bioeth. 2004;4(3):44-9.
48. Heyde CJ. NABR's misinformation cripples animal welfare and scientific integrity. [Internet]. AWI Quarterly. 2002 [acesso 28 out 2013];(51)3:14-15. Disponível: http://bit.ly/2cMb0j9
49. Fagot-Largeault A. Petites et grandes fraudes scientifiques. [Internet]. In: Fussman G, organizador. La mondialisation de la recherche. Paris: Collège de France; 2011 [acesso 27 out 2015]. Disponível: http://bit.ly/2bWEkU7
50. Brasil. Ministério da Ciência, Tecnologia e Inovação, Conselho Nacional de Controle de Experimentação Animal. Normativas do Concea para produção, manutenção ou utilização de animais em atividades de ensino ou pesquisa científica. 2ª ed. Brasília: Concea; 2015. Disponível: http://bit.ly/2bOCmll

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