Science-based assessment of animal welfare: laboratory animals

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Summary

The use of animals in experimental research parallels the development of medicine, which had its roots in ancient Greece. The increasing demand for high-standard animal models, together with a critical view of the way animals are used, has led to the development of a multidisciplinary branch of science we now know as 'laboratory animal science'. The guiding principles are replacement, reduction and refinement (the Three Rs), first proposed by Russell and Burch in 1959. When animals are used, the people involved have an obligation to safeguard their welfare and minimise discomfort; this will also generally be beneficial for both the animal and the experimental outcome. The ability of an animal to cope with the environment and exert control over its life seems to be crucial for animal welfare. In this paper, attention is paid to the assessment of welfare, environmental factors affecting welfare, legislative requirements and future trends such as the production and use of genetically modified animals.

Keywords

Environmental enrichment – Genetically modified animal – Individually ventilated cage – Laboratory animal – Laboratory Animal Science – Three Rs – Welfare.

History of animal experimentation

Humans have been using animals for a very long time, initially for food, for transport and for companionship. The use of animals in experimental research parallels the development of medicine, which had its roots in ancient Greece, where Aristotle and Hippocrates investigated the structure and function of the human body (see their respective *Historia Animalium* and *Corpus Hippocraticum*). These works are based mainly on dissections of animals, since performing autopsies on humans was not permitted at that time. Galen (130 AD to 201 AD), physician to the Roman Emperor Marcus Aurelius, conducted physiological experiments on pigs, monkeys and dogs that provided a basis for medical practices in the centuries thereafter. After Galen, experimental science stopped until the beginning of the Renaissance, when Vesalius took up the empirical approach, starting with anatomical studies; later, physiological studies were also carried out.

The development of Cartesian philosophy in the 17th Century meant that experiments on animals could be performed with little ethical concern. The French philosopher René Descartes (1596 to 1650), for example, stated that living systems could be understood on purely mechanical principles. The difference between humans and animals was that a human had a mind, a prerequisite for awareness and consequently for the capability of feeling pain, whereas animals could not think and were more like machines. Jeremy Bentham (1789), however, opposed Descarte's views by stating that 'The question is not, can they reason? Nor, can they talk? But can they suffer?'

The discovery of anaesthetics and Darwin's publication *On the origin of species* in 1859, emphasising the biological similarities between humans and animals, contributed to an increase in animal experimentation. Claude Bernard

then published his book 'Introduction à l'étude de la médecine expérimentale' in 1865, which introduced methodology as a tool for the design of physiological experiments. The development of microbiology resulted in a further escalation in the use of animals when Koch's postulates stated that the pathogenicity of micro-organisms could be proven by successfully infecting healthy, susceptible animals (6, 66).

In the 20th Century the development of pharmacology, toxicology and immunology caused yet another increase. This continued until the early 1980s, when the number decreased, probably because of public awareness and strict legislation on animal use, the development of animal ethics committees, and the improved quality of the animals used. During the last decade, however, the number of animals used has again increased, mainly due to the potential advantages that may accrue from genetic modification.

The use of laboratory animals

Today, 75 to 100 million vertebrates are used per year worldwide in research, teaching and testing for a wide range of purposes, including 10.7 million vertebrates in Europe (66). Drug research, testing of vaccines and other biologicals, and cancer research account for about 70% of the animals used, while the remaining 30% are used for purposes such as fundamental research, for diagnostic purposes, for teaching, etc. (Fig. 1). Mice and rats are the most frequently used species (Fig. 2).

In many countries it is mandatory to grade the level of discomfort for the experimental animal as minor (e.g. single blood sampling), moderate (e.g. recovery from anaesthesia) and severe (e.g. toxicity testing). In Europe for example, records indicate 50% of laboratory animals experience minor discomfort, 30% moderate discomfort, and 20% severe discomfort.



Fig. 1 Distribution of the purposes of animal use in research



Fig. 2 Distribution of vertebrate animal species used for research, testing and education

Development of laboratory animal science

The increasing demand for high-standard animal models, together with the more critical view taken of the use of animals for experimental purposes, led to the development of laboratory animal science in the 1950s. This is a multidisciplinary branch of science aimed at contributing to the quality of experiments in which animals are used and at improving their welfare. It encompasses the biology of laboratory animals, their environmental requirements, genetic and microbiological standardisation, prevention and treatment of disease, experimental techniques, anaesthesia, analgesia and euthanasia, alternatives to their use, and ethics. The guiding principles are replacement, reduction and refinement (the Three Rs), first proposed by Russell and Burch in 1959 in their book *The principles of humane experimental technique* (49).

'Replacement' means the substitution of living animals by in vitro techniques (e.g. cells, tissues), computer models and other alternative methods. 'Reduction' means a decrease of the numbers of animals used by standardising in terms of genotype and microbiological quality, and by standardisation of the experimental procedures and of the environment in terms of food and climate in the animal room. The value of statistical assessment prior to the experiment (i.e. power analysis) in order to calculate accurately the number of animals needed has also been noted. 'Refinement' means a decrease in discomfort by meeting the behavioural and physiological needs of the animal through adequate housing and husbandry, by provision of adequate anaesthesia, analgesia and care, guaranteeing the skills of the researcher/animal staff, improving experimental procedure and determining a

humane endpoint where the animal can be euthanised to prevent unnecessary suffering (1, 66).

Welfare of laboratory animals

Animal experiments should only be performed when no alternative is available and when the benefit of the experiment outweighs the suffering of the animal. When animals are used, there is a legal and moral obligation to safeguard welfare and minimise discomfort, since this is generally beneficial for both the animal and the experimental outcome. Discomfort and stress both before and during the experiment can lead to non-specific effects such as endocrinological and immunological changes, thus jeopardising results (64).

What is good animal welfare?

This is a complex issue and part of a continuing scientific and philosophical debate. One of the first definitions of welfare was published as minimal standards for farm animals in 1965 by the Brambell Committee and known as the 'five freedoms' (13):

- a) freedom from thirst, hunger and malnutrition
- b) freedom from discomfort
- c) freedom from pain, injury and disease
- d) freedom to express normal behaviour
- e) freedom from fear and distress.

It is arguable whether these five freedoms in fact represent an adequate basis for welfare. The ability of an animal to cope with the environment it is in, and thus exert control over its life, may be more important (68). This is in line with Broom and Johnson's definition of animal welfare as 'its state as regards its attempts to cope with its environment' (14). Predictability and controllability are key concepts in this respect, but some uncertainty in terms of unpredictability and uncontrollability is equally important (69). A predictable and controllable environment might lead to boredom, while total unpredictability and uncontrollability might be stressful to the animal. This means that the state of welfare can be defined as a state of balance between positive and negative experiences similar to those of the animals' wild counterparts (61).

While welfare may be considered to be a subjective experience, it has a biological function that is related to the fitness and survival of the animal, and researchers have suggested that welfare is compromised when the animal's evolutionary fitness is reduced (1, 14).

Assessment of welfare

Assessing welfare is also a complex problem, and a number of approaches have been taken to try and resolve it. The question is whether the animals are physically and psychologically healthy and whether they get what they want (for example, do they need or want more space and will their health improve if more space is provided?). In respect to health, early warning signs are obviously important. Needs can be defined as requirements, fundamental in the biology of the animal, to obtain a particular resource or respond to a particular environmental or bodily stimulus (14), whereas 'wanting' is related to an incentive motivation.

Preference tests and the behaviour of animals in correlation with their choice can provide information on what an animal wants (2, 4, 12, 21, 22, 58). Assessment of wellbeing should ideally be performed in a positive way, such as by measuring pleasure, by preference tests or by behavioural observations in the home cage. In order to measure pleasure, anticipatory behaviour expressed by an increase in activity prior to an announced reward may be a useful tool to elucidate welfare in terms of the positive and negative experiences (51, 61). Anticipatory behaviour has been described as a typical arousal with goal-directed activity that occurs in the appetitive phase when the actual reward is not yet present (e.g. food, water, sexual contact, access to enriched housing) (61).

Such tests, however, have some limitations; for example, the animal's ad hoc choice might not reflect long-term priorities, the animal may be forced to choose between non-valued commodities or the choice may be too complex. Nevertheless, when the right 'question' is asked in terms of the animal's sensory capacity, cognitive ability and natural history, it is reasonable to assume that natural selection has equipped the animal to make such choices (27). Measuring the strength of preference by examining the strength of motivation for a certain option makes preference tests even more valuable (50, 59).

Motivated behaviour together with physiological data may provide a useful indicator of animal priorities and physical health, and of the effects of the environment, husbandry and experimental procedures performed on the animal (32). Behavioural observations in the home cage can be used to study differences in the behavioural repertoire after changing the living conditions of animals, such as the provision of cage enrichment or a social partner. Animal well-being is in general related to a broad behavioural repertoire; to evaluate well-being requires a thorough knowledge of the specific behaviour and biological needs of that particular species (5).

Another way of assessing the well-being of an animal is to take the negative approach by measuring its failure to cope, leading to discomfort and/or stress, and by measuring pain. Until recent times this approach was frequently used. Behavioural, physiological and post-mortem parameters are suitable for measuring discomfort. Behavioural parameters that are utilised include abnormal behaviours such as stereotypies, posture, sudden fear or aggression, vocalising, a decrease in grooming leading to chromodacryorrhea (a red secretion around the eye and nose) in rats and mice, and activity changes. Physiological changes include weight loss, reduced food intake, diarrhoea, respiratory and cardiovascular signs, and changes in stress-hormone levels and immunological parameters (8). More recently radio-telemetry has enabled heart rate, blood pressure and body temperature to be measured in stress-free, awake and freely moving animals (34). Post-mortem parameters are valuable in assessing animal welfare retrospectively, with the results being beneficial for the surviving animals; examples of postmortem parameters include fatty deposits, organ size, infections, stomach ulcers and dehydration (8).

Environmental conditions

Housing systems for laboratory animals have often been designed on the basis of economic and ergonomic aspects (such as equipment, costs, space, work load, and ability to observe the animals and to maintain a certain degree of hygiene) with little or no concern for animal welfare. The environment of an animal, however, consists of a wide range of stimuli, including the social environment of conspecifics, contraspecifics and humans, as well as the physical environment of the cage and its contents (56).

Housing and husbandry have a major impact on the laboratory animal throughout its life, not only during, but also before and after the experiment. Traditional care and maintenance do not usually consider the species-specific needs in relation to housing and feeding regimes; yet the variability in specific needs not only differs between species but also within species due to the genetic background. While laboratory animals have partially adapted to captive life, they still show similarities to their wild counterparts (5, 11, 52). The environment of captive animals should cater for physiological and behavioural needs such as resting, nest building, hiding, exploring, foraging, gnawing and social contacts.

Moreover, many laboratory animal species such as rodents and rabbits are highly susceptible to predators, and are thus likely to show strong fear responses in unfamiliar situations if they cannot shelter. This is shown by attempts to flee, biting when handled, or sudden immobility to avoid being detected. Careful handling from a young age, together with conditioning to experimental and husbandry procedures, will probably reduce these stress responses considerably (29), and for this reason cages should be provided with shelter or hiding places. Ideally, the animal should feel secure in a complex, challenging environment that it can control (47). A sense of security can be achieved by providing nestable and manipulable nesting material, hiding places and compatible cage mates.

In practice, however, laboratory animals are usually housed throughout their lives in relatively barren cages, and given unrestricted access to food. This frequently results in adverse effects on the behaviour and physiology of the animals, and in a shortened lifespan due to overfeeding and inactivity (38, 55, 57). Standardisation of environmental conditions has been designed to reduce individual differences within animal groups (intraexperimental variation), ultimately facilitating the detection of treatment effects, and to reduce differences between studies (inter-experiment variation), ultimately increasing the reproducibility of results across laboratories (45, 66). Nevertheless, despite rigorous attempts to equalise conditions among sites, tests with different inbred mouse strains, simultaneously carried out in three recommended laboratories, revealed significant effects from their respective sites for nearly all variables tested (18, 67). It therefore seems that barren, restrictive and socially deprived housing conditions interfere with the development and function of brain and behaviour (10, 20, 48, 70), and restrictions such as those imposed by the standard rodent cage are potentially stressful (37, 39). In other words, the barren environment that has been devised to minimise uncontrolled environmental effects on the animals may ironically be a primary source of pathological artefacts.

Current thinking is that appropriate structuring of the cage/pen environment may be more beneficial than provision of a large floor area, although a certain area is necessary to provide a structured space. Except for locomotor activity (e.g. playing), animals do not actually use space, but instead use resources and structures within the area for specific behaviours. It is difficult to scientifically specify the minimum cage sizes for maintaining laboratory animals; much depends on the strain, group size and age of the animals, their familiarity with each other, and their reproductive condition. Cage sizes recommended in current European guidelines on accommodation for laboratory animals are generally based on scientific evidence; where such evidence is lacking or insufficient, they are based on what is described as best practice (17), which has been agreed upon by researchers, veterinarians and animal staff.

Environmental enrichment

One of the possible ways to improve the living conditions of laboratory animals is to give animals opportunities to perform more species-specific behavioural repertoires through providing enrichment of their environment. This can be defined as any modification in the environment of captive animals that seeks to enhance their physical and psychological well-being by providing stimuli that meet their species-specific needs (3, 43). This approach has been increasingly introduced into laboratory animal research facilities (44). From a welfare point of view, it seems to be a good development, as there is general agreement that the well-being of the animals improves with the provision of environmental enrichment. For example, beneficial effects of environmental enrichment have been described in animals with brain damage and disturbed motor function: an increased arborisation of dendrites has been found in the brains of these animals (41). The effects of environmental enrichment are dependent on the type of enrichment used. In the field of neuroscience, enrichment mainly refers to social housing in a large, complex cage containing different toys that are changed frequently in order to induce changes in the brain and behaviour. In animal welfare research, enrichment focuses on specific needs such as nest building, hiding and gnawing, in order to improve the well-being of the animals.

Enrichment of the animal's environment can be focused on both the social environment (social partners, including human beings) and the physical environment consisting of sensory stimuli (auditory, visual, olfactory and tactile) and nutritional aspects (supply and type of food). The animal's psychological appraisal of its environment in terms of controllability and predictability can be improved by structuring the cage with nest boxes, tubes, partitions and nesting material (56). Van de Weerd *et al.* (58) showed that tissue, for example, was strongly preferred by mice as a component of their cage (Fig. 3); dogs and rabbits on the other hand required a higher position in the enclosure, such as a platform or shelf, for control of their environment (28, 53) (Fig. 4). Enrichment items need to be designed and evaluated on the basis of knowledge gained in enrichment studies (65). Besides meeting the needs of the animal, enrichment items should be practical and inexpensive, and pose no risk to humans, the animals used or the experiment. There is some concern, however, as to whether environmental enrichment conflicts with the standardisation of experiments. The question is: 'Do enriched animals show more variability in their response to experimental procedures because they show more diverse behaviours?' Some researchers think they do. In complex environments, for example, animals are not just responding to one stimulus but to many variable stimuli at once, and this can result in increased variation among subjects (24).

The counter-argument is that because an animal can perform more of its species-specific behaviour in enriched environments, it may be able to cope better with novel and unexpected changes and thus in fact show a more uniform response. If animals from enriched housing conditions are therefore likely to be physiologically and psychologically more stable, it follows that they may be considered as more refined models and so ensure better scientific results. In practice, however, results from different studies seem to indicate that the effects of enrichment on the variability in results depend on the parameter being measured, the strain of animal and the type of enrichment (60).

It seems clear, therefore, that environmental enrichment should comprise a well-designed and critically evaluated programme that benefits the animals as well as the experimental outcome; it should not be a process of randomly supplying objects that staff consider attractive for the animals. Enrichment needs to be regarded as an essential component of the overall animal care programme, and just as important as nutrition and veterinary care. The key component of the enrichment programme is the animal-care staff, whose members must be motivated and educated (54).



Fig. 3 Paper nesting material as cage enrichment for laboratory mice *Photo:* T.P. Rooymans



Fig. 4 Platforms and toys as cage enrichment for laboratory dogs Photo: Novo Nordisk A/S, Denmark

In summary, evaluating enrichment in terms of the animal (i.e. by assessing the use of and preference for a certain enrichment, and the effect on behaviour, the performance of species-typical behaviour and physiological parameters) is essential. Equally important is evaluating the impact of enrichment on the scientific outcome. In practice, results from different studies seem to indicate that the effect on the outcome will depend on the parameter measured, the type of enrichment used and the strain of the animal (7, 58).

Legislative aspects of housing and care of laboratory animals

Specifications of housing of laboratory animals in Europe are given in two documents issued in 1986. One is the European Convention for the protection of vertebrate animals used for experimental and scientific purposes (Convention ETS 123) from the Council of Europe, with its Appendix A: Guidelines for the accommodation and care of animals (Council of Europe 1986) (15). The other (25) is the similar European Union Council Directive on the approximation of laws, regulations and administrative provisions of the Member States regarding the protection of animals used for experimental and other scientific purposes (Directive 86/609/EEC), with its Annex II: Guidelines for the care of animals. Article 5.1 of the Convention requires that 'Any restriction on the extent to which an animal can satisfy its physiological and ethological needs shall be limited as far as practicable', while Article 5b of the Directive requires such restrictions to 'be limited to the absolute minimum'.

These guidelines are based mainly on empirical considerations and are now under revision. Space regulations should allow housing of gregarious animals in harmonious groups. Increasing the complexity of the cage is more important than increasing floor area as such, as the inclusion of structures will provide more opportunity for activity and will increase useable space (16). In the United States of America (USA), guidelines on accommodation and care of laboratory animals are included in the 'Guide for the care and use of laboratory animals of the National Research Council' (42), although mice, rats and birds do not come under the official legislation in the USA. The use of environmental enrichment to improve the well-being of laboratory animals is widely promoted and is currently incorporated in European legislation (17, 33). Many other countries have similar legislation and regulations on this matter.

Other organisations besides governments are involved in developing guidelines and regulations on animal use. In Europe the 2001 position paper of the European Science Foundation, an association of the major science-funding organisations, endorsed the principles of the Three Rs and the need for laboratory animal welfare research. The aims of the European Science Foundation are to advance the cooperation of scientists in Europe, to promote the mobility of researchers and to advise national member organisations on science policy issues (26).

Future trends

Two specific trends that affect the way laboratory animals are used are briefly discussed. They are the use of genetically modified animals and of individually ventilated cage systems.

Genetically modified animals

Genetically engineered or modified mice are those with induced mutations, including mice with transgenes, with targeted mutations (knockouts) and with retroviral, proviral or chemically induced mutations (19, 31, 62). Transgenic technology focuses on the introduction or exclusion (knockouts) of functional gene material in the germline of an animal, thus changing the genetic characteristics of an organism and its progeny. The most frequently used methods for genetic transformation of the germline are microinjection of deoxyribonucleic acid (DNA) into the pronucleus of fertilised oocytes, and the injection of transfected embryonic stem cells into normal mouse blastocysts resulting in a subsequent generation of chimaeras. These techniques have led to the rapid development of a variety of animal models designed for the study of gene regulation, gene expression, pathogenesis and treatment of human and animal diseases (e.g. Alzheimer's disease, growth hormone disturbances, poliovirus vaccine testing in humans and mastitis in cows).

The process of transgenesis by microinjection may itself compromise welfare, as the donor animals, vasectomised males and foster mothers needed for the production of the offspring may experience discomfort from procedures such as early mating (from three weeks onwards), anaesthesia, surgery and injections. Furthermore, at the level of integration of the microinjected DNA into the genome, unintentional mutations that lead to welfare problems may occur. Detrimental side effects may result when the newly introduced gene expresses itself, as in the example of the giant mouse with an overproduction of growth hormone and suffering from chronic kidney and liver dysfunction (46). The presence of both functional and non-functional microinjected DNA has been shown to increase the body weight and mortality of mouse pups in the first two to three days after birth, although no significant differences in behaviour or morphological development were observed during later stages of development (62).

Yet another concern is the increase in the use of genetically modified animals (e.g. an increase in numbers of mice used per year of more than 23%). This is not only due to an increase in numbers used in research but also to an increase in the numbers of mice necessary to create each genetically modified line. Non-transgenic and wild-type littermates that are suitable neither for research nor for further breeding may also result (23). Ethical concerns have been raised suggesting that the integrity of such animals has been compromised; additional comment has been made with respect to the patentability of transgenic animals such as the oncomouse.

While these issues are important, transgenic technology has great potential for increasing the understanding of the role of genes, and may produce suitable models for the study of human and animal diseases. The rewards and possibilities for those working in this field are enormous. However, the science is still at an early stage and the welfare implications for the animals have to be carefully monitored, at least until the second generation of offspring (19, 40, 62, 63). A surveillance system (e.g. score sheets) can be helpful in identifying welfare problems. Humane endpoints should be established in order to euthanise severely affected animals (8, 40, 63). Data banks from existing genetically modified animals will be useful to help predict potential impairments in new genetically modified lines yet to be created.

Individually ventilated cage systems

Individually ventilated cage (IVC) systems, which were first used 30 years ago, are now in favour, especially for housing transgenic rodents. Typically, each cage can be ventilated with 25 to 120 air changes per hour, with the air blown into the cage at relatively high speed (Fig. 5). The advantages of the system are the improved protection of the animals against micro-organisms at cage level, protection of the animal-care staff against allergens, the improved microclimate and the reduced need for cage



Fig. 5 Individually ventilated cage rack Photo: T.P. Rooymans

cleaning. However, health monitoring and inspection of the animals can be difficult when such systems are used, as can any necessary procedures and cage cleaning, and the high intra-cage ventilation rate could induce chronic stress and heat loss with the draught (9, 35). This has the potential to affect welfare and must be considered. In respect of rats for example, their physiology and behaviour were not affected when there were fewer than 80 air changes per hour (35); with mice, the location of the air supply in their cage, the ventilation rate and the presence of nesting material were important when considering the impact on their well-being (9). When introducing IVC systems in the animal facility, attention should be paid to the provision of nesting material, inspection and to handling of the animals in cases where cage cleaning is limited.

Conclusions

The scientific study of the welfare of laboratory animals has resulted in useful findings, but often has simultaneously generated conflicting results. No single parameter can yet be conclusive for the assessment of welfare; multiple parameters need to be investigated in order to be able to interpret implications for the animals (5, 30, 36).

With regard to environmental conditions, it is advisable to focus on specific needs of the animals and to implement relatively simple enrichment, which is preferably standardised for each species. This approach will influence variability much less than complex cages such as those used in neuroscience research where the objective has been to induce changes in the brain and in learning and memory abilities. Even when enrichment increases variation within the experimental study, it is important not to overstate this, but instead balance this variation against the improved well-being of the animals.

More data are needed to provide information related to the effects of specific enrichment programmes on the animal, on specific animal species, strains and models, and on experimental results.

It is very important to describe the type of enrichment sufficiently in the Material and Methods section of scientific publications to ensure the reproducibility of experimental results. Only then can the controls and variables in the scientific experiment be accurately defined and measured.

As animal welfare is a prerequisite for reliable experimental results, it is essential to seek for methods and procedures that will improve the well-being of the animals. Animal welfare and good science are inextricably connected.

Évaluation scientifique du bien-être animal appliquée aux animaux de laboratoire

V. Baumans

Résumé

L'utilisation des animaux en recherche expérimentale va de pair avec l'essor de la médecine, dont l'origine remonte à la Grèce antique. La demande accrue de modèles animaux de grande qualité conjuguée à la critique des conditions d'utilisation des animaux ont conduit à la création d'une science pluridisciplinaire désormais connue sous le nom de « science des animaux de laboratoire ». Les principes directeurs en sont le remplacement, la réduction et le raffinement (les « trois R », énoncés par Russell et Burch en 1959. Les personnes qui manipulent les animaux sont tenues de préserver leur bien-être et de réduire autant que possible le désagrément occasionné ; en général, cette attitude aura des effets positifs tant sur l'animal que sur les résultats de l'expérimentation.

La capacité d'un animal à s'adapter à l'environnement et à exercer un contrôle sur son existence semble être capitale pour son bien-être. Cet article est axé sur l'évaluation du bien-être, les facteurs environnementaux qui ont une incidence sur le bien-être, les exigences réglementaires et les tendances futures telles que la production et l'utilisation d'animaux génétiquement modifiés.

Mots-clés

Animal de laboratoire – Animal génétiquement modifié – Bien-être – Cage ventilée – Enrichissement environnemental – Science des animaux de laboratoire – Trois R.

Evaluación por métodos científicos del bienestar de los animales de laboratorio

V. Baumans

Resumen

El uso de animales en la investigación experimental va en paralelo al desarrollo de la medicina, cuyas raíces se hunden en la Grecia clásica. La creciente demanda de modelos animales de calidad, junto con las críticas vertidas sobre el modo en que se utilizan los animales, ha llevado a la aparición de una rama multidisciplinar de la ciencia denominada 'ciencia de los animales de laboratorio' que se rige por tres principios cardinales: la sustitución, la reducción y el perfeccionamiento (o "tres erres" por sus iniciales en inglés: '*replacement, reduction, refinement*'), formulados por Russell y Burch en 1959. Quien utiliza animales tiene la obligación de proteger su bienestar y causarles el menor sufrimiento posible, lo que además suele ser positivo para el propio proceso experimental.

Desde el punto de vista del bienestar, la capacidad del animal para reaccionar a las condiciones de su entorno y ejercer el control de su vida parece revestir una importancia capital. El autor se centra en la evaluación del grado de bienestar,

los factores ambientales que influyen en él, los requisitos legislativos y las tendencias que se anuncian para el futuro, como son la obtención y utilización de animales modificados genéticamente.

Palabras clave

Animal de laboratorio – Animal modificado genéticamente – Bienestar – Ciencia de los animales de laboratorio – Enriquecimiento del medio – Jaula con ventilación individual – Tres erres.

References

- 1. Barnard C.J. & Hurst J.L. (1996). Welfare by design: the natural selection of welfare criteria. *Anim. Welf.*, **5**, 405-434.
- Baumans V. (1997). Environmental enrichment: practical applications. *In* Animal alternatives, welfare and ethics (L.F.M. Van Zutphen & M. Balls, eds). Elsevier Science BV, Amsterdam, 187-191.
- Baumans V. (2000). Environmental enrichment: a right of rodents! *In* Progress in the reduction, refinement and replacement of animal experimentation (M. Balls, A.M. Van Zeller & M.E. Halder, eds). Elsevier, Amsterdam, 1251-1255.
- Baumans V. (2004). Methods for evaluation of laboratory animal well-being. *Alternatives Lab. Anim.*, **32** (Suppl. 1), 161-162.
- Baumans V. (2004). The welfare of laboratory mice. *In* The welfare of laboratory animals (E. Kaliste, ed.). Animal welfare and nutrition series. Kluwer Academic Publishers, Dordrecht, 119-152.
- 6. Baumans V. (2004). Use of animals in experimental research: an ethical dilemma? *Gene Therapy*, **11** (Suppl. 1), 64-66.
- Baumans V. (2005). Environmental enrichment for laboratory rodents and rabbits: requirements of rodents, rabbits and research. *In* Enrichment strategies for laboratory animals. *ILAR Journal*, 46 (2), 162-170.
- Baumans V., Brain P.F., Brugère H., Clausing P., Jeneskog T. & Perretta G. (1994). – Report of the FELASA Working Group on pain and distress. *Lab. Anim.*, 28 (2), 97-112.
- Baumans V., Schlingmann F., Vonck M. & Van Lith H.A. (2002). – Individually ventilated cages: beneficial for mice and man? *Contemp. Top. Lab. Anim. Sci.*, **41** (1), 13-19.
- Benefiel A.C. & Greenough W.T. (1998). Effects of experience and environment on the developing and mature brain: implications for laboratory animal housing. *ILAR Journal*, **39** (1), 5-11.
- Berdoy M. (2002). The laboratory rat: a natural history. Oxford University, Oxford. Website: www.ratlife.org (accessed on 13 July 2005).

- Blom H.J.M., Van Vorstenbosch C.J.A.V.H., Baumans V., Hoogervorst M.J.C., Beynen A.C. & Van Zutphen L.F.M. (1992). – Description and validation of a preference test system to evaluate housing conditions for laboratory mice. *Appl. anim. Behav. Sci.*, **35** (1), 67-82.
- Brambell Committee (1965). Report of the Technical Committee to enquire into the welfare of animals kept under intensive livestock husbandry systems. Command paper 2836. Her Majesty's Stationary Office, London.
- 14. Broom D.M. & Johnson K.G. (1993). Stress and animal welfare. Animal behaviour series. Chapman and Hall, London, 74.
- Council of Europe (CoE) (1986). European Convention for the Protection of Vertebrate Animals used for experimental and other scientific purposes (ETS 123). CoE, Strasbourg.
- 16. Council of Europe (CoE) (1997). Resolution on the accommodation and care of laboratory animals. Adopted by the Multilateral Consultation, 30 May, Strasbourg. CoE, Strasbourg. Website: www.coe.int/T/E/Legal_Affairs/Legal_ cooperation/Biological_safety%2C_use_of_animals/Laborator y_animals/Res%20accommodation.asp (accessed on 13 July 2005).
- 17. Council of Europe (CoE) (in preparation). Revision of Appendix A, European Convention for the protection of vertebrate animals used for experimental and other scientific purposes (ETS 123). CoE, Strasbourg. Website: www.coe.int/T/E/Legal_affairs/Legal_cooperation/Biological_ safety%2C_use_of_animals/Laboratory_animals/draft%20rev ision%20of%20Appendix%20A.asp (accessed on 19 July 2005).
- Crabbe J.C., Wahlsten D. & Dudek B.C. (1999). Genetics of mouse behavior: interactions with laboratory environment. *Science*, 284 (5420), 1670-1672.
- Crawley J.N. (1998). What's wrong with my mouse? Behavioral phenotyping of transgenic and knockout mice. Wiley-Liss, New York.
- Cummins R.A., Livesey P.J. & Evans J.G.M. (1977). A developmental theory of environmental enrichment. *Science*, 197 (4304), 692-694.

- Dawkins M.S. (1990). From an animal's point of view: motivation, fitness and animal welfare. *Behav. Brain Sci.*, 13, 1-9.
- Dawkins M.S. (2004). Using behaviour to assess animal welfare. *In* Proc. Universities Federation for Animal Welfare (UFAW) International Symposium: science in the service of animal welfare, 2-4 April 2003, Edinburgh. *Animal Welfare*, 13 (supplement).
- Dennis M.B. Jr (2002). Welfare issues of genetically modified animals. *ILAR Journal*, 43 (2), 100-109.
- 24. Eskola S., Lauhikari M., Voipio H.M., Laitinen M. & Nevalainen T. (1999). Environmental enrichment may alter the number of rats needed to achieve statistical significance. *Scand. J. Lab. Anim. Sci.*, **26** (3), 134-144.
- 25. European Commission (1986). Directive on the approximation of laws, regulations and administrative provisions of the Member States regarding the protection of vertebrate animals used for experimental and other scientific purposes (86/609/EEC). European Commission. Off. J. Eur. Union, L 358, 18/12/1986, 1-28. Website: europa.eu.int/comm/food/fs/aw/aw_legislation/scientific/86-609-eec_en.pdf (accessed on 13 July 2005).
- European Science Foundation (2001). Use of animals in research. ESF Policy Briefing, 15, 1-6. Website: www.esf.org/ publication/115/ESPB15.pdf (accessed on 13 July 2005).
- 27. Fraser D. (1996). Preference and motivational testing to improve animal well-being. *Lab. Anim.*, **25** (1), 27-31.
- Hubrecht R.C. (2002). Comfortable quarters for dogs in research institutions. *In* Comfortable quarters for laboratory animals (V. Reinhardt & A. Reinhardt, eds), 9th Ed. Animal Welfare Institute, Washington, 56-64.
- Hurst J.L. (1999). Introduction to rodents. *In* UFAW handbook on the care and management of laboratory animals (T. Poole, ed.), vol. 1, 7th Ed. Blackwell Science Ltd, Oxford, 262-273.
- Hurst J.L., Barnard C.J., Tolladay U., Nevison C.M. & West C.D. (1999). – Housing and welfare in laboratory rats: effects of cage stocking density and behavioural predictors of welfare. *Anim. Behav.*, 58 (3), 563-586.
- Jackson Laboratory (2005). Mouse Genome Informatics. Website: www.informatics.jax.org/ (accessed on 5 August 2005).
- King L.A. (2003). Behavioral evaluation of the psychological welfare and environmental requirements of agricultural research animals: theory, measurement, ethics and practical implications. *ILAR Journal*, 44 (3), 211-221.
- Kornerup Hansen A. & Baumans V. (2004). Housing, care and environmental factors. *In* The welfare of laboratory animals – book series Animal welfare and nutrition (E. Kaliste, ed.). Kluwer Academic Publishers, Dordrecht, 37-50.
- Kramer K. (2000). Applications and evaluation of radiotelemetry in small laboratory animals. PhD thesis, Utrecht University, Utrecht.

- 35. Krohn T.C. (2002). Method developments and assessments of animal welfare in IVC systems. Thesis, The Royal Veterinary and Agricultural University, Frederiksberg. Website: www.scanbur-bk.com/products/PDF%20Folder/ PHDreport.pdf (accessed on 13 July 2005).
- Ladewig J. (1998). Behaviour of laboratory animals under unnatural conditions. Arch. Toxicol., 20 (Suppl.), 41-46.
- Ladewig J. (2000). Chronic intermittent stress: a model for the study of long-term stressors. *In* The biology of animal stress (G.P. Moberg & J.A. Mench, eds). CAB International, Wallingford, 159-169.
- Mattson M.P., Duan W., Lee J. & Guo Z. (2001). Suppression of brain aging and neurodegenerative disorders by dietary restriction and environmental enrichment: molecular mechanisms. *Mechan. Ageing Dev.*, **122** (7), 757-778.
- 39. Mench J.A. (1998). Why it is important to understand animal behaviour. *ILAR Journal*, **39** (1), 20-26.
- Mertens C. & Rülicke T. (2000). A comprehensive form for the standardized characterization of transgenic rodents: genotype, phenotype, welfare assessment, recommendations for refinement [in German]. *ALTEX*, 17, 15-21.
- Mohammed A.H., Zhu S.W., Darmopil S., Hjerling-Leffler J., Ernfors P., Winblad B., Diamond M.C., Eriksson P.S. & Bogdanovic N. (2002). – Environmental enrichment and the brain. *In* Progress in brain research (M.A. Hofman, G.J. Boer, A.J.G.D. Holtmaat, E.J.W. Van Someren, J. Verhaagen & D.F. Swaab, eds), vol. 138. Elsevier Science BV, Amsterdam, 109-133.
- National Research Council (1996). Guide for the care and use of laboratory animals, 7th Ed. National Academy Press, Washington, DC, 140 pp. Website: www.nap.edu/ readingroom/books/labrats/ (accessed on 13 July 2005).
- Newberry R.C. (1995). Environmental enrichment: increasing the biological relevance of captive environments. *Appl. anim. Behav. Sci.*, 44, 229-243.
- Olsson A.S. & Dahlborn K. (2002). Improving housing conditions for laboratory mice: a review of environmental enrichment. *Lab. Anim.*, **36** (3), 243-270.
- 45. Olsson A.S., Nevison C.M., Patterson-Kane E.G., Sherwin C.M., Van de Weerd H.A. & Würbel H. (2003). – Understanding behaviour: the relevance of ethological approaches in laboratory animal science. *Appl. anim. Behav. Sci.*, **81**, 245-264.
- Poole T.B. (1995). Welfare considerations with regard to transgenic animals. Anim. Welf., 4, 81-85.
- Poole T.B. (1998). Meeting a mammal's psychological needs: basic principles. *In* Second nature: environmental enrichment for captive animals (D.J. Shepherdson, J.D. Mellen & M. Hutchins, eds). Smithsonian Institution Press, Washington, 83-96.
- 48. Renner M.J. & Rosenzweig M.R. (1987). Enriched and impoverished environments. Springer-Verlag, New York.

- 49. Russell W.M.S. & Burch R.L. (1959). The principles of humane experimental technique. Methuen, London.
- Sherwin C.M. & Nicol C.J. (1997). Behavioural demand functions of caged laboratory mice for additional space. *Anim. Behav.*, 53, 67-74.
- 51. Spruijt B.M., Van den Bos R. & Pijlman F.T. (2001). A concept of welfare based on reward evaluating mechanisms in the brain: anticipatory behaviour as indicator for the state of reward systems. *Appl. anim. Behav. Sci.*, **72** (2), 145-171.
- 52. Stauffacher M. (1995). Environmental enrichment, fact and fiction. *Scand. J. Lab. Anim. Sci.*, **22**, 39-42.
- 53. Stauffacher M. (2000). Refinement in rabbit housing and husbandry. In Progress in the reduction, refinement and replacement of animal experimentation, developments in animal and veterinary sciences (M. Balls, A.M. Van Zeller & M. Halder, eds). Elsevier Science BV, Amsterdam, 1269-1277.
- 54. Stewart K.L. & Bayne K. (2004). Environmental enrichment for laboratory animals. *In* Laboratory animal medicine and management, international veterinary information service, B2520.0404 (J.D. Reuter & M.A. Suckow, eds). International Veterinary Information Service, Ithaca, New York.
- 55. Van de Weerd H.A., Baumans V., Koolhaas J.M. & Van Zutphen L.E.M. (1994). Strain specific behavioural response to environmental enrichment in the mouse. J. experim. anim. Sci., 36 (4-5), 117-127.
- Van de Weerd H.A. & Baumans V. (1995). Environmental enrichment in rodents. *In* Environmental enrichment information resources for laboratory animals. *AWIC Resour. Series*, 2, 145-149.
- 57. Van de Weerd H.A., Van Loo P.L.P., Van Zutphen L.E.M., Koolhaas J.M. & Baumans V. (1997). – Nesting material as environmental enrichment has no adverse effects on behavior and physiology of laboratory mice. *Physiol. Behav.*, 62 (5), 1019-1028.
- Van de Weerd H.A., Van Loo P.L.P., Van Zutphen L.E.M., Koolhaas J.M. & Baumans V. (1997). – Preferences for nesting material as environmental enrichment for laboratory mice. *Lab. Anim.*, **31** (2), 133-143.
- Van de Weerd H.A., Van Loo P.L.P., Van Zutphen L.F.M., Koolhaas J.M. & Baumans V. (1998). – Strength of preference for nesting material as environmental enrichment for laboratory mice. *Appl. anim. Behav. Sci.*, **55**, 369-382.

- Van de Weerd H.A., Aarsen E.L., Mulder A., Kruitwagen C.L.J.J., Hendriksen C.F.M. & Baumans V. (2002). – Effects of environmental enrichment for mice: variation in experimental results. *J. appl. Anim. Welf. Sci.*, 5 (2), 87-109.
- 61. Van der Harst J. (2003). Tools to measure and improve welfare of laboratory rats: reward-related behaviour and environmental enrichment. Thesis, Utrecht University, Utrecht.
- Van der Meer M., Baumans V., Olivier B. & Van Zutphen L.F.M. (2001). – Impact of transgenic procedures on behavioral and physiological responses in post-weaning mice. *Physiol. Behav.*, **73**, 133-143.
- Van der Meer M., Rolls A., Baumans V., Olivier B. & Van Zutphen L.F.M. (2001). – Use of score sheets for welfare assessment of transgenic mice. *Lab. Anim.*, 35 (4), 379-389.
- 64. Van Herck H., Baumans V. & De Boer S.F. (1994). Assessment of discomfort in laboratory animals. *In* Autoimmune disease models, a guidebook (J. Cohen & A. Miller, eds). Academic Press, New York, 303-320.
- Van Loo P.L.P., Blom H.J.M., Meijer M.K. & Baumans V. (2005). – Assessment of the use of two commercially available enrichments by laboratory mice by preference testing. *Lab. Anim.*, **39** (1), 58-67.
- 66. Van Zutphen L.F.M. (2001). History of animal use. In Principles of laboratory animal science (L.F.M. Van Zutphen, V. Baumans & A.C. Beynen, eds). Elsevier, Amsterdam, 2-5.
- 67. Wahlsten D., Metten P., Phillips T.J., Boehm S.L. II, Burkhart-Kasch S., Dorow J., Doerksen S., Downing C., Fogarty J., Rodd-Henricks K., Hen R., McKinnon C.S., Merrill C.M., Nolte C., Schalomon M., Schlumbohm J., Sibert J.R., Wenger C.D., Dudek B.C. & Crabbe J.C. (2003). Different data from different labs: lessons from studies in gene-environment interaction. J. Neurobiol., 54 (1), 283-311.
- Webster J. (1994). Animal welfare: a cool eye towards Eden. Blackwell Science Ltd, Oxford.
- 69. Wiepkema P.R. & Koolhaas J.M. (1993). Stress and animal welfare. *Anim. Welf.*, **2**, 195-218.
- Würbel H. (2001). Ideal homes? Housing effects on rodent brain and behaviour. *Trends Neurosci.*, 24 (4), 207-211.