Environmental Enrichment for Laboratory Rodents and Rabbits: Requirements of Rodents, Rabbits, and Research

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Abstract

Environmental conditions such as housing and husbandry have a major impact on the laboratory animal throughout its life and will thereby influence the outcome of animal experiments. However, housing systems for laboratory animals have often been designed on the basis of economic and ergonomic aspects. One possible way to improve the living conditions of laboratory animals is to provide opportunities for the animals to perform a species-specific behavioral repertoire. Environmental enrichment should be regarded both as an essential component of the overall animal care program and equally important as nutrition and veterinary care. The key component of an enrichment program is the animal staff, whose members must be motivated and educated. It is critically important to evaluate environmental enrichment in terms of the benefit to the animal by assessing the use of and preference for a certain enrichment, the effect on behavior and the performance of species-typical behavior, and the effect on physiological parameters. At the same time, it is necessary to evaluate the impact on scientific outcome, how the enrichment influences the scientific study, and whether and how the statistical power is affected. The result will depend on the parameter measured, the type of enrichment used, and the animal strain. In this article, goals of enrichment are defined and discussed. Animal behaviors and needs are described, along with the translation of those needs into environmental enrichment programs. Specific types of environmental enrichment are outlined with examples from the literature, and an evaluation of environmental enrichment is provided.

Key Words: animal needs; environmental enrichment; housing; rabbits; rodents; variation

Introduction

Environmental conditions such as housing and husbandry have a major impact on the laboratory animal throughout its life and will thereby influence the outcome of animal experiments (Baumans 2004; Van de Weerd et al. 2002). However, housing systems for laboratory animals have often been designed on the basis of economic and ergonomic aspects (e.g., equipment, costs, space, workload, ability to observe the animals, and ability to maintain a certain degree of hygiene) with little or no consideration for animal welfare (Baumans 1997, 2004; Olsson et al 2003; Van de Weerd et al. 1997a,b). The traditional care and maintenance of laboratory animals does not usually include species-specific needs in relation to their environment. The variability in the specific needs, however, is different not only between species but also, due to variability in the genetic background, among strains of a species (Van de Weerd et al. 1994).

Behavioral Repertoire of Rodents and Rabbits

Laboratory rodents and rabbits have partially adapted to captive life, but still reveal similarities to their wild counterparts (Baumans 2004; Berdoy 2002; Stauffacher 1995). For that reason, the environment of the laboratory animal should accommodate innate physiological and behavioral needs such as social contacts, resting, nest building, hiding, exploring, foraging, and gnawing. For example, rodents and rabbits are very susceptible to predators and are thus likely to show strong fear responses in unfamiliar situations when they cannot find shelter. Examples of this behavior include attempts to flee, to bite when handled, or to become suddenly immobile to avoid being detected. Ideally, the animal should feel secure in a complex, challenging environment that it can control (Poole 1998).

One possible way to improve the living conditions of laboratory animals is to provide opportunities for the animals to perform a species-specific behavioral repertoire. Opportunities may result from providing environmental enrichment, which can be defined as follows: any modification in the environment of captive animals that seeks to enhance its physical and psychological well-being by providing stimuli meeting the animals' species-specific needs (Baumans 2000; Newberry 1995). Environmental enrichment applies to heterogeneous methods of improving animal welfare and includes everything from social companionship to toys (Young 2003). Environmental enrichment can influence the animal's behavior, physiology, and brain anatomy. For example, Hebb (1947) showed that rats from enriched environments were better able to solve problems in the "Hebb-Williams maze." Animals that have been kept in

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enriched captive environments have improved learning abilities; increased cortical thickness and weight; increased size, number, and complexity of nerve synapses; and a higher ratio of RNA to DNA (Renner and Hackett Renner 1993; Shepherdson 1998; Widman et al. 1992). In this context, environmental enrichment is used as an experimental tool in neurobehavioral research.

Environmental enrichment programs were initially developed in zoos in an effort to enhance the environment of captive animals (Shepherdson 1998). The use of environmental enrichment to improve the well-being of laboratory animals is widely promoted and is currently incorporated in European legislation (Council of Europe, Revision of Appendix A, ETS 123, in preparation).

Goals of Environmental Enrichment

The goals of environmental enrichment are to improve the quality of the captive environment so that the animal has a greater choice of activity and some control over its social and spatial environment (Newberry 1995; Stauffacher 1995). Enrichment should pose no risks to the animals (i.e., cause injuries or excessive aggression), to the humans (i.e., jeopardize the health and safety of the animal staff), or to the experiments (i.e., cause undesirable interference or an excessive increase in the number of animals used). The design of enrichment items should be based on knowledge of behavioral needs and data available from enrichment studies and should be scientifically tested prior to marketing and implementation (Van Loo et al. 2004).

The implementation of specific environmental enrichment approaches should be based on the following important tenets:

- Improving the quality of the captive environment so that the animal has a greater choice of activity and some control over its social and spatial environment (Newberry 1995; Stauffacher 1995);
- Increasing behavioral diversity;
- Reducing the frequency of abnormal behavior;
- Increasing positive utilization of the environment;
- Increasing the animal's ability to cope with challenges (Young 2003).

Environmental enrichment has been introduced increasingly into laboratory animal research facilities (Olsson and Dahlborn 2002). From a welfare perspective, this increase appears to be a positive development because it is generally accepted that the animal's well-being improves with the provision of environmental enrichment.

When animals are deprived of the possibility to perform species-specific behavior, they may show signs of suffering such as behavioral disorders, chronic stress, or other pathological conditions (Würbel et al. 1996). It has been shown that barren, restrictive, and socially deprived housing conditions interfere with the development and function of brain and behavioral functions. Beneficial effects of environmental enrichment have been described in animals with brain damage and disturbed motor function, and an increased arborization of dendrites in the brain has been identified (Mohammed et al. 2002). In addition, investigators have observed genetic differences between mouse strains that have been obscured by standard laboratory rearing compared with enriched environments (Henderson 1970; Würbel 2001).

Animal Needs

Animals have physiological and behavioral needs. Physiological needs include eating, drinking, and sleeping, and should logically include some provision of shelter. Behavioral needs include performing behavior necessary for the maintenance of a normal physiological and psychological state (Poole 1998), such as social behavior, exploration, foraging, grooming, digging, nest building, and seeking shelter. These behaviors, which are performed by rodents and rabbits in the wild as well as in captivity, may be considered essential innate behaviors.

To assess the needs of animals and identify what they want in their environment, it is necessary to know and understand the animal's natural behavior. Studying the behavior of ancestors of current laboratory rodents that still live in the wild provides a good starting point (Baumans 2004; Berdoy 2002; Sörensen 2004); however, not all naturally occurring behaviors or all aspects of life in a natural environment are desirable or necessary in the laboratory. Nevertheless, it is generally agreed that environmental enrichment is beneficial for the well-being of laboratory animals and that it should be applied whenever appropriate or practical (Kaliste and Mering 2004). It is not so much a matter of bringing natural behaviors into the laboratory as it is of bringing crucial features of the environment into the laboratory so that natural behaviors may be expressed and reinforced (Blanchard and Blanchard 2003).

Translation of Animal Needs into Environmental Enrichment Programs

To assess the preference of an animal for a certain feature, one can use well-designed choice tests (Blom et al. 1995; Van de Weerd et al. 1997a; Van Loo et al. 2001) preferably combined with consumer-demand tests, which show how much an animal is willing to "pay" for that feature. This method provides a way of prioritizing animal needs (Dawkins 1983; Mason et al. 2001; Sherwin and Nicol 1997). Enrichment programs should focus on behavior that is strongly motivated, such as social behavior, foraging, nest building and exploring.

The key component of an enrichment program is the animal staff, whose members must be motivated, educated, and empowered to implement the enrichment program

(Baumans 1997; Young 2003). Including the input of participating animal caregivers and researchers is recommended in establishing an enrichment group within an institution, along with management representatives, veterinarians, and invited outside experts (Stewart and Bayne 2004; Young 2003). Enrichment should be regarded both as an essential component of the overall animal care program and equally important as nutrition and veterinary care. All decisions related to enrichment should be included in the standard operating procedures of the institution (Stewart 2004) to standardize the approaches and to facilitate acceptance by researchers, animal staff, and management. Moreover, records and/or databases should be established with data to support the introduction and evaluation of different types of enrichment.

Types of Environmental Enrichment

Environmental enrichment should comprise a well-designed and critically evaluated program that benefits the animals as well as the experimental outcome. It should not be a process of randomly applying objects that staff consider attractive for the animals. The types of enrichment, typically categorized as social and physical enrichment, are described below (Van de Weerd and Baumans 1995; Young 2003).

Social Enrichment

Social enrichment includes socialization of animals both in contact and not in contact (termed "noncontact") with conspecifics and/or contraspecifics, including humans. The respective descriptions appear below.

Social Contact Enrichment

Gregarious species should be housed in groups or in pairs with conspecifics. Ideally, animals housed together should be littermates, but this arrangement might not be possible in the majority of cases due to group size and possible bias in the study. However, the group composition should be stable and harmonious (Love 1994; Morton et al. 1993; Stauffacher, 1997a; Turner et al. 1997), although it may be necessary to provide visual barriers or hiding places to minimize aggression (Stauffacher 1997b, 2000; Van de Weerd and Baumans 1995; Van Loo et al. 2002). Even in harmonious groups, it is necessary to allow individuals to initiate contact by approach or to avoid contact by withdrawal from sight.

For social animals, a social partner is the most challenging enrichment factor. Whereas enrichment objects are static and of interest only for specific activities, a social partner always creates new and unpredictable situations to which the animal must react. A social partner leads to an increase of alertness and exploratory behavior and provides diversion, occupation, and probably also some feelings of "security" in stable harmonious groups (Stauffacher 2000).

Procedure-induced stress-like responses are less frequent and of shorter duration in group-housed rats than in those housed singly (Sharp et al. 2002, 2003). In 1997, the Multilateral Consultation of the Council of Europe adopted a resolution related to the accommodation and care of laboratory animals, which specified that "group housing, even pair housing, is preferable to individual housing for all gregarious species normally manifesting social behavior, as long as the groups are stable and harmonious" (Council of Europe 1997) For other important guidelines, according to the *Guide for the Care and Use of Laboratory Animals* (NRC 1996), "Animals should be housed with a goal of maximizing species-specific behaviors and minimizing stress-induced behaviors. For social species, this normally requires housing in compatible pairs or groups" (p. 22). Finally, contact with humans (e.g., handling, training, and socializing) usually benefits both the animals and the outcome of experiments because it engages the animal on a cognitive level and allows positive interaction with animal caretakers, technicians, and scientists (Baumans 2004; Shepherdson 1998; Van de Weerd and Baumans 1995).

Social Noncontact Enrichment

Social noncontact enrichment includes visual, auditory, and olfactory communication with conspecifics or contraspecifics (e.g., through bars or mesh). In the resolution of the Council of Europe on the accommodation and care of laboratory animals, it is stated that when group housing is not possible, "consideration should be given to accommodating conspecifics within sight, sound or smell of one another" (Council of Europe 1997; NRC 1996). However, it should be noted that this approach might be aversive to animals when they are exposed to these stimuli without the possibility of escaping.

Physical Enrichment

Physical enrichment includes complex enclosures and both sensory and nutritional stimuli. These sources of enrichment are described briefly below.

Complexity

Appropriate structuring of the cage/pen environment is typically more beneficial than provision of a larger floor area; however, a minimum floor 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 an area for specific behaviors. Most rodents and rabbits attempt to divide their living space into separate areas for feeding, resting, and excretion. The divisions also allow the animals to control their environment, including light levels (Baumans 1997, 1999; Blom

1993; Manser et al. 1998; Sherwin 1997; Stauffacher 1997a; Townsend 1997). Such divisions may be facilitated by structures within the cage (e.g., shelters, nest boxes, nesting material, tubes, and platforms that provide withdrawal areas and lookout possibilities).

The provision of nesting material has been shown to enhance breeding results in mice and rats (also see below). Several reports (Nolen and Alexander 1966; Norris and Adams 1976; Porter and Lane-Petter 1965) have indicated that providing nesting material can lead to a reduction in preweaning mortality and a greater number of surviving pups. However, at least one study (Eskola and Kaliste-Korhonen 1999b) has reported that nesting material did not affect breeding results.

Nesting Material

Rats, mice, hamsters, and gerbils. Nesting material is important for rats, mice, hamsters, and gerbils because it enables the animals to create appropriate microenvironments for resting and breeding (Figure 1). It is also important to provide nest boxes or other refuges for rats.

Guinea pigs. Guinea pigs are cursorial rodents that do not burrow; in the wild, they may live in burrows made by other animals. Refuges such as nest boxes, tubes, or shelters should be provided within the cage or pen to allow the animals to climb onto or hide under them. Hay will satisfy the need for roughage, and wood sticks can be used for chewing and gnawing.

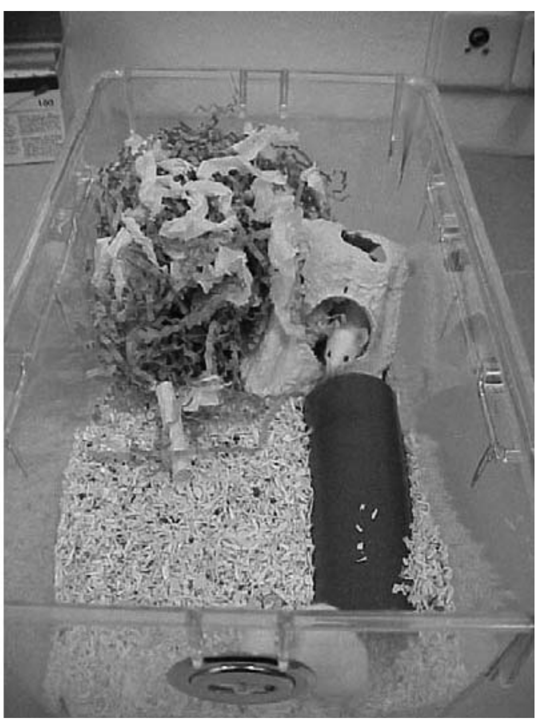

Figure 1 Example of environmental enrichment for mice. Photograph by M. K. Meijer.

Gerbils. In the wild, gerbils build extensive tunnel systems, and in the laboratory they often develop stereotypic digging behavior unless they are provided with adequate facilities (Waiblinger and Köning 2004; Wiedenmayer 1997). For this reason, gerbils need comparatively more space for building or using burrows of sufficient size, and they require a thick layer (20 cm long) of litter for digging and nesting and/or a burrow substitute. Nesting material (e.g., hay or straw) and wood sticks can be used for chewing and gnawing.

Hamsters. The wild ancestors of the hamster were largely solitary. Housing the animals in groups is possible, but special care should be taken in forming socially harmonious groups, and aggressive animals (especially females) should be separated. At a minimum, enrichment should include nesting material, a refuge area (e.g., a tube or hut), roughage, and gnawing objects.

Rabbits. Suitable enrichment for rabbits includes at a minimum roughage, hay blocks, or chew sticks, as well as an area for withdrawal and lookout (e.g., a platform). For breeding does, nesting material and a nest box or other refuge should be provided. In floor pens for group housing, the provision of visual barriers (Figure 2) is recommended (Council of Europe, Revision of Appendix A of the Convention ETS 123).

Breeding animals are not the only animals to use nesting material. It has been shown that laboratory mice will readily use nesting material and perform nest-building behavior (Eskola and Kaliste-Korhonen 1999a; Van de Weerd et al. 1997a, 1998), and that they spent 10 to 20% of their time-budget manipulating nesting material (Van de Weerd et al. 1997b). Toys can have a beneficial effect on the animals in developing exploratory behavior and locomotor and visual performance. One reason animals play is to practice the behavioral skills they need for survival (Young 2003). However, toys have a limited time of attraction—typically 1 day (Young 2003). A certain level of exploration can be maintained by changing toys frequently, but toys that are related to food or nesting retain their attraction longer.

Sensory Enrichment

Sensory enrichment includes visual, auditory, olfactory, tactile, and taste stimuli. Possibly the most satisfying enrichment for rodents and rabbits is visual, auditory, olfactory, and tactile communication with conspecifics or contraspecifics, either directly or through bars. Mirrors provided in mouse cages have not appeared to fulfill the criteria for an enrichment item, as in studies with primates (Sherwin 2004), possibly because albino mice have poor vision.

It has been suggested that a constant background noise during daytime (e.g., 85-dB volume radio music) has some benefits in facilitating breeding and decreases the excitability of animals by reducing the startle effect of sudden noises (NRC 1996). Behavioral results suggest that new age music has an overall calming effect on mice, compared with classical, pop, or no music. However, mice still showed a dis-

Figure 2 Example of enriched housing for rabbits. Photograph by Novo Nordisk A/S, Denmark.

turbance reaction (freeze or flight) during exposure to loud noise, irrespective of background music (Van Loo et al. 2004). Nevertheless, the use of radios in animal facilities during daytime may benefit the animal staff, which could in turn have beneficial consequences for the animals (Sherwin 2002; Van Loo et al. 2004).

Cage cleaning is a necessary routine procedure in laboratory animal facilities; however, removal of the olfactory cues disturbs the social hierarchy of the animals in the cage, often resulting in a peak in aggression among male mice. It has been shown that olfactory cues from nesting and bedding material have affected aggression in a different way: Transfer of nesting material reduced aggression, whereas sawdust containing urine/feces intensified aggression (Van Loo et al. 2000). Providing the animals with different food items (e.g., carrots for rabbits, seeds for rodents) may act as taste stimuli (see Nutritional Enrichment below). However, this approach might interfere with good laboratory practice (GLP[1]) requirements. It is possible to achieve tactile stimulation by providing nesting material, shelter, and the opportunity to dig.

Nutritional Enrichment

Animals tend to be highly motivated to make use of enrichment involving food items. Reports of many studies have provided such data. It is also important to note that GLP requirements might demand a definition and analysis of the materials used.

Frequency and schedule have an impact on the animal. Krohn and colleagues (1999) reported that feeding rabbits immediately before dark, in their active period instead of in the morning, reduced stereotypic behavior remarkably. Presentation of food and giving the animal the opportunity to forage (e.g., scattering food in the bedding) appear to prevent boredom because in nature, a large part of the time-budget is spent on this activity, even though these food items might be contaminated by feces and urine when the animals have no opportunity to compartmentalize their environment. Mench (1998) has reported that animals preferentially search for food even when it is readily available because this behavior affords them information about the location and quality of potential foraging sites. Additional food items such as hay, straw, or grass cubes can satisfy the need for roughage and for chewing in guinea pigs and rabbits (Baumans 1997). Rodents and rabbits use soft wood sticks for gnawing. Rats gnaw on aspen blocks, especially when they are housed without bedding (Eskola et al. 1999a; Kaliste-Korhonen et al. 1995). Hamsters (Niethammer 1988) and gerbils (Brain 1999) routinely store food and should be provided with food pellets inside the cage.

Evaluation of Enrichment

Factors such as genotype, age, sex, and individual variation influence exploration and animals' responses to novelty (Mench 1998), as do housing conditions in general (Jahkel et al. 2000; Olsson et al. 2003; Prior and Sachser 1995; Rilke et al. 1998; Van de Weerd et al. 1994). Whenever environmental enrichment is added to an animal enclosure, new material that is involved (e.g., paper or wood) might also influence the animal and the experiment. For example, volatile compounds of bedding material and enrichment items have been shown to affect the animals (Vesell 1967).

[1]Abbreviation used in this article: GLP, good laboratory practice.

Standardization of environmental conditions serves to reduce individual differences within animal groups (intraexperiment variation) ultimately facilitating the detection of treatment effects, and to reduce differences between studies (interexperiment variation) ultimately increasing the reproducibility of results across laboratories (Olsson et al. 2003; Van Zutphen et al. 1993). Nevertheless, Crabbe and coworkers (1999) and Wahlsten and colleagues (2003) have shown that despite rigorous efforts to equalize conditions among sites, different inbred mouse strains tested, which originated simultaneously from three well-recommended laboratories, revealed significant effects from their respective sites for nearly all variables examined.

For the reasons described above, it is critically important to evaluate environmental enrichment in terms of the benefit to the animal by assessing the following factors: the use of and preference for a certain enrichment; the effect on behavior (e.g., absence of abnormal behavior); the performance of species-typical behavior; and the effect on physiological parameters (e.g., body weight, heart rate, stress-related hormones, and immunology). At the same time, it is necessary to evaluate the impact on scientific outcome—whether the enrichment influences the scientific study (e.g., nutritional studies in the case of nutritional enrichment). Moreover, it is imperative to assess whether and how the statistical power is affected. The power of an experiment and the sample size might be increased, reduced, or unchanged due to the provided enrichment.

Based on the definition of animal well-being as the ability of the animal to cope successfully with its environment (Broom 1986), it follows that animals from an enriched environment may be better able to cope with environmental variations such as differences in housing conditions between breeder and animal facility and during experimental procedures. This result is consistent with the refinement principle of Russell and Burch (1959). In addition, because enriched animals have been shown to be less reactive to stressful experimental situations, there should be less variation between results, which should ultimately reduce the number of animals used (Baumans 1997; Stauffacher 1997b; Van de Weerd et al. 2002) and fulfill the reduction principle of Russell and Burch. Furthermore, as animals from enriched housing conditions are expected to be physiologically and psychologically more stable, they may be considered as more refined animal models, ensuring better scientific results (Bayne 1996; Benn 1995; Dean 1999; Rose 1994; Spinelli and Markowitz 1985; Van de Weerd 1996; Van de Weerd et al. 2002). When housing conditions do not meet the demands of a particular species, one cannot expect reliable and reproducible results (Fortmeyer 1982). Mice from enriched environments have been shown to have an increased behavioral repertoire and less stress (Newberry 1995; Van de Weerd et al. 2002), and to be less fearful and easier to handle (Van de Weerd et al. 1997b, 2002).

Despite the reports described above, concern persists that enrichment conflicts with the standardization of animal experiments because the potential exists for animals from an enriched environment to show more variability in their response to experimental procedures. The concern is that this characteristic may lead to more variation in results and to an increase in the number of animals used (Eskola et al. 1999b; Mering et al. 2001; Tsai et al. 2003). Although the objective is to minimize all sources of variation to achieve highly accurate and reliable results in animal-based research, the possible variation introduced by environmental enrichment might not be a negative factor because it might mean that the animals are allowed to express more of their behavior and that the experiment performed on a nonstressed and healthy animal has indeed led to more reliable results (Young 2003).

Moreover, other findings suggest that enrichment does not necessarily increase variation in results. It has been shown that nesting material did not influence the behavior and physiology of mice to a great extent (Augustsson et al. 2003; Van de Weerd et al.1997a,b). Mice provided with objects and nesting material habituated faster to open field tests and did not show effects on the circadian rhythm of behavioral patterns (Wainwright et al. 1994). In some pharmacological experiments, mice and hamsters housed in enriched cages showed a more sensitive response to anxiolytic drugs (Baumans 1997) and fever (Kuhnen 1997, 1999). Group-housed rabbits did not show any immunosuppression (Turner et al. 1997). However, several different reports on the effect of enrichment on experimental results have emphasized that in addition to the beneficial effects of enrichment on animals, it is important to evaluate specific parameters, the type of enrichment, and the animal strain.

Thus, enrichment has been reported to increase, decrease, or not affect variability, depending on the parameter studied (Eskola et al. 1999b; Tsai et al. 2003; Van de Weerd et al. 2002). Investigators have described strain differences in mice with respect to their response to enrichment (Nevison et al. 1999; Van de Weerd et al. 1994). With regard to the type of enrichment, it is advisable to focus on specific needs of the animals and to implement relatively simple enrichment because that approach will influence variability much less than a complex cage as used in the field of neuroscience in order to induce changes in the brain and in learning and memory abilities. Finally, 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 we accurately define and measure the controls and variables in the scientific experiment.

Conclusion

It is incumbent upon individuals who use and care for laboratory animals to seek to improve the quality of the captive environment. To that end, well-designed and carefully communicated enrichment approaches are required. Even when enrichment increases variation within the experimental study, it is important not to overstate the variation but instead, to balance the variation against the improved well-being of the animals. More data are needed to provide information related to the effects of specific enrichment programs on the animal, on specific animal species, strains and models, and on experimental results. These results must be based on approaches designed and implemented by successful enrichment programs.

Future scientific work is likely to involve many genetically modified strains of rodents. Because it is clear that a single approach to enrichment is not suitable for all species, we can anticipate the likelihood that a single approach to enrichment will not be suitable for all strains of rodents. For this reason among others, scientists are urged to compile, document, and publish pertinent data to dispel the myths and define the variations related to environmental enrichment.

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