Environmental enrichment for laboratory animals

Satish T. P.

Satish T Panchal received BVSc and MVSc (Pathology) from Anand Agricultural University, Anand, Gujarat. He is extensively involved in the field of laboratory animal medicine, toxicology and pathology since 12 years. He worked in various organizations viz. Jai Research Foundation, Torrent Research Center and Alembic Research Centre prior to joining Sun Pharma. Currently, working as group leader at the Department of Biological Research Toxicology, Sun Pharma Advanced Research Co. Ltd., Vadodara.

Satish T.P.
Department of Toxicology, Sun Pharma Advanced Research Co. Ltd.
Vadodara 390010, Gujarat, India

Corresponding author:
Satish P, Department of Toxicology, Sun Pharma Advanced Research Co. Ltd.
907/4, GIDC – Makarpura, Vadodara – 390010, Gujarat
Phone : 09825319421, email : satish.panchal@sunphrama.com

Abstract

Laboratory animals (Rat, Mice, Rabbit, Guinea pig and Dog) are extensively used in pre-clinical safety and efficacy research over several decades. Their use has led to significant contribution in drug discovery and development which ultimately has helped in finding cure for several human diseases. Hence, it is necessary to care the laboratory animals with the highest standards to ensure the scientific data obtained from these animals is not miss represented. Presently followed housing in research setup provides little for their behavioral and physiological needs, which compromises the welfare of the animals and can affect the validity of research results if stress has affected the animals. Environmental enrichment (EE) is the alteration of an animal's housing environment to create an opportunity to express species-typical behavior, which will reduce abnormal behaviors. The EE will also enhance animal welfare. In this review, various types of EE of laboratory species is discussed with an aim to integrate them into routine laboratory animal husbandry.

Keywords: environmental enrichment, welfare, stress, animal behavior

Introduction

Environmental enrichment is defined by Beaver (1989) as "additions to an animal's environment with which it can interact." Laboratory animals spend most of their lives in their cage so any positive change in the environment of home cage can lead to improvement in overall enrichment and welfare of animals. Hence, EE is a key component of animal welfare since it influences the animal's overall well-being, reduces stress, provides opportunities for activity, and encourages appropriate behaviors (Sparling *et al.* 2009). Several aspects of rodent enrichment have received a significant amount of attention during the last few years, which has lead to great advances in developing a beneficial enrichment program (Galef, 1999; Hutchinson *et al*. 2005). Restriction of opportunities for physical activity in the currently used standard cages for housing laboratory animals often results in inactive and obese animals, which are more prone to stress and anxiety. Exercise benefits in improving animal health by positively affecting animal welfare, hence system which can enhance animal's physical activity would be beneficial form of EE. Physical activity can offset the deleterious effects of a sedentary life combined with overeating and may prevent stress-induced immune suppression and consequent disease. Animals under experimental condition should have freedom from hunger, thirst, discomfort, pain, injury, disease, fear and distress and should have freedom to express normal behavior. This can be accomplished only by providing ready access to feed, water, appropriate environment, prevention or rapid diagnosis and treatment, sufficient space and facilities and company of animal's own kind (Moraska and Fleshner, 2001).

Humans are also part of social environment of laboratory animals and handling the animals is a very important aspect

of the daily care and use of animals. It is also beneficial to train animals to become used to routine care and management practices. Most of the animal models are social animals in nature and get benefited from the living association of same species or humans. Welfare of animals is improved by interactions among animals. Animals gain variety of experience during the growing phase which usually determines the social behavior of animals and hence conditions of animal holding in a breeding colony will affect on the animal's future well-being. Vaious aspects of enrichments viz. social environment, nutritional environment and sensory environment of various laboratory species are described below.

Enrichment for rodents

Enough considerations should be given for environmental factors like lighting, heating, ventilation and caging to fulfill social needs of rodents (rat and mice) used in research, teaching, or testing. In the case of individually housed animals, daily observation gives other form of social contact for the animal and will also facilitate easy handling in that the animal becomes familiar to the human presence. Group housing in mice (especially for breeders) will be successful if the social arrangement is limited to one male with more than two females and monitored for pecking order. Female mice from the same litter can be group housed (Wolfensohn and Lloyd, 1998). However, this may not be always possible for male mice as they often exhibit increasing aggression and dominance behavior (Brain, 1992), interestingly male rats unlike male mice easily get used to group housing situations (Barnett, 1975). Several evidences indicate that individually housed mice may have a poor immune system and developed tumors faster when singly housed (Schwartz *et al.* 1974; Riley, 1981). Therefore, when feasible, rodents should be housed socially in compatible groups. Hierarchy and relationship between dominant and submissive individuals should be given consideration while group housing mice.

Depending upon the involvement of strain, subordinate mice can be killed in group housing, hence it is necessary to routinely observe for signs of incompatibility and separate the mice in question. For deciding the degree of socialization, strain specific variations in behavior and vendor should also be evaluated (Haemisch & Gärtner, 1994). Wistar rats housed in group of three or less revealed reduced stereotypic behavior compared to individually-housed animals (Hurst *et al.* 1997; 1998). Individually housed rats which can smell, see and hear nearby housed animals also seem to be benefited. In some studies individually housed animals had abnormal levels of cholesterol, triglyceride and glucose (Perez *et al. 1997).* Rodents get highly aggravated to make use of food item as enrichment. In the wild, rodents spend a major portion of time searching for food whereas under laboratory condition food is mostly provided *ad libitum* and is easily available to animals. Food items scattered in the cage gives animal an opportunity to forage and prevents boredom as in nature. It has been found that rats preferred earned food though free food was available (Carder and Berkowitz, 1970). Rodents are nocturnal and sleep during the day. Also, albino rodents (unpigmented eyes) are susceptible to retinal degeneration if exposed to higher light intensities than normally recommended in animal rooms. The light intensity experienced by the rodents is reduced by the presence of wire bar lids, food, water bottles, and microisolator tops. It can also be further decreased by use of reduced lighting, with timer-controlled supplemental lighting when people are working in the room; by use of opaque cages; by not housing animals on the top shelf near the light source; and by providing nesting material or a nest box so the rodents can move away from the light (Mortell, 2001).

Enrichment for dogs

In case of dog, benefits of socialization with other dogs and care takers have been demonstrated experimentally (Hubrecht, 1993). Abnormal behavior such as fearfulness, aggression due to fear, hyperactivity, immobility and timidity are observed in case pups are not adequately socialized during the sensitive period of their life i.e. 4-14 weeks of age (Scott *et al.* 1967). Such pups suffer from isolation syndrome later in life and do not make good research subject due to the distress experienced by them while performing common laboratory procedures. Physiological parameters monitoring of such animals may fall outside normal limits (Vanderlip *et al.* 1985). Socially-housed dogs interact with kennel-mates and spend more time sniffing the pen, probably as a result of the increased diversity of scents (Hubrecht *et al.* 1992), and considerable reduction in stereotypic circling is evident, as compared to individually-housed animals. Individually housed dogs vocalize more, sleep less and exhibit more stereotypy than group-housed dogs (Hetts *et al.* 1992), and it was concluded that social isolation might well be even more harmful than space limitations. The maximization of visual contact with other dogs, pair housing, periods of exercise are all considered beneficial. In addition, it also seems that proximity with humans, by means of grooming, handling and petting while performing any procedures, are important aspects of human interactions in improving the wellbeing of laboratory dogs. In many kennels, cages are very simple without any complexity, and in some countries it is still legal to house dogs in small cage. It is doubtful that such small cage will provide dogs' physiological and psychological needs. Housing system should allow the dog to maneuver or masticate safe objects, and provide opportunities for human and dog interaction (Hubrecht, 1993). Pens/cages should be divided into sleeping and exercise areas which provide complexity, allows the dog to defecate/urinate away from its resting area (Fox, 1986). Solid partitions between cages provide seclusion and prevent injuries, but can detach the dog from its surrounding environment. A good pen design should allow the dog to fulfill its inquisitiveness about events taking place outside its' home cage.

A provision of platform can be made at a height that allows the dog to see over the partitions while lying down (Hubrecht, 1993). Sometimes, dogs have to be housed individually for research or quarantine purposes, in which case greater care should be taken to provide extra human interaction time and an enriched environment. Animal welfare regulation requires that dogs over three months old (except lactating and nursing females with pups) must be given exercise regularly if they are housed individually in an area that is less than two times required floor space. Group housed dogs do not require exercise provided total floor space equals the sum of required spaces for the dogs if housed individually. Forced exercise

programs are not considered ethical. If group of dogs are released together for exercise, group should remain as stable as possible because acceptance of new dog in group varies and group may attack new member. It is also necessary to frequently observe the pack to ensure safety of the group. Interestingly, presence of one or more persons in exercise area helps achieving positive social interaction. Indoor-outdoor pens are an option in some cases and offering dogs' access to the outside is an enriching experience for them. Dogs are very motivated by toys or chews that have an appetizing aroma or taste; therefore, it receives a lot of attention. Such items increase their active period and decrease destructive behavior.

Enrichment for rabbits

Social organizations of the rabbit are influenced by how they are housed in experimental settings. It is reasonable to suggest that they should be housed in groups since wild rabbits live in groups containing at least one other rabbit of the same sex (Cowan, 1987), except matured males (Gunn & Morton, 1995). Irrespective of type of housing used for rabbits, occasionally they should be provided exercise and social interaction which may allow them to have control over their immediate environment (Batchelor, 1991). Floor pen housing allows easier way to achieve contacts with others, wherever cages are used, group housing of compatible animals in a large cage is recommended. Socialization has been reported to reduce abnormal physiological and psychological behavior. If group housing is followed, cage furniture should be designed in such a way that animals have some form of refuge. Group housing also facilitates normal behavior, such as reciprocal grooming and chasing, jumping, rearing but groups should not be composed of more than 6-8 mature animals since monitoring turn out to be difficult (RSPCA, 1993). The cages should be large enough to allow rabbits to sit on hind legs and lie down with full stretch (Gunn & Morton, 1995) and should have visual contact with other rabbits. If they are grouped at younger age they tend to get adjusted to the social group better whereas older animals, particularly males have tendency to fight and intimidate younger ones (Davys, 1994). Since they are social in nature, they mix well at an early age; there may be problems with removal or replacement of adults in an established group (Love and Hammond, 1991). As enrichment, rabbit should be provided with woodchip litter or shredded paper or straw bedding. Rabbits prefer straw or shredded paper and avoid sawdust or wood shavings (Turner *et al*. 1992). Hay should be provided for foraging and building nest and nest boxes for females under reproduction, designed in such a way that it makes impossible for delivered females to see each other and trigger infanticide behavior. Shelves may be provided to offer comfortable resting and refuge places. Rabbits generally spend about 20% of the time gnawing hard objects like wood sticks (Stauffacher, 1992). Rabbits get easily frightened and react to environmental noises, hence care takers and experimenters should work calmly and quietly in rabbit rooms. For enrichment and supply roughage, rabbits may be supplied variety of food treats.

Enrichment of G.pigs

Guinea pig is a social animal and hence should be always housed in group unless there is a veterinary or experimental requirement. To avoid aggression and stress, group should be as stable as possible with adequate space for housing and nursing. Females can be kept in groups and also males (till four months age) whereas housing in pair is not recommended for boar above four months of age. To avoid the social tension due to presence of several males, a male should be housed with 3-4 females and pups should be removed at two - three weeks to prevent overcrowding. Adult animals can be housed in the unisex group provided that male groups have no olfactory or visual contact with females. Smell of female urine turns the friendliest males into enemies and will not tolerate each other's presence. Exchanging males for breeding purposes are easily accepted by females without any aggression similarly strange female can also be easily introduced without any aggression (Raje & Stewart, 2000) as long as none of them is nursing. Cage design should be such that they have place of refuge to improve their welfare. Place of refuge is utilized by them for sleeping together and for pregnant females to deliver (Gray, 1988). They do well on pelleted diet but for enrichment, fresh hay can be supplemented and animal express great pleasure in eating and burrowing inside the hay (Sutherland and Festing, 1987). To prevent G. pigs developing overgrown front teeth, they must be engaged in regular gnawing behavior by providing hard food pellets, carrots and softwood sticks (Scharman, 1991). Guinea pigs get easily frightened and react to environmental noises and hence care takers and experimenters should work calmly and quietly in Guinea pig rooms.

Summary

Any modification in the environment of the laboratory animal that enhances its psychological and physiological well being by providing stimuli, meets animal's species-specific needs. Fulfilling animals' need is not equal to going back to nature/ wild or to bring wild behavior in laboratory but is to bring crucial features of wild environment into the laboratory e.g. foraging. Social needs of animal can be met by housing them with conspecifics and by providing good care takers whereas physical needs can be fulfilled with the provision to rest, explore, groom, dig, nest or hide. Other needs of animals like eating, foraging, light, temperature, humidity, defection and urination should also be appropriately met. Several researches conducted in past supports that EE in the form of nesting material and foraging behavior and physical activity or exercise are effective in enhancing the welfare of laboratory animals. More research is still required in establishing feasible and cost effective EE methods which does not affect the research and at the same time improves animal welfare. This review presents a fair scientific evaluation of important topic like this and it will be helpful in developing enrichment programs and in planning the studies that produce positive effects on laboratory animals' welfare and research outcomes.

References

Barnett, S (1975). The Rat. University of Chicago Press, Chicago.

Batchelor GR (1991). Group housing on floor pens and environmental enrichment in sandy lop rabbits (I). *Anim. Techno.* 42, 109-120.

Beaver BV (1989). Environmental enrichment for laboratory animals. *ILAR News*. 31(2): 5-11.

Brain PF (1992). Understanding the behaviours of feral species may facilitate design of optimal living conditions for common laboratory rodents. *Anim. Techno.* 43: 99-105.

Carder B, Berkowitz K (1970). Rats preference for earned food in comparison with free food. *Sci.* 169:1273-1274

Davys JS (1994). The floor pens for laboratory animals a mixed blessing? *Anim. Techno.* 45 : 95-100.

Fox MW (1986). Laboratory Animal Husbandry**:** Ethology**,** Welfare, and Experimental Variables*.* State University of New York Press: Albany, NY. pp. 1-98.

Galef BG Jr. (1999). Environmental enrichment for laboratory rodents: animal welfare and the methods of science. *J. Appl. Anim. Welf. Sci*. 2(4):267-280.

Gray G (1988). Guinea pigs. Humane Innovations and Alternatives in Animal Experimentation 2. 48-49.

Gunn D, Morton DB (1995). Inventory of the behaviour of New Zealand White rabbits in laboratory cages. *Appl. Anim. Behav. Sci.* 45: 277 – 292.

Haemisch A, Gärtner K (1994). The cage design affects intermale aggression insmall groups of male laboratory mice: strain specific consequences on social organization, and endocrine activations in two inbred strains (DBA/2J and DBA/J). *J. Exp. Anim. Sci*. 36 : 101-116.

Hetts S, Clark JD, Calpin JP, Arnold CE, Mateo JM (1992). Influence of housing conditions on beagle behaviour. *Appl. Anim. Behav. Sci.* 34:137-155.

Hubrecht RC, Serpell JA, Poole TB (1992). Correlates of pen size and housing conditions on the behaviour of kennelled dogs**.** *Appl. Anim. Behav. Sci.* 34:365-383.

Hubrecht RC (1993). A comparison of social and environmental enrichment methods for laboratory housed dogs. *Appl. Anim. Behav. Sci.* 37: 345-361.

Hurst JL, Barnard CJ, Nevison CM, West CD (1997). Housing and welfare in laboratory rats: welfare implications of isolation and social contact among caged males. *Anim. Welfare.* 6: 329-47.

Hurst JL, Barnard CJ, Nevison CM, West CD (1998). Housing and welfare in laboratory rats: welfare implications of isolation and social contact among caged males. *Anim. Welfare* 7: 121-36.

Hutchinson E, Avery A, Vandewoude S (2005). Environmental enrichment for laboratory rodents. *ILAR J.* 46(2):148-161.

Love JA, Hammond K (1991). Group-Housing Rabbits. *Lab. Anim.* 20 (8): 37 -43.

Moraska A, Fleshner M (2001). Voluntary physical activity prevents stress-induced behavioural depression and anti-KLH antibody suppression. *Am. J.Phy.Reg. Integra. Comp. Physiol.* 281: 484-489.

Mortell N (2001). Practical environmental enrichment for rats and mice: the results of a survey. *Anim. Technol*. 52: 1-17.

Perez C, Canal JR, Domimgues E, Campillo JE, Guillen M, Torres MD (1997). Individual housing influences certain biochemical parameters in the rat. *Lab. Anim.* 31: 357-61.

Raje SS, Stewart KL (2000). Group housing female guinea pigs. *Lab. Anim.* 29(8) : 31-32.

Riley V (1981). Psychoneuroendocrine influences on immunocompetence and neoplasia. *Sci.* 212: 1100-1109.

Scharmann W (1991). Improved housing of mice, rats and guinea pigs: A contribution to the refinement of animal experiments. *ATLA*, 19: 108-114.

Schwartz R, Sackler A, Weltman A (1974). Adrenal relationships and aggressiveness in isolated female mice. *Experientia*. 30: 199-200.

Scott JP, Shepard JH, Werboff J(1967). Inhibitory training of dog: Effects of age at training in basenjis and shetland sheepdogs. *J. Psychol.* 66(2):237-252.

Sparling JE, Mahoney M, Baker S, Bielajew C (2009). The effects of gestational and postpartum environmental enrichment on the mother rat: A preliminary investigation. *Behav. Brain Res.* 208(1):213-23.

Stauffacher M (1992). Group housing and enrichment cages for breeding, fattening and laboratory rabbits. *Anim. Welfare*. 1: 105-125

Sutherland SD, Festing MFW (1987). The Guinea-pig. In The UFAW Handbook on the Care and Management of Laboratory Animals, Sixth Edition Poole TB (ed), Churchill Livingstone, New York, NY. pp 393-410.

Turner RJ, Selby JI, Held SDE, Howells KJ, Eveleigh JR, Wootton RJ (1992). Preferred substrates for penned laboratory rabbits. *Anim. Technol.* 43: 185-192.

Vanderlip SL, Vanderlip JE, Myles S (1985). A socializing program for laboratory-raised canines. Part 2: The puppy socialization schedule. *Lab. Anim.* 14(2):27-32.

Wolfensohn S, Lloyd M(1998). Handbook of laboratory animal management and welfare. 2nd ed. Blackwell Science. Oxford, United Kingdom.

■