ISSN: 2583-9365

Effect of enriched environment on reproductive performance and body weight gain in Wistar rats

Satish D. Patel

Satish Patel received his B.V.Sc and M.V.Sc (Pharmacology & Toxicology) degree from Anand Agricultural University, Anand, Gujarat. He has more than 15 years experience in laboratory animal science and experimentation. He has vast experience in maintaining the crucial transgenic and mutant strains of laboratory animals. He is extensively involved in AAALAC accreditation as an attending veterinarian. He worked at different capacity in various organization viz. B.V.Patel PERD Centre, Hester Pharmaceuticals, Cadila Pharmaceuticals and Novopharm prior to joining Zydus Research Centre. Currently, working as Senior Scientist and In-charge, Animal research facilities, Zydus Research Centre, Ahmedabad.

Satish D. Patel, Samir R. Patel, Urvesh D. Patel, Guru P.Y. and Mukul R. Jain
Animal Research Facility
Department of Pharmacology and Toxicology
Zydus Research Centre, Cadila Healthcare Limited
Gujarat, Ahmedabad

Corresponding author:
Dr. Satish D. Patel
Senior Scientist & In-charge, Animal Research Facility, Zydus Research Centre
Cadila Healthcare Limited, Moraiya,Ta-Sanand, Ahmedabad, Gujarat, India
Phone No.: 09825615088, Fax: +91 02717 665355, Email: satishpatel@zyduscadila.com

Abstract

Environment is known to play an important role in animal health, reproduction and its growth. In order to understand the influence of different kind of enrichment items on breeding performance and growth rate, this experiment was carried out on Wistar rats. The male and female rats of mature age were randomly selected and these animals were provided with different kind of enrichment items in cages such as fat rat hut, tunnel and crawl ball during the breeding period. Control animals were not provided with any enrichment item in cages. Reproductive parameter (fertility index, survival success, M/F ratio) were evaluated in all four group of animals. The results indicated that, the presence or absence of enrichment items did not affect the reproductive performance and survival success of the pups. The pups continued to remain exposed to the same kind of enrichment item, to evaluate the effect of enrichment on body weight gain. The male rats with fat rat hut as an enrichment item showed significantly less body weight gain compared to control, whereas female rats with crawl ball and tunnel as enrichment items showed significantly more body weight gain compared to that of control. The result of this study indicates that, male and female may have different discrimination for enrichment items according to their shape, size and surface area; however these enrichment items have no effect on reproductive parameter.

Key words: Environmental enrichment, reproduction, rats

Introduction

Enriched environment in breeding and experimental animals (rodents) is extensively accepted and practiced worldwide as a means of improving animal well-being. It has been proved that animals, including farm animals, zoo animals and laboratory animals, can maintain natural behavior when they live in an enriched environment (Mellen and Shepherdson 1997, Loveridge 1998). Different type of practices are adopted to provide the enriched environment to rodents, for example, clean mixed bedding material, small toys (igloos, tunnels, ball, hut, nesting materials etc.), novelty food and social contact by human handling. Providing physical enrichments in rodent cage may have mood changing effect which ultimately leads to happiness, reduced stress and good exercise to rat and mice. Enriched environment in cage can give social support in stressful conditions which has been pointed in numerous studies as being a positive factor for the health of animals particularly social one (Huls *et al.,* 1991; Sachser *et al.,* 1998; von Holst, 1998).

Some researchers fear that animals with enrichments in cage show more variability in their responses to experimental procedures due to dissimilar behavior. In complex environments, animals are responding not just to one stimulus

but to many variable stimuli at once (Appleby, 1997), which may cause increased variation within subjects (Eskola *et al.,* 1999) or enhance the deviation in experimental data (Gartner, 1999; Tsai *et al.,* 2002). Some studies depicted that animals housed in cage with enrichments would be expected to be more stable physiologically and psychologically and may, therefore, be considered more refined animal models, ensuring better scientific results (Bayne, 1996; Benn, 1995; Van de Weerd and Aarsen, 2002). Van de Weerd *et al.* (1997) reported that an enriched environment will not affect the results of experiments.

Rat is the primary species used in research for over a century which lead to significant findings in medicine and science. It has also been reported that enriched environment differentially affects growth and activity of preadolescent and adolescent male rats (Zaias *et al.,* 2008). Standard caging provides little for their behavioural and physiological needs (Johnson *et al.,* 2004), which compromises the welfare of animals and can challenge the validity of research results if stress has intruded upon the rats as models of normal biological and psychological functioning. Caging with enrichment item is an alteration of an animal's environment and provide an opportunity for positive species-specific behaviour, while reducing abnormal behaviours (Key, 2004), to enhance animal welfare. However, very few studies described the effects of the enrichment devices on the rising and survival of animals (Inglis *et al.,* 2004).

Therefore, it felt necessary to collect more information about the effects of enriched housing before it is routinely introduced into experimental design. Thus present study was planed to evaluate the effect of different types of enrichment items on reproductive performance and growth rate of male and female Wistar rats.

Materials and Methods

Animals

Wistar male (20) and female rats (40) of 8 weeks of age were selected randomly and housed in Animal Research Facility (AAALAC Accredited) of Zydus Research Centre, Ahmedabad. All animals were further divided in four groups (Group-I animals were not provided any enrichment items and served as controls; Group-II, III and IV animals were provided with tunnel, crawl ball and fat rat hut, respectively as an enrichment items), where each group comprised of 5 mating pairs (one male and two female; total 15 animals) during in-house breeding programme. The size of three types of enrichment items were as follow: Tunnel: 6" long with 3" diameter; Crawl ball: 4" diameter with three 2.5" opening and Fat Rat Hut: 6.75" wide X 3.38" high X 6" long.

Housing and cage Environment

The animals were housed in an individual cage using IVC system. The animal room was maintained at temperature of 23 ± 2 ºC, with 30-70% relative humidity and 12-12 h light-dark cycle. Animal cages (42.5 X 26.6 X 19 cm) were of polysulphone material. Approximately 150-200 gram paper bedding material was used for bedding. Bedding with cage was changed once a week. All material including cages, enrichment items and bedding material were sterilized before use. Figure 1 shows types of enrichment items used with cages.

Food and water

Reverse osmosis followed by ultra violet treated purified water in sterile drinking bottles and gamma irradiated rodent pelleted feed (Pranav Agro, Vadodara, Gujarat, India) were provided *ad libitum* to all animals.

Experiment design

All pairs of male and female rats were kept under mating in trio (one male and two female) for 14 days. After 14 days, female rats were observed for pregnancy by abdominal palpation. Pregnant females were transferred to clean sterilized cage with and/or without enrichment items (as per groups mentioned above) along with tissue paper as a nesting material. The delivery and number of pups born were recorded. The weaning of pups was done at the age of 21 days. The survival success was calculated according to Potgieter and Wilke (1997) as follows: Survival success = (Avg. litter weaned X 100) / (Average litter born alive). Male/female ratio, fertility index (No. of pregnant female/Total no. of female put for mating) were also calculated. The pups born from the breeding were used for another experiment to observe the effect of same enrichment items on growth rate of male and female Wistar rats.

One hundred twenty eight weaned male and female rats were divided in to four groups (Group-I animals were not provided any enrichment items and served as controls; Group-II, III and IV animals were provided with tunnel, crawl ball and fat rat hut respectively as an enrichment items) each containing 16 male and 16 female rats. Following one week of acclimatization, from 4 weeks of age, body weight was recorded every week until 8 weeks of age. The body weight of all animals were taken on 4^{th}, 5^{th} , 6^{th}, 7^{th} and 8^{th} week of age to observe body weight gain in male and female Wistar rats housed with or without enrichment items.

Statistical Analysis

Data were presented as Mean ± SEM. The significance between the data from the enriched groups (test) and those of the non-enriched group (control) were analyzed by Students' t-test.

Results

Evaluation of reproductive parameters (Table 1) of Wistar rats housed with different types of enrichment revealed absence of harmful effect on animal's fertility as well as survival of pups before weaning. None of the enrichment items used for environmental enrichment caused any

reproductive stimulation which could lead to a higher number of pups. Higher number of pups was weaned from the cage where tunnels were used, followed fat rat hat, cages with no device and crawl ball respectively.

In the present study, the body weights of male rats housed with fat rat hut were significantly lower ($p < 0.01$) compared to animals of non-enriched group (Figure 2). However, there was no significant ($p > 0.05$) difference between body weight gain of male rats housed with tunnel and crawl ball when compared to those of non-enriched group (control). In contrast, the body weight gain in female rats housed with tunnel and crawl ball were significantly ($p < 0.05$) higher compared to non-enriched female rats. The body weight gain in female rats housed with fat rat hut was similar ($p > 0.05$) to those in the non-enriched environment (Figure 3).

The shape of enrichment plays a crucial role in liking and disliking of animals. We have chosen different types of enrichment with different shape (Crawl ball: Round; Tunnel: Tubular; Fat rat hut: Concave) to evaluate the response of animals as well as impact of three types of enrichment on some parameters as mentioned earlier. The species and sex difference may have its liking and disliking of different enrichments. Reduced body weight gain in the animals of different groups may be indicative of response of animals to types of enrichment.

Discussion

The standard cage used for housing laboratory rats would often result in sedentary and overweight animals because of restricted opportunities for physical activity. The benefits of physical activity in improving health would have positive implications for animal welfare, so a system that enhances physical activity in the form of environmental enrichment would be an advantageous. It is reported that an exercise can counteract the deleterious effects of a sedentary life combined with overeating (Holloszy, 1988), and may prevent stress (Moraska and Fleshner, 2001).

From the result of present study, it was observed that the enrichment having large surface area may be responsible for positive discrimination in terms of reduced body weight due to exercise in male rats. While, round and tubular shape of enrichment having less surface area and its discrimination may be responsible for higher body weight gain in female rats. Zaias *et al.* (2008) also reported that environmental enrichment reduces feeding and weight gain in rat. Primary considerations for decreased weight gain include more frequent home cage activity, competition for food, altered metabolism and decreased food consumption. But in case of this experiment, large surface area of enrichment can provide an opportunity for more number of male rats to play thereby providing than more exercise. Different strains of animals can respond differently to enrichment as observed in mice (van de Weerd *et al.* 1994). Carvalho *et al.* (2009) reported that changes observed by introducing tubular devices as an enrichment item of micro-environments of out bred mice of the Swiss strain do not affect their development and reproductive performance. However, Tsai *et al.* (2002) observed variation in some parameters like body weight, organ weights and haematology in different strains of mice due to enriched environment. Physiological variables (body-weight, reproductive function, heart rate, hormonal levels and immune status) can also be indicative of responses to changes in laboratory environments. The effects of enrichment may all be influenced by the type of enrichment, the duration of experiments, and the sex and the strain of animals.

Based on these results, conclusion can be made that the enriched environment may enhance the animal welfare, reduce stress and provide tools for enhancing the physical activity in rodent cage without affecting their development and reproductive performance. However, long term effect of types of enrichment on physiological and psychological status of rat needs to be evaluated for different species and strain of laboratory animals.

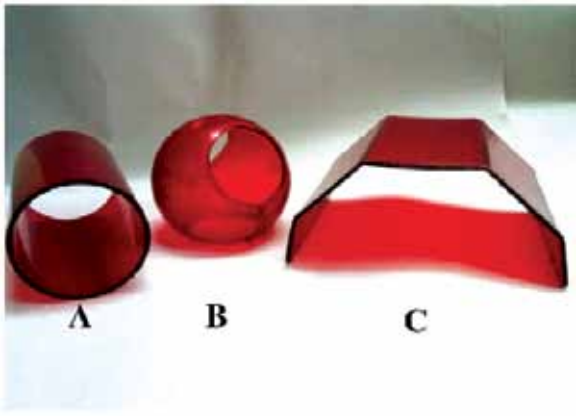

Fig 1: Enrichment items used in the experiment.
A: Tunnel; B: Crawl ball; C: Fat Rat Hut.

Table 1: Reproductive performance of animals housed with different types of enriched environment items (n = 10)

Groups	Pups Born	Pups Died Before Weaning	Pups Weaned	Male	Female	M/F ratio	Survival Success	Fertility index
I (Control)	10.44 ± 1.07	0.11 ± 0.11	10.33 ± 1.70	4.67 ± 0.78	5.67 ± 0.94	0.82 ± 0.45	98.99 ± 1.01	0.90
II (Tunnel)	12.43 ± 0.61	0.43 ± 0.20	12.00 ± 0.58	5.43 ± 1.02	6.57 ± 0.95	0.83 ± 0.41	96.69 ± 1.57	0.70
III (Crawl ball)	8.89 ± 1.25	0.11 ± 0.33	8.78 ± 3.73	4.56 ± 2.19	4.22 ± 1.92	1.08 ± 0.35	98.89 ± 1.11	0.90
IV (Fat rat hut)	11.86 ± 0.77	0.14 ± 0.14	11.71 ± 0.64	6.57 ± 0.72	5.14 ± 1.01	1.28 ± 0.33	99.11 ± 0.89	0.70

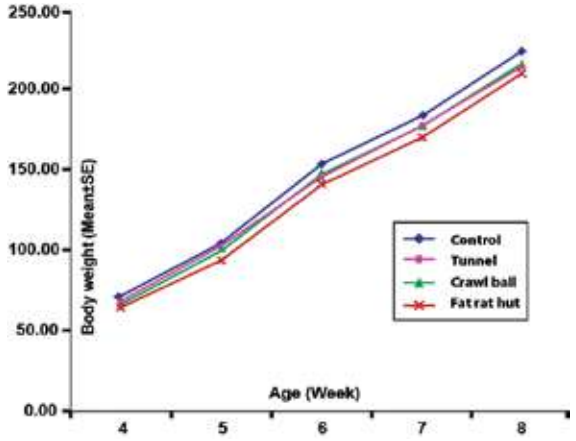

Fig 2: Body weight (Mean±SE) versus age (weeks) of male Wistar rats housed without and with enrichment items. ** P < 0.01

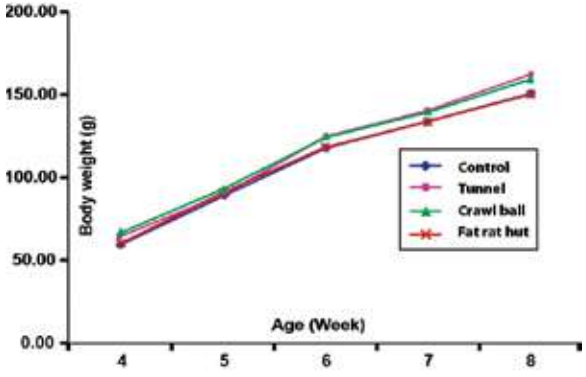

Fig 3: Body weight (g) versus age (weeks) of female Wistar rats housed without and with enrichment items. *P < 0.05, ** P < 0.01

References

Appleby MC (1997). Life in a variable world: Behaviour, welfare and environmental design. *App. Anim. Behav. Sci.* 54: 1–19.

Bayne K (1996). Normal and abnormal behaviors of laboratory animals: What do they mean? *Lab. Ani.* 25: 21–24.

Benn DM (1995). Innovations in research animal care. *J. Am.Vet. Med. Assoc.* 206: 465 - 468.

Carvalho AFU, Araujo AJ, Farias DF, Rocha-Bezerra LCB, Cavalheiro MG (2009). Development and reproductive performance of Swiss mice in an enriched environment. *Braz. J. Biol.* 69: 153-160.

Eskola S, Lauhikari M, Voipio HM, Laitinen M, Nevalainen T. (1999). Environmental enrichment may alter the number of rats needed to achieve statistical significance. Scandin. *J. Lab Ani. Sci.*26, 134–144.

Gartner K (1999). Cage enrichment occasionally increases deviation of quantitative traits. In Tur-Mari J, Orallana-Mariana JM (Eds.), Animals research and welfare: Partnership (p. 207). London: Laboratory Animals.

Holloszy JO (1988). Minireview: exercise and longevity: studies on rats. *J Gerontol.* 43 B 149-151. Cited by: Spangenberg EMF, Augustsson H, Dahlborn K, Essen-Gustavsson B, Cvek K (2005). Housing-related activity in rats: effects on body weight, urinary corticosterone levels, muscle properties and performance. *Lab. Ani.* 39: 45-57.

Huls WL, Brooks DL, Bean-Knudsen D (1991). Response of adult New Zealand White rabbits to enrichment objects and paired housing. *Lab. Ani. Sci.* 41: 609-612.

Inglis CA, Campbell ER, Auciello SL, Sarawar, SR (2004). Effects of enrichment devices on stress-related problems in mouse breeding. San Diego: Torrey Pines Institute for Molecular Studies. Available from: <http://caat.jhsph.edu/programs/AWE/2004/final.htm>. Access in: 30 de Julho de 2006.

Johnson SR, Patterson-Kane EG, Niel L (2004). Foraging enrichment for laboratory rats. *Ani. Welfare*. 13: 305-312.

Key D (2004). Environmental enrichment options for laboratory rats and mice. *Lab. Ani.* 33: 39-44.

Loveridge GG (1998). Environmentally enriched dog housing. *Appl. Ani. Behav. Sci.* 59: 101-113.

Mellen JD, Shepherdson DJ (1997). Environmental enrichment for felids: an integrated approach. *Internation. Zoo Yearbook* 35: 191-197.

Moraska A, Fleshner M. (2001) Voluntary physical activity prevents stress-induced behavioural depression and anti-KLH antibody suppression. *Am. J. Physiol.- Regulat. Integrative Compar. Physiol.* 281: R484-489.

Potgieter FJ, Wilke PI (1997). Effect of different bedding materials on the reproductive performance of mice. *J. South African Vet. Assoc.* 68: 8-15.

Sachser N, Dürschlag M, Hirzel D (1998). Social relationships and the management of stress. *Psychoneuroendocrinol.* 23: 891-904.

Tsai PP, Pachowsky U, Stelzer HD, Hackbarth H (2002). Impact of environmental enrichment in mice. 1: Effect of housing conditions on body weight, organ weights and haematology in different strains. *Lab. Ani.* 36: 411–419.

Van de Weerd HA, Aarsen EL (2002). Effects of Environmental Enrichment for Mice: Variation in Experimental Results. *J Appl. Ani. Welfare Sci.* 5: 87–109

Van de Weerd HA, Van Loo P L P, Van Zutphen LFM, Koolhaas JM, Baumans V (1997). Nesting material as environmental enrichment has no adverse effects on behavior and physiology of laboratory mice. *Physiol. Behavior.* 62: 1019–1028.

Van de Weerd, HA, Baumans V, Koolhaas JM, Van Zutphen, LFM (1994). Strain specific behavioural response to environmental enrichment in the mouse. *J. Exp. Ani. Sci.* 36: 117–127.

Von Holst D (1998). The concept of stress and its relevance for animal behavior. *Advances in the Study of Behavior*. 27: 1-131.

Zaias J, Queeney TJ, Kelley JB, Zakharova ES, Izenwasser S (2008). Social and Physical Environmental Enrichment Differentially Affect Growth and Activity of Preadolescent and Adolescent Male Rats. *J. Am. Assoc. Lab. Ani. Sci.* 47: 30–34.