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Should Cages of Laboratory Rats Be Enriched Physically or Socially?

[1]*Usama A. Abou-Ismail,* [1]*Ragab A. Darwish and* [2]*Sameh G.A. Ramadan*

[1]Department of Animal Husbandry, Faculty of Veterinary Medicine,
Mansoura University, Gomhoria St., Mansoura, Egypt
[2]Department of Husbandry and Development of Animal Wealth,
Faculty of Veterinary Medicine, Menofia University, Sadat Branch, Egypt

Abstract: Experiments of environmental enrichment usually compare between group-housed rats in enriched and unenriched cages or between group-housed rats in enriched cages and single-housed rats in unenriched cages. This bias is mainly to maximize the chance of enrichment both socially and physically. The present study was designed to assess whether the addition of physical and social environmental enrichment causes different effects on the behaviour, performance and welfare of male laboratory rats from weaning through adolescence. 30 newly-weaned male Wistar rats were housed in either standard cages (SC), physically enriched cages (PEC) or socially enriched cages (SEC) for five consecutive weeks. Animals were received in three batches and each experimental treatment was replicated two times within each batch. Results revealed that rats housed in the (PEC) displayed higher levels of measures suggestive of improved welfare such as sleep, grooming, feeding, moving, exploration, body weight, weight gain, relative weight of thymus, spleen and testes and being in-the-open part of the cage and lower levels of measures suggestive of compromised welfare such as stationary, bedding-directed behaviour and being under-hopper compared to rats in the (SEC). It appears that physical method of enriching conventional cages can have significant effects on behaviour and growth of laboratory rats and may therefore improve their welfare.

Key words: Animal Welfare · Laboratory Rats · Enrichment · Behaviour · Performance

INTRODUCTION

Research with laboratory animals are important to investigate vital issues such as the ontogeny and ageing of mammals, mechanisms of diseases and their prevention or treatment, or health risks in our living environment [1]. Therefore as laboratory rats offer an important tool for scientists seeking to clarify the functions of biological systems, as well as to develop new treatments to human diseases and safer living environment, their housing conditions should be improved. Housing rats in standard laboratory conditions constitutes a confined, non-stimulating, barren environment for them. This restrictive housing situation may compromise the welfare of the animals and can cause stress in various ways. For example, the restriction of behaviour may act as a stressor itself, when highly motivated behaviours are thwarted [2, 3]. Moreover, restrictive conditions limit the ability of the animals to predict and control their physical and social environment and can therefore be stressful [4-6]. Improving housing conditions may not only improve the overall well-being of the animals [7, 8] but also the accuracy of experimental results [9, 10]. Environmental enrichment, generally referred to as any treatment that provides cognitive and/or physical stimulation beyond that which would be received in standard housing conditions [11], has been shown as a widely accepted practice of improving the housing conditions and thus the welfare of laboratory animals.

Experimental studies investigating the effects of environmental enrichment on animals' behaviour and physiology tend to adopt one of two approaches. The first approach is to keep animals in enriched conditions for a particular length of time and to compare

Corresponding Author: U.A. Abou-Ismail, Department of Animal Husbandry,
Faculty of Veterinary Medicine, Mansoura University, Mansoura, Egypt.
Tel: +2 (0)1062557996, Fax. +2 (0)502379952.

the way they perform, with the conspecifics housed in unenriched conditions [12-15]. Whereas, the second approach is to compare how a previously "unenriched" animal performs when it experiences an enriched housing situation [16-18].

Recent years have thus witnessed a surge of interest in methods of environmental enrichment for rodents housed in laboratory conditions. Five types of enrichment practices have been increasingly used in enriching standard cages of laboratory rodents. The first practice is the social enrichment of the environment by housing animals together with conspecifics in pairs or in groups [19-21]. Humans are also a part of the social environment of laboratory animals and handling animals is a very important aspect of this daily care routine [22]. It is also beneficial to train animals to become used to routine handling and procedures [23]. The second practice is the physical enrichment of the environment by increasing cage size or adding physical structures and additional accessories to the cage [24-28]. The third practice is the nutritional enrichment of the environment by scattering food items in the substrate or bedding so that the animals spend time searching for it [29]. It has been shown that rats prefer earned food although free food was available, when the work demands were not too high [30]. The fourth practice is the psychological enrichment of the environment by increasing the possibilities for animals to control their environment [4, 13]. It has been shown that providing shelters or refuges gave the animals the opportunity to withdraw from frightening stimuli as well as too much light [31-33]. Plastic tubes [34] old drinking bottles [35] and huts [33] are simple solutions for shelters. Whereas, the fifth practice is the sensory enrichment of the environment by providing aids that can reduce reactivity of animals to sudden background noises, such as playing radios softly during the day [36-38].

Although these five practices of environmental enrichment have been implemented in studies investigating the effects of enriching conventional cages of laboratory rodents on their behaviour and welfare, it appears that they are usually represented in two forms only; namely animate (social enrichment) (i.e. enrichment through the provision of social contacts with conspecifics) and inanimate (physical enrichment and other types) (i.e. enrichment through the provision of structures and substrate changes to cages, toys, cage furniture, exercising devices, climbing accessories, addition of manipulanda, novelty food, auditory and olfactory stimulation) [39-41]. Devices such as igloos, tunnels, nesting materials, climbing devices such as ladders, gnawing objects and other structures are commonly used as physical enrichment items in rat cages. Used alternatively or in conjunction with physical manipulation of the cage environment are increased handling of rats and social enrichment (for example, multiple housing of rats) [42, 43].

The environment has a major impact on rat physiology and behaviour, especially in the early periods of life. Varying the rearing environment results in individual variation and, hence, alters responses during experiments. Several studies have shown that environmental enrichment and a complex environment may decrease or increase the variability in research results [44-47]. Such an increase in the variation of experimental animals leads to an increase in the number of animals needed in an experiment. This contradicts with the overall goal of reducing the number of animals required for experiments. However, housing animals in adequately complex environment to ensure their normal development and welfare is thought to be necessary for producing animals with better coping abilities and greater tolerance of stressful manipulations in experiments [48]. The well-being of rats with reduced variation might be achieved by rearing the animals in similarly enriched environments, i.e. through the standardisation of the housing environment of laboratory animals, where enrichment tools are consistently included.

Therefore, little information is available regarding the respective roles of social and physical factors on altering behaviour, performance and welfare of laboratory rats. The present study was designed to assess whether the addition of physical and social environmental enrichment causes different effects on the behaviour, performance and welfare of male laboratory rats from weaning through adolescence. Comparing environments with potentials to differ socially and physically would require housing rats singly versus housing them in groups (see housing conditions). One would therefore argue that because rats are social animals housing them singly would be stressful.

Single housing of some rodent species such as laboratory rats has been demonstrated to have adverse effects on their behaviour and health [49, 50] and therefore to be stressful [51, 52]. Moreover, when single housing involves isolation rather than separation (as when individuals do not have the ability to see, smell or touch other rats) it can be more stressful [51]. Nevertheless, single housing is still used worldwide for logistical and ethical reasons, for example, to reduce the number of animals used, to avoid pseudoreplication, following surgery, in case of electroencephalogram recording of phases of sleep in rats, or paradoxically to remove social stress [53-55]. Furthermore, it is sometimes the case that social housing could escalate aggression to the extent that injuries or wounds may occur and that in turn makes the full time social housing of the injured individuals ubiquitously unimplemented. On the other hand, it should be made clear that the stress of housing rats singly can be overcome if the rats' ability to control their environments is improved by providing them with opportunities to communicate without direct physical contact. Research has shown that housing laboratory rats singly in cages that permit communication between them (through auditory, olfactory and visual interactions) may be of sufficient significance not only to remove stress of social isolation but also to alleviate the social pressure of housing in groups and therefore to improve welfare of singly-housed rats [56-58]. Therefore, in this experiment cages with elevated cage lids (21 cm) were used for housing rats (see later) to provide singly housed rats and others, with opportunities to communicate with other rats in the same experimental room. These cages may not only remove stress of single housing but can also be considered as a physical (increasing cage height) and a psychological method (allowing communication with other rats) of cage enrichment.

MATERIALS AND METHODS

Animals and Husbandry: This experiment was carried out using 30 rats. The subject animals were newly weaned male rats, 35-50 g weight at arrival, of the Wistar strain (Al-Alamia, El-Gharbia, Egypt). Animals were received in three batches and each experimental treatment was replicated two times within each batch.

Cages were cleaned once a week, in which all rats were removed from their cages and rehoused in entirely clean cages with new bedding (saw dust) and nesting (shredded paper) material. All cleaning and handling procedures were carried out by the same experienced caretaker in order to minimize any possible effects, due to unfamiliar caretaker, on behaviour and hence avoid stress. Cages were cleaned at fixed times every week and two days before behavioural observation took place to reduce any possible effects of the cleaning regimen on behaviour and physiology of the rats.

All cages were arranged on a table (200 cm length × 50 cm width × 90 cm height) alternating the position for each of the six cages so as to control for the possible effect of the position of the cage on the table in relation to the environmental cues (proximity to the red light or white light).

Pelleted food (Rat chow®, Oil and Soap Manufacturing Company, El-Gharbia, Egypt) and tap-water (two bottles fitted in each cage) were provided *ad-libitum* and were refreshed daily. Water bottles were changed every week. The experimental room was maintained under a 12:12 h light:dark schedule with the white light on between 03:00 and 15:00 and continuous dim red light (two 60 Watt bulbs, Serma Electrical, Egypt) enabling observation during the dark period, at a constant temperature (20±2°C).

Housing Conditions: Rats were housed in one of the following conditions for five consecutive weeks:

- "Standard cages" (SC): Standard cages (48.5 cm length × 33 cm width) with elevated metal bar cage lids (21 cm height) that were used for housing of single rats.
- “Physically enriched cages” (PEC): Standard cages with elevated cage lids that were supplied with many physical structures including retreats (20.5 cm L × 15.7 cm W × 11.5 cm H Guinea pig huts, red-tinted, Lillico, UK), nylabone (Regular size, original flavour, (36g), Lillico, UK), crawl ball (115 mm, with 3 × 58 mm holes, red-tinted polycarbonate, Lillico, UK) and ladders (9 step wooden ladder 35.5 cm, local pet store, El-Gharbia, Egypt) and were used for housing of single rats.
- “Socially enriched cages” (SEC): Standard cages with elevated cage lids that were supplied with none of the physical structures but were supplied with two rats in addition to the experimental rat (focal rat) and these cages were therefore used for housing rats in groups of three.

Behavioural Assessment

Ethogram: Observation was carried out in two sessions per day for each of the three housing conditions every week. The first session took place during the light phase; starting at 14:00 hrs and ending at 14:30 hrs. The second session was carried out while the white light was off (during the dark phase of the day); starting at 15:30 hrs and ending at 16:00 hrs.

Behaviour of the rats in the three housing conditions was recorded in real time using instantaneous sampling method with 4-s intervals between each consecutive focal animal, with the observer moving

Table 1: Ethogram for recorded behaviours

Behavioural category	Behavioural component	Description
A- General activities:	1- Feeding	Eating food from food hopper
	2- Drinking	Drinking water from waterspouts
	3- Non-intake maintenance	Self-grooming and pandiculation (stretching and yawning)
	4- Movement activities	Movement and/or climbing the cage lid
	5- Exploratory behaviour	Sniffing cage wall, cage top and sniffing air outside the cage
	6- Bedding-directed behaviours	Sniffing bedding, eating bedding, bedding manipulation and burrowing
B- Sleep:	1- Sleep	Lying unalert with both eyes closed- apparently asleep
C- Awake non-active:	1- Awake non-active	Stationary
D- Other behaviour:	1- Social interaction	Aggressive and non-aggressive social interaction for rats in SEC
E- Position in the cage:	1- Underneath-hopper	When the whole body of the rat, excluding its tail, is entirely underneath the food hopper or waterspouts at the moment of the scan
	2- In- the-cage	When the whole body of the rat, including its tail, is entirely in the open part of the cage at the moment of the scan

between cages every 4 seconds. Each sample interval was dictated by an audio cue via headphones and the behaviour recorded onto a check sheet. The behaviour of the individual rat (in the first two housing conditions) and those of the focal rat (in the third housing condition) was sampled [59, 60] and its position within the cage (under food hopper or in-the-open part of the cage) was also recorded (Table one) [61].

Weight Changes and Weight of Internal Organs: Rats were weighed weekly using equilibrated scales (Sartorius, AG, Gottingen, Germany) throughout the five week experimental period. At the end of the experimental period rats were euthanized by cervical dislocation. Immediately after euthanasia each rat was dissected and selected internal organs, including the thymus gland, spleen, adrenal glands, kidneys and testes were removed and stored on ice in sterile balanced salt solution. The organs were subsequently dried, trimmed and weighed (in g).

Statistical Analyses: SPSS (version 12.0 for windows) was used for all statistical analyses. Data were checked for normality and homogeneity of variances to test for the suitability of using parametric tests. A GLM-repeated measures model was used to compare between the three experimental treatments for the different behaviour variables collected because data were collected from the same cage/group at several different time points. All data are presented as EMM (estimated marginal means) ± SE. Differences between the three experimental groups in the final body weight, total weight gain and weight of the different internal organs were tested using a One-way analyses of variance (ANOVA) test.

RESULTS

Behaviour

Main Effects: Housing laboratory rats in different conditions significantly changed some of their behavioural activities including: Average % scan drinking ($F_{2,14}$=10.16, $P < 0.01$) (Figure 1), grooming ($F_{2,14}$=5.66, $P < 0.01$), stationary ($F_{2,14}$=11.35, $P < 0.001$), bedding-directed behaviour ($F_{2,14}$=13.11, $P < 0.001$) (Figure 2), under-hopper ($F_{2,14}$=284.16, $P < 0.001$) and in-the-cage ($F_{2,14}$=352.94, $P < 0.001$).

Interactions

Housing Condition* Observation Session: Average % scan sleep showed a significant treatment*session ($F_{2,14}$=9.46, $P < 0.001$) (Figure 3), increasing significantly in the light phase in the PEC rats; and both average % scan exploration ($F_{2,14}$=3.99, $P < 0.01$) and moving ($F_{2,14}$=11.43, $P < 0.01$) (Figure 4), increasing significantly in the dark phase in the PEC rats as compared to both SC and SEC rats.

Housing Condition*Observation Week: Average % scan feeding showed a significant treatment*week ($F_{8,24}$=4.53, $P < 0.05$) (Figure Five), increasing significantly in the PEC rats in the 1st, 4th and 5th week.

Weight Changes and Weight of Internal Organs: The output of the One-way ANOVA test showed that housing laboratory rats in different conditions significantly changed performance parameters measured in this study, including: body weight (g) ($F_{2,15}$= 3.81, $P < 0.05$) and weight gain (g) ($F_{2,15}$= 4.58, $P < 0.05$) (Figure 6), with the rats in the PEC weighing heavier and gaining more weights over the five weeks of the experiment than rats in the SC and SEC.

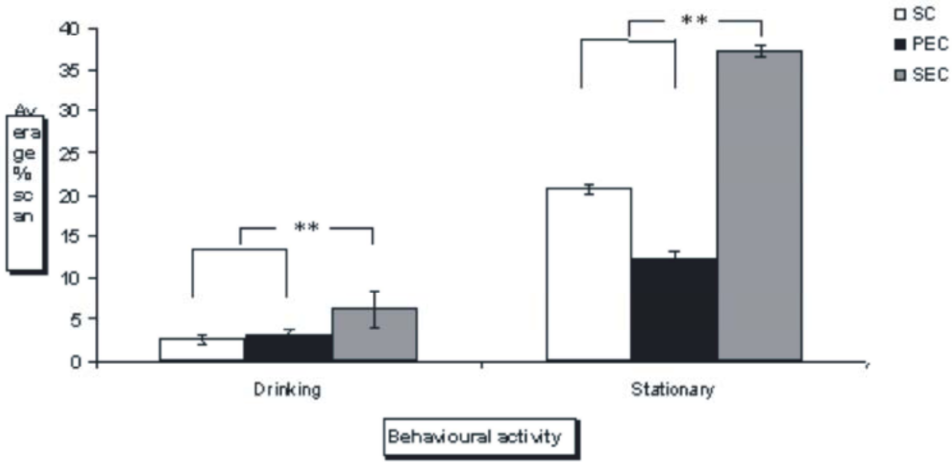

Fig. 1: EMM ± SE "Average % scan drinking and stationary" by the rats in the three housing conditions.

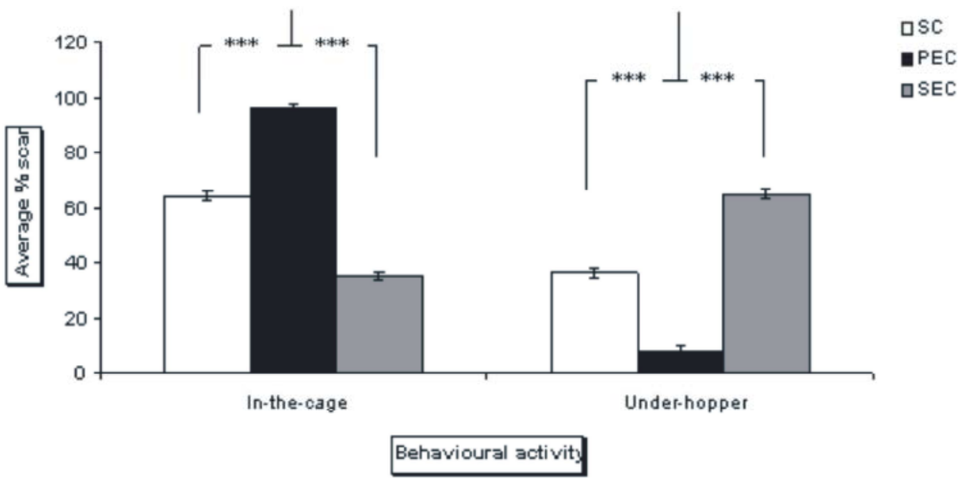

Fig. 2: EMM ± SE "Average % scan in-the-cage and under hopper" by the rats in the three housing conditions.

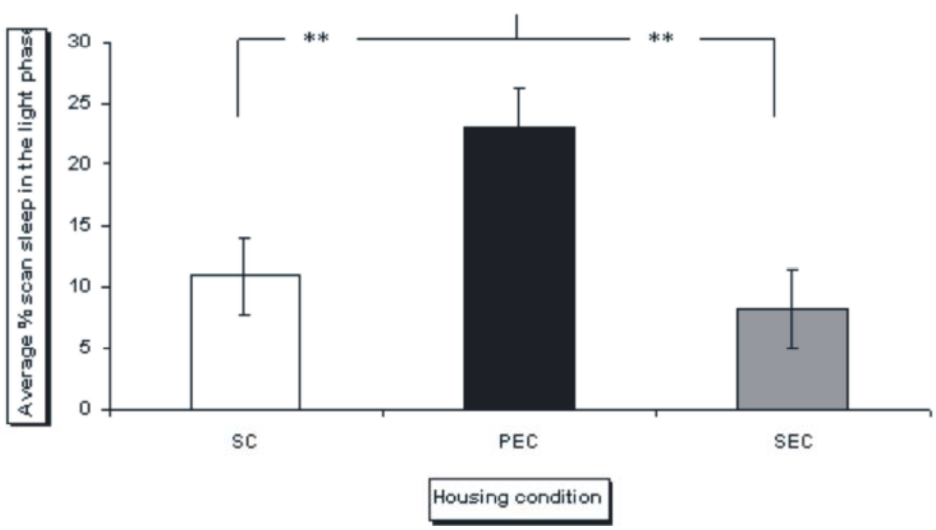

Fig. 3: EMM ± SE "Average % scan sleep" in the light phase by the rats in the three housing conditions.

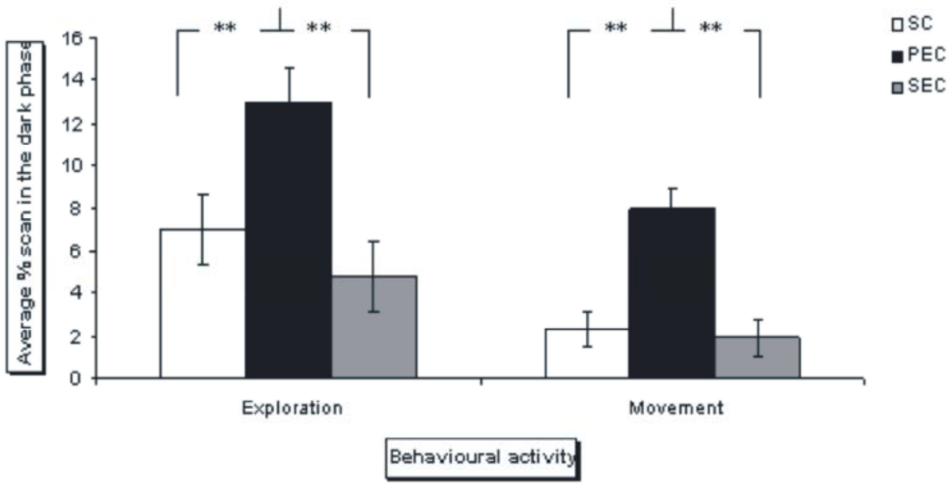

Fig. 4: EMM ± SE "Average % scan exploration and moving" in the dark phase by the rats in the three housing conditions.

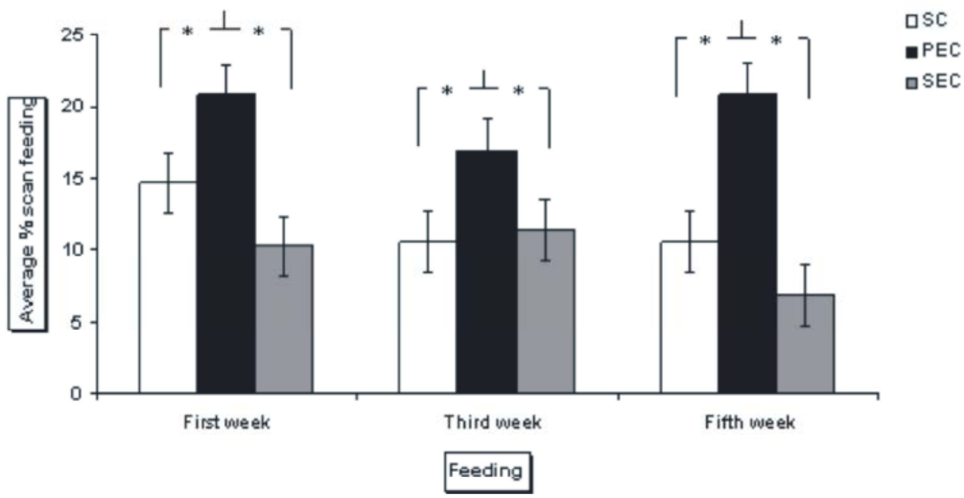

Fig. 5: EMM ± SE "Average % scan feeding" in the 1st, 3rd and 5th week by the rats in the three housing conditions.

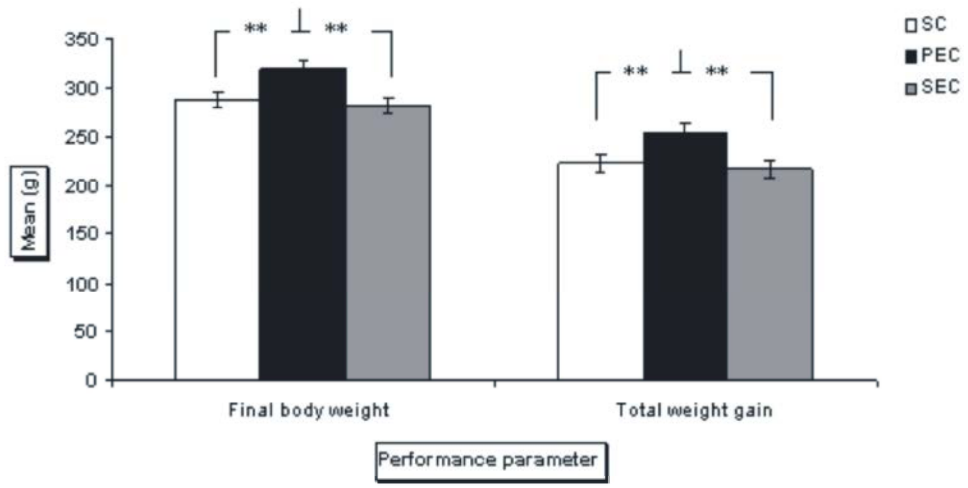

Fig. 6: EMM ± SE "Average final body weight (g) and total weight gain (g)" by the rats in the three housing conditions.

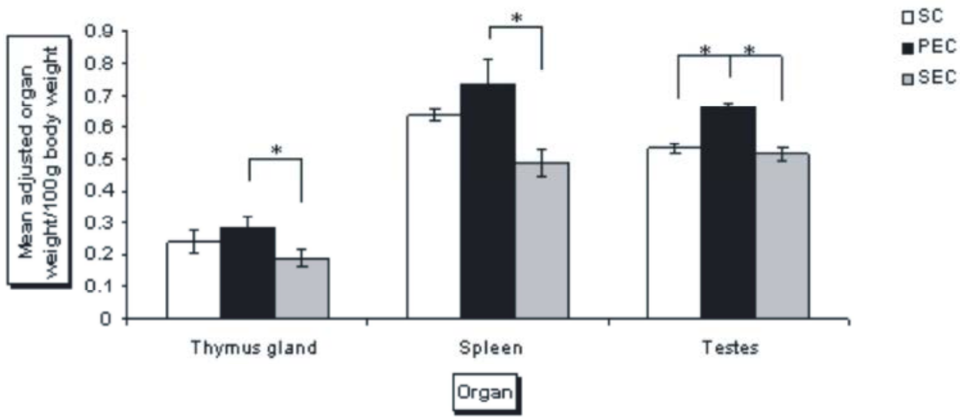

Fig. 7: EMM ± SE "Average relative organ weight (g)" by the rats in the three housing conditions.

Housing rats in different housing conditions had a significant effect on the relative weight of their thymus (g) ($F_{2,15}$= 3.71, P <0.05); spleen ($F_{2,15}$= 6.14, P <0.05) and testes ($F_{2,15}$= 6.11, P <0.05) (Figure 7) with the rats housed in the PEC having heavier thymuses, spleens and testes than rats housed in the SC and SEC. On the other hand, housing laboratory rats in PEC did not significantly change the relative weight of their adrenals ($F_{2,15}$= 0.65, NS) and kidneys ($F_{2,15}$= 0.37, NS).

DISCUSSION

When we looked at the effect of physical versus social method of enriching conventional cages of laboratory rats we found that the level of several behaviours including sleep, self grooming, movement activities, exploration, feeding and being in-the-open part of the cage increased in rats housed in (PEC). The increase in the level of these behaviours reflects the findings for rats housed singly in cages enriched with multiple enrichment items [33]. Research on environmental enrichment has shown effects on sleep in captive-kept rodents. In laboratory rats, experiments attached some effects to environmental modification during the early post weaning period, such changes that were thought to be beneficial. These beneficial effects of environmental enrichment included increasing in sleep time (both rapid eye movement sleep and slow wave sleep and total sleep time) and shortening in sleep latency [62-64]. These changes were considered beneficial because they were associated with other indicators of improved mental and physical well-being such as improved learning and higher brain (hypothalamus and cerebral cortex) weight [63, 65]. Sleep has been shown to be a non invasive welfare indicator and high levels of it may therefore indicate good welfare in laboratory rats [66]. This high level of sleep displayed by rats in the (PEC), relative to those in either (SC) or (SEC), could be due to the increased level of their movement and exploration but also due to the increased activity directed towards the enrichment objects. It could also be due to the ability of rats in the (PEC) to control their environment by avoiding the disruptive effect of the white light.

On the contrary, it should be acknowledged that sleep may also increase due to the exposure to boring (monotonous or unstimulating) environments, [67, 68] during the course of some diseases such as hypersomnia (excessive daytime sleepiness or prolonged night-time or both) [69] or due to psychopathology such as endogenous depression [69]. However, these two possibilities do not appear to be the cause of high sleep levels displayed by the rats in (PEC) because they were physically fit, maintained their high level of sleep over the weeks of the experiment and displayed higher levels of other indicators of good welfare such as exploration and being in the open part of the cage and higher body weights and weights of spleen and thymus glands.

Similarly, high levels of self grooming, movement activities, exploration and feeding might indicate improved welfare. Research has shown that chronic stress decreases general activity levels and locomotor behaviour, [70-72] self-grooming, [73, 74] food intake [71, 75] and exploration [60,76]. The higher levels of movement and exploration by the rats housed in (PEC) could be due to the increased complexity of their environment. It has been illustrated that, when given the choice, rats prefer high complexity in their environment and that they spend more time active (moving and exploring) in the complex environment [77]. The high levels of feeding could be due to the higher activity levels performed by these animals. It could also reflect the need of these animals to gnaw. On the other hand, higher levels of feeding displayed by rats in (PEC) could also indicate the higher metabolic rate of these animals to maintain a stable body temperature especially in the rest, as individually housed rats could not sleep together to keep each other warm and save energy. The later two possibilities can be ruled out because (PEC) rats displayed higher body weights compared to rats in the other housing conditions (see later). However, the high level of self-grooming activity in the rats of the (PEC) may be due to the higher amount of sleep in these animals. This high level of self-grooming activity can also reflect the higher amount of sleep that animals in this housing condition achieved and could therefore be due to longer sleeping time. Self-grooming was reported to be the second activity of the laboratory rat that occupies the longest duration of their time budget after sleep. Indeed, it is the most time consuming activity of the laboratory rat's awake time [78, 79]. Self-grooming was reported to be concentrated around sleeping time. It takes place after sleeping, but also occurs when the animal prepares for sleep.

The finding of increased time spent in-the-open part of the cage might indicate improved ability of the animals to exert better control over the environment and hence good welfare. Good ability of animals to cope with and to control, the environment is a necessary requirement for good welfare [6]. This could be due to the increased compartmentalization of the cages by the provision of multiple physical structures into them which, in turn, might have provided various resources for the rats to hide from the disruptive effect of the white light, particularly in the light phase of the dark/light cycle and intensified their thigmotactic nature.

On the other hand, housing rats in (SEC) increased levels of some behaviours including awake non-active behaviour (stationary) and bedding-directed behaviour. This relative increase in the level of bedding-directed behaviours in the (SEC) could be due to the fact that rats in these cages had not enough cage structures (objects) to interact with. The only available cage structure in these cages was the bedding substrate, therefore this method of cage enrichment limits the available options of the rats for interaction. The finding of reduced bedding-directed behaviours in groups of rats housed in physically enriched cages is similar to that reported by previous experiments [14]. Higher levels of awake non-active behaviour together with lower levels of sleep have been shown to accompany chronic stressful situation in both humans [80] and laboratory rats [81, 82].

In accordance with the direction of the behavioural data, the findings of the changes in the performance parameters (body weights and weight gains) and the changes in the weight of internal organs could also indicate that long-term housing of rats in the (SEC) appeared to decrease their ability to cope with and to exert control over the environment. Rats housed in (PEC) showed higher body weights and weight gains and higher weights of spleen, thymus and testes compared to their counterparts housed in either (SEC) or (SC). Measures of body weight and weight gain can be easily monitored with minimal disturbance to the animal and can therefore be a useful way of monitoring the welfare state of animals subjected to experimental procedures [83]. The increased weights and weight gains in the (PEC) rats could be due to their increased feeding, but could also be due to their increased sleep behaviour. One of the many theories that have been proposed for the function of sleep is the protective theory that is: the function of sleep is to protect the organism from excessive wear and tear. This finding indicates that long-term housing of juvenile laboratory rats in (SEC) appears to decrease their ability to cope with the environment. Body weight and weight gain have been reported to decrease after chronic physical [84] and social stress [73, 85].

Similarly, the weight of internal organs is a simple procedure that may be very instructive in providing good information about whether the animal has been experiencing stress [83, 86]. It has been reported that stress can decrease the weight (reduce the lymphatic tissue mass) of lymphoid organs such as thymus (thymus atrophy) and spleen [87, 88]. The findings of increased weights of spleen and thymus in the rats experienced housing conditions enriched with physical objects are similar to those reported by previous work on the effects of environmental enrichment on laboratory mice [24].

Single housing of laboratory rats in standard laboratory cages has been shown to impact their behaviour, physiology and to compromise their welfare [19, 33, 90]. Social grouping of laboratory rats in conventional laboratory cages has also been demonstrated to cause social pressure and to impair welfare, [60, 91] although rats still seek social contact. The differences observed between the experimental treatments in the current study were significant between (PEC) on one side and both (SC) and (SEC) on the other side. This means that social housing of laboratory rats in standard cages appeared to have an affect similar to that of single housing on their welfare. This finding contradicts with that of other studies [28] who reported that social housing of laboratory rats can be effective and can be used alone as a method of cage enrichment. A possible reason for this contradiction could be the type of the laboratory cage used for housing rats. In the current study, cages with elevated cage lids (21 cm) were used for housing rats. These cages might have allowed some degree of visual, auditory and olfactory communication between singly-housed rats and animals in other cages which, in turn, might have improved the welfare of singly-housed rats. Housing laboratory rats singly but in cages with elevated lids that permit communication between the singly-housed rats and animals in other cages, but in the same experimental room, appeared not only to remove stress of social isolation but also to alleviate the social pressure of housing in groups and therefore to improve welfare of singly-housed rats [58]. Similarly, visual interactions between rats housed singly in cages with a view to other neighbouring rats and to the rest of laboratory holding room has been shown to improve the welfare of rats and to be preferred to housing in cages without this view [57]. Housing rats singly in cages permitting social communication (visual, olfactory and auditory) was preferred to housing them in pairs [56].

In a call for more sensitive treatment of laboratory animals, it has been pointed out that many researchers ignore the status of housing conditions despite the fact that stress evoked by such conditions can jeopardize the value of their data [92]. We posit that there are many of us who have great sensitivity for our animal subjects but are forced nonetheless to isolate them in order to obtain the data we need. Housing rats singly in cages with elevated cage lids enriched with multiple physical objects and adjacent to other laboratory cages in the same experimental room is only one, but a significant way of improving their welfare.

CONCLUSION

Enriching standard cages of laboratory rats physically can help them to increase the control over their environments and to promote their species-specific behaviour. This was evident by the findings that rats in these housing conditions exhibited high levels of behavioural indicators of good welfare such as sleep, exploration, movement activities and being in-the-open part of the cage and low levels of behavioural indicators of bad welfare such as stationary, bedding-directed behaviour and being under hopper. Therefore, laboratory rats could benefit from housing in physically enriched cages with elevated lids that permit visual and olfactory communication between rats compared to socially enriched cages.

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