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LONG-TERM EFFECTS OF USING VARIOUS ENRICHMENT OBJECTS ON MULTIPLE MEASURES OF WELFARE IN SINGLY-HOUSED RATS

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ABSTRACT

Single housing of laboratory rat may be recommended in some situations such as toxicological and nutritional studies and also to prevent the spread of infectious diseases. However, as single housing of laboratory rat has been shown to be stressful, modification of the housing environment are needed to improve the welfare of these animals. The aim of this experiment was to investigate how long-term enrichment of laboratory cages of singly-housed rats using multiple physical items affects various measures of welfare such as behavioural, weight changes and the weight of internal organs. 24 rats were housed singly in either enriched or unenriched cages. Behaviour was sampled every week and so was body weight and weight gain over a six week experimental period. Behaviours of the rats in the elevated plus-maze were recorded in the seventh week whereas, weight of internal organs were recorded post-mortem. Long-term single housing of rats in super-enriched cages increased levels of indicators of good welfare including sleep, exploration and feeding behaviour, body weights, weight gains and the relative weights of thymus gland and spleen, and decreased levels of indicators of poor welfare such as stationary behaviour and relative weight of adrenal glands. Thus, enriching conventional cages of singly-housed rats with multiple physical structures appeared to improve their ability to control the environment and to promote their species-specific behaviour; potentials that can ultimately result in good welfare.

Key words: Laboratory Rats, Multiple Enrichment, Single Housing, Welfare.

INTRODACTION

Laboratory rodents spend a major proportion of their life span in the laboratory cage, and therefore improving this environment may not only improve their overall well-being **(Rodent Refinement Working Party, 1998)** by improving their ability to cope with the environment, but also

the accuracy of experimental results **(Sherwin, 2004)**. This is in turn likely to provide a valid animal model for research **(Poole, 1997)** and can ultimately result in a reduction in the number of animals used.

Environmental enrichment defined as "the modifications of the environment resulting in an improvement in the biological functioning of the captive animals **(Newberry, 1995)** is an important tool of improving housing conditions of laboratory rodents. Experiments have demonstrated wide beneficial effects of environmental enrichment on group-housed laboratory rodents **(Chamove, 1989; Tsai et al., 2003).** However, despite this consensus over the effects of environmental enrichment in rats, very few studies have considered how enriching laboratory cages of singly-housed rats by adding multiple physical structures may affect their welfare.

Although group housing is the recommended housing situation for laboratory rats (Patterson-Kane et al., 2002), it may not, under certain circumstances, be achievable. For example, in nutritional (metabolism and digestibility) and toxicological studies in which researchers need to know how much animals eat, metabolise and excrete, single housing of the subjects may be necessary. 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. For large animals such as farm animals, primates and zoo animals, to prevent the spread of infectious disease single housing is also recommended. Moreover, some animals are normally solitary and can only be housed singly, such as laboratory hamsters. It could therefore be interesting to look at the effect of enriching housing conditions for singly-housed animals.

There have already been some studies that looked at the effect of environmental enrichment on some behaviours of singly-housed rat such as exploratory behaviour and general activity (**Denny, 1975)**, on the interest of the rats towards enrichment items **(Townsend, 1997)** and also on the development of their brains in the enriched environment **(Bennett et al., 1969).** However, the fact that none of these studies looked at how long-term enrichment can affect the ability of the singly-housed animals

to cope with their environment by looking at their behavioural, physiological, pathological and psychological responses to the housing condition highlights the need for more research. In addition, most of experiments that looked at the effect of environmental enrichment on behaviour of laboratory rodent relied on supplying cages with a single physical item. There is evidence from research that increasing the extent of enrichment by increasing the number of items supplied to the cages of group-housed animals may augment the effect of enrichment **(Marashi et al., 2004; Abou-Ismail, 2010).** It may thus be worth studying how much environmental enrichment can do in improving the welfare of singly housed animals particularly in laboratory rats.

This experiment was therefore carried out to study the overall long-term effects of enriching cages of singly-housed rats, by adding various physical structures that are thought to stimulate rats' specific behaviours, on multiple measures of welfare such as behaviour, physiology, psychology and pathology.

MATERIALS AND METHODS

Animals

This experiment was carried out in the Department of Hygiene and Preventive Medicine, Faculty of Veterinary Medicine, Kafrelsheikh University. The experiment was conducted in a standardized laboratory animal room. The room was maintained under a 12:12 h light:dark schedule with the white light on between 0200 and 1400 and continuous dim red light (two 60 Watt bulbs, Serma Electrical, Egypt) enabling observation during the dark period, at a constant temperature (20±1 ^{o}C).

The experiment was carried out with two batches of rats in which each experimental treatment (see later) was replicated six times within each batch. The subject animals were 24 newly weaned male rats, 35- 50 g weight at arrival, of the Wistar (outbred) strain (Al-Alamia, El-Gharbia, Egypt). The rats were four weeks of age on arrival and were fed on pelleted food and tap watered (two bottles fitted in each cage) ad-libitum.

Rats were housed singly in cages supplied with sawdust as bedding and a handful of shredded paper as a nesting material. Cages were cleaned once

a week in which rats were re-housed in clean cages with new bedding and nesting material.

Housing conditions

Rats were arbitrarily housed in one of the following two conditions:

1) "Enriched cages" (EC): standard polypropylene cages (48 cm length × 30 cm width × 21 cm height) that were supplied with 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), ladders (9 step wooden ladder 35.5 cm, local pet store, El-Gharbia, Egypt) and nestlets (5 cm × 5 cm sterilized cotton fibre pads, Lillico, UK) **(Abou-Ismail et al., 2010)**.

2) "Unenriched cages" (UC): standard polypropylene cages (48.5 cm length × 33 cm width × 21 cm height) that were not supplied with any additional cage structures.

Behavioural assessment

Ethogram

The observer entered the experimental room 10 minutes before the scheduled start of the observation to allow the rats to habituate to his presence **(Hurst et al., 1999).** Observation was carried out every week in two sessions per day (representing one observation week) for the two housing conditions. The first session took place during the light phase (white light was on); starting at 1230 hr and ending at 1330 hr. The second session was carried out while the white light was off (during the dark phase of the day); starting at 1400 hr and ending at 1500 hr.

Behaviour of the rats in each of the 12 cages, in each batch, was recorded in real time using instantaneous sampling method with 4-s intervals between each consecutive focal animal. Each sample interval was prompted by an audio cue via headphones, and the behaviour recorded onto a check sheet. Each session therefore yielded 75 scans per rat. This meant a total of 150 scans per rat per day (observation week), and a total of 900 scans per rat over the entire experimental period (six observation weeks). The behaviour of each individual rat was sampled and its

position within the cage (underneath food hopper or in the open part of the cage) and state (contacting or away from enrichment) was also recorded **(Abou-Ismail et al., 2010).**

Fear and anxiety measurements (emotional behaviours)

At the seventh week and after behavioural observations were finished, a 5-min elevated plus-maze (EPM) test was conducted for each animal of the two housing conditions. EPM test is widely used in pharmacological research to analyze the level of anxiety in laboratory rodents, and is based on the natural conflict between the tendency of the animal to explore a novel environment and the aversive properties of a brightly lit open area (Menzaghi et al., 1996). The maze had 2 open arms and 2 closed arms (115 × 10 cm). The closed arms had 50 cm high walls. The plus-maze was elevated 100 cm above the floor. The maze was made of wood and was arranged in a manner such that arms of the same type were opposite each other, connected by a central area (15 cm × 15 cm). In order to keep the rats from falling over, the open arms were surrounded by a 0.5 cm high edge. All rats were tested individually in the light phase of the light/dark cycle in the same day between 0900 and 1200. The order of testing was counterbalanced between the two housing conditions to control for possible effects of time of the day on behaviour. Each rat was placed in the middle of the apparatus with its head facing an open arm, and its behaviour was video recorded for 5 min **(Kaliste et al., 2006).** The arms of the plus-maze were wiped with ethyl alcohol (Pharma One, Cairo, Egypt) after each individual rat was tested. The total numbers and durations of entries into closed and open arms, latency to the 1st entry into closed and open arms (seconds), frequency of rearing and grooming behaviours, and the number of head dip was recorded. Analysis was done by an experienced observer who was unaware of which housing conditions each animal belonged to.

Table 1- Ethogram for behavioural elements recorded (**Hurst et al., 1999; Meddis, 1975).**

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- Other behaviour:	1- Awake non-active	Stationary
D- Enrichment-directed:	1- Enrichment-directed	Sniffing, chewing, climbing, and manipulating the enrichment objects.
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

Weight changes and weight of internal organs

Throughout the six week experimental period rats were weighed weekly. Rats were picked from their cage and weighed using equilibrated scales (Sartorius, AG, Gottingen, Germany). At the end of the 7th week of the housing period rats were euthanized by cervical dislocation. Immediately after euthanasia the weight (in g) of each individual rat was recorded using a digital scale (Oertling, OB033, UK). Each rat was then dissected and selected internal organs, including the thymus gland, spleen and adrenal glands were removed and stored on ice in sterile balanced salt solution. They were subsequently dried, trimmed and weighed (in g).

Statistical analyses

Behavioural and weight changes data

We used a repeated measures General Linear Model (GLM) with week (week 1-6) and session (session 1-2) as within subject factors because the behavioural (ethogram) and physiological data (body weight and weight

gain) had been collected from the same cages at two different time points every week. Treatment (EC and UC) was included as a between subjects factor. SPSS (version 12.0 for windows) was used for all statistical analyses. The average % of scans spent in performing each behaviour was calculated by dividing the total number of scans for each behaviour variable by the total number of scans for each individual rat in each session (75 scans), and each figure was then multiplied by 100.

The relative weight gain (%) was determined by dividing the value of the absolute weight gain by the value of the body weight in the previous week, and then the resultant figure was multiplied by 100. Data were checked for normality and homogeneity of variances to test for the suitability of using parametric tests. Data of organ weight showed normality whereas behavioural data showed normality after square root transformation. All data are presented as EMM ± SE.

2.4.2. Elevated plus maze and weight of internal organ data

Data met the assumptions of parametric statistics (normality, homogeneity of variance, linearity). Relative durations of time spent in open (open/total × 100) and closed arms (close/total × 100), and latency to the 1st entry to open and closed arms were determined for each housing condition. Relative frequency of entries into opens (entries to open arms/total arm entries × 100) and closed (entries to closed arms/total arm entries × 100) arms, and frequency of rearing and grooming behaviours and head dip were also recorded for each group. The organ weights were expressed as a ratio of the body weight (relative weight for each organ). Differences between the rats of the two housing conditions in behaviours of the EPM test, final body weight and the relative weight of internal organs were tested using an independent *t*-test.

RESULT AND DISCUSSION

Behaviour

Main effects

Several behaviours showed an effect of housing conditions, average % scan: sleep ($F_{1,21}$=6.81, P<0.05); stationary ($F_{1,21}$=38.64, P<0.001) (see figure one); moving ($F_{1,21}$=9.62, P<0.05); bedding-directed behaviour ($F_{1,21}$=26.24, P<0.001) (see figure 2); under hopper ($F_{1,21}$=691.57, P<0.001) and in-the-cage ($F_{1,21}$=691.57, P<0.001) (see figure 3). The

values of sleep behaviour, movement activities and being in-the-cage were higher in the EC whereas those of stationary, bedding-directed behaviour and being under hopper were higher in the UC.

Interactions

Average % scan non-intake maintenance behaviour (self-grooming) showed a significant treatment*session ($F_{1,21}$=5.41, P<0.05), increasing significantly in the light phase in the EC; and both average % scan feeding ($F_{1,21}$=5.37, P<0.05) and exploration ($F_{1,21}$=5.43, P<0.01), increasing significantly in the dark phase in the EC (see figure 4).

Elevated plus maze:

Housing rats in enriched versus unenriched cages had a significant effect on their behaviours in the EPM including: relative time spent in open arms (sec) (t_{22}= 3.71, P<0.001); relative time spent in closed arms (sec) (t_{22}= -3.71, P<0.001) (see figure 5); relative open arm entry (t_{22}= 3.45, P<0.001); relative closed arm entry (t_{22}= -3.45, P<0.001) (see figure 6); and latency (sec) to 1st entry to open (t_{22}= -2.78, P<0.01) and closed arm (t_{22}= 5.99, P<0.001).

Weight changes and weight of internal organs

The output of the repeated measures-GLM showed that housing laboratory rats in enriched versus unenriched cages significantly changed weight changes parameters measured in this study, including: body weight (g) ($F_{1,21}$=111.68, P<0.001) and weight gain (g) ($F_{1,21}$=25.98, P<0.01) (see figure 8), with the rats in the EC weighing heavier and gaining more weights every week than rats in the UC.

Similarly, housing rats in enriched versus unenriched cages had a significant effect on the weight of internal organs recorded in this study including: final weight (g) (t_{22}= 5.20, P<0.001); relative adrenal weight (g) (t_{22}= -3.14, P<0.05); relative thymus weight (g) (t_{22}= 3.50, P<0.01); relative spleen weight (g) (t_{22}= 3.41, P<0.05) with the rats housed in the EC weighing more, having heavier thymus and spleen but lighter adrenal than rats housed in the UC (see figure 9).

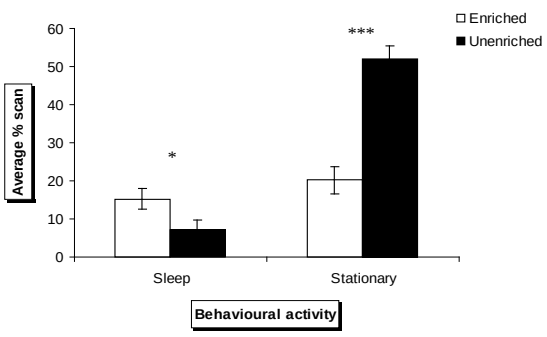

Figure 5: EMM ± SE 'Average % of time spent in the open and closed arm of the elevated plus maze' by the rats in the two housing conditions. *** P <0.001

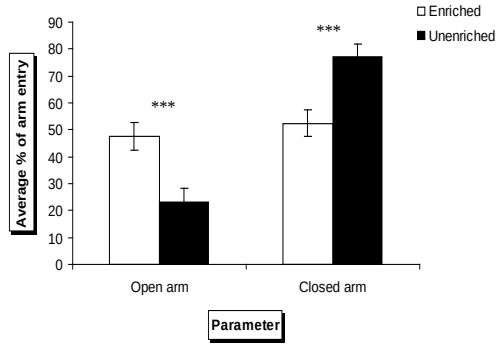

Figure 6: EMM ± SE 'Average % of open and closed arm entry of the elevated plus maze' by the rats in the two housing conditions. *** P <0.001

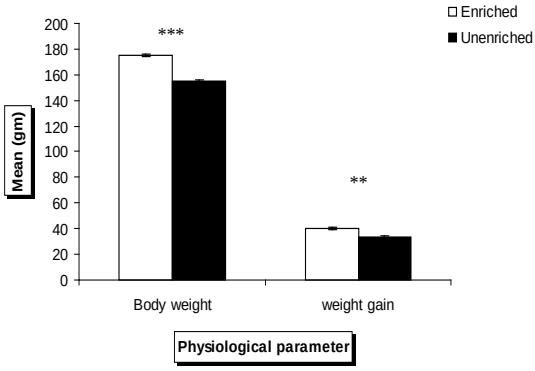

Figure 3: EMM ± SE 'Average % scan under hopper and in-the-cage' by the rats in the two housing conditions. *** P <0.001

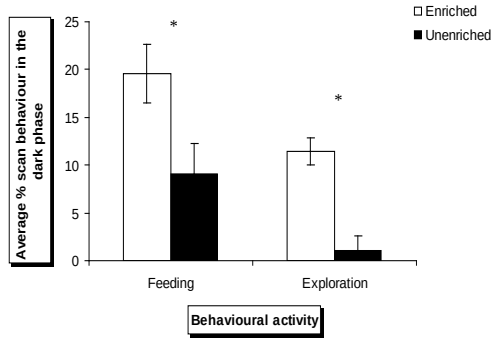

Figure 4: EMM ± SE 'Average % scan feeding and exploration' by the rats in the two housing conditions. * P <0.05

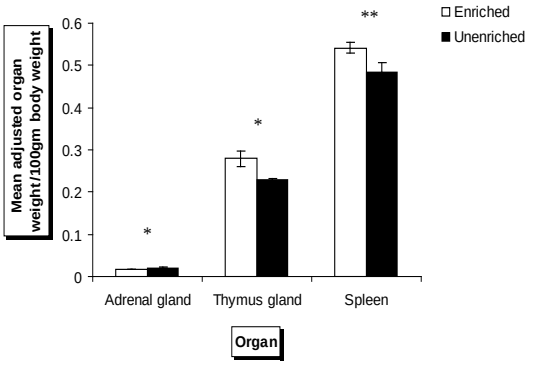

Figure 5: EMM ± SE 'Average % of time spent in the open and closed arm of the elevated plus maze' by the rats in the two housing conditions. *** P <0.001

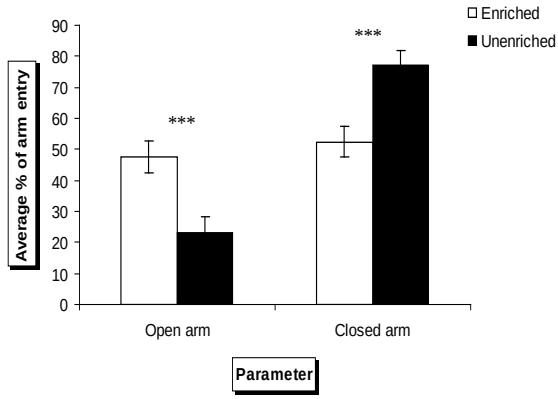

Figure 6: EMM ± SE 'Average % of open and closed arm entry of the elevated plus maze' by the rats in the two housing conditions. *** P <0.001

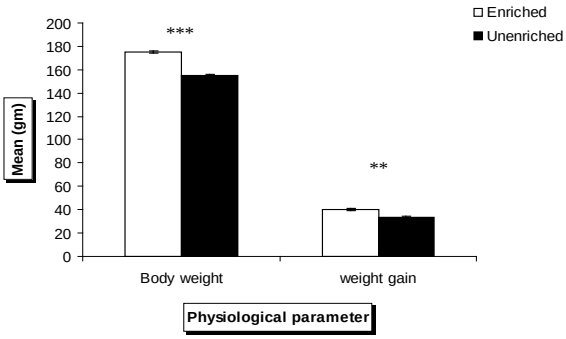

Figure 7: EMM ± SE 'Body weight and weight gain (g)' by the rats in the two housing conditions. ** P <0.05 *** P <0.001

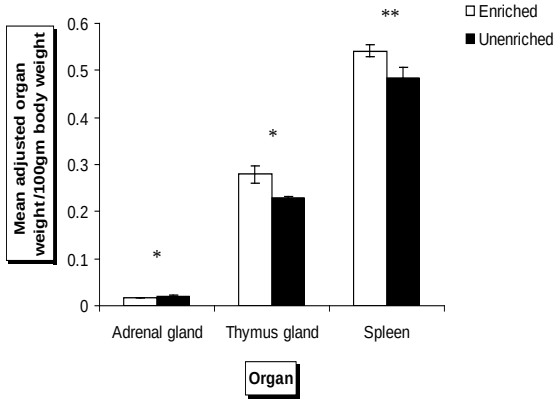

Figure 8: EMM ± SE 'Average relative adrenal, thymus and spleen weight (g)' by the rats in the two housing conditions. * P <0.05 ** P <0.001

DISCUSSION

Behaviour

Our results demonstrate clear differences between rats in the different housing conditions. Rats housed in the enriched conditions displayed higher levels of sleep, movement activities, exploration and both intake (feeding) and non-intake maintenance (self-grooming) behaviours and lower levels of stationary and bedding-directed activity as compared to rats in the unenriched conditions. Moreover, rats in the enriched cages were found to be in-the-cage (in the open part of the cage) more frequently and under hopper less frequently as compared to rats in the unenriched cages. These findings demonstrate that housing laboratory rats in conventional laboratory cages (standard unfurnished cages) is stressful compared to housing them in cages enriched with multiple physical structures.

High levels of sleep behaviour have been shown to indicate good welfare in laboratory rats **(Abou-Ismail et al., 2007)**. Research on both humans and laboratory rats has shown that chronic stress can affect both sleep quantity and quality. In humans, the activity of HPA axis has been shown to influence some features of sleep patterns, and that this may be due to the increased level of CRF, ACTH and cortisol. HPA axis hyperactivity (as in chronic stress, aging and depression) reduces sleep quality and causes sleep disturbances **(Bradbury et al., 1998).** Similarly, in laboratory rats, typically, in a chronic stressful situation, total sleeping frequency and duration decrease, with sleep liable to have more interruptions (**Knat et al., 1995)**. It has been shown that physical stress reduces both sleep quality and quantity (Bradbury et al., 1998). Chronic psychological stress (e.g. subordination) also appears to reduce sleep quantity **(Hurst et al., 1999).**

This high level of sleep displayed by rats in the EC 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 EC to control their environment by avoiding the disruptive effect of the white light. It was shown that unavoidable light constitutes a stressful condition for a nocturnal animal (laboratory rats) and that it resulted in a marked decrement of both types of sleep (rapid eye movement sleep and short wave sleep) **(Fishman and**

Roffwarg, 1972). The provision of multiple physical structures to the cage may have allowed the rats to use some of these structures (such as the retreat and crawl ball) to hide from the direct effect of the white light.

Laboratory rats are well known as thigmotactic (edge-users) preferring to spend most of their resting and sleeping time in contact with the surrounding walls of their environment **(Anzaldo et al., 1994, 1995).** Adding multiple physical structures to the conventional cages might have increased the walls and edges in the cage therefore improving the rats' ability to display more natural behaviour such as sleep. Similar finding of increased sleep behaviour in rats housed in enriched laboratory cages, but in groups, has been reported by **Orok-Edem and Key (1994).**

Rats in the EC displayed also higher levels of exploration and movement activities as compared to rats in the UC. Research work has shown that chronic stress decreases general activity levels and locomotor behaviour (Blanchard et al., 2001), and exploration **(Menzaghi et al., 1996).** These higher levels of movement and exploration by the enriched housed rats could be due to the increased complexity of their environment. **Denny et al. (1975)** 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. This finding of increased levels of movement and exploration by the rats in the EC are in accord with those of **Townsend (1997) and Marashi et al. (2004).**

Rats in the EC displayed higher levels of both intake and non-intake maintenance behaviour as compared to rats in the UC. Research work has reported an inhibition or a reduction in the self-grooming time after chronic stress; a repeated social defeat **(Van De Poll et al., 1982),** chronic stress by anxiogenic drugs **(Maldonado and Navarro, 2001),** chronic psychological stress (predatory stress) **(Blanchard et al., 1998)** and also in the subordinate animals after long period of grouping **(Hurst et al., 1996).** Similarly, a reduction in food intake has been found after chronic stress **(Blanchard et al., 2001).** This high level of self-grooming activity in the enriched housed rats may be due to the higher amount of sleep in these animals. 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 **(Saibaba et al., 1996).** Self-grooming was reported to be concentrated around sleeping time. It takes place after sleeping, but also occurs when the animal prepares for sleep. However, the high level of feeding displayed by the enriched housed rats could be due to the higher activity levels performed by these animals.

Rats in the EC exhibited lower levels of bedding-directed behaviours than rats housed in the UC. This relative increase in the level of bedding-directed behaviours in the UC could be due to the fact that rats in these cages had no enough cage structures (objects) to interact with. The only available cage structure in these cages was the bedding substrate; thereby these conventional cages limit the available options of the rats for interaction. On the other hand, rats in the EC may have performed bedding-directed behaviours less because they spent time interacting with the various different enrichment objects in their environment. Similar finding of reduced bedding-directed behaviours in groups of rats housed in enriched cages was reported by **Orok-Edem and Key (1994).**

The finding that rats in the EC were present more frequently in-the-open part of the cage and less frequently underneath-hopper compared to rats in the UC could be due to the increased compartmentalization of the EC by the provision of multiple physical structures into them. This 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. This might, in turn, have improved the ability of these animals to exert better control over their environments compared to their counterparts in the UC. Good ability of animals to cope with, and to control, the environment is a necessary requirement for good welfare **(Wiepkema and Koolhaas, 1993).**

Rats in the EC directed various behaviours towards the enrichment objects used in the study. The provision of enrichment objects appeared to have fulfilled the animals' "needs" including the choice to rest or sleeps in the open part of the cage during a certain time of the day (e.g. the light phase of the light/dark cycle), seek a refuge, forage and gnaw. "Needs" are requirements that are fundamental to the biology of an

animal e.g. to obtain a particular resource, respond to a particular environmental, or bodily stimulus (**Broom and Johnson, 1993).** In addition, the type of cage modification implemented in this study was of affordable cost, practical to use, clean and easy to replace, did not compromise the physical health of the rats, nor did it prevent ease of checking the animals.

Elevated plus maze:

Our results showed that rats experienced the EC explored the open arms of the maze for longer time and the closed arms of the maze for shorter time as compared to those experienced the UC. The EC rats also entered the open arms more frequently and the closed arms less frequently, and showed short latency to open arm entry as compared to the UC rats. Taken together, the results of the EPM indicate that increasing the extent of enrichment of conventional cages of laboratory rats appeared to decrease the level of stress they experience.

Tests for measuring anxiety, such as elevated plus-maze are generally accepted as a reproducible measure of anxiety in laboratory rodents **(Kantor et al., 2000)**. It has been shown that anxious animals are found to prefer, and are more active in, the closed arms over the less secure open arms; such behaviour which is indicated by less time spent on and low frequency of entries to the open arms as well as low latency to open arm entry **(Menzaghi et al., 1996).**

It appears therefore that long-term housing of laboratory rats in standard unfurnished cages is stressful. In accordance with our results, Batchelor, (1993) has mentioned that laboratory rats housed in conventional laboratory cages are ethologically, physiologically and psychologically aberrant and cannot be considered as normal animals. More importantly, **Sherwin, (2004)** showed that reduced external validity of the research and therefore the benefit gained from the research has been shown to arise when laboratory rodents are housed in standard laboratory cages.

Single housing of laboratory rat has been shown to be stressful **(Dronjak et al., 2004)**. Experiments have pointed out that individual housing

enhances anxiety-like behaviour **(Jankowska et al., 1991).** However, there are also data that have indicated that individual housing perse did not increase the anxiety-like behaviour **(Nakayasu and Ishii, 2008)**. Thus, simply, individual housing perse of laboratory rat may not be stressful **(Arakawa, 2003)** but housing them in standard laboratory cages for long term may be stressful.

Weight changes and weight of internal organs:

Our results showed that rats in the EC had higher weights and weight gains compared to rats in the UC. Moreover, the EC rats had higher relative weight of spleens, thymuses and lower relative weights of adrenal glands as compared to the UC rats. The increased weights and weight gains in the EC 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 **(Everson et al., 1989).** This finding indicates that long-term housing of juvenile laboratory rats in conventional laboratory cages appears to be stressful. Body weight and weight gain have been reported to decrease after chronic physical and social stress **(Hurst et al., 1996; Stefanski et al., 2001).**

In accordance with the direction of the data of behaviour, weight changes and elevated plus maze, the findings of the changes in the weights of the internal organs could also indicate that long-term housing of rats in the UC appeared to be stressful. The increase in the weight of the adrenal gland (adrenal hypertrophy) is generally thought to result due to the increased activity of the gland particularly the cortex (cortical hypertrophy) **(Manser, 1992).** This increase in the adrenal cortex weight has been suggested to happen under the frequent stimulation and the increased activity of the adrenocorticotrophic function of the pituitary gland which results from the stimulation of the HPA axis during chronic stress **(Christian, 1955).** Similarly, stress can decrease the weight (reduce the lymphatic tissue mass) of lymphoid organs such as thymus (thymus atrophy) and spleen **(Blanchard et al., 1995).**

The type of cage modification implemented in this study was of affordable cost, practical to use, clean and easy to replace, did not compromise the physical health of the rats, nor did it prevent ease of

checking the animals. The modification regimen provided all the required physical features of enrichment items suggested by previous studies **(Van de Weerd and Baumans, 1995; Pritchett and Corning, 2003).** Importantly, this particular type of cage modification provided the rats with ample opportunities to cope with and to exert control over their environment; characteristics that resulted in improved welfare in the animals experiencing it. It has been suggested that for an efficient environmental enrichment program to improve the welfare of the animals experiencing it, the enrichment should enhance the expression of desirable behaviours such as species-specific behaviours, decrease undesirable behaviours such as abnormal behaviour, or do both **(Kitchen and Martin, 1995; Van de Weerd and Baumans, 1995).** As, adding some physical structures to the laboratory cages should not be considered enrichment until it produces good long-lasting changes in welfare **(Line and Morgan, 1991),** the regimen used in this study appeared to have met this requirement and can therefore be called enriching.

CONCLUSION

Long-term enrichment of conventional cages of newly weaned laboratory rats with multiple physical structures appeared to improve the ability of these animals to control their environment and to promote their species-specific behaviour; potentials that can ultimately result in good welfare. Long-term single housing of rats in super-enriched cages increased levels of indicators of good welfare and decreased levels of indicators of poor welfare. The findings of this experiment showed that laboratory rats housed in enriched cages demonstrated improved welfare and were less stressed compared to those animals housed in conventional laboratory cages. The results, more importantly, demonstrated that when single housing of laboratory rats is necessitated their laboratory cages should be enriched with multiple physical structures in order to improve their welfare. These findings thus strongly support the need of the current conventional housing systems of laboratory rats, particularly singly-housed rats, for re-evaluation to help provide better environment for the animals that can in turn result in an improvement in their welfare.

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التأثيرات طويلة المدي لإستخدام أدوات دعم متعددة علي القياسات المختلفة لمستويات الإراحة في الجرذان منفردة المسكن

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يوصي بالإسكان المنفرد للجرذان المعملية في بعض الأحيان كما في دراسات السموم و التغذية و أيضا لمنع إنتشار الأمراض المعدية. وحيث أن الإسكان المنفرد للجرذان المعملية مجهد لها فإنه يتطلب تعديل بيئة الإسكان لتحسين مستويات الإراحة في تلك الحيوانات.

تم إجراء هذه التجربة لدراسة التأثيرات طويلة المدي لدعم الأقفاص المعملية وذلك بإضافة أدوات عديدة في الأقفاص علي القياس المختلفة لمستويات الإراحة مثل السلوكيات، التغيرات في وزن الجسم وفي أوزان الأعضاء الداخلية للجرذان منفردة المسكن . تم إ سكان الجرذان المستخدمة في هذه التجربة وعددها 24 جرذ منفردا في أقفاص إما 'مدعمة' أو 'عادية'. تم تسجيل السلوكيات وأوزان ومعدلات نمو الجرذان كل أسبوع خلال فترة الأسابيع الستة للتجربة . تم تسجيل سلوكيات الجرذان في المتاهة المتعامدة المرتفعة في الأسبوع السابع في حين تم تسجيل أوزان الأعضاء الداخلية بعد إماتة الجرذان.

كشفت النتائج أن الإسكان المنفرد طويل المدي للجرذان في أقفاص فائقة الدعم أدي إلي زيادة في مستوي مؤشرات الرفاهية الجيدة كسلوكيات النوم والإستكشاف والتغذية، أوزان الجسم ومعدلات النمو والوزن النسبي للغدة التيموسية والطحال ، وإلي إنخفاض في مستوي مؤشرات الرفاهية السيئة كسلوكيات الثبات والوزن النسبي للغدة الكظرية . وبالتالي، فإن دعم الأقفاص الم عملية التقليدية للجرذان منفردة المسكن بإستخدام أدوات عديدة يمكن أن يؤدي إلى تحسين في قدرتها علي السيطرة علي البيئة وإلي تعزيز سلوكياتها الخاصة ، الإمكانيات التي يمكن أن تؤدي إلي تحسين مستويات الرفاهية.