

LONG-TERM EFFECTS OF SINGLE VERSUS GROUP HOUSING OF LABORATORY RATS ON THEIR BEHAVIOUR, PERFORMANCE AND WELFARE

Mohamed, M. Fouda; Usama, A. Abou-Ismael and Ahmed, F. Abou-Elnaga

Department of Animal Husbandry, Faculty of Veterinary Medicine,
Mansoura University, Gomhoria St., Mansoura, P.O. box 35516, Egypt.

ABSTRACT

Social isolation of laboratory rat which is usually referred to as 'isolation syndrome' has been shown to affect corticosterone levels, metabolism, growth, and behaviour. It is not however known whether housing rats singly in close proximity to social groups such as in cages with elevated lids that allow visual, auditory and olfactory communication improves their welfare. The aim of this experiment was to investigate how housing of single rats in cages with elevated lids in close proximity to group-housed rats might affect their behaviour, performance and measures of anxiety. 18 rats were housed either singly (SH) (N= 3) or socially in groups of five (GH) (N=3) in standard laboratory cages with elevated lids (21 cm height) permitting visual, auditory and olfactory communication for six weeks. The results showed that housing rats singly in cages permitting some degree of social communication appeared to remove social pressure of group housing and to improve the welfare of these animals. SH rats showed higher levels of sleep and self-grooming behaviour and were more frequently observed in the open part of the cage as compared to GH rats. SH rats had also lower adrenal gland weight and higher thymus and spleen weight, and entered the open arms of the elevated plus maze more frequently compared to GH rats. It could be concluded that, through making small changes in the procedures and housing environments, the welfare of singly-housed rats can be improved.

Key words: Laboratory Rats, Single Housing, Group housing, Communication, Welfare.

INTRODUCTION

Isolating an animal refers to the situation where the animal is physically fully demarcated from conspecifics without physical, visual, olfactory and auditory contact (e.g. Krohn et al., 2006). Animals housed in separate cages

in the same room are, although deprived of physical and visual contact, still in olfactory and auditory contact, and thus not totally isolated. During the fifties and sixties several studies claimed to show physiological and behavioural differences between individually and group housed rats. The so-called 'isola-

tion Syndrome' characterised by changes in corticosterone levels, metabolism, growth, and behaviour was introduced, rather as a model for psychoneurosis than through any concern for animal welfare. It is often stated as common knowledge in laboratory animal science text books that individual housing as well as isolation of rats has an effect on physiology and behaviour. It is however, unclear whether this effect actually impairs welfare of singly-housed animals.

Singly-housed rats have been shown to develop 'odd' behaviours such as bar biting and tail chasing (**Baenninger, 1967; Hurst et al., 1998**), eat more (**Levitsky, 1970**), put on less weight (**Hatch et al., 1963**), be more aggressive (**Patterson-Kane et al., 2002**), have heavier adrenal glands (**Sandstrom and Hart, 2005**) and under-perform in cognitive tests (**Patterson-Kane et al., 1999**) relative to socially housed conspecifics. As a consequence of these findings, many major animal science regulatory bodies (for example, UK Home Office) strongly discourage single housing of rodents in animal research. 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, or paradoxically to remove social stress (**Nyska et al., 2002; Verwer et al., 2007**).

However, despite the widespread belief that single housing impairs welfare, single housing does not consistently evoke greater stress hormone responses (**Morinan and Leonard, 1980**) or result in heavier adrenal gland weights than does social housing (**Baldwin et al., 1995**). Furthermore, singly-housed rats

are not always cognitively impaired (**Wongwitdech and Marsden, 1996**) nor do they always eat and weigh more than socially housed conspecifics (**Baldwin et al., 1995**).

One explanation for these conflicting findings is that single housing effects vary depending on the severity of the isolation (**Krohn et al., 2006**). For example, single-housed rats spend more time investigating a barrier between neighbours the more that barrier allows social contact (**Hurst et al., 1997, 1998**). Although 'isolation' is frequently used in paper titles, it almost always means individual housing, i.e. social physical isolation. So, it is impossible to compare the effects from isolation (that comprises deprivation of the subject animal from communication with other animals) with the effects of individual housing (that allows the subject animal a certain degree of communication with other animals) to reveal any differences between the two housing types.

Also, differences in cage sizes could play a significant role in changing behaviour, physiology and organ weights (**McGlone et al., 2001; van Loo et al., 2001**). Cage sizes seemed to be selected rather randomly. Individually-housed rats were caged with a floor area ranging from 286 cm² to 1353 cm², while group-housed rats had floor areas from 930 cm² to 5625 cm² and stocking densities from 183 cm² to 948 cm² per animal (e.g. **Baenninger, 1967; Hatch et al., 1963, 1965**). In a very few studies the same cage sizes were used for individually and group-housed animals, and in only one study the same stocking density was used for both (**Takemoto et al., 1975**). Whether or not the

different cage sizes may have had an influence on the results is unclear, but some of the discrepancies found may have been caused by different cage sizes as opposed to different housing conditions.

The differences in results of experiments on singly and group-housed animals could be due to the effect of animal sex (**Bartolomucci et al., 2009**). Females housed singly in cages separated by a wire mesh were found to spend significantly more time close to the companion animal than males (Krohn et al., 2006). Finally, different strains are known to react differently in behavioural and physiological tests (**Cunliffe-Beamer et al., 1981; Dahlborn et al., 1996**). Some strains may be very sensitive to individual housing, whereas others are unaffected (Vadiei et al., 1990). Even rats of the same strain, but from different breeders show differences in behavioural and clinical chemistry (**File and Vellucci, 1979**). Therefore, comparison of results from different studies on different strains is difficult.

This experiment was carried out to study the overall long-term effects of housing laboratory rats singly in cages with elevated lids that allow some degree of visual, olfactory and auditory communication with other rats in the same room, as a method of indirect social enrichment, on the behaviour, body weight and weight gain, weight of internal organs and measures of anxiety in these animals. It could be hypothesized that if visual, olfactory and auditory communication between neighbouring rats is allowed, it is possible that single-housing of rats would become less stressful than previously considered.

MATERIALS AND METHODS

Animals

This experiment was carried out in the Department of Animal Husbandry, Faculty of Veterinary Medicine, Mansoura University, in the period from December to January, 2009. 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 0100 and 1300 and continuous dim red light (two 60 Watt bulbs, Serma Electrical, Egypt) enabling observation during the dark period, at a constant temperature ($20\pm 2^{\circ}\text{C}$).

The experiment was carried out using one batch of 18 rats in which each experimental treatment (see later) was replicated three times. The subject animals were 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 ad-libitum.

All cages were supplied with sawdust as bedding material and were cleaned once a week in which rats were removed and rehoused in clean cages with new bedding material. Cages were arranged on an elevated metal rack to allow clear observation.

Experimental treatments

Rats were arbitrarily assigned to one of the following two experimental treatments:

1) **"Single housing" (SH)** : Rats were housed singly in standard cages (48cm length x 30cm width) with elevated cage lids (21cm height).

2) **"Group housing" (GH)** : Rats were housed in groups of five in standard cages (48 cm length x 33 cm width) with elevated cage lids (21 cm height).

All rats were introduced to their particular experimental treatments at four weeks of age and were kept under the same housing condition until they were ten weeks old; the age at which data collection was stopped and the animals were euthanised.

Behavioural assessment

Ethogram

In order to let the rats habituated to the presence of the observer, the observer entered the experimental room 10 minutes before the observation started (e.g. 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 1100 hr and ending at 1200 hr. The second session was carried out while the white light was off (during the dark phase); starting at 1400 hr and ending at 1500 hr.

Behaviour of the rats in each of the six cages was recorded in real time using instantaneous sampling method with 10-s intervals between each consecutive focal animal (a single rat in the SH conditions and five rats in the GH conditions). Each sample interval was prompted by an audio cue via headphones, and the behaviour recorded onto a check sheet. Each session therefore yielded 20 scans per rat. This meant a total of 40 scans per rat per day (observation week), and a total of 200 scans per rat over the entire experimental period. The behaviour of each individ-

ual rat was sampled and its position within the cage (underneath food hopper or in the open part of the cage) was also recorded (Abou-Ismaïl et al., 2010).

Fear and anxiety measurements (emotional behaviours)

At the sixth week and after behavioural observations were finished, a 5-min elevated plus-maze (EPM) test was conducted for each animal of the two experimental treatments. The elevated plus maze is a rodent model of anxiety that is used as a screening test for putative anxiolytic and anxiogenic compounds (Pellow et al., 1985) and as a general research tool in neurobiology to assess the level of anxiety (Rodgers, 1997). The model is based on rodents' aversion of open spaces (Trett et al., 1993). This aversion leads to the behaviour termed thigmotaxis, which involves avoidance of open areas by confining movements to enclosed spaces or to the edges of a bounded space (Carobrez and Bertoglio, 2005). In EPM this 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 elevated plus-maze was constructed of wood with two open arms and two closed arms of the same the size (50cm x 15cm) and with 50cm high wall. The maze was arranged in a manner such that arms of the same type were opposite to each other, connected by a central area (15cm x 15cm), and the entire maze was elevated to a height of 50cm above the floor. In order to keep the rats from falling over, the open arms were surrounded by a 0.5cm high edge. The rats were placed individually in the center of the maze facing an open arm. Subject behaviours were recorded by a video camera for

5 minutes (Pellow et al., 1985). The total number of entries to open and closed arms and the time spent in the open and closed arms was recorded. An arm entry was defined as an animal entering the arm with all four feet.

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 6th week of the housing period rats were euthanised 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

SPSS version 16.0 was used for all statistical analyses. Average % scan for each behavioural pattern was calculated by dividing the total number of the activity by the total number of scans and the resultant value was multiplied by 100. Data of the rats of the GH con-

ditions were averaged to be comparable to those of the rats of the SH conditions. A General linear model (GLM)-repeated measures was used to test for the main effect of experimental treatments on the observed behavioural variables because the data were collected from the same subject at different times (sessions and observation weeks). 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. All data are presented as estimated marginal means (EMM) \pm SE.

Elevated plus maze and weight of internal organ data

Relative durations of time spent in open (open/total \times 100) and closed arms (closed/total \times 100) were determined for each experimental treatment. Relative frequencies of entries into open (entries to open arms/total arm entries \times 100) and closed (entries to closed arms/total arm entries \times 100) arms, were also recorded for each experimental treatment. The organ weights were expressed as a ratio of the body weight (relative weight for each organ). Differences between the rats of the two experimental treatments in behaviours of the EPM test, final body weight and the relative weight of internal organs were tested using an independent t-test.

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 (body care behaviours)	Self-grooming and pandiculation (stretching and yawning).
	4- Movement	Locomotion in the cage.
	5- Exploratory behaviour	Sniffing cage wall, cage top and cage floor.
	6- Air-out	Sniffing air outside the cage.
	7- Air-in	Sniffing air inside the cage.
	8- Bedding-directed behaviours	Digging, sniffing bedding, bedding manipulation (pushing bedding material forwards or backwards with nose, forepaws or hind legs) and burrowing.
B- Sleep:	1- Sleep	Lying unalert with both eyes closed- apparently asleep.
C- Abnormal behaviour:	1- Tail chasing	Chasing of own tail in circles.
	2- Bar biting	Chewing at any part of the cage bars.
D- Other behaviour:	1- Awake non-active	Stationary.
	2- Agonistic and social interaction	Upright, aggressive over (pinning cage mate on its back), aggressive groom, biting, chase, mounting, pull tail and allogrooming, and social sniffing (collected for GH conditions only).
	3- Out of sight	Behaviour of the rat cannot be observed.
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, excluding its tail, is entirely in the open part of the cage.

RESULTS

Behaviour

Main effects of experimental treatment

There was a significant effect to the experimental treatments on the position of the rats in the cage. Rats of the SH condition were more frequently seen in-the-cage than rats of the GH condition ($F_{1,6} = 82.81$, $P < 0.001$). In contrary, rats of the GH condition were more frequently seen under-hopper than rats of the SH condition ($F_{1,6} = 82.81$, $P < 0.001$), (Figure 1). Similarly, rats of the GH condition showed higher levels of feeding ($F_{1,6} = 119.58$, $P < 0.001$) and movement ($F_{1,6} = 89.29$, $P < 0.001$) than those of the SH condition (Figure 2).

Interactions

Housing condition*observation week

Rats of the GH condition drank more ($F_{4,24} = 5.994$, $P < 0.05$) and self-groomed less ($F_{4,24} = 8.585$, $P < 0.01$) than those in the SH condition in the 4th observation week (Figure 3). Rats of the GH condition showed higher levels of exploration in the 3rd observation week ($F_{4,24} = 8.539$, $P < 0.05$) (Figure 4), and bedding-directed behaviour ($F_{4,24} = 9.16$, $P < 0.001$) in both 2nd and 4th observation weeks as compared to those in the SH condition (Figure 5). On contrary, rats of the SH condition slept more than those in the GH condition in the 2nd, 3rd and 5th observation weeks ($F_{4,24} = 7.47$, $P < 0.001$) (Figure 6).

Housing condition*observation session

Rats of the GH condition drank more ($F_{1,6} = 14.93$, $P < 0.01$) and were observed to be less stationary ($F_{1,6} = 22.73$, $P < 0.01$) than rats of the SH condition in the dark phase (Figure 7).

Elevated plus maze:

Housing rats in SH versus GH conditions had a significant effect on the relative closed arm entry ($t_{16} = -2.86$, $P < 0.05$) (Figure 8). Whereas, there was no significant effect to the experimental treatments on the other measures of anxiety including relative open arm entry ($t_{16} = 1.80$, NS), relative time spent in open arms (sec) ($t_{16} = 1.63$, NS) and relative time spent in closed arms (sec) ($t_{16} = -1.63$, NS).

Weight changes and weight of internal organs:

There was no significant effect to the experimental treatments on the body weight of the rats ($F_{1,6} = 75.33$, NS). There was however a significant effect to the experimental treatments on the relative weight of internal organs. Rats of the SH condition had lighter adrenal glands ($F_{1,6} = 1.42$, $P < 0.01$) but heavier spleen ($F_{1,6} = 4.34$, $P < 0.001$) and thymus ($F_{1,6} = 7.16$, $P < 0.01$) than those of the GH condition (Figure 9).

DISCUSSION

Behaviour :

The results demonstrate clear differences between rats in the different experimental treatments. Rats of the SH condition displayed higher levels of sleep, self-grooming activity and awake non-active behaviour, and lower levels of intake maintenance behaviours (feeding and drinking), movement activities, exploration and bedding-directed behaviours as compared to rats of the GH conditions. Moreover, rats of the SH conditions 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 of the GH conditions.

An explanation for why GH rats fed and drank more than SH rats could be the increase of their activity levels such as movement and exploration but also their social interaction (both aggressive and non-aggressive). Whereas, the increase in the level of bedding-directed behaviour by the GH rats could be explained as an attempt to escape. Social housing of laboratory rats in standard laboratory cages has been shown to cause social stress and to increase specific form of behaviours termed as 'escape-related' (**Hurst et al., 1999**). Although chronic stress (crowding stress) has been shown to have an anorexic affect (reduces food and water intake) (**Gómez et al., 1996**), it has been stated that the increase in water intake, such as polydipsia (excessive water drinking) may appear as an abnormal behaviour, as a sign of stress, due to chronic confinement (**Fraser and Broom, 1997**). On the other hand, SH rats may have performed bedding-directed behaviours less because they spent more time performing other behaviours such as sleep and self-grooming.

Rats and mice are energy consumers, as the animals change their food consumption to keep the weight if required (**Adolph, 1947**). Higher food consumption may be expected in individually housed animals due to the increase in space and the lack of heating from cage mates. However, one study found no differences in food consumption in rats (**Szenasi et al., 1988**) housed individually, while another study found a decreased food consumption (**O'Connor and Eikelboom, 2000**) and yet two more studies found an increased food consumption (**Brown and Grunberg 1996; Pérez et al., 1997**) in individually housed rats. It is therefore difficult to draw clear con-

clusions about whether increased or decreased levels of feeding and drinking under single and group-housing condition of laboratory rats is good or bad for their welfare. However, an increase in the level of behaviours indicative of escape attempts such as exploration and bedding-directed behaviours may indicate that the animals are having a decreased ability to cope with their environment and that the housing condition is stressful for them (**e.g. Hurst et al., 1999**).

On the other hand, the high levels of sleep displayed by the SH rats as compared to GH rats may indicate that the welfare of SH rats is better than that of GH rats. High levels of sleep behaviour have been shown to indicate good welfare in laboratory rats (**Abou-Ismaïl et al., 2007**). This high level of sleep displayed by SH rats could be due to their improved ability to control the environment by being under-hopper; the only place in the cage that provides a protection from the disruptive effect of the white light. Such criterion that might have not been available for the GH rats as it may probably be difficult to the five animals to be under-hopper. Even if the GH rats can all get under the hopper, their sleeping bout may get interrupted by the vocalization or movement of cage mates. The high level of self-grooming activity displayed by the SH rats may be due to the higher amount of sleep in these animals. Self-grooming was reported as 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.

Results did not reveal a significant effect to the experimental treatments on either tail chasing or bar biting. An increase in self-directed behaviour, e.g. tail chasing and bar biting, has been observed in individually housed rats (**Hurst et al., 1997, 1998; Baenninger, 1967**). In general, stereotypic behaviour is seen in impoverished environments (**Würbel et al., 1998**), so individual housing may induce stereotypies, but actually no stereotypic behaviour was observed in our study, either because it was not observed or because it was not performed. The absence of stereotypic behaviour may indicate a smaller welfare impact than is supposed from being housed individually.

Hurst et al., (1997) concluded that although single housing may remove social pressure, singly housed animals may still seek social company. Looking at the animals' motivation to seek social company or preference for a cage containing conspecifics, does not show that social company is that important. In two studies on mice, the cage containing a partner was visited just as frequently as other cages containing food, space or shelter (**Sherwin and Nicol, 1996; Sherwin, 1996**). Also, the mouse preferred to rest in the cage containing the food rather than the social company, which may indicate, that the social companionship is not highly prioritised. An explanation of this could be that only visual contact between the two mice was possible. In another study on rats, a rat could choose company in a T-maze and there was only a slight favour for the cage with conspecifics compared to an empty cage (**Patterson-Kane**

et al., 2001), although the rat could be in direct contact with the other rats.

Elevated plus maze

The results of the EPM showed that SH rats displayed low levels of behaviours indicative of emotionality as compared to GH rats. SH rats entered the open arms of the maze more frequently, and the closed arms less frequently compared to GH rats. Although there was no significant effect to the experimental treatments on the time spent in both the open and closed arms of the maze, the findings indicate that the welfare of animals housed singly but in cages with elevated lids that allowed some degree of social communication, as compared to those housed in groups, is improved. Behavioural tests of anxiety such as EPM have been shown as a valid measure of assessing anxiety in laboratory rodents (**Degroot and Treit, 2004**). Anxious animals were shown to enter the closed arm of the maze more frequently and the open arms less frequently compared to non-anxious animals (**Lister, 1987**). There are data that have indicated that individual housing per se did not increase the anxiety-like behaviour (**Nakayasu and Ishii, 2008**). Thus, simply, individual housing per se of laboratory rat may not be stressful (**Arakawa, 2003**) but housing them in cages that deprive them of social communication for long-term may be stressful.

Weight changes and weight of internal organs

The results of this experiment showed that the body weight and weight gain of SH and GH rats did not differ significantly. Although, individual housing may change feeding behaviour it does not necessarily have to change

body weight and weight gain of the animals. Several studies on rats revealed no differences in body weight. In some studies rats housed individually had a higher body weight than group-housed rats (File, 1978; Levitsky, 1970; Lopak and Eikelboom, 2000). In another study no effect on body weight was observed (Sobel et al., 1979).

It is however interesting to note that despite the finding that GH rats were more frequently seen feeding than SH rats there were no significant differences in their weights or weight gain over the experimental period. This lack of significant differences in weight and weight gain between the rats of the two experimental treatments, despite the significant differences in feeding, could be due to that GH rats were more active both physically (moved and explored more) and socially (agonistic interactions between rats), and directed more behaviours towards the bedding materials in their cages than SH rats.

On the other hand, SH rats displayed lighter adrenal weights and heavier spleen and thymus weights as compared to GH rats. Changes in the weight of some internal organs have been shown to accompany stress and therefore to be a valid measure of welfare in laboratory rodents (e.g. Manser, 1992; Abou-Ismael and Mahboub, 2010). In accordance with the direction of some behavioural findings (e.g. sleep) and the data of elevated plus maze, the findings of the changes in the weight of the internal organs could also indicate that long-term single-housing of labora-

tory rats in cages with elevated lids is not stressful and can therefore be considered as a method of social enrichment.

CONCLUSION

There is no strong scientific basis for concluding that individual housing always imposes a major welfare problem in rats, and more and better controlled studies are needed. Although single housing of laboratory rats that involves social isolation of the subject animals has sometimes, under the circumstances of the experiments, been shown to cause stress, 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. Thus, there probably is an effect of being housed individually, but the effect may not be that major, and it seems likely to assume that it could be eliminated or minimised by small procedural and housing changes e.g. housing in cages with elevated lids which can be considered as a method of social enrichment for the animals.

ACKNOWLEDGEMENT

The authors would like to thank Department of Animal Husbandry, Faculty of Veterinary Medicine, Mansoura University for funding the study.

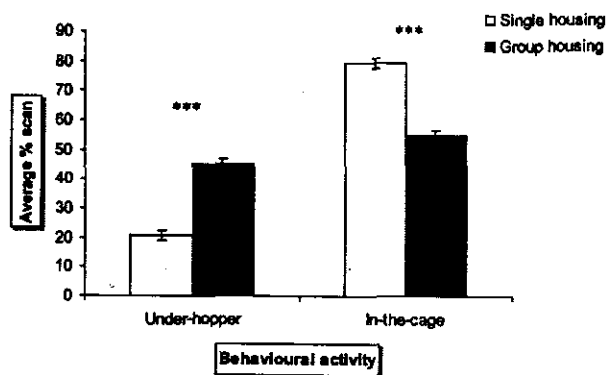


Fig (1) : 'Average % scan under-hopper and in-the-cage' by the rats in the two experimental treatments. *** P <0.001

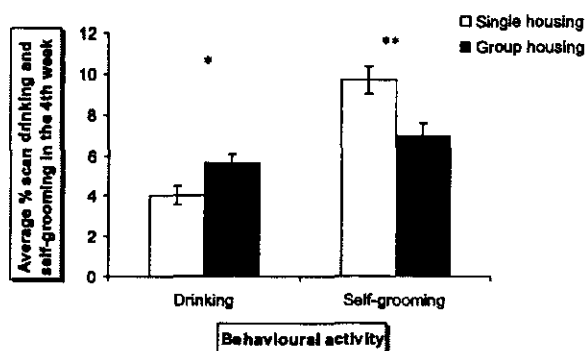


Fig (3) : 'Average % scan drinking and self-grooming' by the rats in the two experimental treatments. *P<0.05 ** P<0.01

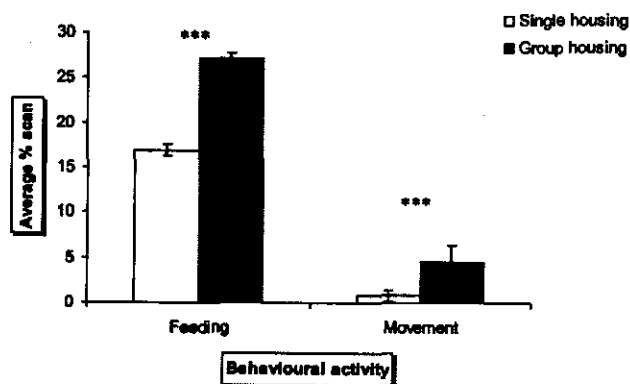


Fig (2) : 'Average % scan feeding and movement' by the rats in the two experimental treatments. *** P <0.001

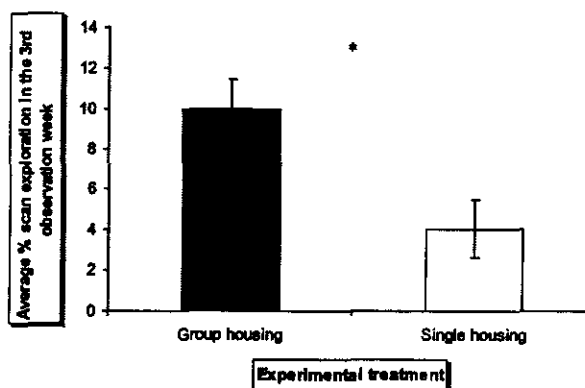


Fig (4) : 'Average % scan exploration' by the rats in the two experimental treatments. * P <0.05

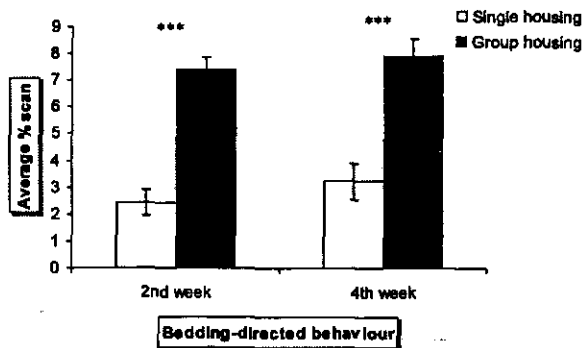


Fig (5) : 'Average % scan bedding-directed behaviour' by the rats in the two experimental treatments. *** P <0.001

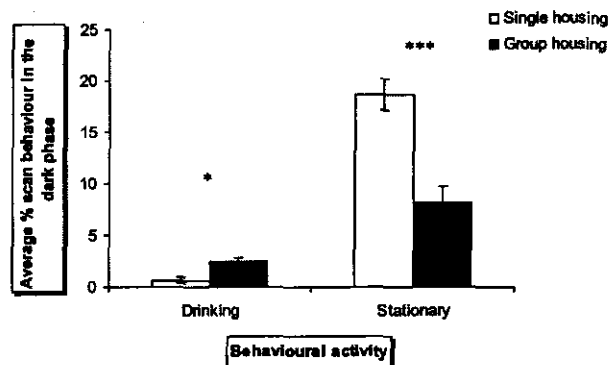


Fig (7) : 'Average % scan drinking and stationary' by the rats in the two experimental treatments in the dark phase. * P <0.05 *** P <0.001

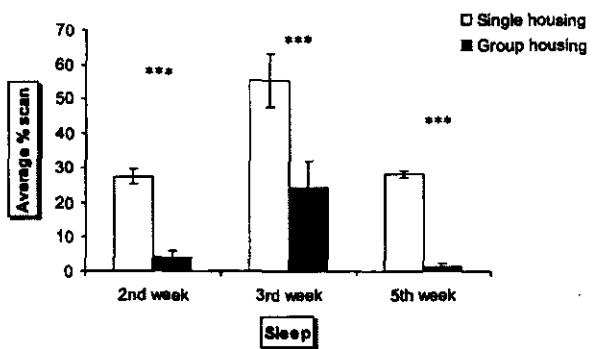


Fig (6) : 'Average % scan sleep' by the rats in the two experimental treatments. *** P <0.001

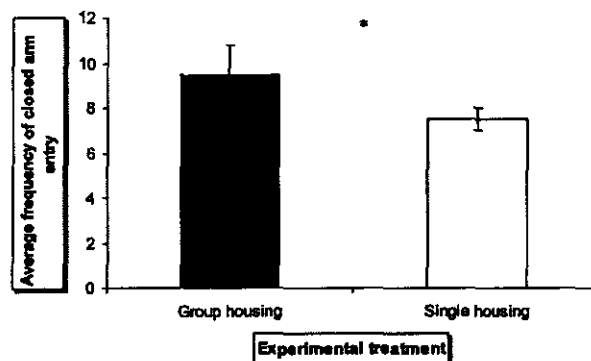


Fig (8) : 'Average frequency of closed arm entry' by the rats in the two experimental treatments. * P <0.05

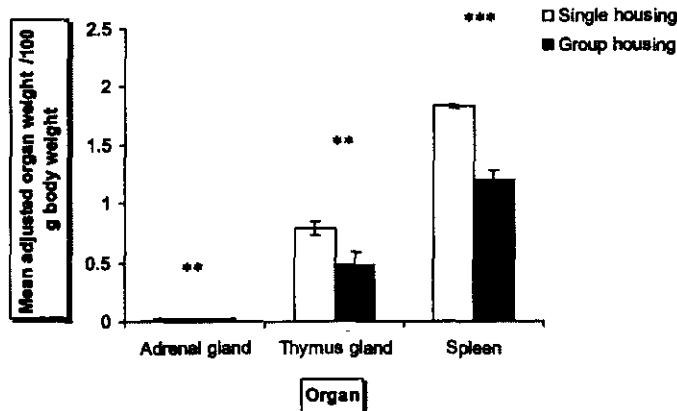


Fig (9) : 'Average relative adrenal, thymus and spleen weight (g)' by the rats in the two experimental treatments. * P <0.05 ** P <0.001

REFERENCES

- Abou-Ismaïl, U. A. and Mahboub, H. D. (2010)** : Long-term effects of using various enrichment objects on multiple measures of welfare in singly-housed rats. The 4th Scientific Annual Conference of Egyptian Society for Animal Management (ESAM).
- Abou-Ismaïl, U. A.; Burman, O. H. B.; Nicol, C. J. and Mendl, M. (2007)** : Can sleep behaviour be used as an indicator of stress in group-housed rats (*Rattus norvegicus*)? *Animal Welfare*, 16, 185- 188.
- Abou-Ismaïl, U. A.; Burman, O. H. B.; Nicol, C. J. and Mendl, M. (2010)** : Effects of enhancing cage complexity on the behaviour and welfare of laboratory rats. *Behavioural processes*, 85, 172- 180.
- Adolph, E. F. (1947)** : Urges to eat and drink in rats. *American Journal of Physiology*, 151, 110- 125.
- Arakawa, H. (2003)** : The effects of isolation rearing on open-field behavior in male rats depends on developmental stages. *Developmental Psychobiology*, 43, 11- 19.
- Bacninger, L. P. (1967)** : Comparison of behavioural development in socially isolated and grouped rats. *Animal Behaviour*, 15, 312- 323.
- Baldwin, D. R.; Wilcox, Z. C. and Bayliss, R. C. (1995)** : Impact of differential housing on humoral immunity following exposure to an acute stressor in rats. *Physiology and Behavior*, 57, 649- 653.
- Bartolomucci, A.; Parmigiani, S.; Gioliosa, L.; Ceresini, G. and Palanza, P. (2009)** : Effects of housing social context on emotional behaviour and physiological responses in female mice. *Scandinavian Journal of Laboratory Animal Science*, 36, 87- 95.
- Brown, K. J. and Grunberg, N. E. (1996)** : Effects of environmental conditions on food consumption in female and male rats. *Physiology and Behavior*, 60, 293- 297.
- Carobrez, A. P. and Bertoglio, L. J. (2005)**: Ethological and temporal analyses of anxiety-like behavior: the elevated plus-maze model 20 years on. *Neuroscience and Biobehavioral Reviews*, 29, 1193- 1205.
- Cunliffe-Beamer, T. L.; Freeman, L. C. and Myers, D. D. (1981)** : Barbiturate sleep time in mice exposed to autoclaved or unautoclaved wood beddings. *Laboratory Animal Science*, 31, 672- 675.
- Dahlborn, K.; van Gils, B. A. A.; van de Weerd, H. A.; van Dijk, J. E. and Baumans, V. (1996)** : Evaluation of long-term environmental enrichment in the mouse. *Scandinavian Journal of Laboratory Animal Science*, 23, 97- 106.
- Degroot, A. and Treit, D. (2004)** : Anxiety is functionally segregated within the septohippocampal system. *Brain Research*, 1001, 60- 71.
- File, S.E. (1978)** : Exploration, distraction, and habituation in rats reared in isolation. *Developmental Psychobiology*, 11, 73- 81.
- File, S. E. and Vellucci, S. V. (1979)** : Behavioral and biochemical measures of stress in hooded rats from different sources. *Physiology and Behavior*, 22, 31- 35.
- Fraser, A. F. and Broom, D. R. (1997)** : *Farm animal behaviour and welfare*. (3rd Ed.) Book craft, Midsomer Norton, U K.
- Gómez, F.; Lahmame, A.; de Kloet, E. R. and Armario, A. (1996)** : Hypothalamic-pituitary-adrenal response to chronic stress in five inbred rat strains: Differential responses are mainly located at the adrenocortical level. *Neuroendocrinology*, 63, 327- 337.

Hatch, A.; Wiberg, G. S.; Balaz, T. and Grice, H. C. (1963) : Long-term isolation stress in rats. *Science*, 142, 507

Hatch, A. M.; Wiberg, G. S.; Zawidzka, Z.; Cann, M.; Airth, J. M. and Grice, H. C. (1965) : Isolation syndrome in rat. *Toxicology and Applied Pharmacology*, 7, 737- 745.

Hurst, J. L.; Barnard, C. J.; Nevison, C. M. and West, C. D. (1997) : Housing and welfare in laboratory rats: Welfare implications of isolation and social contact among caged males. *Animal Welfare*, 6, 329- 347.

Hurst, J. L.; Barnard, C. J.; Nevison, C. M. and West, C. D. (1998) : Housing and welfare in laboratory rats : the welfare implications of social isolation and social contact among females. *Animal Welfare*, 7, 121-136.

Hurst, J. L.; Barnard, C. J.; Tolladay, U.; Nevison, C. M. and West, C. D. (1999): Housing and welfare in laboratory rats: effects of cage stocking density and behavioural predictors of welfare. *Animal Behaviour*, 58, 563-586.

Krohn, T. C.; Sørensen, D. B.; Ottesen, J. L. and Hansen, A. K. (2006) : The effects of individual housing on mice and rats: a review. *Animal Welfare*, 15, 343- 352.

Levitsky, D. A. (1970) : Feeding patterns of rats in response to fasts and changes in environmental conditions. *Physiology and Behavior*, 5, 291- 300.

Lister, R. G. (1987) : The use of a plus-maze to measure anxiety in the mouse. *Psychopharmacology*, 92, 180- 185.

Lopak, V. and Eikelboom, R. (2000) : Pair-housing-induced feeding suppression: individual housing not novelty. *Physiology and Behavior*, 71, 329- 333.

Manser, C. A. (1992) : The assessment of

stress in laboratory animals. Horsham, Sussex: RSPCA.

McGlone, J. J.; Anderson, D. L. and Norman, R. L. (2001) : Floor space needs for laboratory mice: BALB/cJ males or females in solid-bottom cages with bedding. *Contemporary Topics In Laboratory Animal Science*, 40, 21- 25.

Meddis, R. (1975) : On the function of sleep. *Animal Behaviour*, 23, 676- 691.

Menzaghi, F.; Heinrichs, S. C.; Vargas-Cortes, M.; Goldstein, G. and Koob, G. F. (1996) : IRI-514, a synthetic peptide analogue of Thymopentin, reduces the behavioural response to social stress in rats. *Physiology and Behavior*, 60, 397-401.

Morinan, A. and Leonard, B. E. (1980) : Some anatomical and physiological correlates of social isolation in the young rat. *Physiology and Behavior*, 24, 637- 640.

Nakayasu, T. and Ishii, K. (2008): Effects of pair-housing after social defeat experience on elevated plus-maze behavior in rats. *Behavioural Processes*, 78, 477- 480.

Nyska, A.; Hester, S. D.; Cooper, R. L.; Goldman, J. M.; Stoker, T. E.; House, D. and Wolf, D. C. (2002) : Single or group housing altered hormonal physiology and affected pituitary and interstitial cell kinetics. *The Journal of Toxicological Sciences*, 27, 449- 457.

O'Connor, R. and Eikelboom, R. (2000) : The effects of changes in housing on feeding and wheel running. *Physiology and Behavior*, 68, 361- 371.

Patterson-Kane, E. G.; Harper, D. N. and Hunt, M. (2001) : The cage preferences of laboratory rats. *Laboratory Animals*, 35, 74- 79.

Patterson-Kane, E. G.; Hunt, M. and Harper, D. (1999) : Behavioural indexes of

poor welfare in laboratory rats. *Journal of Applied Animal Welfare Science*, 2, 97- 110.

Patterson-Kane, E. G.; Hunt, M. and Harper, D. (2002) : Rats demand social contact. *Animal Welfare*, 11, 327- 332.

Pellow, S.; Chopin, P.; File, S. E. and Briley, M. (1985) : Validation of open: closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. *Journal of Neuroscience Methods*, 14, 149- 167.

Pérez, C.; Canal, J. R.; Domínguez, E.; Campillo, J. E.; Guillén, M. and Torres, M. D. (1997) : Individual housing influences certain biochemical parameters in the rat. *Laboratory Animals*, 31, 357- 361.

Rodgers, R. J. (1997) : Animal models of anxiety: where next? *Behavioural Pharmacology*, 8, 477- 496.

Saibaba, P.; Sales, G. D.; Stodulski, G. and Hau, J. (1996) : Behaviour of rats in their home cages: daytime variation and effects of routine husbandry procedures analysed by time sampling techniques. *Laboratory Animals*, 30, 13- 21.

Sandstrom, N. J. and Hart, S. R. (2005) : Isolation stress during the third postnatal week alters radial arm maze performance and corticosterone levels in adulthood. *Behavioural Brain Research*, 156, 289- 296.

Sherwin, C. M. (1996) : Laboratory mice persist in gaining access to resources: A method of assessing the importance of environmental features. *Applied Animal Behaviour Science*, 48, 203- 213.

Sherwin, C. M. and Nicol, C. J. (1996) : Reorganization of behaviour in laboratory mice, *Mus musculus*, with varying cost of access to resources. *Animal Behaviour*, 51, 1087- 1093.

Sobel, E. H.; Zuppinger, K. A. and Joss,

E. E. (1979) : Effects of isolation, handling and restraint on growth of rats. *Growth*, 43, 1- 6.

Szenasi, G.; Bencsath, P. and Takacs, L. (1988) : No Effect of isolation on blood pressure and daily electrolyte excretion in rats. *Acta Physiologica Hungarica*, 72, 93- 98.

Takemoto, T. I.; Suzuki, T. and Miyama, T. (1975) : Effects of isolation on mice in relation to age and sex. *Tohoku Journal of Experimental Medicine*, 117, 153-165.

Treit, D.; Menard, J. and Royan, C. (1993) : Anxiogenic stimuli in the elevated plus-maze. *Pharmacology Biochemistry and Behavior*, 44, 463- 469.

Vadiei, K.; Berens, K. L. and Luke, D. R. (1990) : Isolation induced renal functional changes in rats from 4 breeders. *Laboratory Animal Science*, 40, 56- 59.

van Loo, P. L.; Mol, J. A.; Koolhaas, J. M.; van Zutphen, B. F. and Baumans, V. (2001) : Modulation of aggression in male mice : influence of group size and cage size. *Physiology and Behavior*, 72, 675- 683.

Verwer, C. M.; van der Ven, L. T. M.; van den Bos, R. and Hendriksen, C. F. M. (2007) : Effects of housing condition on experimental outcome in a reproduction toxicity test. *Regulatory Toxicology and Pharmacology*, 48, 184- 193.

Wongwitdecha, N. and Marsden, C. A. (1996) : Effects of social isolation on learning in the Morris water maze. *Brain Research*, 715, 119- 124.

Würbel, H.; Chapman, R. and Rutland, C. (1998) : Effect of feed and environmental enrichment on development of stereotypic wire-gnawing in laboratory mice. *Applied Animal Behaviour Science*, 60, 69- 81.

الملخص العربي

التأثيرات طويلة المدى لإسكان جرذان التجارب أحاديا مقابل إسكانها في مجموعات علي سلوكياتها وأدائها و مستويات إراحتها

محمد محمد فودة أسامة أحمد أبو إسماعيل
أحمد فتحي أبو النجا

قسم الرعاية وتنمية الثروة الحيوانية - كلية الطب البيطري - جامعة المنصورة

إن عزل الحيوان يشير إلى الحالة التي يكون فيها منفصل جسديا عن باقي الأفراد ، بدون أي إتصال بصري ، سمعي أو عن طريق حاسة الشم. عزل الجرذان المختبرية إجتماعيا والذي عادة ما يشار إليه 'بمتلازمة العزلة' وجد أنه يؤثر على مستويات الكورتيزون ، التمثيل الغذائي ، النمو والسلوك. ومع ذلك فإنه من غير المعروف ما إذا كان إسكان الجرذان منفردة على مقربة من مجموعات كإسكانها في أقفاص ذات أغطية مرتفعة تسمح بالإتصال البصري والسمعي وعن طريق حاسة الشم يحسن من مستويات إراحتها أم لا؟

تم إجراء هذه التجربة لدراسة كيف يؤثر إسكان الجرذان أحاديا في أقفاص ذات أغطية مرتفعة على مقربة من مجموعات على سلوكها ، أدائها ، وقياسات مستوي القلق عندها. تم إسكان الجرذان المستخدمة في هذه التجربة وعددها ١٨ جرذ إما منفردة (ن = ٣) أو في مجموعات من خمسة أفراد (ن = ٣) في أقفاص مختبرية عادية ذات أغطية مرتفعة (ذات إرتفاع ٢١سم) تسمح بالإتصال البصري والسمعي و عن طريق حاسة الشم لمدة ستة أسابيع.

كشفت النتائج أن إسكان الجرذان أحاديا في أقفاص تسمح بقدر من التواصل الإجتماعي أدى إلي إزالة الضغوط الإجتماعية للإسكان الجماعي وإلي تحسين مستويات الإراحة في تلك الحيوانات. أظهرت الجرذان منفردة المسكن مستويات أعلى من سلوكيات النوم وسلوكيات العناية بالجسم كما لوحظ تواجدتها في الجزء المفتوح من القفص أكثر بالمقارنة مع الجرذان التي تم إسكانها في مجموعات. أظهرت الجرذان منفردة المسكن أوزانا أقل للغدة الكظرية وأوزانا أكبر للغدة التيموسية والطحال ، ودخلت الأذرع المفتوحة للمتاهة المرتفعة المتعامدة أكثر بالمقارنة مع الجرذان التي تم إسكانها في مجموعات. ولذلك فإنه يمكن التوصية بإجراء تغييرات صغيرة في إجراءات وبيئة المسكن لتحسين مستويات الإراحة للجرذان منفردة المسكن.