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Effects of Different Resource Distribution on Behaviour and Corticosterone Level of Male Mice (*Mus Musculus*)

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Abstract

Animals housed in a cage with clustered environmental enrichments may deal with higher aggression when they have to compete for depleting resources. In this study, the effects of the way environmental enrichments distributed between individuals were investigated. Sixteen adult male mice (*Mus musculus*) were housed in four different treatments with manipulation in terms of food choices availability and distribution of resources. Each male was observed for their aggressive and stereotypic behaviours for seven minutes twice a day for five days. The faeces were also collected after each treatment for measuring the corticosterone level in order to indicate the stress level of the mice. The results showed reduced aggression ($p < 0.05$), stereotypic behaviour ($p < 0.05$) and corticosterone level ($p < 0.05$) in both dispersed environment and food available environment compared to the clustered environment as well as in the environment where the food choices were not available. From the results, it can be suggested that resources are better arranged in a dispersed way, so that the resources are easier to be shared. Thus, with lesser aggression and stereotypic behaviour, the welfare of the laboratory mice can be improved and easily managed.

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1. Introduction

In recent years, more and more animals were placed in laboratory cages for easier animal management [1]. However, the way resources were distributed was rarely taken into considerations when designing their housing environments [2]. In addition, most of the animals were housed in high density per cage to lower the cost of doing research [3]. As a result, high density of animals per cage with inappropriate resources distribution could cause elevated stress in socially housed mice [3]. Whenever competition increases, competitive interactions

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were high [4] and the mice tend to exhibit aggressive behaviour such as fighting, vigorous sniffing, biting and chasing [5]. These will reduce animal welfare as well as causing loss of profit to the related sector, laboratories and pet owners when it involves injuries and death of the animals.

Although the increase of aggressive and stereotypic behaviour is expected in clustered environment, a research is still need to be conducted for confirmation purposes. For example, several authors were in disagreement whether the aggressive and stereotypic behaviour of animals that are subjected to the same enclosure will be increased or decreased when they are placed in different set up of environmental enrichment. Some of the authors stated that the environmental enrichment may increase aggression when they are kept together, and came in conclusion that the environmental enrichment may significantly decrease their welfare [6]-[8]. Others have found that environmental enrichment decreases or brings no change to the aggressive and stereotypic behaviour level in the male mice [9]-[11]. For example the welfare of pigs and laying hens were maintained when environmental enrichment has been used in their farms in order to reduce aggressive and stereotypic behaviour [12] and [13].

Thus in this study, the short term effects of clustered and dispersed environmental enrichment, and the availability of food choices towards the aggression and stereotypic behaviour of male mice were investigated.

2. Materials and Methods

2.1. Animals

16 male mice aged around 30 days after weaning (*Mus musculus*) were housed in four standard laboratory cages (N = 4 per cage). All mice were allowed to habituate in the cage for two weeks prior to the experiment to get used to the laboratory environment, with free access to food pellet and water ad libitum.

2.2. Experimental Design

Four different environmental enrichments with different distribution of resources were created (Fig. 1). For each environment, two clear cages with a transparent plastic tube as a connecting bridge were prepared. A transparent plastic house, running wheel, foods, water bottle and wood shaving for bedding were provided for each cage. The resources for the first housing environment were clustered in only one cage and the other connected cage was left empty. Meanwhile, the resources in second type of housing environment were dispersed equally in both connected cages. Both clustered and dispersed housing environment were further divided into two depending on the type of food provided as the following:

- Clustered A with no food choice: 100 g of food pellet were provided (Control Group)
- Dispersed A with no food choice: 100 g of food pellet were provided (Control Group)
- Clustered B with food choices available: Cheese biscuit, sunflower seeds, food pellet and dried corn with each food weighing 25 g were provided (Treatment Group)
- Dispersed B with food choices available: Cheese biscuit, sunflower seeds, food pellet and dried corn with each food weighing 25 g were provided (Treatment Group).

Four mice were placed in Clustered A and another four in Dispersed A for five days before they were moved to Clustered B and Dispersed B for five days as well. This study was then replicated using different group of mice.

2.3. Behavioural Observation

The aggressive and streotypic behaviour of all mice in all groups were observed and recorded. The description for both behaviour are shown in Table 1. Behaviours were recorded by direct observation aided with video recording using focal animal sampling for seven minutes for each mouse twice a day.

2.4. Metabolite Corticosterone Measurement

The fecal samples for each environment were collected every morning which was supposed to be excreted during the dark phase on previous day where the mice were most active [14]. The samples collected throughout the 20 days of experiment were then homogenized, added with 1 mL of 80% ethanol in order to purify and analyzed using ArborAssays DetectX Corticosterone Immunoassay Kit before being read by the microtiter reader and analyzed using statistical analyses software (SPSS).

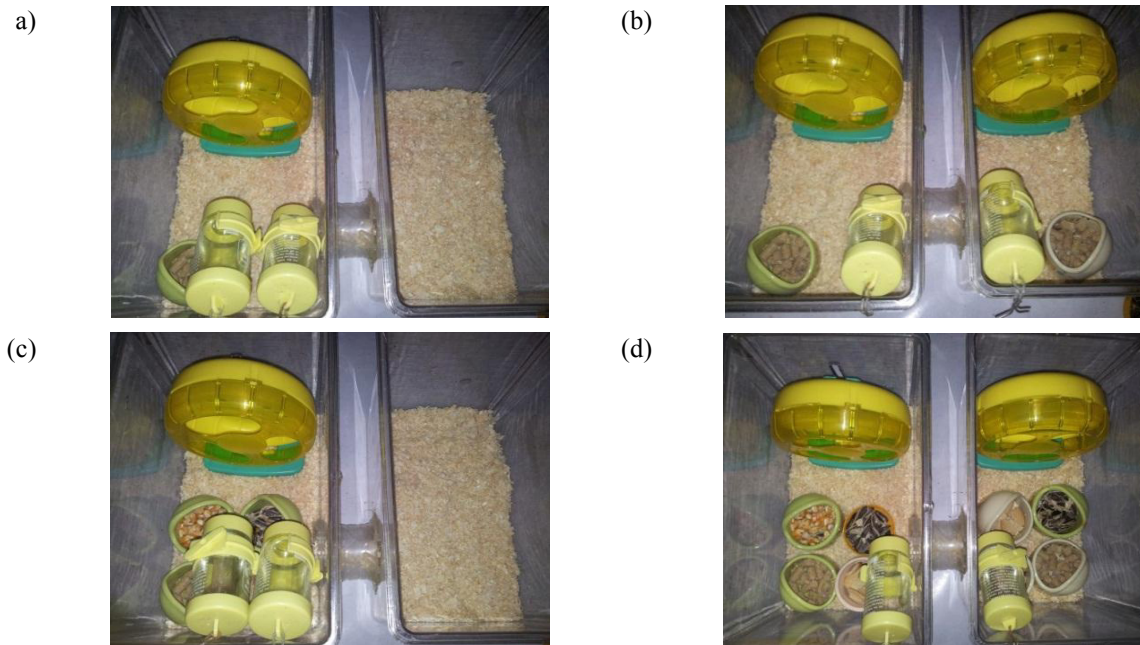


Fig. 1. Setup for the four different treatments. (a) Treatment 1: Clustered A environment with no food choice; (b) Treatment 2: Dispersed A environment with no food choice; (c) Treatment 3: Clustered B environment with food choice and (d) Treatment 4: Dispersed B environment with food choice.

Table 1. Ethogram describing the behaviours observed in both control and treatment groups.

Behaviour	Description
Streotypic	Bar gnawing and route tracing for at least 3 times. Bar mouthing which lasts for at least 5 s (Akre et al., 2011)
Agression	Aggressive behaviour towards cage mate which include chasing, fighting, and biting actions.

3. Result and Discussion

3.1 Aggressive and Stereotypic Behaviour of Male Mice in Four Different Environments

From Fig. 2, the level of aggressive and stereotypic behaviour were observed to produce significant reduction in dispersed environment compared to clustered environment ($p < 0.05$). This is due to more resources were accessible to mice and were equally divided within the two areas of the connected cages. Aggressive and stereotypic behaviour decreases significantly with more resources accessible [15].

When compared within the clustered and dispersed environment, between the environments where food

choices were available and not available, the aggressive and stereotypic behavior decreased significantly ($p < 0.05$) in the environment where food choices were available. This indicated that they can now consume more food, thus need to compete less, resulting in lower stress in the mice [16]. However, within the food choices available in dispersed environment, it showed no significant difference ($p > 0.05$). Here, the stereotypic behaviour was decreased dramatically probably due to a variety of food type available and easier access to foods, as well as physical resources such as running wheel. With more activities can be done by the mice, lesser abnormal stereotypic behaviour was exhibited by the mice [15].

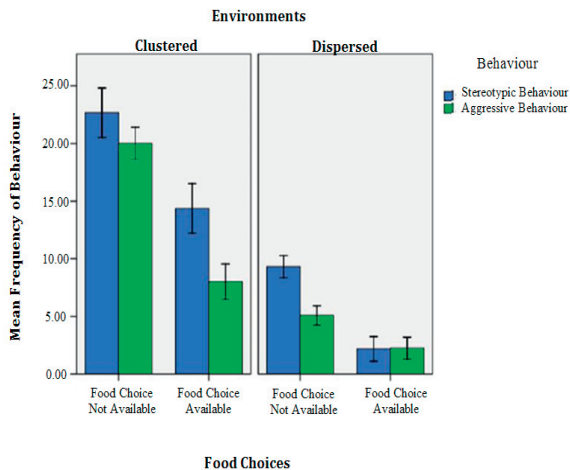


Fig. 2. Aggressive and stereotypic behaviour of male mice in four different environments. (Mean \pm SE)

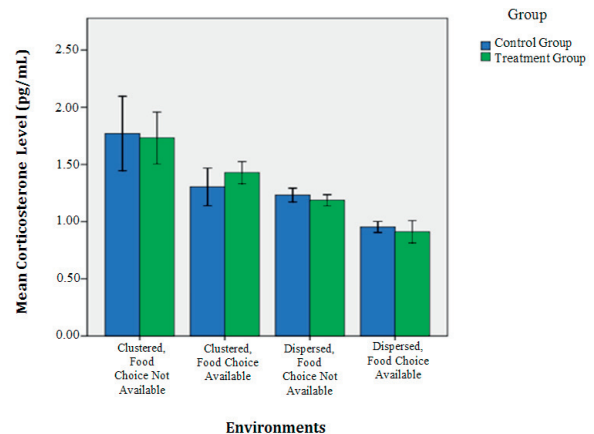


Fig. 3. Corticosterone level of mice in four different environments. (Mean \pm SE)

3.2 The Corticosterone Level in Mice

Fig. 3 shows the final result of Corticosterone levels in mice for each environment. The differences of corticosterone level between environments were significant ($p < 0.05$). The corticosterone level was highest in the clustered environment when food choices were not available and lowest in dispersed environment when food choices were available. This indicates that stress was decreasing across groups, from clustered with no food choices available to dispersed environment with food choice available. When all the resources were clustered, the corticosterone level in the mice increased [15] in positive correlation with aggression and stereotypic behaviour of mice that were also increased (Fig. 3). This is in agreement with a previous study which reported that the higher the stress, the higher is the aggression in living things [17]. However, there was no significant difference in the corticosterone level between the mice in dispersed environmental enrichment where food choices were not available with the mice in clustered group with food choice available food ($p > 0.05$). This indicated that the mice do not have significant decrease in stress when they were transferred from clustered to dispersed environment probably because they were themselves stressed when transferred to new environment regardless of more dispersed environment [18].

4. Conclusions

In conclusion, aggressive and stereotypic behaviour were higher in clustered environment compared to dispersed environment. Both behaviours were also higher in environment where food choices were not available. These results were further proved by the corticosterone level in mice where the highest level were found in mice placed in clustered environment with only food pellet available and the lowest level found in mice placed in dispersed environment with food choices provided. By utilizing dispersed type of environment,

the welfare of laboratory animals may be improved and may eventually reduce the cost in replacing injured or dead animals.

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