A Simple Method for Intracage Mouse Environmental Enrichment Device Assessment

Animal Care and Veterinary Services

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Abstract

The current method of choice to address psychological well being of mice in cage environments is to add devices to the cage with which the mice can interact. Literature on mouse enrichment is relatively sparse, and few of the commercially available enrichments have been properly assessed or even demonstrated to be beneficial to mice by promotion of highly motivated behaviours(1). The purpose of this study was to create a simple yet practical in-house method for individual research animal facilities to identify the most beneficial of intracage Environmental Enrichment device(s) for their strains of mice.

Female mice were placed into five test groups and provided with commercial environmental enrichment devices and fed a calorie rich breeder diet that normally produces excess weight in non breeding females. These mice were weighed twice weekly for a period of ten weeks to see if the test groups would gain less weight than the control group with no intracage device(s). Daily observations were recorded of active and non active behaviors exhibited by the mice in the five tests groups and controls while on study. The results demonstrated statistically significant differences in the extent to which the various groups gained weight within the first three weeks of the ten week study. In addition there were statistically significant between group differences in terms of weight measurements at time points following the initial three weeks. Daily observations revealed minimal fighting and no stress related activities in groups plus controls, and increased active behaviors in test groups compared to controls. A single test group with two environmental enrichment device options had no observed fighting.

From this study it appears that measurement of weight gain combined with daily activity monitoring is one method that can be used in a short term study for the assessment of intracage mouse environmental enrichment devices. The combination of weight measurement with daily activity monitoring provides for a more accurate overall assessment than weight measure alone. Results for the intracage devices chosen on this study allowed for a clear understanding of use by mice and greatest potential of benefit in the area of provision of more than one device per cage.

Introduction

Environmental enrichment, by definition, is the provision of biologically relevant resources or structuring to the cage that facilitate or allow highly motivated natural behaviours, particularly those that allow the animal to control its environment or homeostasis (1). The use of commercially available intracage Environmental

Enrichment devices is the current method of choice to address the normal behavioural needs of mice beyond those of social contact. In the last few years there has been an explosion of new strains of mice through genetic manipulation with transgenic and knockout technologies. A significant number of strains are physically and behaviourally affected by these altered genetics, and are generally more difficult to breed once established. This not only increases the need for facilities to provide well chosen intracage Environmental Enrichment, but to address it in a manner more specific to the needs of particular strain (s) housed. The market is developing numerous such in cage devices, with little or no data on benefit to mice. Providing complete and proper care for mice in research involves selection of intracage devices and/or methods that best suit the strains, age, sex and phenotype of mice housed. These choices are now made with no published data on assessment tools and selection criteria.

The purpose of this study was to create a simple method of mouse intracage device assessment that would allow for application of individual institutional needs of specific strains, sex, groups and genetically altered strains of mice. The specific devices chosen for this study included relevant selection criteria to this institution. Selection criteria could be altered as required by any animal facility while still applying the device assessment method described in this study.

The hypothesis was that non breeding female mice fed a high caloric breeder diet would gain less weight in test groups with more complex cage environments to that of controls animals. Observations were recorded of active and non active behaviors exhibited by the mice once daily while on study to supplement the weight data. The recording of this data was based on the assumption that interaction with intracage device would increase mouse activity.

Materials and Methods

Eight week old female CD-1 females were chosen for the study as they are readily available, gain weight easily and were accepted for transfer to other approved protocols once the study was complete. Ten weeks was chosen for the duration of the study in order to allow adequate time for weight gain, with eight weeks of age of the mice representative of the onset of breeding. Mice were housed in a C.C.A.C. (Canadian Council of Animal Care) approved animal facility in open top cages in a conventional housing room of controlled temperature, humidity and light cycle(12 hours light/12 hours dark). Bedding used was corncob ¼, 1/8 combination (Bed-O-Cob®) and Harlan Teklad S-2335 Mouse Breeder Sterilizable diet #7904 was fed ad lib. Water was supplied in bottles, with cages and bottles cleaned weekly.

Upon arrival the animals were randomly assigned to groups of four mice per cage, eight cages per group in standard shoebox caging for a total of 32 mice per group. Four extra mice arrived with the order, so the extra cage produced was applied to Group D (In house modified "Mouse House" by Techniplast®). All groups plus controls received four Nestlet ™ squares by Ancare® (1" X 1" each) for chewable nesting material . Intracage devices were cleaned twice monthly, with the exception of rings, which were washed with the wires on a monthly basis. Nestlets were changed out weekly with bedding changes rather than transferred to clean cages weekly.

Selection Criteria of Intracage Devices: The most important criterion was clear visibility of the mice. It is essential that animal care technicians could observe the animals interacting with the device without causing a disturbance to their behaviour by movement of the cage. All devices had to be constructed of inert material, allow for sterilization by the autoclave method and not substantially increase the labour investment by technicians to add, remove, clean and sterilize the device. Disposable devices had to follow the regular dirty bedding waste stream, while non disposable devices had to be durable material that resisted chewing and could be safely cage washed. Cost of the devices balanced with predicted shelf life was also considered. Although not directly related to the selection criteria (and not assessed on this study) , an expected benefit of non disposable intracage device selection was the reduction of clogging of exhaust air vents and filters in ventilated racks with nesting material, based on the mice choosing to locate nesting material within the device.

No marketed disposable devices were chosen for this study as none allowed for clear visibility of the mice to the animal care technicians.

1. *Controls Group A*: no intracage device(Figure #1)

2. *Test Group B*: "Fast Trac ® by Bioserve ®non disposable Enrichment device The "Fast Trac"® with yellow or red bottoms was chosen for better technician visibility of mice in the "igloo" portion of the device. (Figure #2)

3. *Test Group C*: "Mouse House"® by Techniplast® corporation non disposable enrichment device (Figure #3)

4: *Test Group D*: "Mouse House"® non disposable enrichment device with an in-house modification of 4 observation holes for better technician visibility as well as a reduced height of the plastic lip on the upper level to eliminate entrapment to the cage wall when utilized with in house ventilated rack caging. (Figure #4**)**

5. *Test Group E* : "Fast Trac ® non disposable Enrichment device and two, three inch diameter Metal "O" rings. (Figure #5)

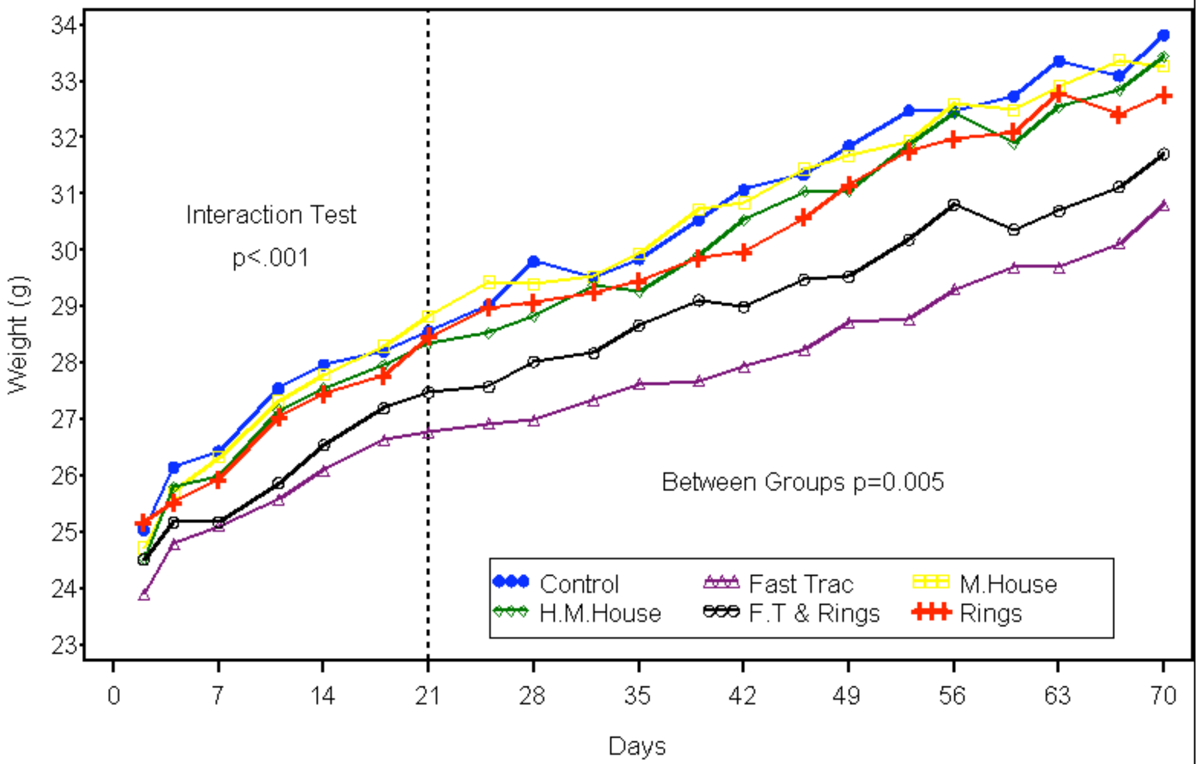
6. *Test Group F* : Metal "O" binder rings (two) of three inch diameter (Figure #6)

Following a one week conditioning period, the environmental enrichment devices were placed in the cages. Baseline weights were taken at two days rather than when devices added (study error). The nocturnal portion of the 12 hour light cycle was altered to begin mid morning over the conditioning period so animals could be observed and recorded once daily at random time slots in the nocturnal part of the light cycle via the use of red lights. Mice were weighed twice weekly to 0.1 gm accuracy with a Mettler/ Toledo PB 3002 Delta Range scale Veterinary care was available as needed.

Based on the experimental design, group sizes were determined and cluster randomization statistical analysis chosen by the department of Epidemiology and Biostatistics. The sum of mean weights within the cage was used to eliminate the need for the effect of the cage in this regression model analysis. The statistical program used was the SAS version 2, which performed a repeated measures ANOVA. This data was defined in the initial three weeks with the Interaction Test ($p< .001$) , and the Tukey post-hoc test comparing mean weight measures for points of significant differences between eight and ten weeks ($p< 0.05$) .

Behaviours were observed and recorded once daily at normal husbandry time(s) during the noctural portion of the light cycle. Behaviour results were grouped into active versus non-active behaviour for comparing possible trends with weight records, and reported as the sum of quantitative behaviours. These behaviours were rated in order of most to least observed behaviours between controls and the five test groups. Observed and charted active behaviours were interacting with cage mates, interacting with the intracage device, moving about the cage and fighting. Observed and charted non-active behaviours were sleeping, grooming, resting awake, eating and drinking.

Results:

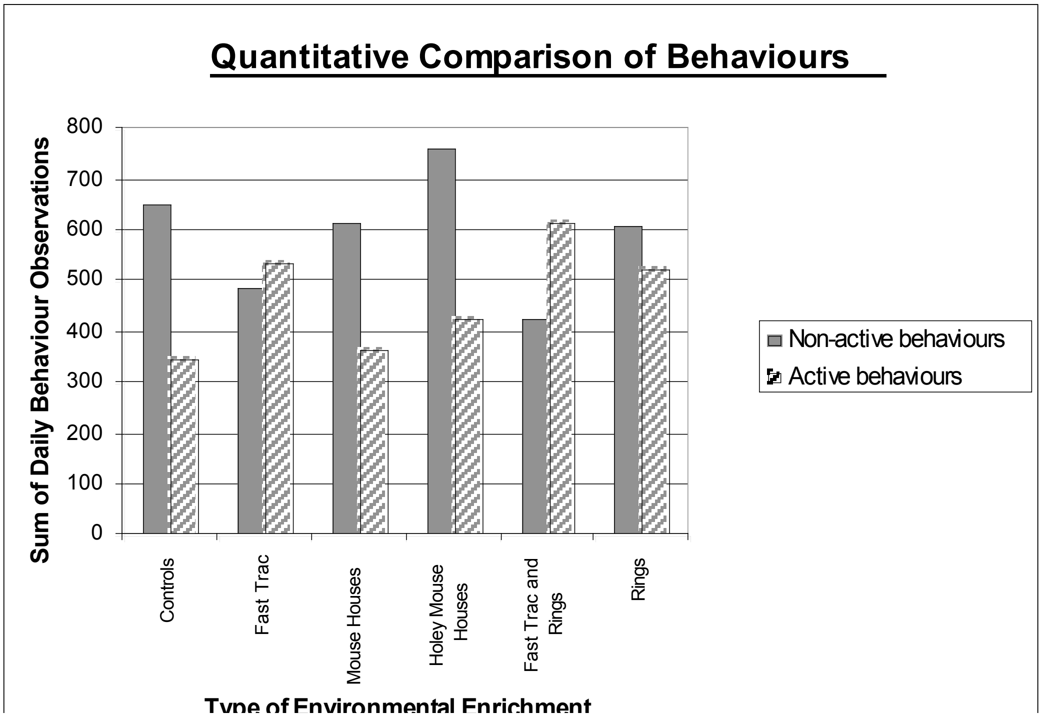


Bi- weekly Weight Averages for Environmental Enrichment Test Groups and Controls

Figure 7

The analysis of the mice weight data between the six experimental groups was conducted using a repeated measures ANOVA, with an overall p-value 0.005 (Figure7). The interaction test revealed that there were significant between-group inconsistencies during the first thee weeks of the study ($p<0.001$). Following an indication from the ANOVA that there were significant differences in weight during weeks eight to ten, a subsequent Tukey post-hoc test illustrated multiple points of significance. Significant weight differences at the $p < 0.05$ level occurred between the Fast Trac® group versus the controls, the Fast Trac® versus the Mouse House® as well as the Fast Trac® versus the Holey Mouse House group(s).

The interaction test performed on the weight data in the first three weeks of this study identifies inconsistent weight measurements over time between groups ($p < .001$) , identifying differing rates of weight gain between groups for that time period.



Quantitative Ranking of Active and Non-Active Behaviours for Environmental Enrichment Test Groups and Controls

Figure 8

Active behavior versus non-active behavior observations indicated the Holey Mouse House to have highest amount of non-active behaviors per day, and the controls to have the second highest non-active behaviors per group per day. The group with the highest sum of active behaviours was the one with two intracage devices (Fast Trac® and Rings) and the group with the least sum of active behaviours was the control group.

Fighting was observed ten times in a total of 3,290 cage observations made over the course of the study. The only group with no observed fighting was the group with two intracage environmental enrichment devices (Fast Trac® and Rings).

When both weight measurement and the single daily active/ non-active behaviours observations are used as assessment tools for evaluation of intracage environmental enrichment for mice on this study, it is observed active behaviors follow a similar trend with statistically significant mean weight data points at time points between eight to ten week period of the study. These time points occurred between the Fast Trac® and controls, Fast Trac® and Mouse House as well as Fast Trac® Holey Mouse house group(s).

Conclusions

This study indicates weight gain measurement of test groups and controls in non breeding female mice on a high caloric diet combined with single daily behaviour observations is a simple, practical method of assessment of intracage devices for mice. This method also allows for the selection of environmental enrichment devices on test to be independent of the assessment method described, therefore allowing for any device of choice to be tested as determined by individual institutional needs.

The division of active versus non-active behaviors was used with the assumption active would best reflect the positive change in activity by the mice with the addition of a device. Future studies should alter the behaviour observations to try and capture the distinction of natural, highly motivated behaviours versus stereotypic and negative behaviours to more accurately address the issue of benefit to mice.

Results for the intracage devices chosen in this study allowed for a clearer understanding of use of the enrichment devices by the mice on study, and illustrate that there may be greatest promise of benefit in the area of application of more than one enrichment item per cage.

Although limited in scope, single daily observations of activity during normal daily husbandry at random times during the nocturnal portion of the light cycle is a practical method for behavioural activity observations.

When both weight measurement, active/ non active behaviors observation are used it is found that the group including two choices of environmental devices within the cage (Fast Trac ® and Rings) is overall most successful. This suggests a combination of intracage devices is worthy of future study.

The interaction test performed on the weight data in the first three weeks of this study does not identify particular time points where differences occur; however it indicates the rate of weight gain over time is different between groups during this time period.

Discussion

This institution concluded that Rings were of benefit both economically (sixteen cents per ring and installed permanently) for mice as a single and in combination environmental enrichment device. They provided greatest visibility of mice to animal care technicians and greatest labour savings. Once installed on the cage wires they remained permanently following the usual monthly change out with no more extra handling required. No floor space requirements allows for more usable space for the mice while allowing for dual intracage device options.

Fast Trac® devices used alone induced high activity in a manner that seemed compulsive. This device was chosen based on the activity combined hiding / nesting space. The results achieved have led this institution to this device for cages of abnormally or stereotypically active strains as an attempt to reduce and /or redirect these behaviours. This device is also the choice within larger cages and higher populations per cage. The fact that it induced a form of hyperactivity in the CD-1 females on this study is not considered a benefit. Fast Trac® also created a squealing, squeaking noise as the mice used them that was intense to humans and could be heard within adjacent hallways. A bat detector used for detection of ultrasonic frequencies was used to confirm this sound was not in the ultrasonic range and therefore not detrimental to the mice. It is assumed a safe, inert and effective lubricant is needed to reduce sound annoyance to humans.

The Mouse House® by Techniplast® and the modified version of the device " Holey Mouse House" groups achieved basically the same results. This device has a manufacturer funded study that indicated calmer interactions of mice when handled. The red plastic inert resin used with the device provides a perceived dark interior for the mouse to escape when desired, while still allowing for see through observation by animal care technicians. This institution has used these devices with success in cages of social stress, such inter-male aggression and easily disturbed breeding strains of mice. The benefit seen with the in house modification of the "Mouse House"® by Techniplast® corporation in our ventilated rack cage system has made it the first choice for this institution.

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References

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