# Behavioural Effects of the Shelter Design on Male Guinea Pigs

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## Abstract

To improve the welfare of group-housed male guinea pigs during the acclimatization period, which is when social groups are formed, different designs of shelters were tested, one shelter having one entrance to a single compartment – a Box for group hiding – and the other having individual entrances to a compartment in the cage – a Garage for single hiding. Both were studied to evaluate whether they had any affect on the behavioral levels. Behavioural and weight data were collected during five of the seven days of the acclimatization period. Data were tested against the Mann-Whitney U and Variance Analysis test. Results demonstrated that males in cages with the garage spent more time inside the shelter (P = 0.0004), while males in cages with the box spent more time resting (P = 0.0000), feeding (P = 0.0043) and drinking (P = 0.0022) on the open floor, and yet there was no difference in individual weight between treatments at the end of the study. Males in cages with garage experienced a more rapid establishment of the social hierarchy (P = 0.0024) by being involved with a lower number of social interactions. The conclusion from the present study is considered to show that males in cages with the garage were able to avoid unnecessarily high levels of stress and aggression caused by territorial defence while the hierarchy was established.

## Introduction

Welfare for laboratory animals has been acknowledged as important not only from an ethical point of view, but also for the trustworthiness of results from physiological, pharmacological and behavioural studies (Reinhardt, 2004; Dean, 1999). The welfare of these animals is of particular importance during the period just after transport to a new animal facility or department. This acclimatization period is intended to decrease the stress and discomfort caused by transport (Meyerson, 1986). To further improve the welfare of the laboratory animals, enriching the individual animal's environment has proven to be an efficient approach (Dean, 1999; Würbel, 2001). The goal of environmental enrichment is to provide the resource that the animal is missing and by doing so increase the animal's capac-

\*Correspondence: Angelica Nordlund AstraZeneca R&D, Djursektionen, Pepparredsleden 3, SE- 431 83 Mölndal, Sweden Fax: +46-31-7763707 E-mail: angelica.nordlund@astrazeneca.com ity to cope with living in captivity and being in experiments (Olsson et. al. 2003; Darlene et. al, 2002). At the present time there are many papers published concerning environmental enrichment for captive animals (Olsson et. al. 2003). However, there are not many studies published concerning enrichment of the environment for guinea pigs (Cavia aperea f. porcellus). Studies have shown that individuals introduced to a cage with an unfamiliar and plain environment will experience more stress, resulting in increased levels of stress hormones (Haemish, 1990; Sachser, 1987), and in those situations the guinea pigs prefer to stay close to feeding and drinking containers or to cage corners (Büttner, 1992; Büttner, 1994; White et. al 1989).

What have been suggested though are shelters, which will provide a place to rest and get away from the otherwise open space of the cage (*Olsson et. al. 2003; Sachser, 1994*). And act as a substitute for the culverts and tunnels which guinea pigs use as a sanctuary in the wild (*King, 1956; Rood, 1972*).

Guinea pigs being truly social animals, form stable dominance hierarchies. The hierarchies are estab-

lished foremost among males by defending their territories and females against other males (Rood, 1972; Beer & Sachser, 1994). In groups with low densities, the hierarchies have been shown to be of the linear rank order type (Sachser, 1994; Rood, 1972; Berryman, 1978). To establish the rank order within the group, levels of aggressive behaviour are usually elevated. Later on, when the hierarchy is established, these levels are reduced to normal levels (Beer & Sachser, 1994) and by then; there will be distinct behavioural differences between dominant and subdominant individuals. The dominant individuals will mainly display aggressive and courtship behaviours whilst low-ranking individuals will mostly be a target of aggression and behave submissively (Sachser et. al, 1998; Büttner, 1994; Sachser, 1990).

Studies on groups of guinea pigs consisting of only males have shown that a shelter can become a subject for defence, here described as territorial defence - the shelter being a part of the cage and hence a part of the territory. Males have been shown to display an outburst of aggression towards males trying to enter, or already situated inside, the shelter (*Fuchs, 1980*). Shelters do seem to be of importance for guinea pigs, but the design of the shelter itself has not yet been studied in detail.

The current study aims to investigate, in order to improve animal welfare, if group-housed male guinea pigs were affected by the design of the shelter for hiding. The hypothesis was that the garage would decrease the levels of stress and aggression by offering one compartment for each individual of the group, as compared to the box. By doing so, the garage would diminish the competition for space inside the shelter.

# Materials and Methods

## Animals

Barrier bred male guinea pigs of the Duncan Hartley strain were obtained from HB Lidköpings Kaninfarm. At the breeder, the pups were housed with their parents the first 2-3 weeks, at which age they were moved into groups of 10-20 animals of similar age. When weighing about 300 grams, they were moved into smaller groups of two to four individuals of the same sex. At about 12 weeks of age and an average weight of 500 grams a total of 96 guinea pigs were transported to the experimental facility. The animals were health-monitored according to Felasa's recommendations during the study and no unwanted agents were found.

# Housing

Animals were group-housed with four animals per cage. The cages were situated in a so-called modified enriched rabbit cage-system and each cage had an area of 4250 cm<sup>2</sup> (42cm high and 85cm wide) (Scanbur, Karlslunde, Denmark), and aspen wood chips (Finn Tapvei Oy, Finland) were used as bedding. Commercial guinea pig diet, K1 (Lactamin, Vadstena, Sweden) and tap water were available ad libitum. The diet was regularly supplemented with autoclaved hay (Granngården, Kungsbacka, Sweden). The animals were kept under constant conditions with a light/dark-cycle of 12h and a photoperiod between 6.00 am and 6.00 pm with 30 min dusk and dawn. The room temperature was 22 C° and the relative humidity 55%.

#### Enrichment items

The cages were enriched with two types of plastic shelters: (a) a Box for group hiding, being a polypropylene shelter with a single entrance into one compartment 6cm in height at the opening and with a total area of 510cm<sup>2</sup>, occupying about 12% of the total floor area of the cage (Figure 1a and 2a); (b) a Garage for single hiding (Scanbur, Karlslunde, Denmark), being a noryl plastic shelter with four separate entrances to four separate compartments 13cm in height at the opening and with a total area of 1382 cm<sup>2</sup>, occupying about 32% of the total floor area (Figure 1b and 2b).

### Experimental design

The experiment was carried out during the sevenday long acclimatization period. The guinea pigs were placed in groups of four per cage with a total





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**Figure 1.** Photos of the guinea pigs in cages with: a) a box for group hiding and b) a garage for single hiding.

of 12 groups per treatment and each group of four individuals that had been transported together from the breeder were placed in the same cage.

Observations took place during the 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup>, 6<sup>th</sup> and 7<sup>th</sup> day of the acclimatization period and two groups per treatment were observed at a time. Individuals were distinguished by different colourations prepared with spray paint (DeLaval marking spray) at arrival. The observer was sitting right in front of the cages during the experiment and since it was nearly impossible to observe through the cage door, a transparent plastic panel was placed at the cage opening to hinder animals from escaping while the cage door was open. During day one no observations took place, but the cage door was left open to allow the animals to get accustomed to the plastic panel.

Every day of observation was divided into three periods, first 8.45-10.15, second 12.30-14.00 and third 14.30-16.00. One at a time, individual one

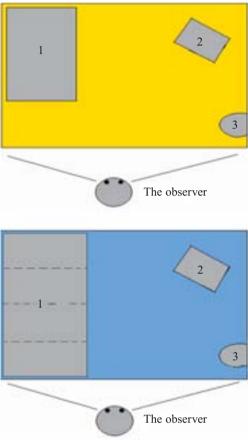


Figure 2. The setup in the cage for the experiment: number one being the shelter, number two being the feeding container and number three the drinking device. Also showing is the placement of the observer while recording on cages with a) the box design of the shelter and b) the garage design of the shelter.

through four in a randomly chosen cage was observed continuously for two minutes, the procedure was then repeated (2x8 minutes), were the whole process being carried out at each of the three daily observation periods (with a four-minutes break after every two cages). Thus each cage was observed for a total of four hours during the 5-dag acclimatization period. Frequency of feeding, drinking, resting (out on the open floor), being inside the shelter (with at least half of its body), social sniff, defensive (including retreat, head up, hiding and submissive crouch), offensive (including strand threat, attack and chase/bite) and sexual (including anal sniff and rumba) behaviour were recorded (*Grant & Mackintosh, 1963; Rood, 1972; Nordlund, 2005*). When a social interaction took place and the winner and loser could be distinguished, one individual acting aggressively and one submissively, this was also recorded. Simultaneously with individually marking, the guinea pigs were weighed using a Mettler Toledo (type R8001), and this was repeated after the last observations.

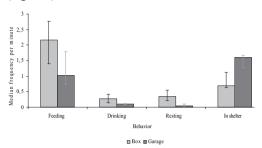
## Statistical analysis

The frequency results for the different behavioural categories were recalculated into frequency per minute to exclude the effects, caused by missing data, on the outcome of the analysis. The data were processed using MINITAB Statistical Software, version 13.20 (©2000, Minitab Inc.). The two treatments were tested in a balanced way throughout the whole experiment and since none of the data sets, except the weighing on day one, were normally distributed; primarily the non-parametric Mann-Whitney U test was utilized. The F test in the Variance Analysis test was used on data concerning the individual weights of the males upon arrival. All results were considered statistically significant at p < 0.05 with degrees of freedom set at 11 (12 groups per treatment).

#### Results

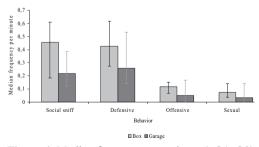
#### Behaviours

With either type of cage, the animals spent more than half of their time feeding and resting, either in the shelter or out on the open floor, and just over a tenth of their time engaging in social interactions. Guinea pigs in cages with the box for group hiding spent significantly more time feeding (P = 0.0043), drinking (P = 0.0022) and resting on the open floor (P = 0.000) than those in cages with the garage for single hiding (Figure 3). However guinea pigs in cages with the garage spent significantly more time being inside the shelter for hiding (P = 0.0004) (Figure 3).



**Figure 3.** Median frequency per minute  $(\pm Q1, Q3)$  for behaviours: feeding, drinking, resting and being inside the shelter for hiding, in cages with the box for group hiding) and cages with the garage for single hiding.

Only low levels of injuries and fighting were recorded for both treatments during the experiment; instead the males tended to retreat or act submissively when approached by an aggressive individual (Figure 4). There was a tendency towards a significant difference in showing the social sniffing behaviour (P = 0.0781) when comparing the two treatments (Figure 4), but the males did not demonstrate any difference between cages with the box or garage shelter when considering the frequency per minute for the other socially interactive behaviours (Figure 4).



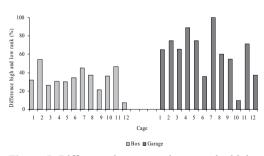
**Figure 4.** Median frequency per minute  $(\pm Q1, Q3)$  for social behaviours: social sniff, defensive, offensive and sexual, in cages with the box for group hiding and cages with the garage for single hiding.

Throughout the entire experiment, there were frequent observations of males being forced, either physically or by vocalization, to leave the shelter and instead having to occupy themselves out on the open floor. These observations were made when behaviours were finally recorded, as well as in cages in the periphery in both treatments and, although not documented, did seem to be more frequent in cages with the box than in cages with the garage.

# Social interactions

Comparing the two types of environmental enrichment by noting the total number of social interactions, where distinction between winner and loser was possible, indicated a tendency towards a significantly higher number of social interactions between males situated in cages with the box (P = 0.0941).

The difference between the individual with the highest percentage of won interactions (considered the highest ranking individual) and the individual with the lowest percentage of won interactions (considered the lowest ranking individual) was also estimated in each cage. Comparing the results from the two treatments illustrated a significantly larger difference between the highest and lowest ranking individual for males in cages with the garage shelter (P = 0.0024) (Figure 5).



**Figure 5.** Difference in per cent between the highest and lowest ranking individual in each cage, results presented for all cages in both treatments.

## Weight

At the start of the acclimatization period the guinea pigs did not differ significantly in weight between individuals. Comparing the two types of environmental enrichments, the shelters did not have any significant effect on the weight gain of the males during the acclimatization period (Mean weight gain  $_{\text{Box design}} = 69.43$ grams and mean weight gain  $_{\text{Garage design}} = 69.43$ grams).

#### Discussion

## **Behaviours**

The unrecorded observations during the present study support previous results of males behaving aggressively in connection to a shelter and perceiving a shelter as a part of their territory to defend when establishing their hierarchy (*Wallner & Dittami, 2003; Fuchs, 1980; King, 1956*).

The time spent by the animals, inside the shelters, as well as their feeding and drinking behaviours, may be explained by their design. Thus the box design only offered a single opening and compartment meaning that all males would have to share the compartment when hiding was needed. However, since males are in the middle of establishing a hierarchy during the acclimatization period, squeezing into and sharing a small area would not be likely unless danger was significant. Males establishing a hierarchy fight for territory and, as the shelter is a part of the cage, it would not be shared without difficulty (King, 1956; Fuchs, 1980). By offering individual openings and compartments for all males of the group, the garage was able to guarantee a place to hide for all individuals independent of their rank and by doing so the levels of stress caused by territorial defence could be reduced design (Wallner & Dittami, 2003). This would not be true in cages with the box, were males were more frequently forced to spend time on the open floor. These individuals instead spent time feeding, drinking and resting on the open floor and were therefore unable to avoid more or less aggressive social interactions as social sniffing.

This did not seem to be the case for all the social

behaviours though. The establishment of the social hierarchy amongst males, in mixed sex groups, are sometimes marked by violent events (Sachser and Renninger. 1993: Sachser et al. 1998). Nevertheless the present study showed very low frequencies of sexual and offensive behaviours for both treatments, while the social sniffing and defensive behaviours were much more frequent. When comparing the results of the social sniffing behaviour from the present study with results from studies where groups consisted of both sexes, the definition of social sniffing should most likely be altered. In groups with mixed sexes the social sniffing is assumed to be what the given name indicates - a social behaviour where an individual simply identifies and socializes with other individuals (Grant & Mackintosh, 1963; Rood, 1972). In groups consisting of only males, especially when they are in the middle of establishing a hierarchy, this behaviour is probably modified into a more aggressive act. This behaviour would instead be relevant when there is no need for very high levels of hostility and it would explain the low levels of offensive and sexual behaviours.

# Social interactions

A large difference between the dominant and the subdominant individual in a group of guinea pigs is an indication of a stable and established hierarchy (Beer & Sachser, 1994; Sachser et al., 1998). Results from comparing the difference between the individual in the group with the highest proportion of won interactions with the individual with the lowest proportion, showed that males in cages with the garage differed more between the high and the low ranking individual of the group. The experiment lasted for seven days, and from the results one could not say that the hierarchies were stable in any of the groups by the end of the acclimatization period. A reason for the non-established hierarchy, published in an earlier study, could be the lack of space for the animals to interact (King, 1956). However it should be recognized that males in cages with the garage came much closer to an established and stable hierarchy, than males in cages with the box. Not only did males with the garage as a shelter get closer to the stable hierarchy, but they also achieved this despite the trend towards initiating fewer social interactions.

# Weight

As expected, since all individuals were assumed to be treated similarly prior to arriving at the animal unit in question, there were no significant differences in weight between the individuals at the day of arrival. Nor were there any differences between the two shelter-treatments when studying the weight gain during the acclimatization period. This might seem like a strange outcome since behavioural results showed a significantly higher frequency of feeding for males in cages with the box than in cages with the garage. A possible explanation is the definition of the behaviours. An individual registered as in the shelter had "at least half of the body inside the shelter" and with the photo of the garage in mind one can easily see that an individual can be eating but still be registered as inside the shelter. The occurrence of males being able to feed from the open floor while situated somewhat inside the box design of the shelter is not likely to happen, since the opening of the box is much too small. This gives the garage another advantage - the ability to eat peacefully without being disturbed by other males in the cage.

# Conclusion

The findings from the present study showed that the shelter designed as a garage for single hiding gave the guinea pigs a chance to avoid unnecessarily high levels of stress caused by territorial defence during the acclimatization period, as compared to the box design. By providing separate compartments for each individual in the cage, males were able to feed while being protected by the shelter and there also seemed to be fewer incidents of males being forced to leave the safety of the shelter. Also, not only were the stable hierarchies established in a less aggressive manner in the cages with the garage, but also more rapidly compared to cages with the box.

There are more aspects of this study to explore though. A prolonged study would show how long it would actually take before the hierarchies are established and stable.

# Acknowledgement

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