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Sveriges Lantbruksuniversitet **Skara 2004** **Studentarbete 27**
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Angelica Nordlund

Degree project in Ethology, 20 credits (30 ECTS credits),
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CONTENT

1. SUMMARY

For animals, such as the guinea pig, which are obtained from breeders, it is of legal as well as of scientific concern that the animals have an acclimatization period just after arriving at their new animal facility. During this period, the guinea pigs have a chance to decrease the levels of stress, caused by the process of moving. This study aims to improve the housing conditions of guinea pigs during the acclimatization period, by evaluating if the design of shelter for hiding affects the guinea pigs' levels of behavioural stress and aggression. The present study was done at AstraZeneca R&D in Mölndal, where guinea pigs are used as an animal model in the process of developing new drugs.
In the experiment 104 male guinea pigs of the Duncan Hartley strain were used, living in groups of four. Two different shelter designs were used: one type with one entrance and one compartment called "box for group hiding", BGH, and the second type with four entrances and four compartments called "garage for single hiding", GSH. Behavioural data was collected by video recorded and direct observations during the 1^{st}, 2^{nd}, 3^{rd}, 6^{th} and 7^{th} day after arrival. Each day of direct observations was divided into three periods of observation, during which frequencies of 17 different behaviours were recorded. All individuals were observed for a total of about one hour. Data from video recorded observations were collected during two 3 hour periods. Data was tested using the Mann-Whitney U test, the Variance Analysis test and the Simple Linear Regression.
Results showed that males living in cages with BGH ate ($p < 0.01$), drank ($p < 0.005$) and rested on the floor ($p < 0.000$) more often than those in cages with GSH. Instead the animals in cages with the garage were more often situated inside the shelter ($p < 0.000$). It was also shown that males living in cages with GSH not only established their hierarchy in a faster manner, but also with one third less interactions, compared to the cages with BGH. By doing so the garage is thought to have decreased the levels of aggression considerably.
The conclusion from this study was that the males in cages with the garage were considered to have decreased the levels of behavioural stress and aggression to a larger degree than males in cages with the box. This was accomplished by avoiding needlessly high levels of social interactions, and still establish their hierarchy in a more effective manner.

SAMMANFATTNING

För djur, som marsvinet, vilka anskaffas hos uppfödare, finns det ett vetenskapligt intresse såväl som ett lagligt krav på att djur får en acklimatiseringsperiod just efter ankomst till den nya djurenheten. Under den här perioden har marsvinet en chans att minska stressnivåerna som orsakats av flyttningsprocessen. Den här studien syftar att förbättra inhysningen för marsvin under acklimatiseringsperioden genom att utvärdera om designen av gömstället i marsvinens burar påverkar marsvinens beteendemässiga stress och aggressionsnivåer. Studien gjordes på AstraZeneca R&D i Mölndal, där marsvin används som modell vid forskningen efter nya mediciner.

I experimentet användes 104 marsvinshanar av Duncan-Hartley stammen, grupperademed fyra hanar i varje bur. Två designtyper av gömstället användes: en typ med en öppning och ett hålrum kallad ”box för gruppgömme” (box for group hiding), BGH, och en annan typ med fyra öppningar och fyra hålrum kallad ”garage för singelgömme” (garage for single hiding), GSH. Beteendedata samlades in via filmade såväl som direkta observationer, under första, andra, tredje, sjätte och sjunde dagen efter ankomst. Varje observationsdag var uppdelad i tre perioder, en på förmiddagen och två på eftermiddagen. Under varje observationsperiod samlades frekvenser för olika beteenden in. Alla individer observerades under ungefär en timme totalt. Data från de filmade observationerna samlades in under två stycken tre timmars perioder. Den insamlade datan analyserades med hjälp av Mann-Whitney U test, variansanalys test och enkel linjär regression.

Resultaten visade att hanar i burar med BGH åt ($p < 0.01$), drack ($p < 0.005$) och vilade på golvet ($p < 0.000$) oftare än vad hanarna gjorde i burar med GSH. Istället befanns sig djuren i burar med garaget oftare inne i gömstället ($p < 0.000$). Det visade sig även att hanarna i burarna med GSH inte bara etablerade deras hierarki på ett snabbare sätt utan dessutom med en tredjedel mindre antal sociala interaktioner, jämfört med hanar i burar med BGH. Därigenom antas aggressionsnivåerna hos hanar i burar med garage ha minskat.

Slutsatsen från den här studien var att hanarna i burarna med garaget anses ha minskat de beteendemässiga stress- och aggressionsnivåerna i större utsträckning än vad hanarna i burarna med boxen gjorde. Det var åstadkommet genom att hanarna kunde undvika onödigt höga nivåer av sociala interaktioner, men ändå etablera deras hierarki på ett mer effektivt sätt.

2. INTRODUCTION

2.1 Background

When using rodents for scientific purposes, such as the guinea pig, the animals are either bred at the laboratory or obtained from a breeder. The transport from the breeder, getting accustomed to their new environment, mates and surroundings can be factors affecting the levels of stress, to which the animals are exposed (Haemisch, 1990). To decrease these levels of stress, there is a legal demand that animals used for scientific purposes must have an acclimatization period, just after arrival at the new animal facility (Meyerson, 1986; Barnard and Hurst, 1996). To improve the housing conditions of the guinea pigs during the acclimatization period, a study at AstraZeneca R&D in Mölndal, was made. The outcome of the study was supposed to further depress the exposure of stress related factors during the acclimatization period, for the guinea pigs.

2.2 Wild cavies (*Cavia aperea*) and domestication

The guinea pig (*Cavia aperea* f. *porcellus*) is the third most commonly used rodent for laboratory experiments in Sweden (the Swedish Animal Welfare Agency, 2004). These animals were domesticated from the cavy about 3000 years ago in South America and in the end of the sixteenth century the guinea pigs were introduced to Europe (Rood, 1972; North, 1999). The wild cavy live in large groups and are abundant in northeastern Argentina, where the suitable habitat is usually an area covered by grass and scrubs with usable places to take refuge. Normally these animals are poor burrowers so they place their dens in tunnels made by other animals or culverts along the roads (King, 1956; Rood, 1972). These refuges serve as the base from which exploration starts and they are where the animals return afterwards (Rood, 1972).

When animals are domesticated, some of their social behaviours may be modified, caused by the change from natural selection to selection made by man. The domesticated species will retain the characteristics that are selected for, under the conditions imposed by the domestication. For guinea pigs the process of domestication has led to behavioural alterations such as reduced aggressiveness, tameness and fewer exploratory behaviours- all traits which help the animals to adjust to their man-made environment (King, 1956; Meyerson, 1986; Sachser, 1993; Künzl and Sachser, 1999).

2.3 Social hierarchies

Guinea pigs, as well as the wild cavies, are truly social animals that form stable dominance hierarchies within the group. The hierarchies are established foremost among males defending their territories and their females against other males. A rank order is also recognized among females but there is no inter-gender rank order (Nicholls, 1922;

Rood, 1972; Beer and Sachser, 1994). In low-density groups the hierarchy amongst males has shown to be of the linear rank order type, however, when the group size increases other types of hierarchies have also been observed (Sachser, 1994; Jacquez and Norusis, 1973a; Rood, 1972). To establish the rank orders within the group, males show elevated levels of agonistic behaviours, and when the dominance hierarchy is established these levels are reduced (Sachser, 1986; Beer and Sachser, 1994). Not only do the males show agonistic behaviours towards each other, but also courtship and sexual behaviours in the same way as males court females. These behaviours are not always homosexual, but some males even act like females – so-called pseudo-females (Beer and Sachser, 1994; Hawke *et al*, 2002). There is evidence, in fact, that the pseudo-females have a better welfare, in terms of fewer injuries and over all lower stress levels than the subdominant males of a group (Beer and Sachser, 1994). The organization of the different social positions is not always a highly aggressive occurrence; the levels of agonistic behaviours seem to depend on the social experiences of the individuals. For a peaceful establishment of the different ranks, previous engagements in agonistic encounters with an older dominant individual appear to be required and it is around the time of puberty that this essential experience must be attained (Sachser and Renninger, 1993; Sachser *et al.,* 1998). If there is such an experience, the subdominant individual has the social skills needed to adapt to a lower rank, in a less stressful and aggressive way (Levinson *et al*., 1979; Sachser, 1986; Sachser, 1994; Stefanski and Hendrichs, 1996). It has been shown that when moving the dominant male from one group to another, neither the body size nor the age mattered when encountering the dominant male of the strange group; the only thing that seems to matter are the previous experiences of earlier interactions (Sachser and Pröve, 1984).

When the rank has eventually been established, the dominant animals will mainly display aggressive and courtship behaviours whilst low-ranking individuals will mostly be a target of aggression and behave submissively. Middle-ranking animals will be those receiving but also initiating interactions – for example aggressive attacks (Beer and Sachser, 1994; Sachser *et al*. 1998). Most of the aforementioned studies have been experiments with mixed-sex groups; nevertheless there have also been studies with males only. These have shown that placing more than eight males in one cage should be reconsidered, since the encounters amongst the males have proved to be very aggressive and have in some cases even led to deaths (Sachser and Lick, 1989; Beer and Sachser, 1994).

2.4 Stress-induced sickness behaviour

High levels of stress can lead to reduction in feeding, exploration and sexual behaviours, and these conditions have been shown to be comparable to those during sickness (Hennessy *et al,* 2004). Further studies have also revealed that mammals, and especially rodents, show a relationship between certain behavioural coping patterns and specific activations of the endocrine system (Sachser, 1993; Haemisch, 1990). These activations involve the sympathic adrenomedullar system (SAM) and pituitary adrenocortical system (PAC) and, like social interactions, have an intense influence on these systems. These

systems will play a key role in adjusting an individual to social and non-social stressors by supplying the reactivity needed (Henry, 1982; Sachser, 1987; Sachser, 1990; Kaiser *et al*, 2003).
The SAM system is evoked when access to necessities such as food, water, shelter, territories and mates are challenged. The reaction is diverse, depending on the necessity in question, and ranges from anger and agonistic behaviours to alert-behaviours triggered by fear. The result from awakening the SAM system is an active way of coping with the problem. The PAC system is instead turned on by helplessness and typical results are such submissive behaviours as immobility and avoiding other animals (Henry, 1982; Sachser, 1990; Sachser, 1994). The SAM and PAC systems will help the animal adjust to social and non-social situations as mentioned above. However if the activation of these systems becomes much too long and intense, the result will instead become a suppressed immune system (Olsson *et al.* 2003). Studies on guinea pigs and tree shrews have shown that extreme fear as a result of aggression can lead to sickness, injuries and in some cases even death by kidney or heart failure (Henry, 1982; Sachser *et al*., 1994; Sachser, 1994).

In stable social systems, where the individuals all have experience of being low in rank, establishment of the dominance relationship will be a predictable event. When the rank-order is recognized later on, there will be distinct behavioural differences between dominant and subdominant individuals, while differences in endocrine levels may disappear. As a consequence the lower rank in the hierarchy will not lead to unnecessary increases in the endocrine stress responses (Sachser, 1987; Sachser *et al*., 1998).

2.5 Welfare and enrichment

Barnard and Hurst (1996) stated that good welfare for animals during experiments is not only important from an ethological point of view but essential for the experiment itself, where physiological, pharmacological and behavioural functions of the animals are of concern. These, like the welfare of the animals, can have a direct effect on the trustworthiness of the final results. One way to improve the animal's welfare is to modify its' housing conditions. This is an area of research called applied environmental enrichment. Olsson *et al.* (2003) reviewed a number of studies where different improvements are suggested for various species of rodents; for example, nesting materials, objects for chewing, wheels for running, structures for climbing and shelters. The goals with these enrichments are to increase the animal's capacity to cope with being in captivity and involved in experiments (Olsson *et al,* 2003).

In guinea pigs, a species of rodent that have proven to be active intermittently both day and night (White *et al.* 1989), it has been shown that merely exposing an individual to an unfamiliar and plain environment can result in elevated activity in the PAC system (Haemisch, 1990). Under these laboratory conditions, the animals often prefer to stay in a corner of the cage or close to the wall near food and water. These places are thought to be substitutes for shelters and thus, for the guinea pig, shelter would likely be considered one of the limiting resources (King, 1956; Büttner, 1992; Büttner, 1994). Although the shelters are meant as a place to rest and get away from the stress of the otherwise open

space of the cage, studies have shown occurrences of resting being terminated by outbursts of aggression (Fuchs, 1980). This was observed among males and as a result of the aggression; some of the animals had to leave the shelter, and consequently avoid the resting site and instead went off feeding (Fuchs, 1980).
It appears that the shelter for hiding is an important enrichment for the guinea pigs. However, the design of the shelter has not yet been much studied in guinea pigs, and especially during the acclimatization period.

3. AIM

This study aims to improve the housing conditions for guinea pigs, during the acclimatization period, and was done in collaboration with AstraZeneca R&D in Mölndal. The purpose of the experiment was to evaluate if the levels of behavioural stress and aggression, during the acclimatization period, for group-housed male guinea pigs were affected by the design of the shelter for hiding.
Two design-types for the shelter were used: one type, called Box for Group Hiding – BGH, had one entrance and one compartment, whilst the second type was called Garage for Single Hiding – GSH and had four entrances and four compartments.
The hypothesis was that the GSH would decrease the levels of stress and aggression by offering one compartment for each individual of the group, as compared to the BGH. By doing so, the garage would diminish the competition for space inside the shelter. With this hypothesis in mind the following questions were of interest for the experiment; for each, a null hypothesis was considered but not presented here:

- Are there higher frequencies of the social, resting and locomotion behavioural categories in BGH than in GSH?

- Are there higher frequencies of the feeding, drinking, being in the facility and popcorn behavioural categories in GSH than in BGH?

- Is there a linear trend during the acclimatization period for the frequencies of the different behaviours and does that differ between BGH and GSH?

- Do the social interactions decrease more rapidly in frequency for GSH than in BGH during the acclimatization period?

- Is there a higher frequency of visits inside and on the roof of the facility in GSH than in BGH?

- Is there a higher frequency of visits to some compartments compared to others in GSH?

- Is the weight gain larger in groups with GSH than in groups with BGH?

- Is there a higher number of started interactions or a higher level of aggression amongst males in BGH than GSH, while establishing the hierarchy?

- Do the guinea pigs use the cage and the facilities for hiding in the same way when there is no observer present?

4. MATERIAL AND METHODS

4.1 Animals

The guinea pigs used in the experiment were of the Duncan-Hartley strain and obtained from the HB Lidköping Kaninfarm outside Lidköping. Only males were chosen for the study. This, since the guinea pigs were supposed to be used for a research study at AstraZeneca R&D, after the acclimatization period was finalized, in which only males are used. Before transport to the laboratory, the animals lived with their parents the first 2-3 weeks, at which age they were moved into groups of 10-20 animals of similar age. At a weight of about 300 grams, they were moved into smaller groups of two to four individuals of the same sex. Later on these groups of guinea pigs were transported, in cardboard crates with wood shavings, by car from the breeder to the animal facility; the transport took approximately one and a half hours.

All animals except one, showed no signs of injury during the experiment. The individual that was hurt was observed in one of the cages with GSH during day six of the acclimatization period. The animal was limping but was still moving around feeding and drinking like the rest of the group, and did not seem to be particularly bullied by the rest of the males. After examination, it was decided that the animal could continue in the experiment and stay in the cage for the rest of the acclimatization period. The animals were introduced into the experiment at an age of about 12 weeks weighing approximately 500 grams.

4.2 Maintenance

The experiment took place at one of the animal units of the Department of Integrative Pharmacology at AstraZeneca R&D in Mölndal. The animals were kept under constant conditions with light: dark cycle of 12h and a photoperiod between 0600-1800 hours, with 30 minute dusk and dawn. The room temperature was around 22 º C and the relative humidity approximately 55 %. Commercial guinea pig diet, K1 (Lactamin, Vadstena) and tap water was available *ad libitum* and the diet was regularly supplemented with autoclaved hay (Granngården, Kungsbacka). Water bottles were replaced every day, and every 4^{th} day the room was cleaned and the cages changed. The cages that were utilized had walls made of stainless steel and plastic flooring (4250 cm^2). Sawdust (from aspen, Finn Tapvei Ky Finland) covered the floor, on top of which the food container and the shelter were placed. The water bottle was mounted onto one of the walls of the cage. The animals were placed in groups of four in each cage.

4.3 Treatments

Two types of boxes for hiding were used in the experiment: 1) Box for group hiding - BGH: a coloured plastic box (polypropylene) with a single entrance and one compartment and a floor area of 510 cm^2 occupying about 12 % of the total floor area of the cage; 2) Garage for single hiding – GSH: a grey plastic box (noryl plastic) with four different entrances and four compartments and a floor area of 1382 cm^2 occupying about 32 % of the total floor area of the cage (Figure 1, Table 1).

a)  b) 

Figure 1. *Photos of guinea pigs in cages with a) BGH and b) GSH .*

Table 1. *The main difference between the two types of facility for hiding, showing the percentage of total floor area as well as the actual floor area the facility is occupying, the floor area for each of the compartments and the number of compartments and entrances for each treatment.*

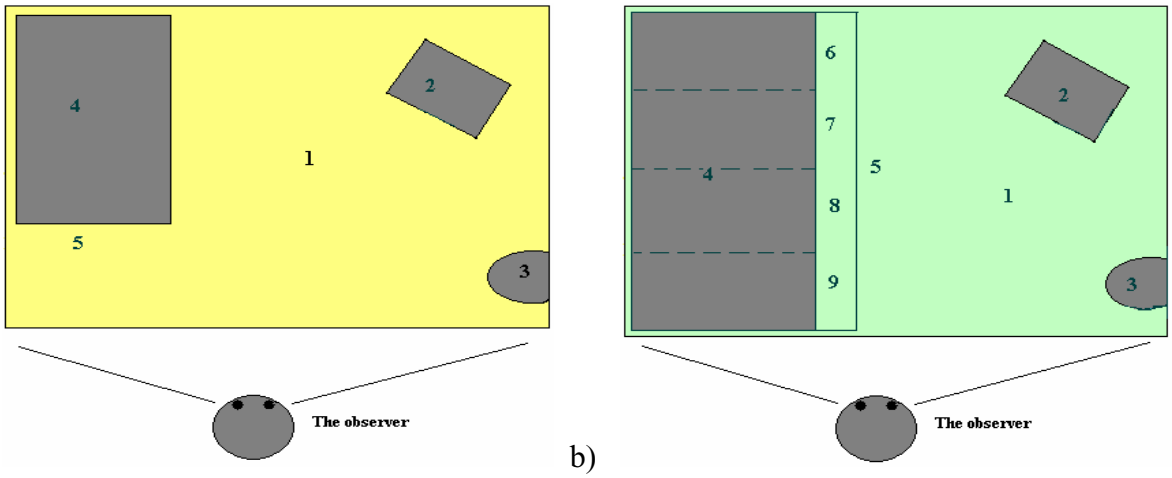
Type of facility	Floor area of facility (cm^2)	Floor area of compartment (cm^2)	No of entrances	No of compartments	% out of total floor area
BGH	510	510	1	1	12
GSH	1382	295	4	4	32

4.4 Experimental design

The experiment was conducted during the acclimatization period, which at AstraZeneca's animal units is seven days, including the day of arrival. In total, 96 individuals were studied. The animals were divided into 12 cages per treatment; the four individuals that had travelled together in the transportation crate from the breeder were placed in the same cage. The experiment lasted six weeks; during each week one test group of 16 animals (i.e. 4 cages, 2 cages per treatment) was observed. During five out of the seven days of the acclimatization period observations took place, and data from 100 hours of direct observations was gathered. All males were marked with different colours of spray paint normally used for livestock (green and blue spray paint, DeLaval, no: 906968-12 and 906968-13; red spray paint, Kruuse no: 240445), so that individual identification was possible. Since the observations had to take place with the cage doors open (it was nearly impossible to observe through the cage door), a transparent plastic panel was placed at the cage opening so that none of the animals could escape. During day one no observations took place, but the door was left open in order to allow the animals to get accustomed to having only the plastic panel at the opening.

4.5 Recordings

On day one the animals were individually marked and at the same time weighed using a Mettler Toledo Type PR 8001. The weighing was repeated at the end of day seven after the final observations were done. The observations took place on the 2^{nd}, 3^{rd}, 4^{th}, 6^{th} and 7^{th} day of the acclimatization period. Each day of observation was divided into three periods, first 8.45-10.15, second 12.30-14.00 and third 14.30-16.00. During these periods the frequencies of 17 different behaviours (Table 2) and also the position in the cage (Figure 2) for each of the behaviours were recorded. When an interaction took place the individuals who were considered the winner and the looser was distinguished and recorded.



a) b)

Figure 2. *Figures showing the different positions in the cages that were recorded, with a) BGH (1-5) and b) GSH (1-5) and the compartments in the cages with GSH (6-9). Placement 1, the individual is situated on the floor; Placement 2, the individual is feeding on concentrate; Placement 3, the individual is drinking; Placement 4, the individual is on the roof of the shelter; Placement 5, the individual is inside the shelter; Placement 6-9, the individual is inside one of the compartments of the garage.*

Every period started with a ten-minute break, during which the animals had time to adjust to the observer being in the room. Throughout the remaining part of the period, each cage was observed for 16 minutes at a time, having a four-minute break after every two cages. All cages were marked and observed in a random order. One at a time, each individual in the cage was observed continuously for two minutes in two sets. To tell time, a stopwatch which beeped in two minute intervals was used, each beep indicating a change of focal animal. All recordings were documented using a tape recorder (SONY TCM-16).

4.6 Video recorded observations

Two groups of four males each were observed during a 3-hour period to get an idea of how the facilities for hiding were used when the observer was not in the room. It was also of interest to see how the observer affected the animal's normal behaviour. One group was placed in a cage with the box for group hiding and the second group in a cage with the garage for single hiding and each cage was video recorded at two different occasions. The group of males in cages with BGH had the facility in the cage for at least one day, while the group with GSH got the garage facility placed in the cage just before video recording. The observations were made using a camera with a 24-hour time lapse (Panasonic WV CL 920), a tape recorder (Panasonic AG 6024) and an IR lamp (Bischke Type IR 20) to permit a better view of the activity in the darker parts of the cage. The camera was placed in approximately the same position as the observer was sitting in the earlier recordings, and the IR lamp just to the right of the camera. The cage door was left open and the transparent plastic panel was once again used and an extra piece of plastic was added where the garage was situated to hinder escape from the facility roof. The cages that were videotaped were those closest to the floor so that none of the animals would get hurt in the event of an escape. To keep track of the people passing in and out of the room and their reason for being in the room, a notebook was placed at the door where time and cause for entrance was noted. The technical equipment was placed in the guinea pig room the morning of recording and at 1300 hours the tape started recording and continued for the following three hours.

Table 2. *Ethogram: The nomenclatures of the behaviours recorded and the categories the behaviours belonged to (Grant & Mackintosh, 1963; Rood, 1972).*

Behavioural categories	Behaviours	Descriptions
Feeding	Feeding hay	Standing on all fours or lying down eating hay from the floor
	Feeding concentrate	Standing on all four legs, or on the hind legs with front legs on the food container eating commercial guinea pig food
Drinking	Drinking	Licking the nipple of the water dispenser
Locomotion	Locomotion	Walking or running around the cage, at least two or three steps
In facility	In facility	The animal is situated inside the hiding facility with at least half the body out of sight of the observer; eating hay with only the head outside the compartment was also considered being the facility
Social Sniff	Sniff	Sniffing, including licking the body regions (not anal) of the opponent
Resting	Resting	Laying down on the floor or on top of the roof of the facility sleeping or resting with eyes closed or almost closed
Popcorn	Popcorn	One or a series of frisky upward leaps and with occasional sharply turns while in the air, resembling gambols of lambs, occurring in sexually aroused males or as play
Defensive	Retreat	Directed movement away from the opponent at walking or running pace moving a minimum of three steps away from the opponent
	Head Up	Head thrown back so that the nose points straight up, often right before retreat
	Hiding	Running or walking into the facility for hiding after being scared by an opponent or something else
	Submissive crouch	Crouching with belly to the ground when approached by a dominant opponent
Offensive	Stand Threat	Interactions involving curved body posture of one or both animals, considered an indication of threat or arousal. Vocalization can also be heard, resulting from striking the incisor teeth against each other
	Attack	Approaching or taking a short jump at the opponent with open mouth, considered to be an intense attack
	Chase/Bite	Running after the opponent, sometimes involving biting
Sexual	Anal Sniff	Sniffing or licking around the ano-genital region
	Rumba	Slowly approaching the opponent, rhythmically swinging the hindquarters from side to side and emitting a burbling vocalization with the head stretched forward

4.7 Statistical analysis

The 17 behaviours recorded were grouped into 10 behavioural categories, which were used for the statistical analysis (Table 2). The different positions in the cage were also grouped into categories. Positions one through three became position one since the definition of position two was that the individual was feeding concentrate, and for position three that the individual was drinking. These positions did not give any new information about where the animal was situated in the cage, so it was better to simply define the first three positions as position one. Positions four and five remained the same. When recording the outcomes of the social interactions, there was not always an obvious winner and loser; those data were not included in the analysis. Data from one entire period were missing during test group 3 and also data from a single cage during one period in test group 2 were missing. By recalculating all behavioural and positional data into frequencies per minute instead of plain frequencies, the missing data during these test groups will not affect the outcome of the analysis.

All analyses were made in consultation with Sofia Andersson at the Department of Biostatistics, AstraZeneca R&D in Mölndal. 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 non-parametric tests were utilized. Using MINITAB Statistical Software, version 13.20 (Minitab Inc.), with the assumption that a result from an analysis with a probability, or p-value, of less than 0.05 would be considered statistically significant, the following analyses were made. The recalculated data in total for the different positions and behavioural categories, as well as for total weight-increase, were compared with regards to the two treatments, using the Mann-Whitney U test. In cages with GSH, the compartments were combined in every possible pair to test if there was any difference in total visiting-frequency per minute, by using the Mann-Whitney U test. The mean frequency per minute for all different behavioural categories, as well as positions in the cages, was plotted for each treatment as histograms. The total percentage of visits in each of the compartments of the GSH was calculated and presented.

A Variance Analysis test was made using the data from the weighing at day one to distinguish if there was any significant difference in weight between the animals at arrival. The mean weight on day one, as well as the weight gain after the acclimatization period per treatment, was plotted in histograms. The recalculated data from the behavioural observations were also used to make a Simple Linear Regression to distinguish if there was a trend during the acclimatization period for all behavioural categories. The linear regression was used even though there was no normal distribution; the F-test is considered a robust test, as well as it was being based on a large amount of data (Jacquez and Norusis, 1973b). The total frequency per minute during each day of the acclimatization period for the social interactions, as well as resting and being in the facility, were plotted in graphs. The data from the interactions between individuals, with winners and losers recorded, ended up being too few to calculate reliable rank orders. Instead these data were summed up as percentage of won and lost interactions for all individuals and the total number of interactions per cage.

When the analysis of this study was made multiple tests were done, and with multiple tests comes some uncertainties in the reliability of the results. There are techniques to implement produced by SAS Institute Inc. to create p-values adjusted for multiplicity but none of those techniques were used for this experiment (Westfall & Young, 1993) as they were thought to be beyond the scope of this work.

5. RESULTS

5.1 Behaviours

The animals spent most of their time feeding (32 respective 24 %), moving around (23 respective 24 %) and being inside the facilities for hiding (13 respective 32%) in the BGH and the GSH, respectively. In both treatments about 16% of the time was spent engaging in social interactions. The male guinea pigs performed a significantly higher frequency per minute feeding and drinking in the BGH than in the GSH (Figure 3).
For the feeding as well as the drinking behaviour category the quartiles were approximately the same size when comparing the proportions of the two treatments (Figure 3).

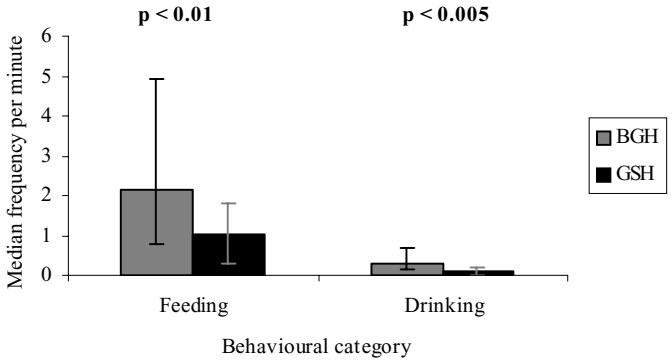


Figure 3. *Median frequency per minute (±Q3, Q1) for feeding and drinking in cages with box for group hiding and garage for single hiding.*

There was also a significantly higher frequency per minute resting and a tendency towards more popcorn in the BGH than in GSH (Figure 4). However, being in the facility for hiding was recorded significantly more often in the GSH than in the BGH (Figure 4). The two treatments did not differ in frequency per minute for the locomotion behaviour (Figure 4). The quartiles for the locomotion, resting and popcorn behavioural categories were about the same, proportionally, for the two treatments (Figure 4). The behaviour category in facility though, had a much larger first quartile in GSH than BGH (Figure 4).

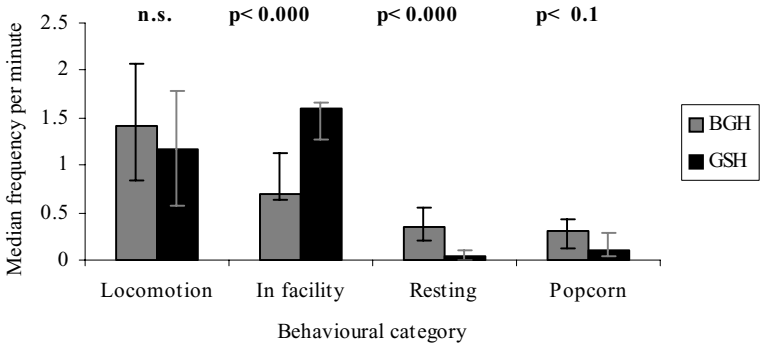


Figure 4. *Median frequency per minute (±Q3, Q1) for the locomotion, being in the facility, resting and popcorn in cages with box for group hiding and garage for single hiding.*

There was a tendency towards a significantly higher frequency per minute for the social sniff behaviour in BGH than GSH (Figure 5). For the remaining social behaviours (defensive, offensive and sexual behaviours) there were no significant difference between BGH and GSH (Figure 5). The quartiles for the social behavioural categories were proportionally the same size for the two treatments (Figure 5).

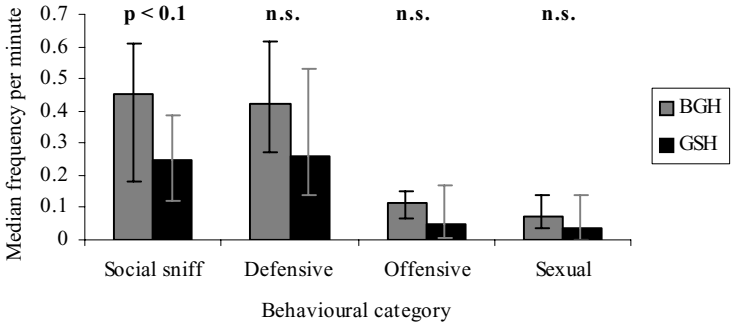


Figure 5. *Median frequency per minute (±Q3, Q1) for the social sniff, defensive, offensive and sexual in cages with box for group hiding and garage for single hiding.*

The animals were more withdrawn than others in a few of the total six test groups, and this was particularly apparent in test group four. During the entire experiment there were regular observations of males being forced to leave the facility for hiding and instead occupied themselves with eating, drinking or resting on the floor outside the facility. These observations were much more frequent in cages with the box for group hiding, than in cages with the garage for single hiding.

5.2 Trends during the acclimatization period

In the cages with BGH, all behavioural categories but locomotion, drinking and resting, had a high percentage of total variation in frequency per minute that was explained by the fitted regression line, R^2 or coefficient of determination (Table 4). Only the behavioural category social sniff, in the cages with GSH, had a comparatively high percentage for R^2. The behavioural categories mentioned above as having a high coefficient of determination, were also the categories with p-values low enough to reject the null hypothesis that the slope of the regression line was equal to zero (Table 4). *b,* also called the regression coefficient of the regression lines, was positive in all behavioural categories, except for being in the facility for hiding, and this was the case in both treatments (Table 4).

Table 4. *Results from the linear regression to find a trend during the acclimatization period, using data from all the behavioural categories in cages with BGH and GSH and an alpha of 0.05.*

	Box for group hiding			**Garage for single hiding**		
Behavioural Categories	p value	R^2, adj (%)	*b*	p value	R^2, adj (%)	*b*
Defensive	0.005	92.7	0.710	0.227	24.5	0.333
Social Sniff	0.007	91.1	0.673	0.050	69.7	0.350
In Facility	0.009	90.0	-0.464	0.435	0.0	-0.202
Offensive	0.027	79.5	0.160	0.451	0.0	0.079
Popcorn	0.028	79.1	0.585	0.078	59.7	0.376
Feeding	0.033	76.6	1.990	0.371	31.1	0.990
Sexual	0.048	70.3	0.128	0.492	0.0	0.042
Locomotion	0.053	68.2	1.690	0.149	40.6	1.120
Drinking	0.221	25.5	0.197	0.500	0.0	0.044
Resting	0.790	0.0	0.032	0.219	26.0	0.018

In most of the interactive behavioural categories (social sniff, defensive and offensive behaviours) the frequencies per minute in all cages increased from day two until day six; however from day six till day seven there was a relatively sudden decrease in frequency (Figure 6-8).
There is an indication of a somewhat more dramatic decrease from day six to seven in the cages with GSH compared to cages with BGH, when comparing frequency per minute for all interactive behavioural categories per minute (Figure 6-9). These indications are strengthened by the results from the linear regression mentioned above, where none of the social categories but social sniff, for cages with GSH had a linear trend (Table 4).
For the behavioural categories resting and being in the facility, there were not many changes in frequencies per minute from day to day in both cages with BGH and with GSH (Figure 10).

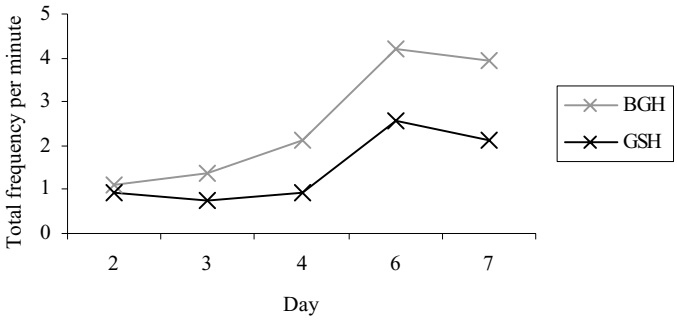


Figure 6. *Total frequencies per minute each day of observations during the acclimatization period, for the behavioural category social sniff in cages with box for group hiding and garage for single hiding.*

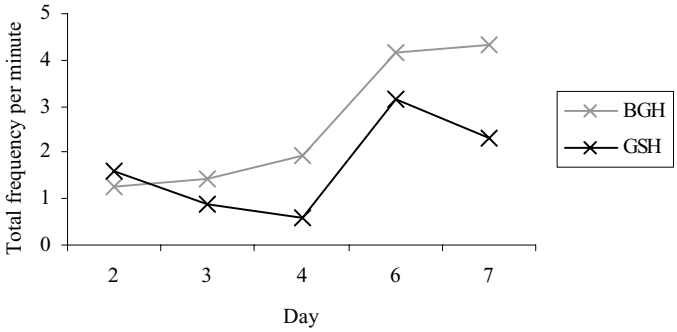


Figure 7. *Total frequencies per minute each day of observations during the acclimatization period, for the behavioural category defensive, in cages with box for group hiding and garage for single hiding.*

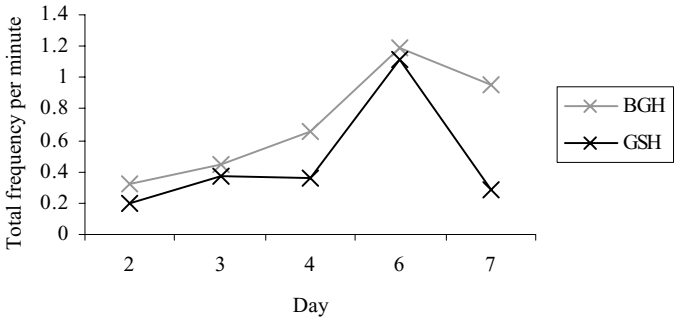


Figure 8. *Total frequencies per minute each day of observations during the acclimatization period, for the behavioural category offensive in cages with box for group hiding and garage for single hiding.*

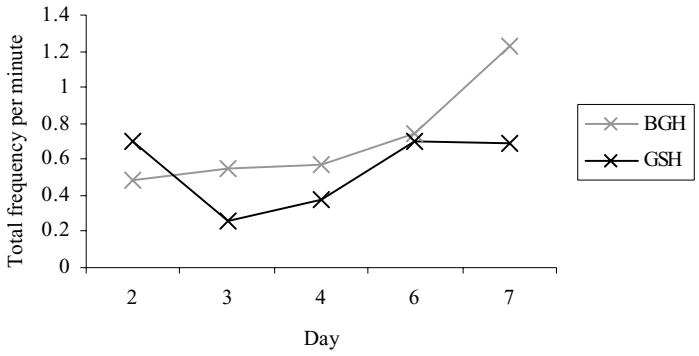


Figure 9. *Total frequencies per minute each day of the observations during the acclimatization period, for the behavioural category sexual in cages with box for group hiding and garage for single hiding.*

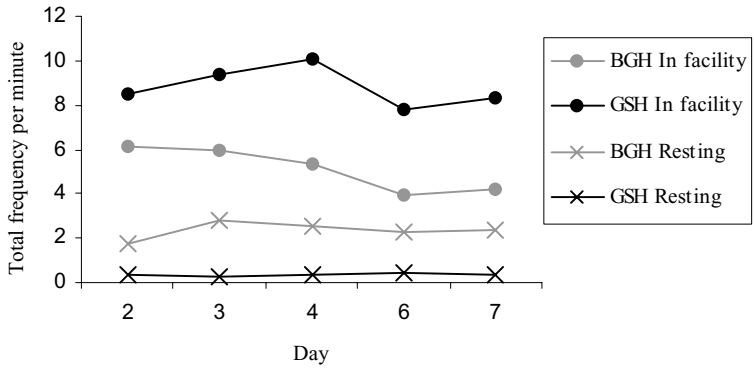


Figure 10. *Total frequencies per minute each day of the observations during the acclimatization period, for the behavioural category in facility and resting in cages with box for group hiding and garage for single hiding.*

5.3 Positions in Cage

There was a significantly higher frequency per minute for being on the floor BGH than GSH (Figure 11). The frequency per minute for being inside the facility for hiding was significantly higher in GSH than BGH (Figure 11). The quartiles for each of the placements in the cage were proportionally the same size (Figure 11). During the whole experiment, the guinea pigs in cages with either treatments had a very low number of visits to the roof of the facilities - not even a hundred in total, and no significant difference between treatments when comparing frequencies per minute (Figure 11). In the box for group hiding, the animals spent six percent of their time resting on the floor and about 13 percent inside the hiding facility, while the animals living in cages with the garage for single hiding spent one percent resting on the floor and 31 percent of their time inside the garage.

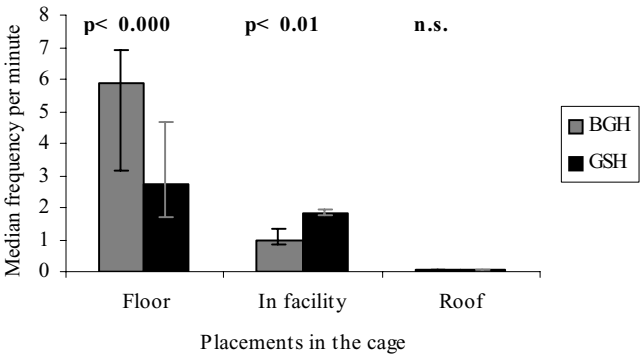


Figure 11. *Median frequency per minute (±Q1 and Q3) for the different positions in cages with box for group hiding and garage for single hiding.*

5.4 Compartments of the Garage for Single Hiding – GSH

There was a significantly higher frequency per minute in compartment six compared to the outer ones, compartments seven ($p < 0.05$), eight ($p < 0.000$) and nine ($p<0.01$). There was also a significant difference when comparing the compartments in the middle, compartments seven and eight ($p < 0.05$). There were no significant differences between the remaining pairs of compartments (n.s.). The compartment that had the highest percentage of visits was the one closest to the inner wall- compartment six, and compartments seven, nine and eight then followed in popularity (Figure 12).

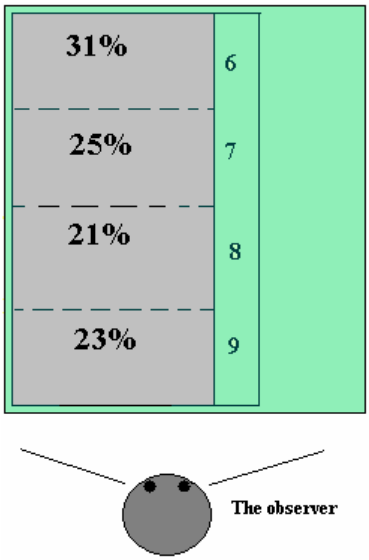


Figure 12. *Percentage for each of the four compartments out of the total frequency per minute spent inside the garage for single hiding.*

5.5 Weight

The result from the F-test in the variance analysis confirmed that the null hypothesis of same weight for all individuals at the day of arrival was not to be rejected (n.s.) and the standard deviation for all test groups and treatments were similar (Figure 13). There was no significant difference in weight gain when comparing the two types of treatments using the Mann-Whitney U test during the whole experiment (n.s.). Though, there was a difference when comparing the test groups with each other. For half of the test groups (one through three) the weight gain was higher than for the rest of the groups (Figure 14). The first and third quartiles for the weight gain during the acclimatization period were about the same for all the test groups and treatments (Figure 14).

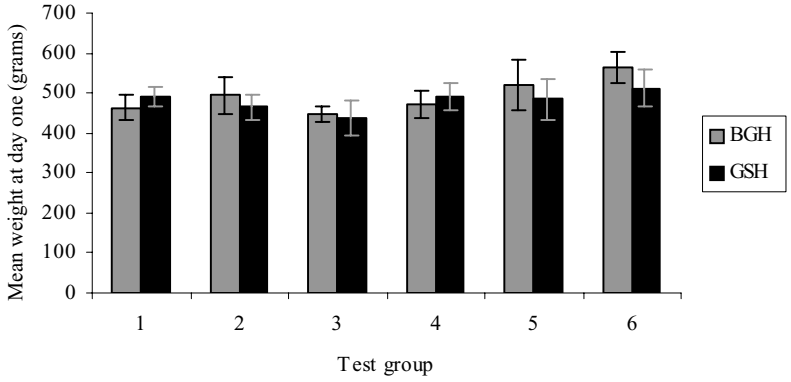


Figure 13. *Mean weight at the day of arrival (±SD) for individuals in all test groups, both in cages with box for group hiding and garage for single hiding.*

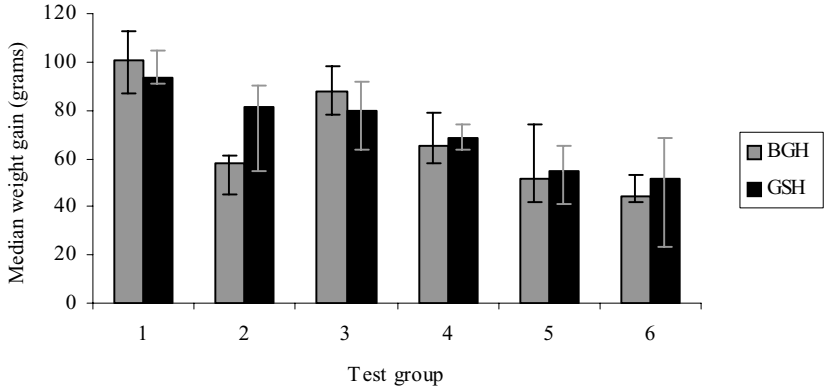


Figure 14. *Median weight gain (±Q1 and Q3) during the acclimatization period for individuals in all test groups both living in cages with box for group hiding and garage for single hiding.*

5.6 Social interactions

The results from social behaviours where winner and loser were registered was assembled in a table as percentage of won and lost interactions per individual, cage and treatment (Appendix I). To present the results from this table graphically, two figures were made using the data (Figure 15 and 16) and the cut-out from the appendix in table 5 helps to show how the figures were made. By adding the number of interactions per cage for all test groups and each treatment it was shown that there was one third more interactions in the cages with BGH than in cages with GSH (Figure 15).

Table 5. *A cut-out from Appendix I showing the resulting percentage of won and lost interactions for individuals in test group 1 and cage two, with the box for group hiding and also the total number of interactions in this cage.*

			Box for group hiding		
			Total no of	Won inter-	Lost inter-
Test group	Cage	Colour	interactions in cage	actions (%)	actions(%)
1	2	Pink	84	34.8	65.2
	2	Green	84	47.7	52.3
	2	Multi	84	51.5	48.5
	2	Purple	84	66.7	33.3

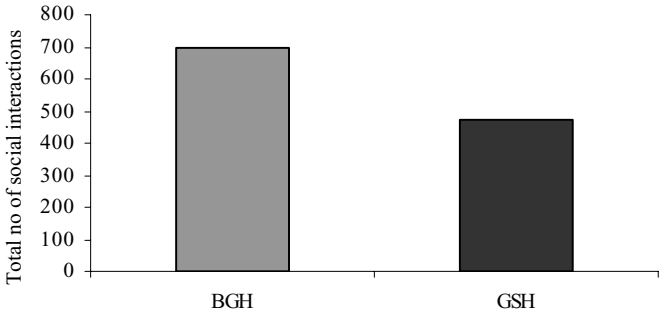


Figure 15. *Total number of social behavioural interactions in cages with box for group hiding and cages with garage for single hiding.*

By using the data in appendix I, it was also possible to calculate the difference between the individual with the highest percentage of won interactions and the individual with the lowest percentage of won interactions, in each cage. In the example presented above, the difference would be calculated by subtracting the percentage of won interactions for the pink individual from the percentage of won interactions of the purple individual (Table 5). The result from these calculations is presented in figure 16 which shows that the difference between the individual with the highest and the individual with lowest percentage of won interactions was higher in the cage with GSH than in cages with BGH in all test groups.

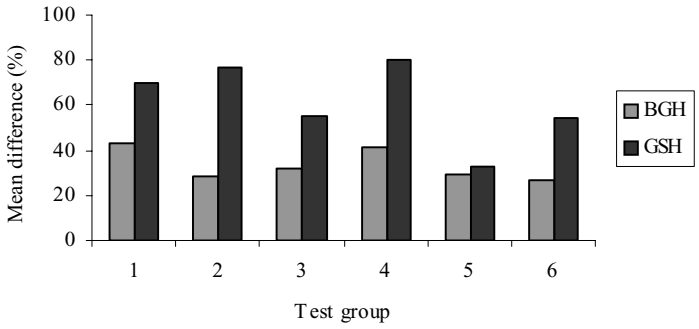


Figure 16. *Mean difference between the individual with the highest percentage of won interactions and the individual with the lowest percentage of won interactions per cage, for males living in cages with box for group hiding and those living in cages with garage for single hiding.*

5.7 Video recorded observations

The recording from the videotaped observations showed, as in the direct observations that males living in the cage with BGH were more often forced to leave the facility for hiding and instead occupy themselves with eating, drinking or resting out on the floor, compared to males living in cages with GSH. It was also apparent that some individuals spent much of their time on the roof of the hiding facility in both cages with BGH and GSH.

6. DISCUSSION

The purpose of this study was to analyse if the design of the shelter for hiding affected the behaviour of male guinea pigs that were living in groups.
During the experiment, the six test groups differed a great deal when regarding the boldness of the animals. For the individuals in the first two test groups, it did not seem problematic for the animals to step on to the roof of the shelter, for example, and stay there for a while even though the observer was present in the room. When the final test groups arrived it was apparent that the individuals were much more withdrawn when the observer entered the room and this was particularly apparent in test group four. These annotations were observed for all the individuals in both treatments and are most likely the cause of the large deviations from the median in the behavioural results. The possible explanation for these fluctuations in timidity among the test groups can be traced back to the breeder and the design of the cages where the animals were bred. There were three kind of cages in which the guinea pigs were kept at the farm; some breeding pairs were kept in grey plastic boxes with four solid walls, making it impossible for the guinea pigs to see more than the arm of the animal technician. The remaining breeding pairs were kept in cages like those used in the present study, making it possible for the guinea pigs to view the entire animal care taker. The young guinea pigs that were kept in groups of 10-20 or two to four individuals were kept in yellow plastic boxes with the same physical properties as the grey boxes. If the majority of the individuals in test group four, for example, had been bred by pairs living in the grey plastic boxes, it would seem reasonable that these individuals would be more scared and withdrawn when faced with the observer and only separated by only a transparent plastic panel. This differs from individuals in a test group bred by pairs living cages like those used in the experiment; for these, the view of humans would not be a new experience.

6.1 Behaviours

Annotations from the direct as well as the video recorded observations confirm what Fuchs found in 1980: One individual in the group frequently placed itself at the opening of the shelter, and by doing so effectively hindered other individuals from entering the shelter, and with aggressive behaviours removed the individuals already inside the shelter. The individuals forced to leave the shelter instead spent their time feeding, drinking and resting on the open floor. The forceful and aggressive behaviours found by Fuchs were more frequent in the cages with BGH than with GSH in the present study, which could provide an explanation of the behavioural results. The behavioural results show that the animals living in cages with the box for group hiding (BGH) spent more time out on the floor feeding, eating and resting compared to males living in cages with the garage for single hiding (GSH). The males living in cages with the garage spent more of their time inside the facility for hiding, compared to the males living in cages with the box. The fact that the males in cages with BGH were not able to use the shelter to the same extent as males in cages with GSH is assumed to also have affected the social

sniffing behaviours. As most of the time for the males in cages with BGH was spent out on the floor, social sniffing would be harder to avoid and did occur more often in cages with the box than in cages with the garage.

The establishment of the social hierarchy amongst males in populations with both sexes are sometimes marked by violent events, especially among males lacking earlier experience (Sachser and Renninger, 1993; Beer and Sachser, 1994; Sachser *et al.,* 1998). Nevertheless the present study shows 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 from the present study with male-only groups establishing a hierarchy, with results from groups consisting of both sexes, the definition of social sniffing would 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 other individuals. In groups consisting of only males that are in the middle of establishing a hierarchy, the definitions of this behaviour are probably modified into a more aggressive act, and the behaviour applied when there is no need for very high levels of hostility. This would explain the low levels of offensive and sexual behaviours.

There were no differences in the frequency of locomotion between treatments, even though there was a clear variation in open floor area for the two types of treatments: 88 % for cages with BGH and 68 % for the cages with GSH. The fact that the feeding, drinking and social sniffing behaviours on the open floor occurred more frequently for guinea pigs in cages with BGH might make the locomotion data seem even more difficult to interpret. An explanation could be the definition of the locomotion behaviour for this experiment; "walking or running all around the cage, at least two or three steps" (page 15). An individual shifting from feeding hay to a social behaviour, for example, might not need to move longer than just a single step and therefore there would not be a registration of movement for this individual. The larger open area of the cage could be the reason for a tendency towards more frequent popcorn behaviours in cages with BGH. The popcorn behaviour is a fairly area-demanding behaviour, where the individual makes "frisky upward leaps and with occasional sharply turns while in the air" (page 15). As the cages with BGH offer much larger areas to perform these upward leaps, it is assumed to be the reason to why there were more popcorn behaviours in the cages with BGH.

6.2 Trends during the acclimatization period

For animals living in cages with the garage-type of shelter, there was only one behavioural category, the social sniffing, that showed a relation between the frequency per minute and the days of the acclimatization period. In cages with the box-type of shelter the results were the opposite - almost all behavioural categories showed this relationship. A possible explanation for these results was presented as the total frequency per minute over time, for each of the social behavioural categories. The cages with GSH showed a more drastic decrease of the defensive, sexual and offensive behaviours from the second-last day to the last day of observations, compared to the animals living in

cages with BGH. These results are thought to illustrate that the males in cages with the garage will, towards the end of the acclimatization period be exposed to lower levels of aggression. Data representing frequency spent resting on the floor or inside the shelter showed that these inactive behaviours did not affect the decreases in defensive, sexual and offensive behaviours for any of the treatments. Decreased levels of aggression are believed to lower the levels of stress for the guinea pigs and consequently the better odds of avoiding the stress-induced sickness behaviours in the long run (Henry, 1982; Sachser, 1990; Sachser, 1994).

6.3 Positions in cage

The positions in the cages where the animals preferred to stay confirmed the results from the behavioural data. In both treatments, the animals chose to spend most of their time on the floor. Males in cages with the garage spent more time inside the shelter for hiding than males in cages with the box. It should be remembered, that not only did the garage for single hiding offer individual entrances and compartments for all males in the groups, but also a much larger over-all area of the shelter compared to the box for group hiding. Consequently, there are three factors that could have affected the outcome, but unfortunately, it would not be possible to separate these factors and independently analyse to what degree they affected the final results.

Almost none of the males in the entire experiment stood on the roof of the shelter; a possible explanation for this could be the noted difference between the test groups in apprehensiveness towards the observer. One could imagine that guinea pigs with less visual experience with humans would be more afraid and nervous about exploring this second level surface, especially since the guinea pigs are thought to experience the roof as a surface where they would be more exposed and vulnerable to the observer, as compared to when they were on the floor. These assumptions were confirmed when watching the video recorded observations, where individuals in cages with both type of treatment spent a lot of their time on the roof and sometimes stayed there for long periods of time. On top of the garage shelter more than one individual was able to rest and explore, compared to the box shelter where only one individual at a time could be situated.

6.4 Compartments of the garage for single hiding – GSH

The males living in cages with the garage-type of shelter preferred the compartments closer to the back wall. This seems to be a predictable result as these compartments are as far away from the observer as possible and also away from what the guinea pigs would probably consider the alien area outside the cage.

6.5 Weight

At the day of arrival all individuals in the present study weighed about the same, independent of treatment and this is the result one would expect. All 96 individuals should have been treated the same way and been fed the same amount of food at the breeding farm, before arriving at the animal unit at AstraZeneca R&D. Nor were there any differences between the two treatments when studying the weight gain after the acclimatization period. This might seem strange because the individuals living in cages with the box for group hiding had been eating and drinking more than the individuals in cages with the garage, when studying the behavioural data. A possible explanation for these results could once again be found in the definitions of the different behaviours. When the individuals were considered to be inside the shelter, the animal had to be "situated inside the hiding facility with at least half the body out of site for the observer, having just the head outside the compartment eating hay was also considered being in the facility". With this in mind, together with the photo of the garage shelter, it is easy to assume that the males with the garage shelter did eat just as much as the males with box shelter, but with at least half of their bodies inside the garage. Thus even though the individuals were eating, they were only registered as being inside the garage. This was also seen during the direct and the video taped observations. Of course it was possible that the males were eating while the observer was not present. The animals living in cages with the garage are then presumed to eat as much as the animals in cages with the box, and this is why the results should show no difference in weight gain between treatments.

6.6 Social interactions

By using the data from the social behavioural interactions, it was possible to state if there was a difference in hierarchies between the two treatments, even though calculating the actual rank order for the males in each cage, was unachievable. The hierarchies were not established and stable for any of the cages by the time that the acclimatization period had passed. This was because the number of interactions between the individuals was so low, but the groups of males had come more or less close to the final set up of the hierarchy.

When a hierarchy is established and stable there will be distinct differences between the dominant and the subdominant individuals. The dominant animal will start most interactions and win most of them; the subdominant individual will start the least number of interactions and lose most of them (Beer and Sachser, 1994; Sachser *et al*. 1998). By calculating the difference between the individual with the highest percentage of won interactions and the individual with the lowest percentage of won interactions in each cage, I developed a way to define how close a group of individuals had come to form a stable hierarchy. The results from the calculations clearly showed that the males living in cages with the garage had a larger difference between the male that was considered the dominant individual, and the male considered to be the subdominant individual, compared to the males living in cages with the BGH.

In the cages with BGH there were about one third more social behavioural interactions compared to animals living in cages with GSH. Combining the very last statement with the data from the differences between the high and low ranking individual in the cages, shows that not only had the males in cages with the garage come closer to establishing a stable social hierarchy, but also done this with one third fewer interactions. These results are interpreted as a decrease in the levels of aggression and stress needed to establish the hierarchy in the cages with garage compared to cages with the box.

6.7 Practical concerns

When the guinea pigs were removed from their cage at times for cleaning or for other reasons, the animal technician had to catch the individuals in the cage. The animal technicians opened the cage door and as quickly and calmly as possible get hold of one individual at a time. Guinea pigs are very easily scared by the faintest noise, especially when the animal technicians hold them. Grabbing hold of the individuals in cages with the BGH was fairly simple since the guinea pigs most often all hide in the shelter and all the animals are in the same. When the guinea pigs lived in a cage with the GSH this process will became a bit more complicated. The animals would most often hide in the shelter here as well, but as the garage had more than one opening, it was more common that the individuals could escape from the compartments when the hand of the animal technician approached the opening. This would in some cases lead to less controlled captures of the guinea pigs and, as a result, higher levels of stress and fear for the guinea pigs. These levels of stress and fear were of course something that would be reduced as the animal technicians over time would learn how to collect the guinea pigs in the least stressful way possible.

7. CONCLUSIONS

7.1 Conclusions

The findings from the present study showed that the garage for single hiding gave the guinea pigs a chance to avoid unnecessarily high levels of stress and aggression during the acclimatization period, as compared to the box for group hiding. By providing separate compartments in the shelter for each individual there were fewer incidents where males were forced to spend time out on the open floor, instead of inside the facility for hiding. The separate compartments also gave the individuals a better chance to avoid social behavioural interactions. 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. Additionally, the frequency of the offensive and sexual behaviours decreased much more towards the end of the acclimatization period in the cages with garage compared the cages with box.

7.2 Future work

As with most research projects, there are more studies to be done in this particular field of ethology. It would be of great importance to investigate if it really was the number of compartments in the shelter, and not the total area of the shelter, that affected the levels of aggression and stress for the individuals in cages with the garage for single hiding.
It would also be of interest to extend the study and investigate if the levels of offensive and sexual behaviours decreased even more in the cages with the garage, when the observations continued for more than seven days. In the extended study, one could also observe how long it would take for the hierarchy to become stable for the animals in the two treatments. Perhaps the guinea pigs need a longer acclimatization period than 7 days? In these supplementary studies, it would be preferable to use a video camera when recoding the observations since the observer did seem to affect the guinea pigs, and by taking samples of stress hormones such as cortical the results would not only be based on observations.

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9. REFERENCES

Barnard, C.J. & Hurst, J.L. 1996. Welfare by design: The natural selection of welfare criteria. Animal Welfare 5: 405-433

Beer, R. & Sachser, N. 1994. Social structure and welfare in all male-groups of guinea pigs. Applied Animal Behavioural Science 40 (1): 254-258

Büttner, D. 1992. Social influences on the circadian rhythm of locomotor activity and food intake of guinea pigs. Journal of Interdisciplinary Cycle Research 23 (2): 100-112

Büttner, D. 1994. Social influences on daily rhythms of guinea pigs and possible explanations by refuge behaviour. Applied Animal Behavioural Science 40 (1): 250-253

Fuchs, S. 1980. Spacing patterns in a colony of guinea pigs: Predictability from environmental and social factors. Behavioural Ecology and Sociobiology 6: 265-276

Grant, E.C. & Mackintosh, J.H. 1963. A comparison of the social postures of some common laboratory rodents. Behaviour 2: 246-259

Haemisch, A. 1990. Coping with social conflict, and short-term changes of plasma cortisol titers in familiar and unfamiliar environments. Physiology & Behaviour 47: 1265-1270

Hawke, S.E., Hennessy, M.B., O'Leary, S.K. & Wilson, S.E. 2002. Social influences on cortisol and behavioural responses of preweaning, periadolescent, and adult guinea pigs. Physiology & Behaviour 76: 305-314

Hennessy, M.B., Deak, T., Schil-Webb, P.A., Wilson, S.E., Greenlee, T.M. & McCall, E. 2004. Responses of guinea pig pups during isolation in a novel environment may represent stress-induced sickness behaviours. Physiology & Behaviour 81: 5-13

Henry, J.P. 1982. The relation of social to biological processes in disease. Social Science and Medicine 16: 369-380

Jacquez, J.A. & Norusis, M. 1973a. Social behaviour in a colony of domestic guinea pigs: Aggression and dominance. Zeitschrift für Thierpsychologie. 46: 200-214

Jacquez, J.A. & Norusis, M. 1973b. Sampling experiments on the estimation of parameters in heteroscendastic linear regression. Biometrics 29: 771-779

Kaiser, S., Nübold, T., Rohlmann, I. & Sachser, N. 2003. Pregnant female guinea pigs adapt easily to a new social environment irrespective of their rearing conditions. Physiology & Behaviour 80: 147-153

King, J.A. 1956. Social relations of the domestic guinea pig living under semi-natural conditions. Ecology 37: 221-228

Künzl, C. & Sachser, N. 1999. The behavioural endocrinology of domestication: A comparison between the domestic guinea pig (*Cavia aperea f. porcellus*) and its wild ancestor the cavy (*Cavia aperea*). Hormones and Behaviour 35: 28-37

Levinson, D.M., Buchanan, D.R. & Willis, F.N. 1979. Development of social behaviour in guinea pig in the absence of adult males. The Psychological Record 29: 361-370

Meyerson, B.J. 1986. Ethology in animal quarters. Acta Physiologica Scandinavica 554: 24-31

Nicholls, E.E. 1922. A study of the spontaneous activity of the guinea pig. Journal of Comparative Psychology 2 (4): 303-330

North, D. 1999. The guinea pig. In: The UFAW handbook on The Care and Management of Laboratory Animals. Blackwell Science Ltd, Oxford. Seventh edition: 387-388

Olsson, I.A.S., Nevison, C.M., Patterson-Kane, E.G., Sherwin, C.M., Van de Weerd, H.A. & Würbel, H. 2003. Understanding behaviour: The relevance of ethological approaches in laboratory animal science. Applied Animal Behavioural Science 81: 245-264

Rood, J.P. 1972. Ecological and behavioural comparisons of three genera of argentine cavies. Animal Behaviour Monographs 5 (1): 3-83

Sachser, N. 1986. Different forms of social organization at high and low population densities in guinea pigs. Behaviour 97: 253-272

Sachser, N. 1987. Short-term responses of plasma norephinephrine, epinephrine, glucocorticoid and testosterone titers to social and non-social stressors in male guinea pigs of different social status. Physiology & Behaviour 39: 11-20

Sachser, N. 1990. Social organization, social status, behavioural strategies and endocrine responses in male guinea pigs. In: Hormones, Brain and Behaviour in vertebrates. Balthazart J ed., Comparative Physiology, Karger Basel 9: 176-187

Sachser, N. 1993. The ability to arrange with conspecifics depends on social experiences around puberty. Physiology & Behaviour 53: 539-544

Sachser, N. 1994. Social dominance and health in nonhuman mammals: a case study in guinea pigs. In Ellis L (Ed). Social Stratification and Socio-economic Inequality, Volume 2. Praeger, Westport, 113-121

Sachser, N. & Lick, C. 1989. Social stress in guinea pigs. Physiology & Behaviour 46: 137-144

Sachser, N. & Pröve, E. 1984. Short-term effects of residence on the testosterone responses to fighting in alpha male guinea pigs. Aggressive behaviour 10: 285-292

Sachser, N. & Renninger, S.V. 1993. Coping with new social situations: the role of social rearing in guinea pigs. Ethology Ecology and Evolution 5: 65-74

Sachser, N., Dürschlag, M. & Hirzel, D. 1998. Social relationships and management of stress. Psychoneuroendocrinology 23(8): 891-904

Sachser, N., Lick, C. & Stanzel, K. 1994. The environment, hormones and aggressive behaviour: A 5-year study in guinea pigs. Psychoneuroendocrinology 19 (5): 697-707

Stefanski, V. & Hendrichs, H. 1996. Social confrontation in male guinea pigs: Behaviour, experience, and complement activity. Physiology and Behaviour 60 (1): 235-241

The Swedish Animal Welfare Agency, 2004. Presentation av den svenska försöksdjursstatestiken år 2003. Dnr: 30-1772/04

White, W.J., Balk, M.W. & Lang, C.M. 1989. Use of cage space by guinea pigs. Laboratory Animals 23: 208-214

Westfall, P.H. & Young, S.S. 1993. Resampling-based multiple testing. Examples and methods for p-value adjustment. John Wiley & Sons Inc. pp: 1-31

APPENDIX I

a) Results from recordings of number of interactions and the winner and looser of each interaction among the individual males in cages with BGH and with GSH.

		Box for group hiding				Garage for single hiding			
Test group	Cage	Colour	Total no of interactions per cage	Won inter-actions (%)	Lost inter-actions (%)	Cage	Colour	Total no of interactions per cage	Won inter-actions (%)	Lost inter-actions (%)
1										
	2	Pink	84	34.8	65.2	1	Pink	70	82.8	17.2
	2	Green	84	47.7	52.3	1	Green	70	36.4	63.6
	2	Multi	84	51.5	48.5	1	Multi	70	30.7	69.3
	2	Purple	84	66.7	33.3	1	Purple	70	17.6	82.4
	4	Pink	54	38.9	61.1	3	Pink	73	86.0	14.0
	4	Green	54	83.8	16.2	3	Green	73	27.3	72.7
	4	Multi	54	29.6	70.4	3	Multi	73	11.4	88.6
	4	Purple	54	30.8	69.2	3	Purple	73	43.8	56.2
2										
	2	Pink	69	60.5	39.5	1	Pink	40	91.6	8.4
	2	Green	69	39.4	60.6	1	Green	40	28.0	72.0
	2	Multi	69	37.8	62.2	1	Multi	40	62.5	37.5
	2	Purple	69	64.0	36.0	1	Purple	40	26.1	73.9
	4	Pink	73	35.9	64.1	3	Pink	38	94.4	5.6
	4	Green	73	54.5	45.5	3	Green	38	78.6	21.4
	4	Multi	73	66.7	33.3	3	Multi	38	34.6	65.4
	4	Purple	73	40.6	59.4	3	Purple	38	5.6	94.4
3										
	2	Pink	131	67.1	32.9	1	Pink	7	100	00.0
	2	Green	131	42.6	57.4	1	Green	7	33.3	66.7
	2	Multi	131	54.2	45.8	1	Multi	7	25.0	75.0
	2	Purple	131	37.0	63.0	1	Purple	7	33.3	66.7
	4	Pink	45	57.7	42.3	3	Pink	92	45.5	54.5
	4	Green	45	59.5	40.5	3	Green	92	65.7	34.3
	4	Multi	45	42.9	57.1	3	Multi	92	30.0	70.0
	4	Purple	45	25.0	75.0	3	Purple	92	48.5	51.5

APPENDIX I

b) Continuing the results from recordings of number of interactions and the winner and looser of each interaction among the individual males in cages with BGH and with GSH.

		Box for group hiding					**Garage for single hiding**			
Test group	Cage	Colour	Total no of interactions per cage	Won inter-actions (%)	Lost inter-actions (%)	Cage	Colour	Total no of interactions per cage	Won inter-actions (%)	Lost inter-actions (%)
4										
	2	Pink	46	65.5	34.5	1	Pink	4	50.0	50.0
	2	Green	46	21.4	78.6	1	Green	4	00.0	100
	2	Multi	46	66.7	33.3	1	Multi	4	50.0	50.0
	2	Purple	46	32.0	68.0	1	Purple	4	100	00.0
	4	Pink	11	50.0	50.0	3	Pink	29	81.1	18.9
	4	Green	11	75.0	25.0	3	Green	29	42.9	57.1
	4	Multi	11	50.0	50.0	3	Multi	29	33.3	66.7
	4	Purple	11	37.5	62.5	3	Purple	29	21.1	78.9
5										
	2	Pink	77	50.9	49.1	1	Pink	14	80.0	20.0
	2	Green	77	35.5	64.5	1	Green	14	25.0	75.0
	2	Multi	77	52.9	47.1	1	Multi	14	66.7	33.3
	2	Purple	77	56.9	43.1	1	Purple	14	44.4	55.6
	4	Pink	68	57.5	42.5	3	Pink	38	52.9	47.1
	4	Green	68	35.9	64.1	3	Green	38	45.0	55.0
	4	Multi	68	33.3	66.7	3	Multi	38	47.1	52.9
	4	Purple	68	69.7	30.3	3	Purple	38	54.5	45.5
6										
	2	Pink	22	33.3	66.7	1	Pink	13	00.0	100
	2	Green	22	50.0	50.0	1	Green	13	50.0	50.0
	2	Multi	22	33.3	66.7	1	Multi	13	50.0	50.0
	2	Purple	22	80.0	20.0	1	Purple	13	71.4	28.6
	4	Pink	17	50.0	50.0	3	Pink	56	69.2	30.8
	4	Green	17	52.9	47.1	3	Green	56	31.6	68.4
	4	Multi	17	45.5	54.5	3	Multi	56	57.7	42.3
	4	Purple	17	50.0	50.0	3	Purple	56	50.0	50.0

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