The effects of group-housing and relative weight on feeding behaviour in rats

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Summary

To meet the growing concern for the well-being of laboratory animals, group-housing is now recommended for rats. The aim of the present study was to examine the effects of group-housing and relative weight within the group on feeding behaviour in rats. Studies of the individual feeding behaviour of group-housed animals have been carried out in many farm animals. In these studies, when effects of group-housing and hierarchy on individual feeding behaviour are studied, the results are often confounded by differences in age, body weight, genetic differences and earlier experience of the animal. All these factors were standardised in the present study. The individual meal patterns of 12 male Sprague-Dawley rats, of the same weight and age, housed singly were compared to their meal patterns after two weeks of housing in groups of three per cage. The feed intake and the feeding behaviour were recorded by computerised balances in combination with time lapse video recordings, during the group-housing period. Although when group-housed the rats made the same number of visits to the food cup as when housed singly, they ate more quickly, ate less per visit, and hence spent less time per day eating. The increase in eating rate was significant for the rats assigned to be the medium weight or lightest in their groups but not for the rats designated to be heaviest in their groups, indicating that the relative weight of the rats had an effect on their eating behaviour.

Sammanfattning

För att möta det ökade intresset för välbefinnandet hos våra försöksdjur rekommenderas idag grupphållning av råttor. Artikeln presenterar en

studie vars mål var att se vilka effekter grupphållning och relativ kroppsvikt inom gruppen har på ätbeteendet hos råttor. Studier av individuellt ätbeteendet hos grupphållna djur har tidigare utförts på lantbruksdjur. Tolkningen av sådana studier kan försvåras av skillnader i ålder, kroppsvikt, genetisk bakgrund och tidigare erfarenheter mellan djuren. I denna studie standardiserades alla dessa faktorer. Det individuella ätmönstret hos 12 lika gamla och stora Sprague-Dawley råtthanar som hölls i ensamburar jämfördes med deras ätmönster efter två veckors grupphållning i grupper om 3 råttor av olika storlek. Foderintaget och ätmönstret registrerades med hjälp av kontinuerlig registrering av foderbehållarens vikt samt videoinspelningar. Resultaten visade att råttorna åt mindre när de hölls i grupp jämfört med när de var ensamma. De åt också snabbare och ägnade följaktligen mindre tid per dygn åt att äta. Antalet ättillfällen och antalet måltider per dygn påverkades inte av grupphållning. Måltiderna blev dock mindre och mindre foder åts vid varje ättillfälle. Ökningen i äthastighet var signifikant för de råttor som blivit grupperade så att de blev mellanstora och små men inte för de råttor som var störst i sina grupper. Detta tyder på att den relativa kroppsvikten inom en grupp påverkar ätbeteendet hos grupphållna råttor.

Introduction

Studies of individual feeding behaviour are carried out for various reasons, e.g. to study the effect of different diet compositions and deficiencies (*Rains et al. 1998*), to study the neurological (*Gietzen 1993*) and endocrine regulation (*Baranyiová & Hullinger 1999*) of food intake and to study

digestive physiology (Tempel et al. 1989; Botermans et al. 2000b). Most of these studies are, as pointed out by Nielsen (1999), carried out on individually housed animals for the convenience of knowing that all food consumed and all excretions can be ascribed to the one experimental animal. This can be appropriate for studies of basic physiology but one must keep in mind the possibility of an effect of social isolation on the feeding behaviour (Hurst et al. 1996; Hurst et al 1997). In the case of laboratory animals, such as rats, being in a social group (or colony) is the normal living condition (Berdoy & MacDonald 1991). Group-housing is recommended by the National Research Council in the US (National Research Council 1996) and by the UK Home Office Code of Practice for housing and care of animals used in scientific procedures in the UK (UK Home Office 1989) to ensure the welfare of the animals. Similarly, farm animals are almost always housed in groups. If we wish to have results applicable in natural and production situations we need to perform studies on grouphoused animals in the laboratory as well. Some attempts at monitoring individual feeding behaviour in group-housed animals have been made in farm animals, in growing-finishing pigs (de Haer & de Vries 1993; Nielsen et al. 1995; Berg et al. 1998; Ramaekers et al. 1999) and in cows (Metz-Stefanowska et al. 1993). In the above mentioned reports the behaviour was recorded using computerised feeding systems with animal identification. In such studies, the interpretation of the results may be difficult because the variation between individuals is often large in comparison to possible treatment effects. In many cases the individual data are the most interesting aspect, but it may be hard to group the animals in such a way to make accurate statistical analyses. Valuable information can therefore be hidden in the results. Differences between individuals in groups may include age, sex, earlier experience, bodyweight, maturity and rank. Differences between high and low ranked individuals are usually coupled to differences in age, sex and bodyweight. In rats there has been evidence for a positive correlation between body weight and rank (Zook & Adams 1975; Militzer & Reinhard 1982; Nott 1993; Smith et al. 1994) even if some authors have found no

such correlation (*Boice 1972*). In other mammals there is often a positive correlation between body weight, age and rank (*Baroso et al. 2000*).

The hypothesis was that group-housing would lead to increased food intake due to social facilitation (Hsia & Wood-Gush 1983; Keeling & Hurnik 1993) and a higher energy demand as a result of increased activity (Hurst et al. 1997). A greater number of visits to the food cup was also anticipated as some of the visits might be interrupted by the other rats in the cage. The main hypothesis concerning relative weight within a group was that the rats that were largest in their groups would not change the feeding behaviour from that in the individual cages while the ones that were smallest in their group would change their behaviour the most, as they adjusted to being able to eat only when the food cup was available. This has been seen in growing-finishing pigs subjected to a high level of competition during feeding (Botermans et al. 2000a).

The purpose of the present study was to study the selective effects of group-housing and relative weight and age in male rats on individual feeding behaviour with the factors sex, age and body weight standardised.

Materials and Methods

Experimental Design

Twelve Sprague-Dawley rats (later called experimental rats) had their individual meal patterns recorded by computer for four days when housed singly. They were then randomly assigned to one of three treatments. 1: To be the oldest/largest rat in a group of three rats, 2: To be the medium aged/weight rat in a group of three or 3: To be the youngest/smallest in a group of three rats. For the groupings an additional 24 male Sprague-Dawley rats (later called companion rats) of two sizes (considerably younger/smaller and considerably older/larger than the experimental rats) were used. The experimental rats that were assigned to be the largest in the group were grouped with two of the small companion rats while the ones that were to be medium weight were grouped with one small and one large companion rat. Consequently, the experimental rats that were to be the smallest were grouped with

two of the large companion rats. In this way the effect of relative weight could be studied without the interference of difference in age, sex, body weight and experience because the rats being compared were the 12 experimental rats which were of the same weight, sex and age. The individual meal patterns were recorded again, for three days, after the rats had been group-housed for two weeks, because it was not the mixing effect *per se* but the effect of being in a social group as well as the relative weight and age in that group, which was the object of the present study. After the recording they were put back into single rat cages and eight days later their meal patterns were recorded again for three days.

Animals and Housing

For this study we used 12 growing male Sprague-Dawley rats (Simonsen Lab inc., Gilroy, CA, USA), of the same age, 92 days at the beginning of the study and body weight, 322 ± 1.33 g (mean \pm SE). The 12 large companion rats (Simonsen inc.) were 470 ±2.33g and 215 days old while the 12 small companion rats (Charles River Laboratories, Hollister, CA, USA) were 160 ± 0.91g and 47 days old when introduced in the study. They were all housed individually in hanging wire cages (25x19x18 cm, depth, width, height) at the start of the study. Throughout the study they were on a 12:12 hour light:dark cycle, with lights off at noon and maintenance carried out between 0830 and 1000 hours. The rats had ad libitum access to a balanced diet (20% casein, 1% vitamin mix, 5% salt mix, 5% corn oil and 69% carbohydrate, cornstarch:sucrose, 2:1, as routinely used in our laboratory) during the remaining 22.5 hours of the day. The temperature in the room was kept between 20-22 °C. The rats were weighed at the start of the study and at the onset and end of each recording session for a total of six measurements. This made it possible to calculate individual daily weight gain (DWG) and food conversion rates separately for each period of the study.

Recording Feeding Behaviour of Individually Housed Rats

The rats were allowed 6 days to habituate to the cages used for recording feeding behaviour. After that, their individual food intake was recorded for four consecutive days using computerised modules as previously described by Castonguay et al. (1982). The modules were made up of the same type of hanging wire cages as used before the trial but with Plexiglas tunnels with holes in the bottom through which the rats could reach food in a glass jar on a computerised balance (Sartorius® AG, Göttingen, Germany). Spillage from the jars was collected on aluminium pans positioned on the balances, providing automatic adjustments for spilled food (Figure. 1). The weight of the food cup, within 0.01 g, was registered by a computer every second, creating a continuous file of the amount of food consumed.

Grouping

After the recording of individual meal patterns in single rat cages the experimental rats were grouped according to the procedure described above. During the first hours after grouping the rats were supervised to make sure that no rat was harmed due to fighting. No incidences of fighting were observed. The rats were grouped in the same cages that later were used for recording so they had 14 days to habituate to the cage.

Recording Feeding Behaviour of Group-housed Rats

The cages used for the group-housed rats were twice the size (25x40x18 cm, depth, width, height)of the individual cages and were new to all the rats when grouped, i.e., they were not grouped in the home cage of any rat. They were fed the same diet as in the individual cages and had free access to two water bottles per cage. The rats had access to one food cup per cage, such that there were three rats per food cup. The food cup was located under a Plexiglas tunnel as in the study with individual



Fig. 1. The experimental equipment to record the feeding behaviour of individually housed rats. The balance was connected to a computer, which registered the weight of the food cup every second.

cages. This meant that only one rat per cage could eat at a time. The individual food intake of the experimental rat was collected by computerised recording of the data for all three rats in the same way as in the first data collection and then by viewing a time-lapse video record and deleting the visits made by the companion rats from the computerised record. To be able to videotape, red light (2*100W) was kept on during the dark hours of the five days prior to recording and of the three recording days. The experimental rat in each group was identified by marking the fur of the head with hair dye (Jet Black, Grecian5TM, Combe Inc., NY, USA).

Second Recording of Feeding Behaviour of Individually Housed Rats

After recording the meal patterns in the group cages the rats were put back into the smaller single rat cages. Eight days after the end of group-housing, their meal patterns were recorded again for 3 consecutive days in the same way as for the

first recording (see above).

Meal Pattern Analysis

The parameters used for describing the feeding behaviour were: daily food intake, time spent eating per day, the rate of eating, number of visits, time per visit, food intake per visit, and number of meals (or feeding bouts) and food intake per meal. The visits to the feeder were grouped together to form meals. A number of studies have been carried out to study the inter-meal interval (IMI) definition (Castonguay et al. 1982; Clifton et al. 1984; Sibly et al. 1990; Tolkamp et al. 1998). The IMI definition, or bout criterion, is the time that has to elapse between two visits to the feeder for the bouts to be considered two separate meals. In this study 5 minutes was chosen based on the reports cited above, and comments by Castonguay (1986). The data in the present study were analysed using a graphic program (LabVIEW[®], National Instruments Corp., Austin, TX, USA) where the visits could be detected visually and the start and

end of a visit could be determined manually along with the time the rat left the food cup. These parameters were identified on the screen and then automatically entered into the data file. If less than 5 minutes elapsed between visits they were counted as one meal for our calculations. When total eating time was calculated only the time marked on the graph, representing the times of the visits was counted. Similarly, when calculating the eating rate only the marked time was used. Thus the time between two adjacent visits considered to be included in one meal was not counted. In the program very rapid variations in weight of the food cup caused by such things as the rats touching the food cup were filtered so that the change in weight of the food cup could be displayed.

The food conversion rate, the amount of food consumed to gain one gram, was calculated for each period in the modules and the times between those periods by dividing the weight of the food consumed by the increase in body weight for each rat during the same period. for a linear time effect (the animals were growing, their weight and food intake increased with time). The linear time effect was calculated from the observations from the single cages. The independent variable in the model was the average deviation of the days in group-housing from the calculated line for each rat, later called the residuals. The 12 residuals were analysed in a one-way ANOVA (proc ANOVA in the SAS package, SAS, Cary, NC, 1982) using the model:

$Y_{ij} = \mu + \alpha_i + e_{ij}$

Where Y_{ij} = variable, μ = overall mean, α_i = treatment (i = 1, 2 or 3 [small, medium or large]) and e_{ij} = residual error (j = 1, 2, 3, 4) with the restriction $\alpha_{1+}\alpha_{2+}\alpha_3 = 0$.

To test the general effect of group-housing we tested $H_0:\mu = 0$ against $H_1:\mu \neq 0$ and to test the effect of relative weight we tested $H_0: \alpha_1 = \alpha_2 = \alpha_3$ against $H_1: \alpha_i \neq 0$ for at least one i.

To test the effect of group-housing on the eating rate within each weight group we tested $H_0:\mu+\alpha_i = 0$ against $H_1:\mu+\alpha_i \neq 0$.

Results

Statistics

For testing the effects of group-housing and relative weight, respectively, the observations during the group-housing period were corrected Group-housing vs. Single Cages The rats ate less but more rapidly when they were housed in groups of three than when they were

Table 1. A comparison of the feeding behaviour of male rats housed with two other male rats	3
and the same rats in single cages before and after the group-housing	

	Single cage 1	Group cage	Single cage 2	P-value
No. of animals	12	12	12	
Daily food intake (g)	19.82±1.48	17.41±1.43	20.86±1.09	< 0.001
No. of visits per day	17.96±3.83	19.00 ± 2.62	18.14±3.27	0.35
No. of meals per day	14.17±2.51	13.89 ± 1.50	14.61±1.90	0.14
Time spent eating (min)	79.40±12.0	52.03±5.79	68.06±7.43	< 0.001
Food per visit (g)	1.16 ± 0.24	0.949±0.13	1.20±0.18	< 0.001
Food per meal (g)	1.44±0.18	1.28±0.14	1.46 ± 0.18	< 0.001
Time per visit (min)	4.62±0.99	2.83±0.43	3.89±0.61	< 0.0001
Eating rate (g/min)	0.257±0.038	0.339±0.033	0.312±0.036	< 0.01

Results are presented as means±SD and the P-value for the analysis of the residuals for the group-housing period.

housed alone (Table 1). Therefore, they spent less time per day cating when they were group-housed. The amount of food eaten per visit and the duration of each visit was significantly lower when the rats were group-housed. The amount of food consumed per meal was also less during grouphousing. No differences in the number of visits to the food cup or number of meals per day could be detected.

There was no difference in DWG during the group-housing when evaluated only for the days in the recording modules (data not shown). When we compared the DWG during the whole experimental period (including the habituation period) of group-housing with the whole period of housing in single cages the DWG was significantly lower during group-housing (1.95 \pm 0.13 g/day vs. 2.75 \pm 0.092g/day, p<0.001).

The food conversion rate was 8.75 g/g on average during the whole study. The variation between rats was very large, with a maximum of 13.0 g/g and a minimum of 6.15 g/g. Variation between the recording sessions within the same rat was also large. Therefore no differences due to the housing conditions could be detected.

Relative Weight in a Group

No significant differences could be detected between rats due to relative weight within their group for the amount of food eaten per day, number of visits, number of meals and time spent eating per day. The duration of visits to the food cup, food consumed per visit and meal were also the same (Table 2). When the rate of eating during group-housing was compared by relative weight there was no significant difference among the different treatments (ANOVA, $F_{2,9}=1.25$, p=0.33). However, the rats that were medium weight and lightest in their groups had significantly higher eating rates during group-housing than when they were housed in single cages while the rats grouped to be largest did not (Figure 2).

Daily weight gain for the whole period in the group-housing and food conversion were also measured. No significant differences were detected but interestingly the animals that were the smallest in the group had a numerically higher daily weight gain and a better food conversion rate during the group-housed period than the rats that were medium weight or largest (Table 2). The variation in food conversion rate was, as mentioned earlier, very large within the different treatments. This variability was greatest for the rats that were medium weight in their groups.

Discussion

In this study we have shown that group-housing of male rats alters their food intake both quantitatively and qualitatively, i.e., the amount of food consumed and the rate of consumption.

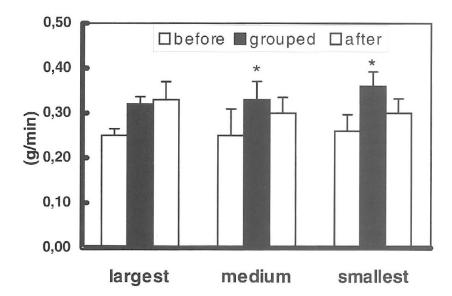
Even if no formal registration of animal activity was carried out, an apparent increase in level of activity was seen and heard during group-housing, especially in the cages with the small companion rats.

Table 2. A comparison of the eating behaviour and performance of male rats of the same weight but with	1
different relative weights in groups of three rats	

	Smallest	Medium	Largest	P-value
No. of groups	4	4	4	
Daily food intake (g)	18.10±1.89	17.13±0.81	17.00±1.51	0.77
No. of visits per day	19.00±4.06	17.58±1.20	20.42±1.40	0.26
No. of bouts per day	14.50±2.32	13.41±1.07	13.75±0.96	0.37
Time spent cating (min)	51.28±7.27	51.61±5.90	53.19±5.70	0.46
Food per visit (g)	0.99±0.15	1.00±0.069	0.86±0.12	0.29
Food per meal (g)	1.28±0.22	1.29±0.12	1.25±0.096	0.22
Time per visit (min)	2.80±0.66	3.01±0.19	2.68±0.35	0.74
Daily weight gain (g)	2.20±0.41	1.81±0.32	1.81±0.62	0.27
Food conversion rate(g/g)	8.09±2.30	11.69±10.47	11.17±5.33	0.24

Results are presented as means±SD and the P-value for the ANOVA of the residuals.

Figure 2. The rate of eating of male rats of the same weight in a single cage, when in groups of three male rats and after returning to single cages (means+SD). Each bar represents the average values for the four rats in each treatment (largest, medium or smallest). The statistical analysis is performed within each treatment on the residual eating rate during group-housing. * = p<0.05.



We had expected the rats to increase their food intake when put in groups in response to the presumed increase in activity (Hurst et al. 1997), which would increase the demand for energy. Increased food intake as result of social facilitation has been seen in finishing pigs by Hsia and Wood-Gush (1983) and in hens by Keeling and Hurnik (1993). Rather, the rats in the present study generally decreased their food intake. This can be interpreted in two ways. 1. If housed alone the rats might eat more to satisfy a need for activity that can be satisfied in other ways when they are housed in groups. 2. When in a group they might not have access to the food cup at their preferred eating time or the absence of interruption required to eat to satiety, or a combination of both. As the group cages were double the size of the single rat cages, the space per rat was only two thirds of that when they were housed individually. The rats consumed less food by reducing the duration of

each visit and meal size while the number of visits and meals per day remained the same. At times, the reduction in meal size was caused by another rat chasing the eating rat away from the food cup. Nielsen (1999) suggested that an increase in eating rate might indicate a high social pressure on an animal. In this study the rats generally increased their rate of cating during the group-housing. The rats grouped to be smallest and medium weight decreased their eating rate again when they went back to individual housing. Even if they ate faster in the group, they still did not eat as much as when housed individually.

In pigs it has been observed that the relatively small pigs in groups of 16 pigs have to adjust their feeding behaviour to fit the times when the feeders are available (*Botermans et al. 2000a*). In another study it was observed that the dominant pigs ate first and the subordinate pigs had to wait (*Vargas Vargas et al. 1987*). We hypothesised therefore

that the rats housed with larger rats would be forced to change their feeding behaviour the most. The fact that they increased their eating rate significantly while the largest did not would suggest that they were under the greatest social pressure. Still, the animals that were the smallest in their group-housing situation appeared to grow at least at the same rate and to have the same food conversion rate as those assigned to be the medium or large members of their groups. This was not significant but a very interesting observation, as it was the opposite of what we had hypothesised before the experiment. The hypothesis that these rats would have a hard time and would have to adjust to the others and perhaps not get access to the food does not appear to be supported by the present results.

The evidence for a correlation between bodyweight and rank is inconclusive, yet it is probable that in the present study, where the differences were very large, that the rats grouped to be largest also were the dominant in their groups and the ones grouped to be the smallest were at the bottom of the hierarchy.

To be able to detect differences in food conversion rate we would need to record the behaviour over a much longer period. This would be possible in automated systems where a transponder, or other monitoring device, identifies the animals every time they eat. Alternatively, if the animals used were younger, and thus growing at a higher rate, differences in food conversion rate and daily weight gain would have been easier to detect.

In conclusion, group-housing alters feeding behaviour and has an impact on the amount of food consumed by the animals. In experimental situations this must be considered when designing and interpreting studies of ingestive behaviour. In addition, in practical animal production we must realise that the performance of the animals can be dependent on the group structure in relation to the feeding system used.

In this study we have demonstrated that it is possible, without too much difficulty, to obtain detailed information about the feeding behaviour of individual rats (or other laboratory animals), even when they are group-housed.

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