

The Effects of Housing on Food and Water Consumption in Da-Rats

by *Krohn, TC. *, Farlov, H. & Hansen, AK.*

Section of Biomedicine, Department of Veterinary Disease Biology, Faculty of Life Sciences,
University of Copenhagen, Frederiksberg, Denmark

Summary

According to European legislation rodents should be housed in stable harmonized groups with enrichment; a principle, which may, however, frequently be disregarded to fulfil the needs of the study. Especially in feeding studies it is necessary to house the animals individually. Literature on single housing shows an inconclusive picture, and it seems as if a range of other factors and not just single housing itself, affect the outcome of the studies on single housed rats. Therefore the aim of the present study was to evaluate the effects of single housing, enrichment and gender on the daily intake of food and water in rats. Twelve male and twelve female rats (DA/Sac) were housed either in groups or individually with or without enrichment and the daily food and water consumption was analyzed by the use of digital feeding stations. There was a significant difference between the two genders of DA rats in their eating and drinking behavior, whereas social environment only seemed to affect the eating and drinking behavior to a minor extent, while enrichment did not have an effect by itself on either of the parameters.

Introduction

According to European legislation rodents should be housed in stable harmonized groups with enrichment (*Council of Europe, 2006*); a principle, which may, however, frequently be disregarded to fulfil the needs of the study. Such suboptimal housing may be single housing, either in a normal cage or in a metabolic cage, to allow the study of effects of feeding and drinking, or it may be housing in enriched cages, eventually combined with single housing, to allow induction of certain neurobiological models (*Robbins et al., 1996*). Single housing has previously been shown to affect daily food intake especially in the first period after being put single (*Brown & Grunberg, 1996; O'Connor & Eikelboom, 2000; Pérez et al., 1997; Szenasi et al., 1988*). Rats are tradi-

tionally regarded as gregarious animals, and a sudden removal from the group will change their lives into a situation in which they are not part of a hierarchy and lack social interaction. This may lead to increased boredom, which again may be visualized by a change in behavioral repertoire (*Hurst et al., 1996*). Studies have shown that males and females do not necessarily react in the same way to group or single housing (*Brown & Grunberg, 1995*), and the two genders do not react the same way to stress in regard to eating behavior and weight gain (*Faraday, 2002; Faraday et al., 2005*).

Lack of enrichment in the cages will affect weight gain, probably because it provides a basic for manipulation and chewing (*Bradshaw & Poling, 1991; Chmiel & Noonan, 1996*), and it supplies security when used for shelter and nest building (*Manser et al., 1998; Townsend, 1997*). A rat unable to build a nest may be frustrated, and if the rat is not able to cope with this environment, it will get stressed (*Belz et al., 2003*), and its behavior may be redirected towards other things, e.g. the food in the food hopper or itself (*Hurst et al., 1998; Hurst et al., 1997*). Lit-

*Correspondence: Axel Kornerup Hansen
Section of Biomedicine, Department of Veterinary
Disease Biology, Faculty of Life Sciences, Ridebanevej
6, 1st floor, DK-1870 Frederiksberg C, Denmark
Tel +45 35 28 27 26
Fax +45 35332755
E-mail akh@life.ku.dk

erature on single housing, and especially when this is combined with enrichment, shows an inconclusive picture, and it seems as if a range of additional factors affect the outcome of the studies on single housed rats (*Krohn et al., 2006*). Therefore the aim of the present study was to evaluate the effects of single housing, enrichment and gender on the daily intake of food and water in rats.

Materials and Methods

Twelve male and twelve female DA/Sca rats, eight weeks of age by the start of study, were used. Six males and six females were housed in four groups of three in Type U1500 cages (Tecniplast, Buguggiate, Italy) with aspen bedding (Tapvei, Kortteinen, Finland). Two of the groups were housed with nesting material (Enviro-Dri®, Lillico Biotechnology, Surrey, UK), a hide (Des.Res™, Lillico Biotechnology, Surrey, UK), a paper tube (GLP Fun Tunnels, Maxi, Lil-

lico Biotechnology, Surrey, UK), a biting stick (aspen brick, size M, Tapvei, Kortteinen, Finland), whereas the other two groups only had bedding. Six males and six females were housed individually in Type 1500 cages, and six of the cages were enriched as described above, while six only with bedding. All cages were changed weekly, and the rats were offered diet (Altromin 1324, Brogaarden, Gentofte, Denmark) and tap-water ad libitum. The cages were placed randomly on shelves in an animal room with temperature at 23±1 °C and humidity at 55 ± 5%. The light regime was from 06:00 a.m. to 06:00 p.m. Immediately upon arrival the animals were anesthetized by 0.2 ml/100g s.c. with a diluted mixture (1:1:2) of Hypnorm®/Dormicum®/sterile water (Hypnorm: Vetapharma Ltd., Leeds, UK; Dormicum: Roche AG, Basel, Switzerland) and an ISO FDXB RFID tag (Pet-ID, The Barn, Danworth Farm, West Susgender, UK) was placed subcutaneously in the neck of the rat.

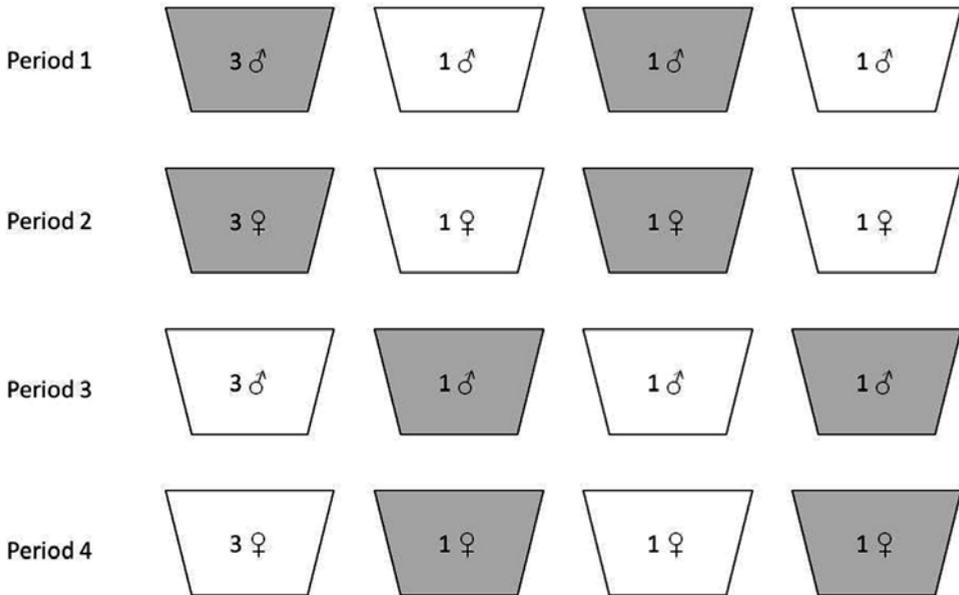


Figure 1. The design of a study for showing the impact of enrichment and single housing on food and water intake in rats. The four periods of the study with four cages in the feeding stations at the time. The enriched cages are indicated with grey, and the unenriched with white. The number of animals and the gender in each cage is shown on the cage figures.

For the study four HM-2 Feed Intake and Activity Monitors (MBRose ApS, Faaborg, Denmark) were used. The cage in the HM-2 was a Type U1500 with the food hopper and water bottle suspended separately on weights automatically registering the consumption of food and water by the individual animal identified automatically by the tag. The animals were housed in the HM-2 stations for three weeks, and the individual food and water consumption was registered. In order to monitor all the animals, experiments were carried out in four periods of three weeks with the animals randomly assigned to the HM-2 stations (Figure 1). All animals were housed identically in the HM-2 stations as in their homecages.

Data were tested for normal distribution by the Anderson-Darling test. Hereafter, the data were tested

by General Linear Model MANOVA on the variables social environment, housing environment and gender, and for the interactions between the three for the parameters eating time per day, eating sessions per day, food intake per day, food intake per session, eating time per session, drinking time per day, drinking sessions per day, water intake per day, water intake per session and drinking time per session (Minitab ver. 14, Minitab Inc., US). All results are given as means \pm SD.

Results

Results are shown in Table 1. The major differences were shown between the two genders, as the males spent more time eating ($p < 0.001$), had more eating sessions ($p < 0.001$), had a higher diet intake

Table 1. The results of food and water consumption for the different housing conditions and the two genders. All results are shown as mean \pm SD. Significant differences are marked with *. ** $p < 0.01$, *** $p < 0.001$.

	Enrichment		Social situation		Gender	
	Enriched (N=12)	No enrichment (N=11)	Single (N=11)	Group (N=12)	Male (N=12)	Female (N=11)
Eating time pr. day (Total in min)	86.5 \pm 18.6	85.2 \pm 19.3	88.0 \pm 18.6	84.0 \pm 19.0	98.2 \pm 16.5	72.5 \pm 8.7 ***
Eating sessions pr. day (total numbers)	18.3 \pm 3.3	19.5 \pm 5.7	20.8 \pm 4.7	17.2 \pm 3.7	22.0 \pm 3.5	15.4 \pm 2.4 ***
Food intake pr. day (g. pr. 100g BW)	6.8 \pm 0.6	6.6 \pm 0.9	6.9 \pm 0.7	6.4 \pm 0.7	6.6 \pm 0.8	6.7 \pm 0.7
Intake pr. session (g. pr. 100g BW)	0.38 \pm 0.08	0.36 \pm 0.1	0.35 \pm 0.09	0.39 \pm 0.09	0.31 \pm 0.06	0.44 \pm 0.05 ***
Eating time pr. session (in sec)	283 \pm 60	268 \pm 57	257 \pm 57	293 \pm 56	272 \pm 73	281 \pm 38
Drinking time pr. day (Total in min)	12.2 \pm 6.2	17.7 \pm 14.7	17.8 \pm 14.5	12.1 \pm 6.5	11.4 \pm 6.7	18.6 \pm 14.0
Drinking sessions pr. day (total numbers)	9.5 \pm 4.2	13.9 \pm 5.4	12.0 \pm 4.9	11.2 \pm 5.7	9.2 \pm 4.0	14.1 \pm 5.3
Water intake pr. day (g. pr. 100g BW)	10.4 \pm 4.5	14.0 \pm 4.8	12.3 \pm 5.2	11.9 \pm 4.8	11.1 \pm 4.6	13.2 \pm 5.1 ***
Intake pr. session (g. pr. 100g BW)	0.56 \pm 0.1	0.5 \pm 0.06	0.49 \pm 0.07	0.57 \pm 0.09	0.50 \pm 0.11	0.57 \pm 0.05 **
Drinking time pr. session (in sec)	77 \pm 33	76 \pm 35	89 \pm 40	65 \pm 24	74 \pm 32	79 \pm 37 **

in each session ($p < 0.001$), less water intake per day ($p < 0.001$) and per session ($p < 0.01$). In relation to single versus group housing, differences were observed only in relation to eating sessions per day with more sessions for the single housed rats ($p < 0.001$), and the single housed had lower water intake per session, but more drinking sessions, compared to group housed ($p < 0.01$). No effects of enrichment could be detected.

There was interaction between enrichment and social environment (single versus group housed) for the time eating per session and water intake per session, and interaction for enrichment and gender for eating sessions per day, intake per session and eating time per session. Finally, there was an interaction between gender and social environment for water intake per session.

Discussion

There was a significant difference between the two genders of DA rats in their eating and drinking behavior, whereas the differences in social conditions seem to have small impact on the eating and drinking behavior. The enrichment does not seem to have impact on the eating and drinking behavior by itself.

The males spent more time eating and had more eating sessions compared to the females, although the females had a higher intake per session, and therefore ended up with the same total food intake per 100 gram bodyweight, which has also been observed in other studies (Faraday *et al.*, 2005; Wellman *et al.*, 2008). The number of eating sessions and the length of each session in the present study is in accordance with previous findings in Sprague Dawley and Lewis rats (Georgsson *et al.*, 2001; Glendinning & Smith, 1994). In general, there are differences between males and females in eating behavior and eating patterns (Kuriyama & Shibasaki, 2004; Leibowitz *et al.*, 1991; VeyratDurebex & Alliot, 1997). For the females the estrous cycle may affect the eating pattern, as they resemble males more in the diestrus than in the estrus (Wellman *et al.*, 2008), but as the results in the present study were

collected over three weeks, the effects of the estrus cycle were reduced. Previously, a study has shown differences in the eating behavior and eating pattern for rats with different positions in the hierarchy of a group (Nott & Sibly, 1993), but that was not seen in the present study, as the standard deviation between single and group housed rats seems equal. In an earlier study, single housed male Wistar rats ate more food compared to pair-housed or group housed rats (Scalera, 1992), but these differences were not seen for the DA rats in this study. A power analysis on food intake per day has shown that a difference of 0.85 g is necessary to significant, and for the eating time per session, a difference of 68 sec would be significant, which are both quite minor.

For all three housing conditions significant differences in the drinking time were found as the group housed rats drank more per session than the single housed rats but in fewer sessions, and the females drank more than the males. The water intake, the drinking session length and the number of sessions in the present study are the same as previously found (Glendinning & Smith, 1994). The single housed rats had more drinking times with lower intake per session. This may be explained by the fact that single housed rats are able to drink without being disturbed by the other animals in the cage. The drinking device is available for these rats all the time, and they are free to drink, whereas the group housed rats must drink, when the device is available and may have to wait for another rat to finish drinking. The increased water intake for the females may be explained by the fact that the females are smaller compared to males and thereby need a higher water consumption. The differences may also be explained by the general differences mentioned earlier between males and females. The power analysis shows that a difference in 12 min would be significant for the drinking time per day, and 6 drinking sessions would be significant for the drinking sessions per day.

It seems as if the effect of enrichment is minor, and is only affecting the animals in their interaction with either the social environment or the gender. This is

surprising, as enrichment normally is regarded as important and in other studies have been shown major effects of enrichment (Belz *et al.*, 2003; Kingston & Hoffman, 1996; Moncek *et al.*, 2004; Sorensen *et al.*, 2004). The effects of enrichment in combination with the social environment need to be studied more, before a conclusion can be drawn.

DA rats seems to react as other strains regarding eating and drinking behavior, although the DA strain often react differently compared to other strains regarding metabolism (Barham *et al.*, 1994; Jablonski *et al.*, 2001; Saito *et al.*, 2004), physiology (Koch *et al.*, 1999; Laruechagiotis *et al.*, 1994) and emotionality (Prior *et al.*, 1997). Therefore, we should not draw conclusions for rats in general, as the present results may only be valid for DA-rats.

In conclusion, the study showed the effects of gender on the eating and drinking in DA rats. In contrast to this, differences in relation to enrichment or single housing had no or minor impact on eating and drinking behavior.

References

- Barham HM, MS Lennard & GT Tucker: An Evaluation of Cytochrome-P450 Isoform Activities in the Female Dark Agouti (Da) Rat - Relevance to Its Use As A Model of the Cyp2D6 Poor Metabolizer Phenotype. *Biochem.Pharma.* 1994, 47, 1295-307.
- Belz EE, JS Kennell, RK Czambel, RT Rubin & ME Rhodes: Environmental enrichment lowers stress-responsive hormones in singly housed male and female rats. *Pharmacol.Biochem.Behav.* 2003, 76, 481-6.
- Bradshaw AL & A Poling: Choice by rats for enriched versus standard home cages: Plastic pipes, wood platforms, wood chips, and paper towels as enrichment items. *J.Exp.Analys.Behav.* 1991, 55, 245-50.
- Brown KJ & NE Grunberg: Effects of housing on male and female rats: crowding stresses male but calm females. *Physiol.Behav.* 1995, 58, 1085-9.
- Brown KJ & NE Grunberg: Effects of environmental conditions on food consumption in female and male rats. *Physiol.Behav.* 1996, 60, 293-7.
- Chmiel DJ & M Noonan: Preference of laboratory rats for potentially enriching stimulus objects. *Lab.Anim.* 1996, 30, 97-101.
- Council of Europe: Appendix A of the European convention for the protection of vertebrate animals used for experimental and other scientific purposes (ETS No. 123), 2006.
- Faraday MM: Rat sex and strain differences in responses to stress. *Physiol.Behav.* 2002, 75, 507-22.
- Faraday MM, KH Blakeman & NE Grunberg: Strain and sex alter effects of stress and nicotine on feeding, body weight, and HPA axis hormones. *Pharmacol.Biochem.Behav.* 2005, 80, 577-89.
- Georgsson L, J Barrett & D Gietzen: The effects of group-housing and relative weight on feeding behaviour in rats. *Scand.J.Lab.Anim.Sci.* 2001, 28, 201-9.
- Glendinning JI & JC Smith: Consistency of Meal Patterns in Laboratory Rats. *Physiol.Behav.* 1994, 56, 7-16.
- Hurst JL, CJ Barnard, R Hare, EB Wheeldon & CD West: Housing and welfare in laboratory rats: time-budgeting and pathophysiology in single-sex groups. *Anim.Behav.* 1996, 52, 335-60.
- Hurst JL, CJ Barnard, CM Nevison & C West: Housing and welfare in laboratory rats: the welfare implications of social isolation and social contact among females. *Anim.Welfare* 1998, 7, 121-36.
- Hurst JL, CJ Barnard, CM Nevison & CD West: Housing and welfare in laboratory rats: Welfare implications of isolation and social contact among caged males. *Anim.Welfare* 1997, 6, 329-47.
- Jablonski P, BO Howden & K Baxter: Influence of buprenorphine analgesia on post-operative recovery in two strains of rats. *Lab.Anim.* 2001, 35, 213-22.
- Kingston SG & GL Hoffman: Effect of environmental enrichment and housing density on immune system reactivity to acute exercise stress. *Phys-*

- iol.Behav. 1996, 60, 145-50.
- Koch LG, SL Britton, JC Barbato, DW Rodenbaugh & SE DiCarlo*: Phenotypic differences in cardiovascular regulation in inbred rat models of aerobic capacity. *Physiol.Genomics* 1999, 1, 63-9.
- Krohn TC, DB Sørensen, JL Ottesen & AK Hansen*: Is individual housing affecting rats and mice - a review. *Anim.Welfare* 2006, 15, 343-52.
- Kuriyama H & T Shibusaki*: Sexual differentiation of the effects of emotional stress on food intake in rats. *Neuroscience* 2004, 124, 459-65.
- Larueachagiotis C, M Goubern, MC Laury & J Louissylvestre*: Energy-Balance in An Inbred Strain of Rats - Comparison with the Wistar Strain. *Physiol.Behav.* 1994, 55, 483-7.
- Leibowitz SF, DJ Lucas, KL Leibowitz & YS Jhanwar*: Developmental Patterns of Macronutrient Intake in Female and Male-Rats from Weaning to Maturity. *Physiol.Behav.* 1991, 50, 1167-74.
- Manser CE, DM Broom, P Overend & TH Morris*: Investigations into the preferences of laboratory rats for nest-boxes and nesting materials. *Lab. Anim.* 1998, 32, 23-35.
- Moncek F, R Duncko, BB Johansson & D Jezova*: Effect of environmental enrichment on stress related systems in rats. *J.Neuroendocrinol.* 2004, 16, 423-31.
- Nott HMR & RM Sibly*: Responses to Novel Food by Rats - the Effect of Social Rank. *Crop Prot.* 1993, 12, 89-94.
- O'Connor R & R Eikelboom*: The effects of changes in housing on feeding and wheel running. *Physiol.Behav.* 2000, 68, 361-71.
- Pérez C, JR Canal, E Domínguez, JE Campillo, M Guillén & MD Torres*: Individual housing influences certain biochemical parameters in the rat. *Lab.Anim.* 1997, 31, 357-61.
- Prior H, H Schwegler & G Ducker*: Dissociation of spatial reference memory, spatial working memory, and hippocampal mossy fiber distribution in two rat strains differing in emotionality. *Behav.Brain Res* 1997, 87, 183-94.
- Robbins TW, GH Jones & LS Wilkinson*: Behavioural and neurochemical effects of early social deprivation in the rat. *J.Psychopharmacol.* 1996, 10, 39-47.
- Saito K, N Sakai, HS Kim, M Ishizuka, A Kazusaka & S Fujita*: Strain differences in diazepam metabolism at its three metabolic sites in Sprague-Dawley, Brown Norway, Dark Agouti, and Wistar strain rats. *Drug Metab.Dispos.* 2004, 32, 959-65.
- Scalera G*: Taste preferences, body weight gain, food and fluid intake in singly or group-housed rats. *Physiol.Behav.* 1992, 52, 935-43.
- Sorensen DB, JL Ottesen & AK Hansen*: Consequences of enhancing environmental complexity for laboratory rodents - a review with emphasis on the rat. *Anim.Welfare* 2004, 13, 193-204.
- Szenasi G, P Bencsath & L Takacs*: No effect of isolation on blood pressure and daily electrolyte excretion in rats. *Acta Physiol.Hung.* 1988, 72, 93-8.
- Townsend P*: Use of in-cage shelters by laboratory rats. *Anim.Welfare* 1997, 6, 95-103.
- VéyratDurebex C & J Alliot*: Changes in pattern of macronutrient intake during aging in male and female rats. *Physiol.Behav.* 1997, 62, 1273-8.
- Wellman PJ, DH Ho & JR Nation*: Differential impact of cocaine on meal patterns in female and male rats. *Life Sci.* 2008, 82, 359-66.