Effects of Alternative Housing Systems on Physical and Social Activity in Male Sprague Dawley and Spontaneously Hypertensive Rats

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Summary

Two alternative rat cages and their effect on home cage physical and social activity were evaluated in male Sprague Dawley (SPD) and Spontaneously Hypertensive (SH) rats for 10 weeks. Rats were housed strain-wise in pairs in ST cages, in groups of eight in Enriched Rat Cage System (ERC) equipped with a shelter and wall-hung ladders, and in groups of eight in four interconnected Scantainer ^{NOVO} cages (NOVO), equipped with shelves. Home cage activity was assessed through direct observations and effects were studied in exercise tests, parameters related to physical activity and in the Elevated Plus Maze (EPM). Effects of within-group variation on the minimum sample size needed to detect a treatment effect were calculated for the different cage types. The home cage activity was highest in NOVO cages, followed by the ERC cages. This was supported by the higher locomotor and exploratory activity in the EPM and an improved performance in the last exercise test, compared to ST-caged rats. Aggressive and submissive interactions were higher in NOVO cages compared to ST cages. The design of the NOVO cages, if connected, might induce both a higher activity level and more aggression. The hypertension and insulin resistance typical of the hypertensive rat model were not influenced by an increased home cage activity. No major effects of alternative cage types were found on within-group variation. The activity was not enough to create a distinct training effect but prevented exercise-related parameters from deteriorating during the study and is therefore still relevant for the health and welfare of the animals. Additional benefits of the alternative cages are qualitative, since they stimulate a wider range of behaviours, social interactions and offer possibilities for the rats to control their situation.

Introduction

The major part of the life of laboratory animals is spent in their home cage. The cage environment and how research animals are kept while they are used for experiments are therefore key areas of concern for laboratory animal welfare. According to the current legislation *(Europe, 2007)* it is allowed to house rats in cages of limited size (minimum 800 cm^2 and 18 cm height). Housing in such limited space does not allow the rats to be physically active and perform normal social interactions, which can lead to the animals being sedentary. Laboratory rodents are routinely fed *ad libitum* and when kept in a restricted space their energy intake can easily

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Published in the Scandinavian Journal of Laboratory Animal Science - an international journal of laboratory animal science

exceed their energy expenditure. These are factors that control body weight and as a result the animals tend to become overweight. Rats are considered inquisitive animals and in the wild they move several kilometres per day exploring their home range *(Barnett, 1963)*. Laboratory rats have been shown to behave like wild rats when released into semi-natural settings, e.g. patrolling the home range along pathways *(Boice, 1977)*. Thus, rats increase their activity when given access to more space.

Today, it is well known that physical activity has many positive effects on the health of rats. It can reduce body weight gain, *(Hoffmann et al., 1987; Kretschmer et al., 2005; Lambert et al., 1996; Narath et al., 2001; Rodnick et al., 1989; Sexton & Laughlin, 1994; Skalicky et al., 2001; Spangenberg et al., 2009)*, improve cardiovascular functions *(Gleeson et al., 1983; Overton et al., 1986; Wisloff et al., 2001)*, insulin sensitivity *(Goodyear & Kahn, 1998)* and immune defence *(Kim et al., 2003; Moraska & Fleshner, 2001)*, attenuate blood pressure *(Evenwel & Struyker-Boudier, 1979; Tipton et al., 1983)* and increase muscle oxidative capacity *(Holloszy & Booth, 1976)*. Physical activity can also affect mental properties by reducing anxiety-like behaviours in rodents *(Fulk et al., 2004)*, prevent or reduce depression-like behaviours *(Greenwood et al., 2003; Moraska & Fleshner, 2001)* and improve learning, memory and neurogenesis *(van Praag et al., 2000)*. A varied repertoire of natural and social behaviours and benefits of physical activity have been seen in rats housed in a large floor pen *(Spangenberg et al., 2005)*. Therefore, it seems reasonable to conclude that housing them in bigger groups in larger cages can enhance the welfare of rats. In addition to increased space, the cage environment should include resources that stimulate active behaviours, such as grids for climbing, as well as shelters for protection which increases the rats' control of their environment and improves their ability to adapt and thereby their welfare *(Broom, 1991)*.

To justify the use of larger cages, the space has to be utilised equally to previous housing systems, which makes it is necessary to increase the group sizes. Hence both the increased cage space and social interactions in larger groups can affect home cage activity. It was previously shown that increasing the group size from two to eight rats per cage (keeping the same amount of cage space per rat by interconnecting ST-cages) resulted in increased social interactions, improved performance in an exercise test and more exploratory activity in the Elevated Plus Maze test *(Spangenberg et al., 2009)*. Thus, the social interactions in larger groups of rats can affect home cage activity to the extent that it can be measured in an exercise test *(Spangenberg et al., 2009)*. The cage, rather than the animal, is commonly considered as the experimental unit since the social environment can differ between cages *(Festing & Altman, 2002)*. This is one of the reasons for keeping animals in small groups. Larger cages with larger groups enables housing both treated and control animals in the same cage, hence exposed to the same environment, and thereby using the animal as the experimental unit. This benefits both Refinement by improved animal welfare thanks to a better cage environment and Reduction by being able to use fewer animals.

Since the type of housing can affect many different physiological parameters, changing the cage type could alter the animal model. It is important to consider whether alternative cages, or enrichment, will increase variation within experimental groups *(Sorensen et al., 2004)* or alter basal levels of physiological parameters. Blood pressure and plasma insulin levels can be affected by increased physical activity and therefore the Spontaneously Hypertensive (SH) rat model is of special interest. It has previously been shown that moderate physical activity can attenuate the hypertension in SH rats. We have shown in earlier studies that rats kept in large floor pens had lower body weights and suggested that this was due to lower body fat content *(Spangenberg et al., 2005)*. We have however been unable to reliably measure body composition repeatedly during a study until a recent collaboration that made it possible to use magnetic

resonance imaging for this very purpose.

The aim of this study was to evaluate two different alternative rat cages and their effect on home cage physical and social activity in male Sprague Dawley (SPD) and Spontaneously Hypertensive (SH) rats, in comparison to standard cages. The parameters used by Spangenberg *et al. (2009)* proved useful for evaluating effects of cage environment. The same parameters were therefore used in the present study; the rats' activity was assessed in home cage behavioural studies, the Elevated Plus Maze test for exploration and risk assessment behaviours, and by physiological parameters related to physical activity such as body weight and fat content, plasma insulin levels and lactate response to exercise. Further, handling tests were performed to assess whether increased agility affected the rats' willingness to be handled. An additional aim was to assess whether housing in larger groups and cages influence the animal model by altering baseline levels of physiological parameters or affect the within-group variation.

Materials and Methods

Animals and housing

The experimental protocol was approved by the Uppsala committee for ethical review of animal studies. For practical reasons, this work was divided into two parts. Study 1 was performed with 24 male Sprague-Dawley (Crl:CD) outbred rats (SPD) and 24 male Spontaneously Hypertensive (SHR/NCrl) inbred rats (SH) (both from Charles River, Germany), for ten weeks. They were seven weeks old at arrival, the age when SH rats start to develop hypertension. The mean body weight was 232.8 ± 8.4 g for SPD rats and 209.5 ± 6.6 g for SH-rats. They were randomly assigned to three cage types per strain; 1) eight rats were housed in pairs in standard Makrolon type IV cages (floor area 2240 cm^2, height 20 cm) (ST), equipped with two black plastic tubes (∅ 6.5 cm, 14-16 cm long). 2) Eight rats were housed in Enriched Rat Cage System (floor area 6020 cm^2, height 46 cm, Scanbur A/S, Denmark) (ERC), equipped with a shelf (24x60 cm, height 13 cm) and two ladders (8x18 cm and 8x22 cm) on the cage walls. 3) Eight rats were housed in Scantainer ^{NOVO} (floor space 2240 cm^2, height 32 cm, Scanbur A/S, Denmark) (NOVO), equipped with a shelf (16x23 cm) in the back of the cage. On each cage side there was a passage, 7x7 cm, 22 cm above the cage floor making it possible to connect four NOVO-cages (total floor space 8960 cm^2). The bedding used was aspen wood chips (Beekay bedding, Scanbur AB, Sollentuna, Sweden). The rats we acclimatised for one week before the start of the study, weighed once weekly and given free access to standard pelleted diet (SDS RM 1, Scanbur AB, Sollentuna, Sweden) and tap water. Food intake was recorded weekly at cage cleaning and the mean food consumption during the study was 26 ± 2g/ SPD rat/day, and 19 ± 2 g/ SH rat/day. There was 12 hours of light between 00:00 and 12:00, a room temperature of 22-23 °C and 23-43 % relative humidity.

To verify the development of hypertension in the SH rats, systolic blood pressure (SBP) was non-invasively recorded once a week (Table 1) with a tail-cuff (ML125 NIBP), connected to a PowerLab (both AD Instruments Pty, Australia) and a computer with the software Chart5 for Windows. The rats were manually restrained and the starting order was randomized for every session. At week 9, SH rats had a systolic blood pressure of 197 ± 34 mmHg and SPD rats 151 ± 15 mmHg.

Study 2 was performed as a part of an ongoing evaluation of the Scantainer NOVO system at Novo Nordisk A/S, Denmark. Hence, it was performed on site in Denmark where they had access to the magnetic resonance imaging equipment. The study was carried out on 16 male Sprague Dawley (NTac:SD) outbred rats (SPD) (Taconic Europe, Denmark), approximately 4 weeks old and with a body weight of 63.8 ± 5.3 g at arrival. Eight animals were housed pairwise in standard Makrolon type IV cages (ST) and the remaining eight were kept in the NOVO-system as in study 1 (NOVO), for 12 weeks. The rats were kept on aspen bedding (Tapvei, Kortteinen, Finland) in a climate controlled room at 20 ± 2ºC, 45 ± 10% relative humidity, 8-15 air

Table 1. Experimental protocol for study 1. BW= body weight.

WEEK		Physiological recordings and tests	Behavioural studies and tests
Week 1	BW	Acclimatisation to facility	
Week 2			
Day 1	BW	Laddermill	
Day 2		Laddermill	
Day 3		Laddermill	
Day 5		Tail cuff habituation	
Day 6		Tail cuff habituation	
Day 7		Tail cuff habituation	
Week 3			
Day 1	BW		
Day 3	BW	Laddermill exercise test 1	
Day 7	BW		
Week 4			
Day 2		Tail cuff recordings	
Day 6			Observations of home cage behaviour
Day 7		Tail cuff recordings	
Week 5			
Day 1	BW	Laddermill	
Day 6			Observations of home cage behaviour
Day 7		Tail cuff recordings	
Week 6		Same procedure as week 5	
Week 7		Same procedure as week 5	
Week 8		Same procedure as week 5	
Week 9		Same procedure as week 5	
Week 10			
Day 1	BW		Elevated Plus Maze test
Day 3	BW	Laddermill exercise test 2	
Day 5		Inclined Plane test	
Day 6			Handling tests
Day 7			Observations of home cage behaviour
Week 11			
Day 1		Tail cuff recordings	
Day 2	BW	Euthanisation	

changes per hour and 12 hours of light from 06:00 to 18:00. They were fed *ad libitum* with a rodent standard diet, (Altromin type 1320, Brogaarden, Gentofte, Denmark) and had access to tap water from an automated watering system (Edstrom Europe, Hereford, UK) that was flushed daily.

Study 1

Home cage behaviour – direct observations

The behaviours of the rats in their home cages were studied through direct observations once weekly (Table 1). All observations were performed by the same person who was well known to the rats. The observer studied one group of eight rats per session. In total, there were six groups; SPD and SH rats in ERC cages, SPD and SH rats in NOVO cages, and SPD and SH rats in four ST-cages per strain. The rats were individually identified by paint marks on the fur of their backs and bellies (DeLaval marking spray, DeLaval Sweden). Each rat in a group was observed continuously for one minute and the frequency of different behaviours (see ethogram in Table 2) performed was recorded. In addition, it was recorded if the same behaviour was performed continuously for the entire 60 s. When all rats in one group had been observed once, the observer then started with the first rat again, after a one-minute break. This was repeated three times during one session, *i.e.* three minutes of observations per rat, and then the observer began to study another group. Each group was studied for one session in the morning, 9.00-12.00 (light), and one in the afternoon, 13.00-16.00 (dark). Thereby both passive and active periods were observed, for a total of 6 minutes/rat and observation day. To visualize the rats in the dark, red spotlights were used. The intra-group observation order was randomised for every session and the between-group order was circulated every week.

Laddermill climbing and exercise tests

Two exercise tests were carried out to assess the effect of the rats' spontaneous home cage activity on lactate response and endurance performance. One exercise test was performed after two weeks of acclimatization and the second test after nine weeks of housing in the different systems (Table 1). The tests were performed on a laddermill, which is a treadmill (Exer 4, Columbus Instruments, Columbus, Ohio, USA) tilted to 50° with wooden rods attached to the belt to facilitate climbing. As reported by Norton *et al. (1990)* the laddermill is a successful alternative to a treadmill when evaluating effects of exercise. Treadmill exercise of rats is a forced activity that generally is achieved by using mild electric shocks at the end of each running lane as reinforcement. When using a treadmill without electric grids in a previous study *(Spangenberg et al., 2009)* it was difficult to persuade the rats to run. In addition, running continuously on a treadmill is far from rats' natural running behaviour which is short bouts resembling sprint exercise *(Rodnick et al., 1989)*. Climbing upwards at slow speeds is an exercise that is probably much more natural and comfortable for the rats which is shown by their willingness to climb *(Norton et al., 1990)* and it is therefore a refinement of the exercise situation. Only mild manual prodding is necessary *(Norton et al., 1990)* which improves the quality of the exercise, and the results obtained. Running wheels are not an option in this type of test since the speed and running time need to be the same for all rats.

Before the first exercise test, all rats were habituated to climbing on the laddermill on three occasions (Table 1), with the ladder moving at 7 m/min for two minutes per occasion. This was also performed once weekly between the exercise tests to maintain the rats' familiarity with the laddermill. The upper end wall of the laddermill was removed, because the rats were more willing to climb towards a free opening, rather than a wall. They were always removed through the upper end opening when they had finished their session. By touching the tail, the rats were encouraged to climb. If they refused to climb despite the prodding, their tails would come into contact with ice-chilled water below the laddermill lane. After each session, the rats were rewarded with honey puff cereals (ICA Honungspuffar, ICA Sverige AB, Solna, Sweden).

Table 2. Ethogram of behaviours studied by direct observations of individually marked rats in their home cages.

Behaviour	Definition
Active	**behaviours**
Walk	Forward movement in walking gait. At least four steps have to be performed for walking to be recorded. A pause in the behaviour of ≥ 2 s gives a new recording.
Run	Forward movement in trotting or galloping gait. At least two leaps or four steps have to be performed for running to be recorded.
Jump	Vertical movement through leaps up or down from shelf/ladder/grid to floor, or vice versa. Leaps can also be performed horizontally from shelf to ladder, or passage (NOVO-cages).
Climb	The rat climbs on objects/structures in the cage, e.g. ladders, grids, tubes. At least three paws have to leave the ground for climbing to be recorded.
Step up/step down	The rat steps on top of the tubes in the ST cages, or steps down from the same. At least three paws have to leave the ground or touch the ground, respectively for the behaviour to be recorded.
Exploratory	**behaviours**
Investigate	The rat sits/stand still sniffing in the bedding material or into the air. If it simultaneously performs rearing or walking those behaviours are considered superior.
Rearing	The rat stands on its hind legs with fore legs hanging free or supporting itself on any structure in the cage.
Stretch Attend Posture (SAP)	The rat stretches its body forward and then retracts to the original position without any forward locomotion.
SAP from shelf	The rat is sitting on the shelf and stretches towards the passage or along the grid-side of the cage lid (NOVO cages) or towards the ladder (ERC-cages).
Passive	**behaviours**
Passive	The rat sits or lies still. The behaviour has to continue for at least 5 s for one recording.
Huddle	Two or more individuals lying together with body contact.
Aggressive and	**submissive behaviours**
Aggressive attack	One individual is attacking another individual, biting around its neck or genitals. It can be followed by a fight where both individuals are standing upright biting and/or striking each other with fore paws, or rolling around on the ground together "wrestling".
Chase	One individual following another in running or galloping gait, maximum one body length apart.
Flight	One individual is being chased by another (see above).
Submissive posture	One individual lying on its side or back, eyes partly or totally closed.
Miscellaneous	**behaviours**
Grooming	Self-grooming with forepaws and licking with its tongue. A pause in the behaviour of ≥ 2 s gives a new recording.
Under shelf	The rat has placed itself under the shelf (ERC cages) and cannot be seen.
Other	Eating, drinking, gnawing on cage structures

Before the start of each exercise test a blood sample was taken from the *vena saphena*. The test consisted of a one-minute "warm-up" at 7 m/min, followed by one minute climbing at the intensities 7 m/min, 9 m/min and 11 m/min, respectively, at the inclination 50°. After every one-minute climbing session the rat was immediately removed from the laddermill to obtain a blood sample within 60 s. After the last blood sample the rat was put on the laddermill once more, to perform an endurance test at 11 m/min. The rat had to climb continuously until it refused to climb three times, despite plenty of prodding.

Elevated Plus Maze

During the last week of the study the rats were subjected to an Elevated Plus Maze test (EPM) (Table 1). The EPM test was chosen since it measures both exploratory activity and risk assessment behaviours and in a previous study effects of different group sizes were found on exploratory activity and risk assessment *(Spangenberg et al., 2009)*. The test arena and procedure was the same as in that previous study. The test time was five minutes and it was videotaped from above with a camera (Philips VR 1200) placed over the central platform. The tapes were analysed for the frequency of visits to each type of arm (open and closed), the total number of arm entries, and the duration of time spent in open arms. An arm entry was defined as the rat entering the arm with at least three paws. The starting order was randomised; all rats were tested during one day and acclimatised to the room for five minutes before the test. The maze was cleaned with water and washing-up liquid after every rat.

Inclined plane test and handling tests

The muscle strength of the rats was assessed in the inclined plane test during week 10 (Table 1). The test box was the same as in Spangenberg *et al. (2005)*. The rat was placed in the box perpendicular to the inclined plane and was not allowed to turn around. Two tests were performed; the first measured the maximal inclination at which the rat could maintain itself on the plane. The test started at 45° and the inclination increased by 1-2°/s. The angle at which the rat could not hold on and started sliding down was recorded as the maximum inclination. This was repeated three times and a mean value was calculated for the statistics. The second part was the endurance strength test, which measured the time that the rats could stay on the plane. It started at 55° for 60 s and the inclination was thereafter increased with 5° for every additional minute until the rat started to slide down. The inclination and time was recorded. Before both tests, each rat was acclimatized to the box for 15 s followed by 5 s at the starting inclination to give it a chance to position itself.

The rats' willingness to be handled was tested during week 10 (Table 1). These tests were performed by a skilled animal technician who was unfamiliar to the rats and ignorant of their different treatments. The tests were 1) Anticipatory reaction to handling 2) Restraining for intra peritoneal (i.p.) injection and 3) Mouth gag cooperation test. They were performed in the same manner as in Spangenberg *et al.* (2009). In test 1 the rat was put in an empty ST cage for 30 s, and then the test person opened the lid and put her hand in for 15 s. The rat was scored as inquisitive (moved towards the hand and sniffed it), passive, fearful or aggressive. In test 2 and 3 the time and number of attempts (maximum five attempts) until the rat accepted the restraining procedures were recorded. In addition, the rat's behaviour was assessed as cooperative, hesitant or unwilling to the procedure *(Spangenberg et al., 2009)*. In test 2 the rats were restrained as when given an i.p injection, but never injected.

Study 2

Body weight and body composition

The animals were weighed once weekly. At arrival and every second week during the study the amount of body fat was determined using magnetic resonance imaging. Unanaesthetized animals were put one at a time in a restrainer tube (∅ approximately 10 cm) that was inserted into the scanner (EchoMRI™, Echo Medical Systems, Houston, US). The procedure took approximately 2 minutes per rat. The amount

of body fat was calculated as a percentage of total body weight.

Euthanasia

The rats in study 1 were euthanized by i.p. injection of pentobarbital (100 mg/ml). Blood was collected by heart puncture to be analyzed for plasma levels of insulin. In study 2, the rats were euthanized by an overdose CO_2/O_2.

Blood analyses

Blood samples taken during the exercise tests were collected in Analox tubes containing a lysing agent (fluoride, heparin and nitrite), and stored at 0° C until analysis. Lactate levels were analyzed in whole blood using the Analox Analyser (Analox Instruments LTD, London W6 0BA, UK, www.analox.com), and expressed as mmol/l. The blood samples taken at euthanasia were stored in ice-chilled water until all rats were euthanized, and thereafter immediately centrifuged for 20 min and the plasma was stored at -20°C for further analyses. The plasma insulin levels were analyzed using a sandwich ELISA (Mercodia, Uppsala, Sweden, www.mercodia.se), and expressed in µg/l plasma.

Statistics

In study 1, comparisons were made using Two-Way ANOVA with strain as factor 1 and cage type as factor 2 and Tukey's test for pair-wise comparisons. Data over time were analyzed using Repeated Measures ANOVA or paired t-test. For physiological parameters where there is a known strain difference, such as body weight and insulin levels, comparisons were made within strains between cage types using One-Way ANOVA (Tukey's test for pair-wise comparisons). Correlations were calculated using Pearson Product Moment correlation. In study 2, differences between housing groups were calculated using unpaired t-test. All statistics were performed using Sigmastat for Windows, 3.0. Results were considered significant at $p<0.05$, and are presented as mean ± SD.

Results

Home cage activity and behaviours

The behaviours recorded during the direct observations in study 1 were analysed according to the categories described in the ethogram (Table 2). No effect of time was found between the seven observation occasions and the results are therefore presented as a mean of all occasions per rat (42 minutes). The only strain effects found were; more passive behaviours in SH than SPD rats, 3.9 ± 0.7 mean observations/rat and day and 3.5 ± 0.7 mean observations/rat and day respectively ($P<0.05$) and the opposite for aggressive and submissive interactions (SPD: 1.5 ± 0.8 mean observations/rat and day, and SH: 0.7 ± 0.5 mean observations/rat and day, $P<0.001$) (Table 3). The remaining results for home cage data are all effects of cage type (pooled strains). NOVO-caged rats had higher levels of active ($P<0.001$) and investigating ($P<0.001$) behaviours compared to rats in ST and ERC cages while the opposite was true for passive behaviours ($P<0.05$, for both) (Table 3). Rats in ERC showed more active behaviours than ST-housed rats ($P<0.05$). The NOVO-caged rats had higher levels of aggressive and submissive interactions than the ERC-housed rats ($P<0.05$) and more miscellaneous behaviours compared to ST-caged rats ($P<0.05$) (Table 3). Jumping and climbing were the most frequent active behaviours in NOVO and ERC cages, respectively (apart from walking) (Figure 1). Step up/down (from the tube) was the most frequent active behaviour in ST cages, besides walking. Since the ST cages did not allow for more than two or three leaps, running was rarely scored in those cages. Rats in ERC cages were often observed under the shelf. Of the passive behaviours performed for the entire 60 s observation period, 14 ± 4 (62 %) were scored under the shelf, 6 ± 2 (26 %) on the shelf and 3 ± 3 (12 %) in the rest of the cage in SPD rats. The corresponding scores for SH rats were 11 ± 4 (43 %), 6 ± 3 (23 %) and 9 ± 1 (34 %), respectively. In the NOVO cages, rats were often found on the shelves when being passive. For SPD rats, 14 ± 6 (72 %) of the passive behaviours performed for

Table 3. Frequencies of behavioural categories from direct observations in the home cages of male Sprague Dawley rats (SPD) and Spontaneously Hypertensive rats (SH) housed in three different cage types. Values are presented as mean ± SD of six minutes per day and rat, once weekly for seven weeks. ST = standard Makrolon type IV cages, NOVO= Scantainer^{NOVO}, 4 cages connected, ERC= Enriched Rat Cage system.

Group	Active	Eploratory	Passive	Aggressive and submissive	Miscelleanous
ST-SPD	1.9 ± 1.2	2.9 ± 1.3	3.7 ± 0.6	1.2 ± 0.5	3.1 ± 0.6
NOVO-SPD	5.0 ± 1.9	4.7 ± 2.1	3.1 ± 0.8	1.7 ± 0.9	4.0 ± 0.4
ERC- SPD	3.3 ± 0.8	2.9 ± 0.8	3.5 ± 0.5	1.5 ± 0.9	3.6 ± 0.7
ST-SH	2.5 ± 0.9	3.2 ± 1.0	3.9 ± 0.7	0.6 ± 0.4	3.1 ± 1.4
NOVO-SH	5.8 ± 1.5	5.1 ± 1.4	3.4 ± 0.6	1.0 ± 0.5	3.9 ± 0.9
ERC-SH	3.4 ± 0.7	3.1 ± 0.9	4.3 ± 0.4	0.3 ± 0.2	3.4 ± 1.7
ST (both strains)	2.1 ± 1.1^{a}	3.1 ± 1.1^{a}	3.8 ± 0.7^{a}	0.9 ± 0.5^{a,b}	3.1 ± 1.0^{a}
NOVO (both strains)	5.4 ± 1.7^{b}	4.9 ± 1.7^{b}	3.3 ± 0.7^{b}	1.4 ± 0.8^{a}	3.9 ± 0.7^{b}
ERC (both stains)	3.4 ± 0.7^{c}	3.0 ± 0.9^{a}	3.9 ± 0.6^{a}	0.9 ± 0.9^{b}	3.5 ± 1.3^{a,b}

^{a,b,c} – different superscripts denote differences between housing types within behavioural category, $p<0.05$.

the entire 60 s observation period were scored on a shelf and 6 ± 6 (28 %) in the rest of the cages. The corresponding scores for SH rats were 11 ± 6 (57 %) and 8 ± 6 (43 %), respectively. Some individuals (SH) were seen resting in the passages between cages. Additional findings of strain differences in cage utilisation were food consumption in the four different food trays in the NOVO cages (one for each single cage). SPD rats had a higher total food intake in the first and fourth cage compared to the third ($P<0.001$), counting from the left (cage 1: 413 ± 168 g, cage 2: 335 ± 89 g, cage 3: 238 ± 56 g and cage 4: 444 ± 79 g). No differences in food intake between individual NOVO cages were found in SH rats (cage 1: 317 ± 112 g, cage 2: 229 ± 46 g, cage 3: 246 ± 80 g and cage 4: 244 ± 100 g).

Body weight, body fat content and plasma insulin

In study 1, the SPD rats in the NOVO cages had a lower body weight than those in ST-cages during week 2 ($P<0.05$) (Figure 2A) and a tendency for the same difference during week 3 ($P= 0.057$). The SH rats had a higher plasma insulin concentration (at euthanasia) compared to SPD rats, 7.7 ± 1.7 ng/ml and 2.7 ± 0.7 ng/ml, respectively ($P<0.001$), which was expected from the hypertensive model. There were no significant differences in insulin levels within each strain between housing systems. A positive correlation was found between plasma insulin concentration and body weight in the SH-rats ($r=0.88$, $p<0.001$).

No difference in body weight was found between the two housing groups in study 2 at any time during the study (Figure 2C). The NOVO-housed rats had lower body fat mass compared to ST-housed rats during the second week of the study ($p<0.05$) (Figure 2D).

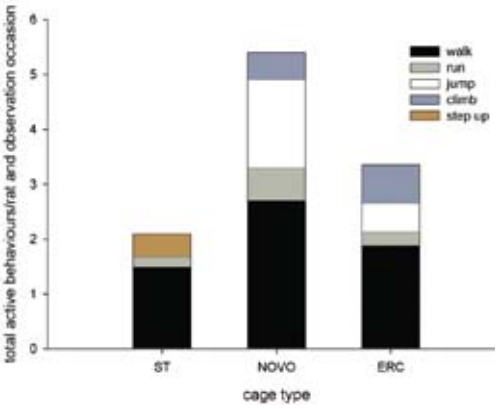

Figure 1. Proportion (%) of different types of active behaviours recorded during seven weeks in male Sprague Dawley and Spontaneously Hypertensive rats kept in three different cage types. ST = standard Makrolon type IV cages, NOVO= Scantainer^{NOVO,} 4 cages connected, ERC= Enriched Rat Cage system. Expressed as mean of total observation occasions per rat.

Exercise tests

Complete data from all rats in the two exercise tests could not be collected, either because the rats refused to climb or it was not possible to obtain enough blood to analyse at every blood sampling occasion. In total, 37 out of 48 rats, 19 SPD and 18 SH rats, performed successfully in both exercise tests.

Effect of cage types was only found in exercise test 2. Rats in ST cages had higher lactate levels at laddermill speeds of 7 and 11 m/min compared to NOVO-caged ($P<0.01$ and $P<0.05$, respectively) and ERC-caged rats ($P<0.05$ for both levels) (Figure 3), and lower endurance than NOVO-caged rats ($P<0.05$) (Table 4). When comparing exercise test 1 and 2, ST-caged rats had higher lactate levels at both 7 and 11m/min in test 2 compared to test 1 ($P<0.001$ and $P<0.05$, respectively) (Figure 3). However, the ST rats also had a greater endurance in the second test compared to the first ($P<0.05$) (Table 4). No differences in lactate levels were found between exercise test 1 and 2 in rats from NOVO or ERC cages, but both groups improved their endurance significantly in exercise test 2 ($P<0.01$ and $P<0.01$, respectively) (Table 4).

Elevated plus maze

In the EPM test, two rats (one ST-SH and one ERC-SH) jumped off the arena and were excluded from the results. The only strain effect found was that SPD rats had a shorter latency to visit open arms compared to SH rats ($P<0.05$). An effect of cage type was found in total and closed arm entries where rats in NOVO and ERC cages had higher frequencies than ST-housed rats in both parameters ($P<0.05$, for both) (Table 5). A positive correlation was found between total arm entries in the EPM and total active behaviours in the home cage ($r = 0.47$, $P<0.01$).

Inclined plane test

No differences were found in maximum inclination test or in the endurance test on the inclined plane between strains or cage types. The mean angle was 66 ± 3° in ST cages, 67 ± 2° in NOVO cages and 67 ± 3° in ERC cages. The first 60 s (at 55°) in the endurance test were successfully completed by eight ST-caged rats, 13 NOVO-caged rats and 12 rats from ERC cages. Only two rats (NOVO) could successfully stay on the plane for 120 s or more (at 60°).

Handling tests

There were no effects of housing types or strains for any of the handling tests performed in study 1. All individuals except five investigated the hand in the anticipatory reaction to handling test. Of the remaining individuals, two rats sniffed the cage but never the hand (both ERC-SPD), one was passive (ERC-SH) and two were scored as fearful (both NOVO-SH). All individuals except one were scored as compliant in the restraining for i.p. injection test, the remaining rat (NOVO-SPD) was scored as hesitant. The restraining was performed in only one attempt and for one second in 45 rats.

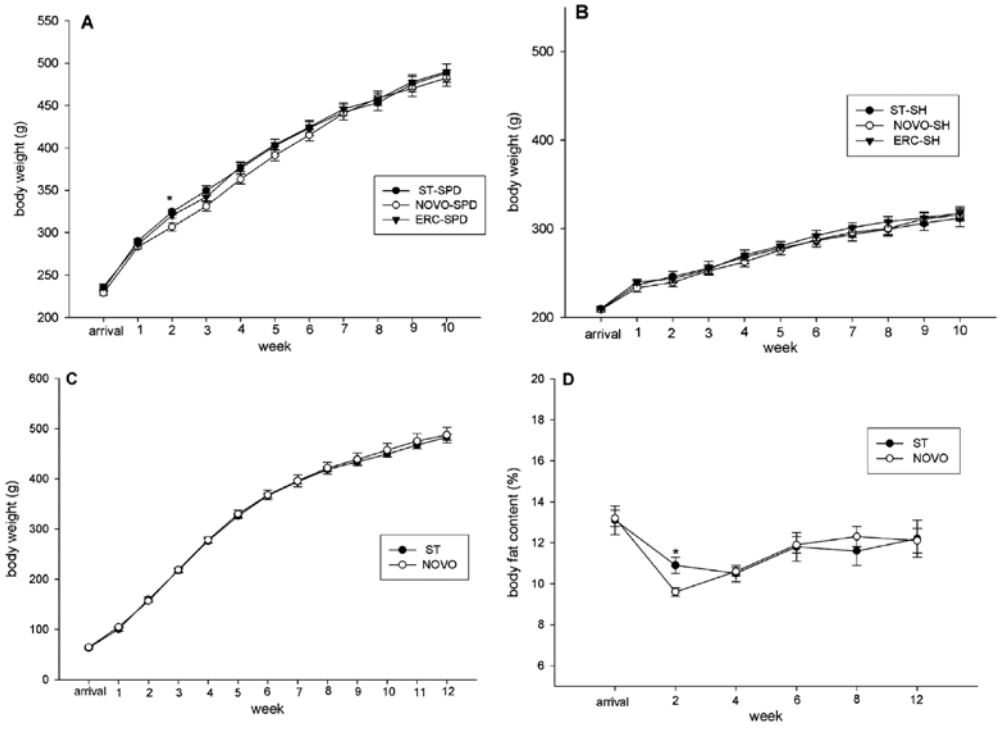

Figure 2A-D. Body weight of male Sprague Dawley rats (SD) and Spontaneously Hypertensive rats (SH) (A and B, respectively) housed in three different cage types for ten weeks. ST = standard Makrolon type IV cages, NOVO= Scantainer^{NOVO,} 4 cages connected, ERC= Enriched Rat Cage system. Body weight and body fat content (C and D respectively) of male SD rats housed in ST and NOVO cages for 12 weeks. All graphs presented as mean ± SEM. * ST > NOVO, $p<0.05$.

The other three rats (one ST-SPD, one ERC-SPD, and the hesitant one, above) completed the test in two attempts with a mean time of 3 s. In the mouth gag cooperation test, 45 rats cooperated within five attempts. Two ERC-SPD rats and one NOVO-SH rat failed the test. They were not the same individuals as has been mentioned in the other handling tests. Rats in ST cages had 2 ± 1 attempts in 20 ± 11 s, NOVO-housed rats had 2 ± 1 attempts in 20 ± 10 s and ERC-housed rats had 3 ± 1 attempts in 27 ± 11 s. The rats that failed had a mean time of 49 ± 10 s.

Variation and sample size

The effect of within-group variation (standard deviation, SD) on the minimum sample size needed to detect a treatment effect with the different cage types was calculated for the physiological parameters body weight and plasma insulin levels and for the behavioural parameter total arm entries in the EPM. The minimum sample size was calculated using a statistical power of 90 %, a significance level of 0.05 and a theoretical group difference in mean values of 20 %. The results show the number of animals needed to reach this difference in mean values with the given group variations. Housing in the alternative cage types resulted in fewer or the same number of animals needed for statistical significance between groups for body weight and

Table 4. Endurance performance exercise tests on a laddermill of male Sprague Dawley rats (SPD) and Spontaneously Hypertensive rats (SH) housed in three different cage types. Presented as mean ± SD. ST = standard Makrolon type IV cages, NOVO= Scantainer^{NOVO}, 4 cages connected, ERC= Enriched Rat Cage system. Data are presented as mean ± SD.

Group	Endurance (s)	
	Exercise test 1	Exercise test 2
ST-SPD (n=5)	46 ± 28	70 ± 49
NOVO-SPD (n=6)	61 ± 27	127 ±50*
ERC-SPD (n=8)	69 ± 39	94 ±42
ST-SH (n=5)	40 ± 24	92 ± 36)
NOVO-SH (n=7)	69 ± 36	132 ± 54*
ERC-SH (n=6)	54 ± 40	95 ± 22*
ST (both strains)	43 ± 25	81 ± 42*[a]
NOVO (both strains)	65 ± 31	129 ± 50**[b]
ERC (both stains)	63 ± 40	94 ± 34**[a,b]

* p<0.05 and ** p<0.01 significant difference from exercise test 1

[a,b] different superscripts denote differences between housing types, both strains included.

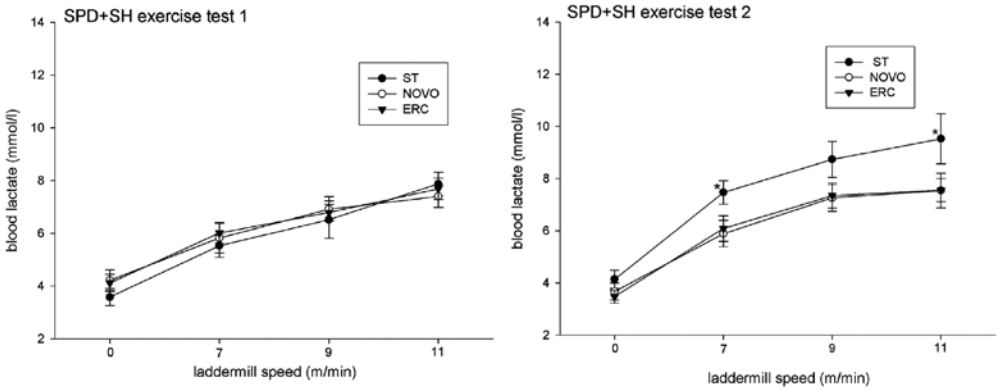

Figure 3. Results from exercise test 1 (left chart) and 2 (right chart) on a laddermill in male Sprague Dawley rats (SD, n=19) and Spontaneously Hypertensive rats (SH, n=18) housed in three different cage types. ST = standard Makrolon type IV cages, NOVO= Scantainer^{NOVO,} 4 cages connected, ERC= Enriched Rat Cage system. Presented as mean ± SEM.

* ST > NOVO and ERC, p<0.05

Table 5. Frequencies of entries into open and closed arms, and latency to first entry into open and closed arms and durations spent in the different zones of the Elevated Plus Maze. Tested on male Sprague Dawley (SPD) and Spontaneously Hypertensive (SH) rats housed in three different cage types. ST= standard Makrolon type IV cage, NOVO= Scantainer^{NOVO} cage, ERC=Enriched Rat Cage system. Data are presented as mean ± SD.

Group	Total arm entries	Arm entries		Latency first entry (s)		% time in open arms
		open	closed	open	closed	
ST-SPD (n=8)	11 ± 4	5 ± 3	6 ± 2	34 ± 47	34 ± 30	30 ± 17
NOVO-SPD (n=8)	15 ± 2	8 ± 2	7 ± 2	8 ± 10	30 ± 23	37 ± 13
ERC-SPD (n=8)	14 ± 3	7 ± 2	7 ± 2	10 ± 13	43 ± 18	36 ± 13
ST-SH (n=7)	12 ± 3	7 ± 3	5 ± 1	25 ± 21	46 ± 41	38 ± 12
NOVO-SH (n=8)	15 ± 2	8 ± 2	7 ± 1	11 ± 11	26 ± 16	33 ± 8
ERC-SH (n=7)	14 ± 2	8 ± 2	6 ± 1	28 ± 20	15 ± 21	35 ± 7
ST (both strains, n=15)	12 ± 3[a]	6 ± 3	6 ± 2[a]	29 ± 36	40 ± 35	33 ± 15
NOVO (both strains, n=16)	15 ± 2[b]	8 ± 2	7 ± 1[b]	9 ± 10	28 ± 19	35 ± 11
ERC (both strains, n=16)	14 ± 3[b]	7 ± 2	7 ± 2[b]	19 ± 19	30 ± 24	35 ± 10

[a, b] denotes differences between groups within parameter $p<0.05$

total arm entries in EPM, in comparison to housing in ST cages (Table 6). For plasma insulin levels however, housing in NOV and ERC cages resulted in a much higher number of animals needed to reach statistical significance in SPD rats, but not in SH rats (Table 6).

Discussion

This study showed that the two larger, alternative cage types induced a higher activity level in the rats without increasing the within-group variation or altering the physiological features of the hypertensive rat model. The increased home cage activity resulted in lower lactate levels and improved endurance in the exercise test as well as increased activity in the Elevated Plus Maze, in comparison to rats kept in standard cages. The effects were similar in the two rat strains of different genetic background.

An improvement of home cage environment for laboratory animals must not result in more animals needed to reach statistical significance in an experiment, due to increased within-group variation (counteracting the aim of Reduction). As has been shown before, changing the home cage environment for laboratory rats and mice affects within-group variation in some parameters but not in others *(Augustsson et al., 2003; Mering et al., 2001; Van de Weerd et al., 2002)*. Wolfer *et al. (2004)* found no effect of home cage enrichment on increased individual variability in behavioural tests and no conflicting results in replicate studies. In the present study, increased within-group variation was only found in plasma insulin levels in SPD rats in NOVO and ERC cages, and not in the other tested parameters. The FELASA Working Group on standardization of enrichment state that it is likely impossible to standardize environmental enrichment for rodents (and thereby enrichment effects on within-group variation) since their needs, and responses to enrichment implements, differ between species, strains, ages, sexes etc. *(Baumans et al., 2006)*. However, implementation of well

Table 6. Estimated required minimum sample size based on within group variation (SD) in body weight and total activity in EPM in Sprague Dawley (SPD) and Spontaneously Hypertensive (SH) rats kept in ST= standard Makrolon type IV cages or NOVO= Scantainer^{NOVO} cage or ERC=Enriched Rat Cage system. Calculated with a statistical power of 0.9, significance level of 0.05 and a theoretical difference in group means of 20%.

	SD-ST cages	SD-NOVO cages	SD-ERC cage	SH-ST cages	SH-NOVO cages	SH-ERC cage
End body weight (group mean)						
Body weight (g)	488.2	482.3	489.8	311.9	315.6	317.5
ST. DEV	30.9	26.8	25.6	26.5	19.8	20.6
N ratio $N_{alternative}/N_{standard}$	-	1	0.75	-	0.8	0.8
N	8	8	6	8	7	7
Plasma insulin levels (group mean)						
Plasma insulin (µg/l)	2.55	3.07	2.43	6.40	9.16	7.45
ST. DEV	1.11	3.34	1.67	5.29	4.13	4.57
N Ratio $N_{alternative}/N_{standard}$	-	9	2	-	0.6	0.7
N	8	72	16	8	5	6
Total arm entries EPM (group mean)						
EPM activity	11.1	15.3	14.4	12.1	15.0	14.3
ST. DEV	3.7	2.3	3.2	2.6	2.1	2.4
N ratio $N_{alternative}/N_{standard}$	-	0.4	0.75	-	0.7	0.9
N	8	4	6	8	6	7

designed environmental enrichment that offers stimulation similar to that in the wild, will likely give a more physiologically and behaviourally "normal" animal *(Baumans et al., 2006)*. Thereby, an improved housing environment will contribute to a better animal model. Effects of enrichment should therefore be acknowledged and used as a valuable source of information rather than to be considered a nuisance.

The cage environment is the most important factor for the welfare of the individual animal, even if it might be exposed to short bouts of aversive experimental situations. We believe that each animal is an individual with its own experiences and that welfare is experienced at an individual level. Hence, welfare needs to be measured at the individual level. Does it affect the individual and its welfare differently to share a cage environment with one or seven conspecifics? If 16 animals are kept as pairs in eight cages it can result in eight dominant and eight subordinant animals. If they instead are kept in groups of eight animals in two cages there will be an effect of the group but it might not influence each individual as strongly as when housed in

smaller groups/pairs. They are protected by the group, so to speak. Thus, the animals can be kept under conditions that favour their welfare and it is still possible to consider the individual animal as the experimental unit, which combines refinement and reduction.

Standardising the composition and group dynamics between groups of rats is basically impossible. Previously we found that two different groups of eight male SPD rats housed in identical cages, differed in the number of social interactions, the social structure in the group and in body weight range *(Spangenberg et al., 2009)*. Hurst *et al. (1999)* found more differences in behavioural and pathophysiological parameters between individual groups of the same size than between groups of different sizes. How groups are created upon arrival at animal facilities, based on scarce information about age and familiarity, could influence the group harmony and thereby affect the welfare of the animals *(Hurst et al., 1999)*, and in turn the research outcome. The way groups are composed and not only the group size, should also be discussed in relation to individual variability.

The handling of the animals is often neglected when discussing effects of enrichment. Rats could be more or less easy to handle as a result of their housing. For example, more agile animals could be harder to catch in the cage and maybe more difficult to restrain. Depending on the test situation, this could also affect the research results and adjustments in handling routines might be necessary. The handling tests in the present study showed no effects of cage types or strains, which is in accordance with previous studies *(Augustsson et al., 2002; Spangenberg et al., 2009)*. The rats were exposed to extensive routine handling (4-5 days/ week) and should therefore have adapted to handling. The hyper-responsitivity to different stimuli reported in SH rats *(McDougall et al., 2005; Tang et al., 1982; Tipton et al., 1983)* was probably balanced by the alleviating effects of handling on emotional reactivity *(Andrews & File, 1993; Ferguson & Cada, 2004; Joffe & Levine, 1973)*. Group housing has been shown to alleviate effects of social stress *(Ruis et al., 1999)* and of stress in relation to routine management and handling procedures *(Sharp et al., 2002)*, compared to single housing. In the present study the rats' cooperation rather than their physiological stress response was measured and group size did not affect these results.

The active behaviours in the home cage were greatly affected by cage type, both in diversity and quantity. Climbing, running and jumping that were observed in NOVO and ERC cages were almost impossible for the rats to perform in ST cages. Similar results were previously seen in pen-housed rats *(Spangenberg et al., 2005)*. A typical activity pattern performed by rats in the NOVO cages was dashes through all four cages by running and jumping between the shelves and cage passages, which was also observed in connected cages in a previous study *(Spangenberg et al., 2009)*. This is similar to what rats do in the wild when crossing open spaces. The inquisitive nature of rats makes them explore their home range regularly. When rats in ERC cages climbed on top of the shelter they got a good view of the entire cage (home range). In contrast, the NOVO-caged rats had to move through all cages to get total information about the present status in the home cage, resulting in more active behaviours. In the ST cages there is not much to explore. Furthermore, the NOVO-caged rats performed more investigating and risk assessment behaviours than ST rats. This was mainly an effect of investigating and rearing and very few SAPs (stretch attend posture) were seen in total. Rats use in-cage shelters *(Lidfors et al., 2002; Townsend, 1997)* which was also seen in the ERC-housed rats in the present study. The shelves in NOVO cages functioned as an elevated resting area which the rats also used, possibly as a consequence of the lack of a shelter. The strain difference in choice of resting area in both alternative cage types shows individual behavioural patterns and coping strategies that can be expressed more easily in these cage types, compared to ST cages.

In general, physical activity must be of a certain intensity, frequency and duration to result in a

training effect *(Henriksson & Sundberg, 2003)*. We have previously shown that moderate treadmill exercise by rats resulted in lower body weight gain and plasma insulin levels, greater relative heart weight, and lower lactate levels and greater endurance in an exercise test compared to control rats *(Spangenberg et al., 2009)*. In the present study, the increased home cage activity by rats in NOVO and ERC cages resulted in better performance and lower lactate levels in the second laddermill exercise test, compared to rats in ST cages. The temporary effects on body weight and body fat content in the NOVO cages during week 2 (study 2) could reflect an early training effect that disappears over time. It can also be an effect of acclimatization to a new cage environment which might indicate that it takes two weeks for rats (at least young ones) to adapt to a larger cage and group. Plasma insulin levels were not affected by the home cage activity in either of the two strains. But as has been shown before, the SH rats had higher plasma insulin levels compared to normotensive rats (SPD in this case) *(Mondon & Reaven, 1988; Reaven & Chang, 1991)*. Thus, the hypertensive model was unaffected by cage type, with regard to insulin levels.

For a sustained training effect, the level of exercise needs to be increased as the fitness of the individual improves *(Tipton et al., 1983; Wisloff et al., 2001)*. In the present study, the cage sizes were constant and the proportional cage size per rat became smaller as the rats grew in size (from 15 to 22 cm in SPD rats). If anything, the opportunity for activity became smaller over time which could explain the overall lack of training effect. It is however important to consider what level of home cage activity is necessary to improve animal welfare. In humans, a daily walk of 30 minutes is stated to have positive effects on health *(Jansson, 2003)*. This is naturally depending on the current fitness status of the individual. In the present study, rats in NOVO and ERC cages had unchanged lactate levels, and in some groups improved endurance, in exercise test 2 compared to exercise test 1. If the fact that rats become heavier and less active with age *(Skalicky et al., 1996)* is taken into consideration, the lactate data could even be regarded as an improvement over time. In contrast, the ST-housed rats showed deterioration (increase) in lactate levels but greater endurance (for both strains combined but not for individual strains) in exercise test 2 compared to test 1. It could be a ”use it or lose it”-effect of the increased activity in the alternative cages and decreased activity in the ST cages. According to Korte *et al.* (2007) too little stimulation of the important regulatory systems of the body (central nervous system, cardio-vascular system, immune and metabolic system) will make those systems less responsive to environmental challenges. Physical activity stimulates all the regulatory systems. Hence, decreased physical activity will make the animals less able to cope with environmental challenges, which reduces their welfare.

Total arm entries in the EPM were higher in rats from NOVO and ERC cages compared to ST-caged rats. As in a previous study by Spangenberg *et al.* (2009), there was a positive correlation between total arm entries EPM and total home cage activity/ social interactions. EPM total arm entries are suggested to reflect a higher level of exploration and locomotion *(Pellow et al., 1985)* and rats in larger groups and cages seem to increase their general level of activity. Elliot & Grunberg (2005) found that social enrichment, in comparison to physical enrichment or single housing, made male rats habituate faster to the Open Field test. The authors state that physical and social enrichment might have different relative effects on performance in behavioural tests. The results of the present study also suggest that an assessment of a general activity level of the rats might be relevant when conducting (and evaluating) behavioural tests based on locomotion. Time spent in the open arms of the EPM reflects the emotional reactivity of the animals and is considered the most sensitive measure in this test *(Pellow et al., 1985)*. No differences between cage types in this parameter were however found in the present study.

NOVO-caged rats showed more aggressive and submissive interactions than those in ERC

cages. The division of the NOVO cage area into sections with passages might stimulate aggressive interactions. The passages can be guarded, which has been observed previously in rats *(Calhoun, 1962)*. No tight passages or other structures that could be guarded were present in the other two cage types. Interestingly, SPD rats in the NOVO system had higher food consumption in the cages at the end of the row (cage 1 and 4) which could indicate that these were more secure or calm places to eat. The NOVO cages could be improved by having two passages between the cages. Thereby subordinates could more successfully avoid encounters with dominants, which is one of their strategies *(Blanchard & Blanchard, 1990)*.

In conclusion, this study describes the effects of housing in two different cage types with similar responses in two different rat strains. The results show that the evaluated alternative rat cages affected home cage activity without increasing within-group variation in the tested parameters or altering baseline levels of physiological parameters related to the hypertensive rat model. The effects of the alternative cages are likely a result of both physical and social activity. Since social animals like rats should be housed in groups, the use of these types of cages should be favoured. In addition, rats have more possibilities to cope with their situation when in a bigger and more complex cage, which is beneficial for the welfare of the individual animal . These results should support the idea that enrichment for laboratory animals need to be functional, not standardised.

Acknowledgements

The authors thank Scanbur A/S for letting us use their cages in study 1, Hans Eklund for excellent care of the animals in study 1, Karin Pettersson for assistance during the exercise tests, Gunilla Drugge and Kristina Karlström for analysing plasma insulin and blood lactate levels, respectively, Novo Nordisk A/S for allowing us to perform study 2 at their facility and Kristina Dahlborn and Birgitta Essén-Gustavsson for valuable comments on the manuscript.

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