Housing Laboratory Dogs and Rats
- Implications of Physical and Social Activity

Elin Spangenberg
Faculty of Veterinary Medicine and Animal Science
Department of Clinical Sciences
Uppsala

Doctoral thesis
Swedish University of Agricultural Sciences
Uppsala 2007

Acta Universitatis Agriculturae Sueciae
2007:103

ISSN 1652-6880
ISBN 978-91-85913-02-2

Tryck: SLU Service/Repro, Uppsala 2007

Abstract

Spangenberg, E. 2007. *Housing laboratory dogs and rats - implications of physical and social activity.* Doctoral dissertation. ISSN 1652-6880, ISBN 978-91-85913-02-2

The major part of the life of laboratory animals is spent in their home cages/pens. Both dogs and rats are social, active, exploratory animals. Keeping them on a restricted area leads to limitations in activity. The aim of this thesis was to evaluate if increased physical and social activity in the home environment improves animal welfare.

In study I, it was shown that male Beagle dogs increased their activity (steps/hour) and frequency of active behaviours when they had access to an outdoor kennel compared to when they were kept only indoors. Male Sprague Dawley rats were kept in several alternative housing systems for 4-10 weeks; a furnished pen (study II), two or four connected Makrolon type IV cages (study III), Enriched Rat Cage System and Scantainer ^{NOVO} cages (study IV), in groups of four (study III) or eight (studies II-IV) with control rats either housed individually (study II) or in pairs (studies III, IV) in standard cages. In study III half of the number of rats from each cage was given moderate treadmill exercise. The cage types in study IV were also tested in Spontaneously Hypertensive rats. Compared to controls, pen-housed rats had lower body weight, increased muscle oxidative capacity and strength and a more diverse behavioural pattern. The treadmill-trained rats had lower exercise blood lactate levels and greater endurance, lower body weight and plasma insulin levels and a greater relative heart weight compared to controls. Rats living in groups of four or eight performed better in an exercise test, had more social interactions and showed more activity in the Elevated Plus Maze compared to controls (study III). In study IV, rats of both strains showed a higher home cage activity with a greater variety of active behaviours in the alternative cages. This resulted in a lower lactate response and a greater endurance in an exercise test in both strains. The hypertensive rat model was not affected by the increased activity.

In conclusion, the outdoor housing in dogs and all alternative housing systems tested in rats has lead to higher physical and social activity with positive implications for animal welfare.

Keywords: laboratory animals, housing conditions, physical activity, natural behaviours, animal welfare, dogs, rats, exercise test

Author's address: Elin Spangenberg, Department of Clinical Sciences, SLU, P.O. Box 7054, SE-750 07 Uppsala, Sweden. Elin.Spangenberg@kv.slu.se

Contents

Appendix

Papers I-IV

The present thesis is based on the following papers; which will be referred to by their Roman numerals:

I. Spangenberg E.M.F., Björklund L., Dahlborn. K. Outdoor housing of laboratory dogs: effects on activity, behaviour and physiology. *Applied Animal Behaviour Science 98 (2006) 260-276*

II. Spangenberg E.M.F., Augustsson H., Dahlborn K., Essén-Gustavsson B., Cvek K. Housing-related activity in rats: effects on body weight, urinary corticosterone levels, muscle properties and performance. *Laboratory Animals 39 (2005) 45-57*

III. Spangenberg E.M.F., Dahlborn K., Essén-Gustavsson B., Cvek K. Effects of physical activity and group size on animal welfare in laboratory rats. *Submitted*.

IV. Spangenberg E.M.F., Dahlborn K., Essén-Gustavsson B., Remes C., Cvek K Effects of alternative housing systems on physical and social activity in male Sprague Dawley and Spontaneously Hypertensive rats. M*anuscript*

Papers I and II are reproduced by kind permission of the journals concerned.

Introduction

Today, millions of laboratory animals are used per year in Europe, and in Sweden we used 83 000 rats and nearly 1200 dogs in research, education and toxicological testing in 2005 (Jordbruksverket, 2007). The major part of the life of these animals is spent in their home cages. The housing of laboratory animals has mainly been designed to meet economic and ergonomic demands, with little concern for animal welfare (Baumans, 2005; Wurbel, 2001). In the last decades the welfare of laboratory animals has raised a great interest and a lot of research has been performed to improve it. Traditionally animal welfare science has focused on the absence of negative welfare parameters, *e.g.* poor production performance, stress and stereotypies, rather than measuring positive states or parameters. One important part of an improved welfare for laboratory animals is the possibility to perform a wide variety of natural behaviours in the home environment, on a daily basis.

Both dogs and rats are social, active and exploratory animals and keeping them on restricted areas leads to limitations of their active and social behaviours. Physical activity positively affects physiological and mental parameters which results in greater welfare and an improved physical fitness. Physical activity in dogs and rats can be induced by interactions with conspecifics or with the environment. However, this implies social housing and enough space and resources to perform activity-related behaviours. The aim of this thesis has been to study effects of alternative housing systems, with increased space, height and complexity, on the home cage activity and assess effects in physiological and behavioural parameters in laboratory dogs and rats.

The laboratory dog *(Canis familiaris)*

Dogs are important animals in biomedical research for studying both human and canine diseases and disorders. As a model for humans, dogs are used in studies of cardiovascular and gastrointestinal diseases, diabetes etc. In addition, the dog is a common species in toxicological studies of candidate drugs for new medicines. It is a requirement by the authorities that candidate drugs are tested on two different species, of which one must be a non-rodent species; hence the dog is often used. There is a greater emotional concern among the public about using dogs as laboratory animals, compared to rodents. The Beagle dog was used in this thesis and it is also the most commonly used breed in biomedical research. It is a gentle, manageable dog of a suitable size and is well characterised in terms of physiology, genetics and diseases.

Dogs are highly social animals and group housing is pertinent for their wellbeing, which is also emphasized in the new European guidelines for housing of laboratory animals (Council of Europe, 2006). Studies of feral dogs showed that they had an average home range size of approximately 11 km^2 with core areas of approximately 6 % of total home range size (Boitani *et al.*, 1995). The core areas contained valuable features like dens, resting sites and retreat sites and were defended as territories. The feral dogs had peaks in activity at dusk, when they travel from the resting site to a feeding site (a dump), and dawn, travelling back to the resting site (Boitani *et al.*, 1995). Approximately 50 % of their time was spent in active behaviour or travelling between sites. The European guidelines do recommend that extra care is put on exercising laboratory dogs outside their pens. This allows the dogs to interact with a more complex environment and with other dogs and humans (Council of Europe, 2006). In addition, the American Animal Welfare Act, states that each facility must have a documented exercise plan for their laboratory dogs (Hetts, 1991). Dogs like to position themselves high, to obtain information about their surroundings. By placing a platform in the pen the dogs can improve their view of the animal room and this will reduce the risk of them developing stereortypies (Hubrecht, 1993). The housing of dogs should also provide separate areas for different activities and places were dogs can retreat and feel secure (Council of Europe, 2006).

The laboratory rat *(Rattus norvegicus)*

The laboratory rat is, second to the mouse, the most used mammal in research. It was domesticated more than 100 years ago and belongs to the species Brown rat *(Rattus norvegicus).* Today, there are a number of strains of laboratory rats available representing a general animal model as well as different disease models such as hypertension, obesity and diabetes. The rat is used within a vast number of research fields, making it a versatile laboratory animal. In this thesis, Sprague Dawley rats, a general strain, and Spontaneously Hypertensive rats, a rat model of hypertension, were used.

The Brown rat is, just as the dog, a social animal and wild rats live in large colonies in complex burrow systems. The social groups consist of both males and females and one colony can be divided into subgroups. Dominance orders emerge mainly between males. Rats are prey and nocturnal animals. They explore their home range regularly in search for food and they are highly active and inquisitive animals (Barnett, 1963). When laboratory rats are released into the wild, they quickly adopt the same behavioural features as wild rats, like following pathways when exploring their home range and digging burrows (Boice, 1977). In fact, laboratory rats have potentials to display all the behaviours seen in wild rats (Boice, 1981). Rats have a behaviour called thigmotaxis, *i.e.* they avoid open areas and stay close to walls and other structures to not expose themselves to predators (Barnett, 1963). When rats have to pass open areas they do it quickly in a sprint-like manner and they usually follow known pathways in their home range. It is therefore important that rats have an environmental complexity in their home cages and that they can divide it into different areas for different activities such as feeding or resting (Council of Europe, 2006). Nest boxes or similar refuges are also important for rats, to use both as shelter and a look-out-point (Council of Europe, 2006).

Housing of laboratory animals

In biomedical studies it is important to control environmental factors to be sure that it is the experimental manipulation of the animals that affects the results and not uncontrolled or unforeseen factors. Therefore, factors such as light, temperature, humidity, ventilation, bedding material and cage material, as well as food and water, are carefully controlled and monitored. Extra attention is also put on health monitoring and protection against infections. It is detrimental to get an infectious disease into the animal facility. In combination with control of environmental and health monitoring factors, economy and management has led to the development of housing systems for laboratory animals that are easy to handle, clean and store, inexpensive and/or make it possible to keep many animals on a restricted area. The housing systems used for the last decades therefore mainly satisfy the basic needs of the animals, *i.e.* to eat, drink and rest. It is rather a consequence of space restrictions and convenience (Brain & Benton, 1979) than an effort to optimise animal welfare (Hetts, 1991). In combination with free access to food, which is a regular management routine, this often results in inactive and overweight animals. Commonly, social species are housed in groups but often on a too small area, with the risk of overcrowding. In laboratory dogs, these systems have resulted in mainly indoor housing of dogs with restricted opportunities for physical activity and activity-related behaviours. It also happens that they for practical reasons are housed individually. Rats are the species among laboratory animals that have the poorest housing standards today. They are commonly housed in cages of limited space and height that restrict their possibilities to perform a variety of their natural behaviours or to be physically active. The housing conditions evaluated in this thesis offers more space and outdoor facilities for dogs and more space and resources in the alternative cages tested for rats. Furthermore, for economical and space optimising reasons, the use of larger rat cages implies housing of larger groups of rats, with an option for more social interactions.

Animal welfare

The difficulty in both defining and measuring animal welfare is that it is not possible to ask the animal if it regards its welfare as good or bad. Therefore, scientists have so far been limited to indirect measurements of parameters with connection to animal welfare. Animal welfare and/or animal well-being have been defined by several scientists with slightly different approaches and starting points. There are different acceptances of the terms in Europe and the US, however, there seems to be a general agreement on that well-being refers to the state of the animal while welfare more addresses a wider aspect, including social and ethical issues (Clark, Rager & Calpin, 1997).

When animal welfare became of interest to scientists, they mainly focused on the function of the animals by describing production parameters in farm animals (growth rate, litter size etc) as effects of housing routines and care. An example of this functional approach is the Five Freedoms from the UK Farm Animal Welfare Council (FAWC, 1993) stating that animals should have freedom from: 1) hunger and thirst, 2) discomfort, 3) pain, injury and disease, 4) freedom to express normal behaviour 5) fear and distress. A somewhat different approach has its starting point in the need for captive animals to perform their natural behaviour and this is the concept used in the Swedish Animal Welfare Act of 1988 (Jordbruksdepartementet, 1988), which states that captive animals should be kept "….in such a way that it promotes their health and gives them the possibility to perform their natural behaviours" (4§). A more "mental" approach is the "coping theory" introduced by Broom (1986) who suggested that "the welfare of an individual is its state as regards its attempts to cope with its environment." To be able to cope with the environment there has to be a certain degree of controllability and predictability (Baumans, 2005) but also a possibility to perform the behaviours needed to get a conception of control. In recent years the cognitive abilities of animals and the influence of cognition on animal welfare has raised interest. In European legislation animals are now defined as "sentient" creatures which makes them conscious individuals with feelings and a value of their own (Korte, Olivier & Koolhaas, 2007). The complicated interaction between an animal's internal emotional state, its cognitive abilities and environmental stimuli is defined in terms of welfare/well-being as "An animal's well-being or quality of life is its internal somatic and mental state that is affected by what it knows (cognition) or perceives, its feelings (affect) and motivational state, and the responses to internal or external stimuli or environments (Clark, Rager & Calpin, 1997). It is said that a state of well-being is when an animal can maintain its physical and psychological homeostasis (Clark, Rager & Calpin, 1997). The cognitive approach moves even further by saying that welfare is not simply the absence of negative affects but also, and maybe even more, the presence of positive emotions (Boissy *et al.*, 2007). Today it is possible to measure effects of positive emotions in animals and their presence might say more about the welfare state of the animal than the simple absence of adverse states (Boissy *et al.*, 2007). A recent science-based animal welfare concept is based on the allostasis theory (Korte, Olivier & Koolhaas, 2007). The definition of allostasis is "stability through change". In the allostasis welfare concept good animal welfare is characterized by "a broad predictive physiological and behavioural capacity to anticipate environmental challenges", which can be guaranteed when "the regulatory range of allostatic mechanisms (central nervous system, cardio-vascular system, immune and metabolic system) matches environmental demands" (Korte, Olivier & Koolhaas, 2007). It is important to note that not only hyperstimulation, but also hypostimulation of the allostatic mechanisms have negative effects on animal welfare. Korte, Olivier & Koolhaas, (2007) dismiss the Five Freedoms as being a more ethical view than science-based approach and that complete freedom is undesirable. They state that fear warns the animal of impending danger, pain protects the animal from injury, the primary function of stress is protective and adaptive and that in the wild, maximum reproduction rather than health is the goal of natural selection. The concept of homeostasis refers to keeping physiological variables at their "set points" which basically implies no environmental challenges, but from an allostasis point of view this leads to hypostimulation and downregulation of the systems and thereby bad animal welfare (Korte, Olivier & Koolhaas, 2007).

The animal welfare approach in this thesis is based on the Swedish Animal Welfare Act, which in turn is based on the concept of natural behaviour. The importance of the performance of species-specific behaviour has therefore been the main focus. However, since the aim is to increase home cage activity and move away from un-stimulating cages/pens that impose inactivity on the animals, the hypostimulation situation of the allostasis animal welfare concept is also highly relevant. By keeping dogs and rats in pens or cages that offers increased physical and social activity and a more varied physical environment, the probability that they will encounter environmental challenges increases. Thereby the probability that their welfare improves will also increase.

Physical fitness and physical activity

Physical fitness (a state of health and well-being) is defined in Wikipedia as "the result of regular exercise, proper diet and nutrition, and proper rest for physical recovery within the parameters allowed by the genome" (2007). Mental and emotional health is also an important part of physical fitness and an individual can be physically fit but still suffer from a mental illness or emotional problems. Physical fitness can be regarded as a triangle made of the three subsections physical, mental and emotional fitness and an "ideal triangle" is balanced in all these areas (Wikipedia, 2007). Regular exercise is thus increasingly discussed as a parameter that improves health in humans. Effects have been found in physical aspects such as body weight regulation or prevention of diabetes type II, as well as in psychological aspects of the human health. Most effects described in humans are also found in animals. Health-promoting aspects of physical activity refer to moderate levels of exercise. In humans, daily walks of 30 minutes are considered a minimum amount of exercise to positively affect health, with additional effects if the exercise is increased (FYSS, 2003). Strenuous exercise as for professional athletes does however put great demands on the body and they often balance on the edge of overexertion of different physiological systems, such as the immune system (Nieman, 2000). Most mammals perform physical activity in one way or the other to search for food or other resources such as mating partners or nesting sites, to patrol territories or home ranges, or to play, mainly as juveniles. The activity can be of a long duration but at a low intensity such as when herds of cattle or elephants move between grazing areas. It can also be short bouts of explosive high intensity movements such as a lion or a cheetah chasing a prey. In the search for an increased level of activity in the home cage/pen of laboratory rats and dogs, the aim is to enable the animals to maintain a normal activity level, instead of a daily life of mainly inactivity that is forced upon the animals due to restricted space and resources. The normal level is by no means static; it varies between individuals, with age and likely also with environmental features. An increased home cage activity would allow the animals to perform a varied repertoire of natural and social behaviours and in the same time there could be beneficial effects of physical activity per se.

Voluntary activity in dogs and rats

The spontaneous activity of dogs and rats in the wild is generally goal-directed. Apart from young and juvenile animals that engage in play-behaviour both species explore their home range in search for food or other resources. If physical activity is a behavioural need for these animals their welfare will be impaired if they are prevented from being active due to restrictions in the housing systems. If their activity level is a secondary effect of the search for food etc, the welfare is still impaired if, as today, the food is readily served.

Just as feral dogs show a diurnal rhythm in activity so do beagle dogs in confinement(Siwak et al., 2003). Further, Siwak *et al.(2003)* showed young Beagles were more active than older ones, but access to outdoor facilities decreased the age-related difference in activity. In this thesis, physical activity was measured in the home pen of the dogs with a pedometer (steps/hour) and as the active behaviours running, walking and jumping.

When voluntary activity in rats has been recorded, it has mainly been in running wheels. When rats are given access to a running wheel they can run up to 10-12 km/day. There is however a great individual variation in rats' running distances in the wheels and therefore they are usually divided into groups of either short, medium, or long distance runner*s* (Afonso & Eikelboom, 2003; Lambert *et al.*, 1996; Narath, Skalicky & Viidik, 2001; Rodnick *et al.*, 1989; Sexton, 1995). Rats can be forced to exercise on a treadmill, usually with mild electric shocks as an aversive stimulus. In treadmill exercise studies it is common that some rats have to be excluded because they simply refuse to run (Lambert & Noakes, 1990; Norton, Jones & Armstrong, 1990; Sexton & Laughlin, 1994). It seems that every rat has it own running level, *i.e.* how much/far it is running per day, that might be controlled endogenously (Afonso & Eikelboom, 2003; Mueller, Herman & Eikelboom, 1999). When stopped from running, the rats compensated for lost amount of running when they got access to the running wheels again (Mueller, Herman & Eikelboom, 1999). This endogenous factor might also direct the amount of activity the rat performs in its home range or home cage. It is not clear what determines this factor, it could perhaps be linked to dominance order, or maybe some rats function as "scouts" in the wild and therefore need to be able to have a greater running endurance. The active behaviours of rats that were studied in this thesis are running ,walking, jumping (both vertically and horizontally) and climbing.

Effects of physical activity on the body

If effects of physical activity are to be measured in animals, it brings both limitations and possibilities regarding what is possible to measure. Just as with animal welfare, we cannot ask the animal if it is exhausted or verbally push it to work a little bit harder. It is however possible to stimulate animals with a reward after every exercise bout. On the other hand, when the exercise period is over, the animal can be euthanized and it is possible to take out entire muscles, the heart, brain or other organs of interest. In this thesis, parameters were chosen that were thought to be affected by the type of physical activity the rats voluntarily can perform in their home cages. In addition, extra care was put on using parameters that would be as mild and non-aversive to the animals as possible. The proposed effect of physical activity in the measured parameters is explained briefly below:

Body weight

The basis of body weight regulation is energy input and energy output. If the energy intake is higher than the energy used, then body weight will increase and vice versa. Factors that affect energy output are physical activity, basal metabolic rate, environmental temperature, and metabolic dysfunctions. As a physiological parameter body weight is easily recorded in both dogs and rats. It is also often used as one of the first clinical signs of an abnormal state in an animal. An advantage with body weight measurements is that it can be recorded repeatedly during a study and give valuable information of effects over time. It can be of interest to study both the absolute body weight and the relative body weight gain. Body fat content is also a relevant parameter and it can be measured in conscious animals by using the magnetic resonance technique. Food- and water intake are closely linked to body weight alterations and deviations in an animal's food- or water intake can indicate a sub-clinical disorder or a disease.

Heart weight

Exercise of a certain frequency, duration or intensity can lead to hypertrophy of the cardiac muscle. The heart adjusts to an increasing work load by pumping out more blood thus increasing both stroke volume and the size of the heart (Berglund, 1988). The increase in size is caused by greater volume and an increased thickness of the walls (Berglund, 1988). Cardiac hypertrophy can be measured in animals by using ultra sound technique or . by simply removing and weighing the heart. at euthanisation.

Metabolic adaptations in muscles

Metabolic systems in muscles for energy production during exercise are anaerobic or aerobic glycolysis, β-oxidation of fatty acids, the citric acid cycle and electron transport chain in the mitochondrias. They all aim at producing adenosine triphosphate, ATP, which is the energy source at the cellular level. To analyse adaptations to an increased level of physical activity the following enzymes have been measured in this thesis; citrate synthase (CS) in the citric acid cycle, representing oxidative capacity, 3-hydroxyacyl-CoA (HAD) in the β-oxidation representing lipid oxidation, lactate dehydrogenase (LDH) in the glycolysis, representing glycolytic capacity and hexokinase (HK) as a marker for the capacity to phosphorylate glucose. Glycogen in the muscle is a main substrate source for energy production during exercise and trained individuals usually have greater amounts (Henriksson & Sundberg, 2003). Muscles contain fibres that have different contractile and metabolic properties (Pette & Staron, 1988). In this thesis both the triceps and the gastrocnemius muscles of rats were studied and they contain regions that consist of different fibre types. There is a red part that mainly contains slow-twitch oxidative and fast-twitch oxidative fibres and a white part that contains mainly fast-twitch low-oxidative fibres (Armstrong & Laughlin, 1983; Fuentes, Cobos & Segade, 1998). The recruitment of these fibre types depend on the intensity and duration of the exercise performed. Low-intensity exercise mainly recruits slow-twitch oxidative fibres and at higher intensities the fast-twitch fibres are used with fast-twitch oxidative fibres usually being recruited before fast-twitch non-oxidative fibres (Armstrong & Laughlin, 1985). In humans and large animals enzymatic activities and glycogen content in the muscle can be measured in muscle biopsies. The rat's muscles are however too small and therefore entire muscles or sections of muscles are removed from the rat after euthanisation.

Metabolic response to exercise and tests of endurance

With an exercise test performed under standardised conditions, it is possible to measure the metabolic response during exercise. Such tests have earlier been performed on treadmills in humans (Larsson *et al.*, 2002) horses (Rose & Hodgson, 1994), and rats (Pilis *et al.*, 1993) to evaluate performance capacity. Repeated tests can detect metabolic changes during a training period. It is well-known that lactate accumulate in muscle and blood at higher intensities of exercise. Blood lactate levels decrease with endurance training and a trained individual can work at greater intensities before lactate is produced in the muscles (Favier *et al.*, 1986 ; Henriksson & Sundberg, 2003). To measure the blood lactate concentration in response to a standardised exercise test can thus give information about the physical fitness of an animal. Exercise capacity can also be analysed in an endurance test where the subject runs at a certain workload on a treadmill until exhaustion. In rats, it is possible to assess their muscle strength in the so called inclined plane test which is based on their ability to climb.

Insulin

The hormone insulin is released from the pancreas into the blood as a response to increased blood glucose levels, such as after a meal. The role of the insulin is to facilitate the transport of the glucose from the blood to muscles, liver and adipose tissue. Exercise can improve the sensitivity of insulin and a trained individual will have lower levels of plasma insulin (Henriksson & Sundberg, 2003). In the metabolic disorder diabetes type II decreased insulin sensitivity, resistance, can develop, resulting in hyperglycemia (Östensson & Henriksson, 2003). Regular physical activity can both improve insulin sensitivity in diabetic individuals and prevent the development of diabetes type II (Östensson & Henriksson, 2003).

Social activity in dogs

Studies of feral dogs showed that those living in meadowlands had a social organisation similar to that of a wolf pack, while feral dogs living in villages kept one another company but no real pack formation was seen (MacDonald & Carr, 1995). Group size was probably regulated by access to food which also has been seen in wolves (Bekoff, Daniels & Gittleman, 1984). It has been stated that social isolation is more detrimental to dogs than what housing on limited space is (Hetts *et al.*, 1992). The social contact with humans is also important to dogs. It has been reported in several studies that the presence of humans has the highest effect on activity of both laboratory dogs (Campbell *et al.*, 1988; Hetts *et al.*, 1992; Hite *et* *al.*, 1977; Hughes, Campbell & Kenney, 1989) and sheltered dogs (Wells & Hepper, 2000). Hubrecht (1993) claimed that quality of life of pups born in laboratory settings can improve if they have regular socialisation with a caretaker. There is no reference of the optimal group size in dogs, and the European guidelines state that dogs should be house in socially harmonious group, minimum as pairs (Council of Europe, 2006). The dogs studied in this thesis were housed in pairs.

Social activity in rats

As has been mentioned, wild Brown rats live in large colonies of both females and males. In the laboratory however, rats are generally housed in same-sex groups, with the exception of breeding colonies and studies that are focusing on interactions between males and females. Important social behaviours in rats are for example allogrooming and huddling, when they sleep together in a pile. In this thesis only males were studied and rats kept singly or in pairs were compared to rats kept in groups of four or eight. Males that have grown up together usually live in harmony, even in an overcrowded area. Adult males that are unfamiliar to each other and are put together usually function well as a group. If a single intruder enters a settled group it will be threatened, chased and attacked if necessary (Adams & Boice, 1989). A dominance order occurs within male rats but it has been stated that females are required in the group to get a true dominance order (Tamashiro *et al.*, 2004). The dominance order is characterised by asymmetry in agonistic behaviours between individuals (Blanchard & Blanchard, 1990; Pellis & Pellis, 1991). There is often a dominant male, usually the largest rat, who seldom loses aggressive encounters with other males in the group. There is no strict hierarchy between subordinates and they have two strategies, either stay close to the dominant male or always avoid him (Blanchard & Blanchard, 1990). Pathophysiological signs of subordination can be shown as increased corticosterone levels, decreased prostate and thymus weights and a suppressed immune defence as a result of disturbed eating, sleeping and sexual behaviour (Blanchard & Blanchard, 1990). This can lead to a premature death. It has been stated that dominance orders are more likely to occur in semi-natural settings (Adams & Boice, 1989) and it can therefore be hard to detect in single-sex groups during laboratory conditions.

It has been suggested that social isolation of rats causes long-term changes in physiology and behaviour (de Jong *et al.*, 2005) and that rats prefer to be in groups even in situations of social stress (Hurst *et al.*, 1999). Positive effects of group housing have been found to diminish effects of the stress model social defeat in rats (Ruis *et al.*, 1999) and effects of routine management and handling procedures (Sharp *et al.*, 2002). The ideal group size of laboratory rats has not been proved and it is rather a result of economical factors and local routines in the different animal facilities.

Emotional reactivity and risk assessment

Being able to react to a potential threat gives the rats a degree of control over their situation, which is of crucial importance for their well-being. Risk- taking is a necessary part of the life of a wild rat, in order to reach resources and ultimately to survive. Risk assessment is necessary to calculate the risks before proceeding. By performing risk assessment behaviours the rat gathers information about the current situation in order to decide whether to go back to normal activities or to perform defensive behaviours (Blanchard *et al.*, 1990). The risk assessment is valuable for the decision making of what behavioural strategy to apply in order to control the situation. Risk assessment behaviours of rats can be; stretched attend posture, rearing, head dip and flat- back approach. The possibility for rats to perform risk assessment in their home cages helps them to meet environmental challenges and thereby improves their welfare. Even in a controlled environment like a laboratory animal facility there can be situations that rats perceive as threatening. Tests of risk assessment or risk taking can give information of the emotional reactivity of the animals.

The animal model

Within the research community there is a strive to work according to the principle of the 3Rs - Reduction, Replacement and Refinement (Russell & Burch, 1959). Reduction of the number of animals used in a study can be reached through proper experimental planning and reduced variation within groups. However, the refinement R must be considered as the most important for the animals used in biomedical research. An important refinement is the improvement of housing conditions since the animals spend most of their time in the home cage. This refinement needs to be thoroughly evaluated to assure that it does not increase the number of animals needed to reach statistical significance within a study. It is a concern among researchers that alternative cages, or enrichment, will affect the animal model, *e.g.* increase variation within experimental groups (Van de Weerd *et al.*, 2002). Studies have shown that the effect of enrichment of cage environment on variation depends upon the parameter measured (Mering, Kaliste-Korhonen & Nevalainen, 2001; Van de Weerd *et al.*, 2002). Enrichment can increase or decrease variation within a group and thereby affect the number of animals needed to reach statistical significance (Mering, Kaliste-Korhonen & Nevalainen, 2001). Enrichment per definition means improvement, but the term has frequently been used to describe a change of the housing environment rather than the outcome (Newberry, 1995). To properly enrich the environment the improvement has to have biological relevance, *i.e.* be designed to meet the behavioural needs of the animal (Newberry, 1995). In this thesis enrichment refers to changing the entire home environment by adding space and variation to the housing of dogs or by using larger cage types that contain resources of biological relevance for the rats. As many parameters in the environment of laboratory animals are strictly controlled (temperature, light air changes, bedding material etc.) there has been a discussion that the enrichment should also be controlled, i.e. standardised. The standardisation of the environment to decrease between-experiment variation has been a sort of dogma and environmental effects on phenotypic expressions are considered a nuisance rather than an effect worth investigation (Wurbel, 2002). As stated by Baumans (2005) “even if enrichment increases variation within a study, it important to not overstate it but rather balance it against the improved well-being of the animals”. Further, it is important to consider whether the statistically significant differences also have biological relevance (Mering, Kaliste-Korhonen & Nevalainen, 2001).

In this thesis the effect of changes of the housing environment on the animal model has mainly been evaluated in study I and IV. In study I, the outdoor kennel used by the dogs cannot be standardised in terms of weather variations, the risk of contaminants such as birds, insects etc. Researchers are hesitant to let laboratory dogs be outside, which has bearings on the dogs’ welfare. It is important, both for researchers and dogs, to study if the outdoor environment does affect the animal model and in what way. The hypertensive rat model the Spontaneously Hypertensive rat (SH) was tested in study IV. It is the result of a spontaneous mutation in the inbred rat strain Wistar-Kyoto. The SH rat develops an abnormally high blood pressure between four and nine weeks of age with an established hypertension around 13 weeks of age (Evenwel & Struyker-Boudier, 1979; Hoffmann *et al.*, 1987). The SH rat also shows elevated plasma insulin levels (Mondon & Reaven, 1988; Reaven & Chang, 1991), which can be a sign of insulin resistance. It has previously been shown that physical activity like voluntary running in running wheels or low-intensity treadmill exercise, can delay the onset of hypertension in SH rats (Hoffmann *et al.*, 1987) or attenuate an the hypertension (Evenwel & Struyker-Boudier, 1979; Overton *et al.*, 1986; Tipton *et al.*, 1983; Veras-Silva *et al.*, 1997). Therefore, it was found of interest to study the SH rat to see if increased home cage activity in alternative housing systems would alter the model.

Aims of the thesis

The overall aim of this thesis was to evaluate whether physical fitness, through increased home cage activity, can be regarded as a part of the animal welfare concept.

Specific aims:

- To study if access to an increased home cage/pen area increases spontaneous activity in laboratory dogs and rats

- To find physiological and behavioural parameters that reflects an improved animal welfare as a result of increased physical activity

- To investigate if exercise training and/or group size affects animal welfare mainly by:
  a) the physical activity
  b) the social activity
  c) both physical and social activity

- To investigate whether alternative housing systems alter the physiological characteristics of the animal model used

- To find important features in the design of a cage to improve housing conditions for laboratory rats

Materials and Methods

Experimental studies

Outdoor housing of laboratory dogs (study I)

In a cross-over study of totally six weeks, eight male Beagle dogs were housed pair-wise either only indoors ($11m^2$) or in indoor pens with daily free access to outdoor kennels ($11m^2$). Each indoor pen was equipped with two stalls (each 1.76x0.85 m) furnished with shelves (0.82x0.84 m) were the dogs could stand, sit or lie down (figure 1). The shelves were placed at a height of 0.58 m. Plastic tubs (0.80x0.80 m) used as beds with wood chips as bedding material were placed under the shelves (figure 1). From approximately one meter above ground, the walls between the pens were made of steel bars which gave the dogs a view over neighbouring pens and also made it possible for them to have nose contact with their closest neighbours (figure 2). There was one outdoor kennel for each indoor pen and it was connected to the indoor pen through a small door in the wall. The doors were unlocked and locked by a magnetic lock in the morning and afternoon every day. The outdoor kennel had concrete floor and walls of concrete or steel grid. The entire outdoor section consisted of kennels for approximately 30 dogs from two different indoor sections. The dogs received visual, olfactory and sound influences from the neighbouring dogs, and olfactory and sound influences from other dogs. They could also have nose contact with the same neighbours as in the indoor pens.

The aim of this study was to assess differences in physiological parameters in dogs housed indoors and with access to an outdoor kennel. In addition, it was evaluated from video recordings how the dogs used the outdoor kennel by recording frequencies of outdoor visits, durations and behaviours displayed outdoors. The effect of the outdoor kennel on physical activity and activity-related behaviours in the dogs was also studied. Blood samples were taken three times per week and analysed for alanine amino transferase (ALT), alkaline phosphatase (ALKP), and cholesterol (CHOL) to study liver functions, creatinine (CREA) and blood urea nitrogen (BUN) for kidney functions, and amylase (AMYL) and lipase (LIPA) for pancreas functions. Further, levels blood glucose (GLU), white blood cells counts (WBC), granulocytes (GRANS), neutrophils (NEUT), eosinophils (EOS), ratio of leukocytes/monocytes (L/M), and platelets (PLT) were also studied. These parameters where chosen because of their relevance for the animal model in the ongoing research at the facility where the dogs were kept. The activity of the dogs was measured using pedometers (Pedometer multi, SILVA AB Sweden) which counted the number of steps the dogs took. The pedometer was attached to a harness and thereby placed on the shoulder of the dog (figure 1), where it recorded all types of movements – running, walking and jumping. Behavioural studies were carried out as direct observations in the pens. One individual at a time was studied for one minute and the frequency of its active or social behaviours was recorded continuously.

Pen-housing of laboratory rats (study II)

Male Sprague Dawley rats were housed in two groups of eight in large pens (each 31500 cm^2) furnished with different items that increased the environmental complexity and that gave an opportunity for increased physical activity (figure 3). These rats were compared with single housed rats in Makrolon type III cages for four weeks (figure 4A). The Makrolon type III cage has a floor area of nearly 1100 cm^2 and a height of 18 cm. The design of this cage obstructs active behaviours like climbing, running or jumping and no social interactions were possible in this study. The pen was chosen to test what the rats would do if they got access to an area that was more then ten times bigger than a standard cage. Basically all the active behaviours that are obstructed by the standard cages are possible to perform in the pen. In addition, the rats were kept in groups of eight in the pen which results in many social interactions compared to single housed rats.

The hypothesis was that an increased level of activity should be reflected in physiological parameters related to physical fitness. The aim of this study was to evaluate whether physical fitness can be regarded as an animal welfare parameter. The active behaviours in the home cage were scored from video recordings. By using instantaneous sampling each rat's behaviours was scored every five minutes during 24 hours. Weekly body weight gain was recorded and muscle strength was assessed on the inclined plane which is a rectangular box with a rubber mat on the floor. It can be elevated in one end to increase the inclination and the rat is supposed to sit still and hold on for as long as possible. The rats were tested in a maximum angle test, where the inclination was increased until the rat could no longer hold on, and an endurance test where the rat was supposed to hold on at predefined angles for one minute at each angle. The level of urinary corticosterone was measured at the end of the study. Urine was collected by placing the rats in empty cages which they often find unpleasant and therefore they tend to urinate. After euthanisation the weight of heart, spleen and kidneys was recorded. The red part of the triceps muscle was analysed for different enzyme activities; citrate synthase (CS), 3-hydroxyacyl-CoA (HAD), lactate dehydrogenase (LDH) and hexokinase (HK) and glycogen content.

Housing of rats in different cage types including a cage with access to running wheel (pilot study, unpublished results)

The pen used in study II was compared to a rebuilt rabbit cage, standard cages (Makrolon type IV) and cages fitted with running wheels. Male Sprague Dawley rats were housed in groups of eight in the pen and the rebuilt rabbit cage, in pairs in standard cages and individually in running wheel cages, for seven weeks. Makrolon type IV cage (ST) has a floor area of 2240 cm^2 and a height of 20 cm (figure 4B). The ST cage is lower than the length of an adult male Sprague Dawley rat (>23 cm, unpublished results) which greatly limits their opportunity to perform the behaviour rearing. While rearing, the rat stands on its hind legs and explores it surroundings, sometimes in a stretched out position. In addition, other active behaviours are, just as in the Makrolon type III cage, obstructed by the size of the cage. It is possible to add a shelter/nestbox, but that leaves little space left for the rats to use. The rebuilt rabbit cage is a former laboratory rabbit cage adapted to rats and it has a height of 50 cm and a floor area of 4320 cm^2 (figure 5). It is equipped with a shelf along the back wall at approximately 35 cm height and has grid on the side walls and the front. This cage type is a feasible alternative to the ST cages in terms of ergonomy and management, as opposed to the floor pen. It was therefore of interest to study the effect of the rabbit cage on the home cage activity and in comparison to the pen. The nearly doubled floor area, compared to ST cages the great height and the grids facilitate the behaviours rearing, jumping climbing, and to some extent running. The shelf is used extensively as a resting and shelter area and if necessary extra shelters/nest boxes can be put on the floor of the cage. Again, housing of groups of eight rats increases the possibility for social interactions in the home cage. The cages fitted with running wheels (figure 6) were used mainly to assess the distance and pattern of voluntary running in the wheels and not as a welfare improvement for the rat compared to the ST cages. The cages and wheels were made of stainless steel and the floor area was 825 cm^2 (grid floor), the height 23 cm and the circumference of the wheels was 100 cm. The rats were single housed in these cages in order to get individual recordings of running distance. The main behaviour possible to perform in the running wheel cages was running.

The aim was to test if the different cage types increased home cage activity and affected physiological parameters related to physical fitness. The parameters recorded were weekly body weight gain, muscle strength on the inclined plane, muscle enzyme activities and glycogen content in a cross-section of the long head of the triceps and of the middle part of the gastrocnemicus, heart weight and time and distance run in the running wheels were recorded.

Housing of rats in cages adjusted to the group sizes and exposure to moderate treadmill exercise (study III)

There are two major factors affecting the animals in a large enriched cage, social contacts in a bigger group and the possibility for spontaneous physical activity. This study focused on social interactions and their effect on the home cage activity. By connecting several ST units together it was possible to house Male Sprague Dawley rats in groups of two, four and eight in cages that still had the characteristics of ST cages, (Single, Double and Quadruple cages, respectively, figures 4B, 7 and 8) for seven weeks. The possibilities for the rats to perform active behaviours in the D and Q cages are similar to those in single ST cages, apart from the possibility to make dashes through the connected cages. The possibility for social activity does however increase with cage- and group size.

The aim of this study was to find effects of physical activity and/or group size on physical fitness and animal welfare. Social interactions were studied in direct observations of groups of rats (two, four or eight) in their home cages. During eight minutes the frequencies of social behaviours were continuously recorded by four observers in four different groups simultaneously. All rats were individually marked to be able to record interactions between specific individuals. The behaviours were recorded as neutral meetings, non-aggressive interactions or aggressive interactions. Half of the number of rats from each cage was given moderate exercise on a treadmill for five weeks, 3 days per week at a maximum of 26 min/day at a speed of 24 m/min. It was intended to correspond to the voluntary physical activity rats could perform in a larger cage. All rats were also tested in two exercise tests on the treadmill, before and after the exercise period. In these tests blood samples were taken for analysis of lactate response during increasing treadmill speeds. The treadmill was also used to test the endurance of the animals. They were made to run until exhaustion after the last intensity in the exercise test. Body weight was recorded weekly and at euthanisation heart and muscle samples were taken and analysed as in the pilot study. In addition, blood was collected for analysis of plasma insulin levels. At the end of the study The Elevated Plus Maze test (EPM) was used to assess effects on emotional reactivity. The EPM is a behavioural test based on rodent natural avoidance of open areas that are perceived as threatening. It is a plus-shaped maze elevated from the ground, with two (opposite) arms protected by walls and the other arms open and unprotected. The rat is left undisturbed to freely explore the arena for a certain time period, commonly five minutes, and the procedure is filmed for future analysis. There is no previous habituation to the arena so it represents a novel environment. Parameters to record are entries into open and closed arms (= total activity), time spent and behaviours performed (*e.g.* risk assessment) in the different arms. In addition, a handling test was performed to assess the rats' willingness to be handled in common experimental situations. This test was based on what was done by Augustsson *et al.* (2002) and three situations were tested; 1) the rats' anticipation reaction to handling, *i.e.* a hand in the cage, 2) their willingness to be restrained as for an i.p. injection, 3) their cooperation in a mouth gag test where they should hold a metal pin in their mouths. The time and number of attempts it took for the rats to accept the different tests were recorded. In addition, they were also scored to be compliant, hesitant or unwilling to the procedure by a skilled animal technician who performed the tests.

Housing of two different strains of rats in commercially available alternative cage types (study IV)

Male Sprague Dawley rats and Spontaneously Hypertensive rats were used to assess effects of two commercially available alternative cages on spontaneous home cage activity. The alternative cages, Enriched Rat Cage (ERC) system and Scantainer^{NOVO} (NOV) both housed eight rats per strain and they were compared with pair-housed rats of both strains in standard cages, for ten weeks. The ERC cage has a design similar to the previously tested rabbit cage, it has a height of 46 cm and a floor area of 6020 cm^2 (figure 9). There is a shelter that also functions as a shelf (height 13 cm) along one of the side walls and two ladders on the other two walls, and the door that constitutes the front is made of grid. As in the rabbit cage, the rats can jump, climb, and run in addition to rest and seek protection in the shelter. The NOV cage is based on the ST cage, floor area 2240 cm^2, but with a greater height, 32 cm and a shelf in the back of the cage, at 20 cm height. Four cages in a row can be connected to each other through passages on the sides of the cage lids (at 20 cm height, figure 10), which is similar to the Q cages in study III.

The passages can be opened and closed manually by the caretakers. Due to the greater height and the connected cages this cage type offers the same opportunities for active behaviours as the ERC cage. The shelves are used as resting areas and places to seek protection. The rats were kept in groups of eight in a row of four cages.

The aim was to evaluate the alternative cages in terms of their effect on physical fitness and animal welfare. In addition, it was of interest to study if the hypertensive animal model was affected by being kept in the different housing conditions. The effects of the cages were assessed in weekly body weight gain, weekly blood pressure levels using the non-invasive tail cuff technique, muscle strength as in study II, heart weight and insulin, corticosterone and glucose levels after euthanisation. Exercise tests, as in study III, were performed in the beginning and end of the study but on a laddermill instead of a treadmill. A laddermill is a modified treadmill where wooden rods are attached to the belt and the inclination of the lanes is greatly increased (Norton, Jones & Armstrong, 1990) (in this case 50°). The rats are made to climb upwards at slow speeds instead of running flat at high speeds. Studies of active behaviours in the home cage were performed as in study I. The EPM test and handling tests were performed as in study III.

Food and water intake

Food intake was recorded per cage (study II-IV) by weighing all food given and then weighing the residuals once per week. The weekly intake was then calculated as a mean per rat and day. Water consumption was also recorded per cage (study II and III) by weighing the residual in each water bottle before it was refilled and weighing the full bottle after refilling. The weekly intake was calculated as for the food intake.

Muscle analyses

For analysing enzyme activities and glycogen content, freeze-dried muscle samples were dissected free from blood, fat and connective tissue and homogenized. Enzyme activities were thereafter measured fluorometrically (Essen-Gustavsson, Karlstrom & Lindholm, 1984; Essen, Lindholm & Thornton, 1980). To determine glycogen content, the muscle tissue was boiled in HCl to hydrolyse the glycogen into glucose residues. Glucose concentration was thereafter measured fluorometrically (Lowry & Passonneau, 1973) .

Hormone analyses

The corticosterone level in urine (study II) was analysed using a RIA kit (ImmunoChem™ Double Antibody Corticosteron ^{125}I RIA kit, ICN Biomedicals Inc., Costa Mesa, CA, USA), and were counted as a ratio of creatinine (analysed using the PAP-method, MPR-1, Creatinine PAP, Roche Diagnostics, Mannheim, Germany), to correct for the volume of urine. In study IV, the levels of plasma corticosterone were analysed from the blood samples taken at euthanisation, using a RIA kit (COAT-A-COUNT, PDC, Diagnostic Products Corporation, Los

Angeles, USA). These blood samples were also analysed for plasma levels of insulin using an ELISA kit (Mercodia, Uppsala, Sweden, www.mercodia.se).

Figure 1. The shelf and bed of the indoor dog pen in study I. The dogs are wearing harnesses with pedometers attached and collars to prevent chewing.

Figure 2. The indoor dog pen used in study 1.

Figure 3. The floor pen for rats used in study II and the pilot study with objects to increase complexity.

Figure 4A-B. The standard Makrolon type III cage (A, left) used in study II, and the standard Makrolon type IV cage (B, right) used in study III, IV and pilot study (ST).

Figure 5. The rebuilt rabbit cage used in the pilot study with a shelf in the back and grid on the side walls.

Figure 6. Cage fitted with running wheel used in the pilot study.

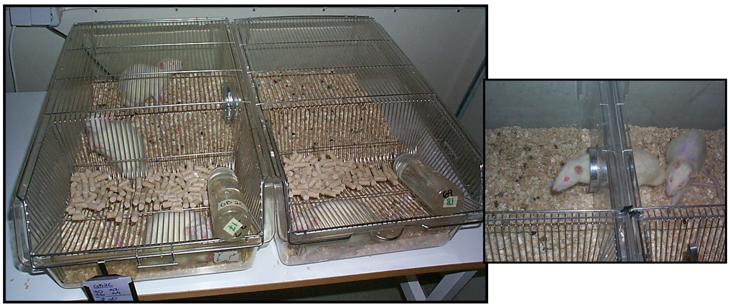

Figure 7. The Double cage (D) used in study II, made of two ST cages and the passage between the two cages.

Figure 8. The Quadruple cage (Q) used in study III, made of four ST cages. The passage between cages is similar to the D cage.

Figure 9. The Enriched Rat Cage system (ERC) used in study IV with a shelter and wall-hung ladders.

Figure 10. The Scantainer ^{NOVO} system (NOV) used in study IV with the shelf in the back of each cage and the passage between cages.

Results and discussion

Outdoor housing of laboratory dogs (study I)

The dogs doubled their activity (steps/hour) with access to the outdoor kennel, compared to only indoor housing (table 1). This was not only a novelty effect since the difference in activity persisted over the three week period of access to the outdoor kennel. A similar effect of an outdoor kennel was found by Siwak *et al.* (2003) where the decline in activity in aged dogs was partly reversed with access to an outdoor kennel. In terms of the outdoor kennel the dogs visited it 102 ± 7 times per day and there were no differences in visits between weeks. The time spent outdoors did however increase the second and third week compared to the first week of outdoor housing, which means that each visit outdoors became longer after the first week. There was a treatment effect in that the dogs that were kept in only indoor housing for the first three week period spent more time outdoors during the period of access to the outdoor kennel, compared to the group that were given the opposite treatment. The dogs also performed more active behaviours, (walking, running, jumping up or down from the shelf) during the entire period of access to the outdoor kennel. In earlier studies of the effect of pen size on activity and behaviour in dogs the results are conflicting. Hughes, Campbell & Kenney, (1989) reported that beagle dogs had a higher activity level in smaller pens, and Campbell *et al.* (1988) found no stress reaction, measured as cortisol and lymphocyte levels, in dogs that were not allowed to exercise with the conclusion that exercise was not needed. Further, Clark, Calpin & Armstrong (1991) stated that physical fitness is not necessarily correlated to pen size and in another study Clark *et al* (1997) found no effect of out-of-cage exercise on the amount of abnormal behaviours in single-housed dogs. However, in agreement with the present study, Bebak & Beck (1993) showed that dogs did use the extra space in a bigger pen. In the present study access to the outdoor kennel significantly affected activity even though the indoor pen was large and furnished which in itself should stimulate activity. As suggested by Siwak et al. (2003) it could be the combination of increased space, a more varied environment and more stimuli as a result of adding an outdoor facility that affects the activity in the dogs. An additional effect on the activity could be that the outdoor area housed a number of kennels for dogs from other sections of the dog facility. At times, this created a high level of activity outdoors which made all dogs run out to “see the action”. This is an example of local enhancement, where the activity of an animal draws the attention of another animal who then moves towards the action (Renner & Rosenzweig, 1986).

The use of a pedometer is a good, simple and inexpensive way to record activity in freely moving dogs. It requires habituation to the harness prior to recordings. There is a risk that the dogs chew their own or each others harnesses. A protection of the harness is necessary for example by applying a substance of bad taste (Tabasco was used in this study) on the harness and to provide the dogs with collars (figure 2). The pedometer also needs to be protected (a metal shield was used in this study) and firmly attached to the harness. This is a straightforward activity recording method than can easily be used on (unfortunately only) larger animals.

Clinical parameters

The access to outdoor housing did not have an overall affect on the clinical blood parameters analysed in the dogs (table 1). ALT, CHOL, WBC, GRANS and NEUT were affected by housing condition but when they were analysed only the last week of the study (to assess long-lasting effects), there was only an effect in ALT, which was higher in indoor housing. All parameters did however stay within the normal ranges for dogs. The fact that it was possible to give the dogs access to outdoor facilities without affecting the model was valuable knowledge for the users of these dogs.

Individual variations

Dogs have different temperaments and it is even suggested that they have certain personality traits that are consistent between individuals (Svartberg *et al.*, 2005). There were great individual variations in how the dogs utilised the outdoor kennel in study I. The amount of visits varied from 47 to 230 between individuals during the study. The total time the dogs could spend outside per day was 500 minutes. The shortest duration spent outside during one day was 32 minutes, which was the sum of 47 visits showing that each visit lasted less than a minute. The longest duration spent outside was 341 minutes in one dog during one day. That is more than 5 hours spent outdoors divided on 130 visits, indicating that each visit was on average more than two minutes. In reality, some visits outdoor were significantly longer. The study was performed in the summertime, and it is likely that winter conditions will decrease the amount of time spent outdoors. There was a great difference in activity within one of the pairs. With access to the outdoor kennel one of those two dogs always had 100 visits outdoor per day. The duration varied between 108 and 235 minutes. It appeared as if though he tried to keep track of what was happening both inside and outside simultaneously. His penmate had a frequency of visits between 45 and 88 and the durations varied between 71 and 255 minutes. He spent more time outside per visit than his penmate, and he did not seem to have a need to constantly check both the indoor and outdoor environment. Neither was he affected by his penmate's behaviour. It has been suggested that the level of activity might ultimately be determined by individual factors. A docile dog may have a low amount of physical activity regardless of pen size and an active dog might get plenty of exercise in a small pen (Clark, Calpin & Armstrong, 1991). It can however be discussed what type of exercise a dog can get in a small pen. Since a small pen will obstruct different types of behaviours the activity likely consists of repetitions of the same movement, which is one of the features of stereotypic behaviour. Further, it can be discussed whether the high frequency of running in and out in the above described active dog, in fact was a stereotypic behaviour. He was however the only one of the eight studied dogs who displayed this behaviour.

Activity patterns in the home cage (study II-IV and pilot study)

The pen-housed rats in study II showed a greater variety of active behaviours including walking, running and climbing. The standard housed rats only displayed walking. In the pilot study, the activity in the running wheel cages was recorded after approximately 4 weeks of housing. The average distance run for 24 hours was 5.1 ± 1.6 km for a mean time of 97 ± 17 minutes. The peak in activity was found 90 minutes after the lights went off. In study IV, SD and SH rats housed in the NOV cages showed a doubled amount of active behaviours compared to rats housed in ST cages. In addition, SD rats in the ERC cage performed more active behaviours than those in ST cages while within the SH-strain, rats in NOV cages performed more active behaviours than those in ERC cages. A common activity pattern seen in the rats in NOV cages was dashes from one end through all four cages by running and jumping between the shelves and the cage passages. This pattern was also seen in the Quadruple cages in study III. In detail, the largest category of active behaviours (apart from walking) was jumping in NOV cages (30 % of observed active behaviours) and climbing in ERC cages (20.5%). This likely reflects the different cage designs. The dashes performed in the NOV cages in combination with jumps from the cage floor up to the passages or vice versa explain a high level of jumping behaviour. The wall-hung ladders and the grid front door of the ERC cages provided opportunities for climbing behaviour. Hence, the activity patterns were slightly different between the two alternative cage types and it could be connected to exploration or home cage patrolling.

In study IV the rats used the area under the shelf (shelter) in the ERC cages extensively, which is similar to another study (Lidfors *et al.*, 2002). This shows that a shelter is an important feature in the home cage of rats, as stated in the guidelines of the Council of Europe (2006). SD rats did however use it more than SH rats. In SD rats, 62 % passive behaviours performed during 60 s were scored under the shelf. This was scored on 43 % of the recordings in SH rats. The SH rats were observed to be passive for 60s in other parts of the cage during 34 % of the recordings , while it was only scored in 12 % of the observations in SD rats. The two strains seemed to have different preferences of where to rest and the design of the ERC cage type gave them the possibility to choose location. Some individuals within the groups were also recognised to have favourite resting spots.

Social interactions (study III and IV)

In study III activity was measured as social interactions and it was higher in groups of four or eight rats, compared to pair-housed rats (table 1). The social structure within each group was established from the number of performed and received aggressive actions based on the findings that dominance in rats is reflected in the asymmetry in agonistic behaviours between individuals (Blanchard & Blanchard, 1990; Pellis & Pellis, 1991). One of the groups of eight showed the most distinct social structure that resembled a hierarchy with a main dominant male that performed many aggressive actions but received few from other individuals. This group had significantly more total social interactions than the other group of eight rats. The latter group did not show a clear social structure and it was not found in any of the groups of four or two rats either. The studies of social interactions were performed on three occasions during the seven week long study. It is possible that more observations and data would help to make the social structures clearer in all group sizes. It has however been stated that true dominance order occurs only in males that are kept with females (Tamashiro *et al.*, 2004) or that it is more likely to occur in semi-natural settings (Adams & Boice, 1989). An alternative explanation could be the different group compositions. Hurst *et al.* (1999) found more differences in behavioural and pathohysiological parameters between individual groups of the same size than between group of different sizes. The two groups of eight rats in study III differed in total amount of social interactions, pattern of social structure and body weight range. The physical environment was the same for these two groups and they received similar amount of handling, consequently the reason for the differences should be found in the social environment. In study IV, there was no difference in aggressive interactions between the different cage types in the SD rats, while the SH rats showed more aggressions in NOV cages compared to ERC cages. In total, the SD rats had a higher level of aggressive interactions compared to SH rats. Interestingly, even though no differences were found in SD rats, the impression during direct observations was that the most aggressive interactions, of all groups, were performed by the SD rats in the NOV cages. Some bite marks were found in these rats and it therefore seems that the type of aggressions was more serious and therefore came across to the observer as a higher level. It does seem to have stimulated aggression in both strains, either in frequency (SH rats) or in character (SD rats). An explanation could be that the environment was divided into sections with passages that could be guarded, which has been reported earlier (Calhoun, 1962). To improve the cage environment the NOV cages could be equipped with two passages between the cages to offer alternative escape routes.

Direct observations are low-cost studies that are time-consuming and demands concentration from the observer. It is important to be familiar with the behaviours that are to be recorded and that all observers score in the same way. The animals need to be habituated to the observer. In big groups it can sometimes be difficult to distinguish individuals, especially in albino rats, and good individual marking is therefore crucial. If the animals are to be filmed in their home environment, the equipment can be expensive. Filming have the advantage of giving records of 24-hour activity and it is possible to study episodes repeatedly. The disadvantage of filming large rat cages equipped with objects is that the view is often obstructed by features in the cages and total awareness of the cage activities is therefore almost impossible. The combination of direct observations and 24-hour filming probably gives the best results. To assess housing conditions it is absolutely necessary to quantify both the amount and type of activity and behaviours the animals perform in their home cages.

Behavioural tests (study III and IV)

Elevated plus maze (study III and IV)

The Elevated plus maze (EPM) was used in study III and IV and it was performed in the same way in both studies. In study III the rats kept in groups of four and eight had more closed arm entries and higher total activity in the EPM, compared to rats kept in pairs. Rats groups of eight had more open arm entries than pair-housed rats. There was an overall positive correlation between total home cage social interactions and total EPM activity. In paper IV, SD rats kept in NOV cages had the highest home cage activity, and a a higher total EPM activity compared to rats in ST cages. They spent significantly more time in the open arms compared to the central arena. A positive correlation was found in the SH rats in paper IV between endurance performance in the second laddermill exercise test and total activity in the EPM. The highest laddermill endurance was found in rats from NOV cages with the most active behaviours, indicating a link between home cage activity and EPM acitivity, just as in study III. Total EPM activity most likely reflects a level of exploration and locomotion (Pellow *et al.*, 1985). The EPM has been validated as a test of emotional reactivity in rats (Pellow *et al.*, 1985) and the closed arms are considered as safe areas and open arms more risky areas that requires less anxiety and more boldness to visit. In study III six rats never entered the open arms of the maze during the test and four of them were housed in pairs. Taken together, these results indicate a link between home cage activity and the level of exploration and locomotion in the rats, also in a novel environment like the EPM. The reduction of anxiety-like behaviours in the EPM as a result of exercise in the study by Fulk *et al.* (2004) was only found in visits to open arms, not in total activity. The increased time spent in open arms (paper IV) and more visits to open arms (paper III) do however indicate a reduced emotional reactivity (Pellow *et al.*, 1985) as a result of increased spontaneous activity due to a larger home cage and group housing.

The EPM is a test that is easy to perform with the use of simple and relatively inexpensive equipment and the analysis of the rats' performance is quite straight forward. It is based on the rodent's spontaneous behaviour and does not require training sessions or the use of aversive stimuli (Pellow *et al.*, 1985). Since it is validated in terms of emotional reactivity in rodents it is a relevant test to use for assessing the welfare of rats housed in cages where spontaneous activity and social interactions can increase.

Handling tests (study III and IV)

The experimental situation usually involves some handling by humans and it can be considered as a mild stressor for the animals (McDougall, Lawrence & Widdop, 2005). It has been shown that environmental enrichment can affect the ease with which the animals can be handled (Van de Weerd *et al.*, 2002). It is therefore important to show that housing rats in alternative systems does not make them more difficult to handle. No effects of group size, treadmill exercise, different cage types or strains were found on handling tests in study III and IV of this thesis (table 1). The handling tests were based on the tests carried out by Augustsson *et al.* (2002) and they found no differences between housing conditions either. However, ameliorating effects of handling have been reported in for example emotional reactivity (Andrews *et al.*, 1995; Ferguson & Cada, 2004; Joffe & Levine, 1973). All rats in study III and IV were handled extensively which might have masked an effect of cage type. Rats in the larger group sizes and cage types were shown to have a reduced emotional reactivity in the EPM test compared to rats in pairs from ST cages. This would indicate that if the extensive handling had affected the results in the handling tests, it would have affected the ST rats rather than rats from larger groups or cage types. The results from this thesis shows that rats housed in larger cages are not more difficult to handle compared to standard housed rats. This is very positive since it facilitates the introduction of alternative housing systems into the animal facilities.

The handling tests are simple and inexpensive and can be performed without extra equipment. It is important to have a skilled person who can assess the performance of the rats. The tests could easily be changed or modified to suite other needs or handling circumstances. Any modification to the rats' daily life should be evaluated for effects on handling since it could be a critical situation for the rats. Other research groups have reported that handling can be a mild stressor as shown by elevated heart rate and blood pressure in the rats (McDougall, Lawrence & Widdop, 2005; Sharp *et al.*, 2002). The handling tests used in this thesis would be further improved if these parameters were added.

Physiological parameters (study II-IV and pilot study)

Body weight, heart weight and food and water intake (study II-IV and pilot study)

Weekly body weight gain was lowered in rats by housing in the pen for four weeks in study II, by having access to a running wheel for seven weeks in the pilot study and by running on a treadmill in a moderate exercise protocol for five weeks in study III (table 1). Previous studies where physical activity has had effect on body weight in rats have had exercise protocols of greater intensity and duration. Treadmill running is often performed 5 days/week and for 60 minutes or more per session, at intensities above 20 m/min, (Fulk *et al.*, 2004; Lambert & Noakes, 1989; Sexton & Laughlin, 1994; Veras-Silva *et al.*, 1997; Wisloff *et al.*, 2002). Voluntary running in running wheels will lead to daily exercise since the rats live in the cages with the wheels, but with great individual variations in running distance as has been mentioned above. In this thesis the rats were approximately running 5 km/day after four weeks of access to running wheels. Interestingly, several studies have found a rapid effect of exercise on decreased body weight gain, *e.g.* after two (Sexton, 1995), three (Overton *et al.*, 1986), or four weeks (Afonso & Eikelboom, 2003; Lambert *et al.*, 1996) of freewheel running. In study IV, the increased home cage activity in the NOV cage only affected body weight during the second week in SD rats.. This temporary reduction in body weight gain coincides in time of housing with lower body fat content, measured with magnetic resonance technique, in six-week old SD rats housed in the same cage type and group size in another study (unpublished results). In paper IV, the rats were nine weeks old when body weight gain was reduced. The fact that the effect on body weight is more pronounced in young animals was also shown in the pen-housed rats in study II. These results indicate an early effect of increased home cage activity on body weight, which disappeared over time in study IV. Rats gain weight more or less their entire life, while cage sizes usually are constant which means that the ratio of cage space/ rat decreases over time. In combination with an age-dependent decrease in activity, it could explain the lack of a persistent reduction in body weight gain.

Cardiac hypertrophy was only seen in the treadmill exercised rats in study III (table 1). Within the running wheel group in the pilot study there was a positive correlation between running distance and relative heart weight. Ventricular hypertrophy in rats has been found after extensive treadmill exercise (Diaz-Herrera *et al.*, 2001; Wisloff *et al.*, 2001; Wisloff *et al.*, 2002) or wheel running (Sexton, 1995), while the rats in study III performed only moderate treadmill exercise. Augustsson *et al* (2002) found a cardiac hypertrophy as a result of voluntary activity in the large pen used in study II. However in that study the rats were kept six weeks longer than in the study presented here.

Food and water consumption have been recorded in papers II-IV and no differences in food intake between cage types has been recorded in any of the studies (table 1). In the Q cages in study III and the NOV cages in study IV food and water was available in all interconnected cages. In one of the Q cages the rat ate more in the first cage compared to the third cage, counting from the left. In study IV the SD rats had a higher total food intake in the first and fourth NOV-cage compared to the third, counting from the left. This could indicate that rats have preferred eating sites, maybe due to circumstances in the surrounding environment outside the cage. Since it was not shown in all groups kept in these cage types it could also be an effect of the group dynamics.

All the weighing parameters – body weight, heart weight and food and –water consumption, are inexpensive and easy to record. In recording food and water intake there is a risk of missing data if food is spilled or incorrect data if a water bottle is leaking. A disadvantage is that as soon as rats are housed more than one per cage it is not possible to get individual data on food or water consumption. In addition, water intake cannot be registered in automated watering systems. Even if individual recordings are not possible, major deviances in consumption could still be noticed. Body weight and food and water consumption can be recorded as often as may be needed, *e.g.* on a daily basis, without affecting physiology or interfere with animal behaviour. In addition, weekly weighing of the animals requires some handling, which aids in preparing them for future experimental situations that usually involve handling.

Metabolic adaptations in muscles (study II, III and pilot study)

The only effect of housing and/or exercise was found in the young rats kept in the pen in study II (table 1). They had a higher CS and HK activity and 28 % more glycogen stored in the red part of the triceps muscle, compared to single housed rats in standard cages. This indicates that the fibres of the red part of the triceps had been recruited during the spontaneous physical activity the rats performed in the pen. The higher oxidative capacity in muscles of the pen-housed rats supports a training effect and sparing of glycogen and thus an improved endurance performance. In study III and the pilot study no significant changes were seen in enzyme activities or glycogen content in muscles of the rats housed in different cages. The range of mean values for the enzyme activities (mmol/kg/min) and glycogen content (mmol/kg) between cages in the pilot study were for triceps: CS; 10-13, HAD; 15-18, LDH; 709-823, glycogen; 144-189 and for gastrocnemius: CS; 21-28, HAD; 18-21, LDH; 1178-1328, glycogen; 151-189.. The spontaneous activity in the cages may thus have been of such low intensity that no adaptations occurred in the muscle of these rats. This may be true also for the rats that were exposed to extra physical activity during the treadmill training in study III. The training intensity was moderate since the rats did not run faster than 26 m/min. These intensities have in a recent study on rats shown to correspond to about 50 % of maximal oxygen uptake (Leandro *et al.*, 2007). At these intensities mainly slow twitch oxidative and fast twitch oxidative muscle fibres get recruited and not many of the fast twitch non-oxidative fibres (Armstrong & Laughlin, 1985). In previous treadmill training studies, an increase in oxidative capacity has been noted in different muscles but the exercise intensity has been higher and the duration of training has usually been over a longer period (Fitts *et al.*, 1975; Leandro *et al.*, 2007; Siu *et al.*, 2003) An explanation for the lack of metabolic changes could also be that cross-sections were taken from the triceps and gastrocnemius muscles in study III and the pilot study. If adaptations did occur in the red muscle part only as seen in study II this may have been difficult to detect as the pieces of muscle analysed also consisted of many fast-twitch non-oxidative fibres. This is supported by the lower oxidative capacity in the triceps muscle in study III and the pilot study compared with the results from the triceps muscle in study II.

The analyses of the muscle enzymes and glycogen content are expensive and labour intensive and demand laboratory skills both in cleaning freeze-dried muscle pieces from connective tissue and in the analysis procedure. However, these are valid parameters to use for studying muscular adaptations to exercise.

Exercise tests using treadmill or laddermill (study III and IV)

The rats in study III that had been trained on the treadmill had both lower blood lactate levels and greater endurance than the untrained rats when performing the exercise test (table 1). Effects of group housing were also found where rats in groups of four and eight had lower blood lactate levels compared to pair-housed rats. The positive effect of a reduction in lactate production is that the capacity for prolonged exercise increases. Exercise tests with increasing intensities have been performed earlier in rats on a treadmill (Pilis *et al.*, 1993). The values for blood lactate during the test in this study are in good agreement with those from Pilis *et al.* (1993) which describes anaerobic threshold in rats. Some problems were encountered when training rats on the treadmill in study III as they varied in their willingness to run on the treadmill and 69 % of the rats succeeded in both exercise tests. Some of the failures were however due to problems with the blood sampling (samples were to be taken within 60 s after the treadmill work). It has been reported that the running style of rats on the treadmill consists of short sprints followed by stops and sniffing of the lane (Wisloff *et al.*, 2001). This running style requires long lanes of the treadmill, which was not the case with the commercially manufactured “rodent treadmill” used in study III. The treadmill was modified by changing the black front wall to a Perspex® front with ventilation holes where the rats could poke out their noses. The aim was to give a feeling of open lanes and stimulate the rats’ curiosity as opposed to forcing them to run towards a black, solid wall. Forced running on a treadmill could be considered a stressful experience for the rats and therefore induce not only positive physiological adaptations but also maladaptive conditions indicative of chronic stress (Moraska *et al.*, 2000). This could be connected to the type of aversive stimuli used. One of the most commonly used aversive stimuli is electric shocks. In this thesis electricity was not used, but a bowl of ice-chilled water was put under the treadmill instead. In addition, the rats were given honey puffs as reward in connection with the running sessions to minimise stress. It is however possible that the stress response could depend upon several factors, for example intensity of the training (Moraska *et al.*, 2000). The exercise protocol performed in study III was of a moderate character and only performed 3 day/ week.

Due to the difficulties to get all rats to run on the treadmill, it was modified to a laddermill for the exercise tests in study IV. The laddermill has been tested on rats that refused to run on the treadmill and they successfully climbed the laddermill using only mild prodding (Norton, Jones & Armstrong, 1990). Since rats are excellent climbers, laddermill exercise might be more suitable than a treadmill exercise. All rats in study IV were willing to climb on the laddermill. The reason why only 77% succeeded in both exercise tests was mainly related to the blood sampling procedure. Rats of both strains kept in the NOV-cages in study IV had the best performance on the laddermill, mainly in the endurance test (table 1). An interesting effect when comparing both strains was that between the first and the second exercise test (7 weeks of housing in the different cage types) the ST housed rats showed elevated lactate levels. For the rats kept in NOV and ERC cages the lactate levels were unchanged between the tests. This indicates that housing in ST cages with limited opportunities for physical activity leads to a deterioration in physical performance but that this deterioration is prevented in activity-stimulating cage types such as NOV and ERC.

The exercise tests demands specific equipment and a number of persons to carry out the tests. The blood sampling is a critical component since it has to be taken within 60 s after the rats were taken from the treadmill/laddermill. It requires a skilful person and habituation to the animals of the procedure prior to the test. There is possibly a component of stress in the exercise test with repeated blood sampling and being pushed to perform on the treadmill/laddermill. Another complication is the variation in performance of the rats, which however was improved with the laddermill. It can however give valuable information about metabolic responses to exercise and endurance capacity if it is performed in well habituated animals with skilled staff.

Muscle strength (study II, IV and pilot study)

The pen-housed rats had a greater maximum strength and a greater endurance in the inclined plane test compared to single rats in standard cages (table 1). In the pilot study, rats kept in the rebuilt rabbit cage performed better than rats kept in ST cages in both tests on the inclined plane. Interestingly the running wheel rats had a very poor performance and all other groups performed better in the maximum strength test. Similar effects of running wheels were found by Johansson & Ohlsson (1996). The test uses the rats' climbing skills and in the running wheels no climbing was possible. In contrast, if the rats tried to climb in the wheel it would just turn. Therefore the results from the inclined plane likely reflect a lack of technique rather than a lack of muscle strength. No differences were found between rats in the three cage types in study IV in either maximum strength or endurance. The best performing individuals were however housed in NOV cages.

The advantages of using the inclined plane test to assess muscle strength are that it is based on rats' natural climbing ability, non-invasive, and it is easy to perform at a low cost. Rats that have been active and climbed in their home cage tend to have a greater performance in the test. The backside of using this method is that it seems to be influenced by the individual rat's temperament and possibly also by the level of handling. Rats that are handled extensively, *i.e.* 4-5 days/week, seem to be less intimidated by humans and some of them are difficult to persuade to sit still and hold on to the plane. They might just immediately let go and never try to hold on, despite prodding. Previously the inclined plane test has been used to assess motor functions after neurological injuries (Johansson & Ohlsson, 1996; Rivlin & Tator, 1977). It can be modified further to give better results better results when measuring muscle strength.

Hormones (study II-IV)

The moderate treadmill exercise in paper III resulted in plasma insulin levels that were 36 % lower in trained compared to control rats (table 1) which is in agreement with earlier findings (James, Kraegen & Chisholm, 1984). No effects of cage types were found in either of the strains used in study IV and the SH rats developed high insulin levels in accordance with the normal course of development of the model (Mondon & Reaven, 1988; Reaven & Chang, 1991). Glucocorticoids are often used as markers for stress, however, the physiological effect of glucocorticoids is to make energy available for activity and/or emergency situations. Plasma corticosterone measures the acute situation while urinary corticosterone reflects the pre-collecting situation, *i.e.* the housing condition. Neither urinary corticosterone (study II) nor plasma corticosterone (study IV) were affected by housing conditions (table 1). In the study by Augustsson *et al.*(2002) the urinary corticosterone levels were higher in the pen-housed rats compared to those in standard cages and the authors suggested an effect of increased activity. Voluntary wheel running has also been shown to increase corticosterone levels that however dropped to normal levels after four weeks of running (Fediuc, Campbell & Riddell, 2006).

Blood pressure (study IV)

Systolic blood pressure was recorded in both SD and SH rats in study IV No differences were found between cage types for either strain (table 1). and the development of hypertension in SH rats was according to the pattern previously described (Evenwel & Struyker-Boudier, 1979; Hoffmann *et al.*, 1987). Several studies have reported lowering effects exercise on blood pressure (Evenwel & Struyker-Boudier, 1979; Hoffmann *et al.*, 1987; Overton *et al.*, 1986; Tipton *et al.*, 1983) . They are however all effects of more extensive exercise on a treadmill, in running wheels or by swimming and not results of only home cage activity. The SH strain is known to be hyperresponsive to a number of external stimuli (Tang, Gandelman & Falk, 1982; Tipton *et al.*, 1983) and it can be discussed whether the tail-cuff recordings, as performed in this study, reflects the effect of the home environment or the effect of the recording situation. It has been shown that handling of SH-pups during the neonatal period can ameliorate the hypertension (Ferguson & Cada, 2004; Tang, Gandelman & Falk, 1982) and that SH rats show an adaptation in their cardiovascular responses to repeated handling (McDougall, Lawrence & Widdop, 2005). Handling has also been reported to reduce emotional reactivity in rats (Andrews & File, 1993; Ferguson & Cada, 2004; Joffe & Levine, 1973). All rats in study IV were handled extensively and it could have erased some effects of the different cage types. Some of the SH rats were very easy to handle and were sitting perfectly still during the recordings which could be an effect of acceptance of the situation or of severe stress. Since these rats showed decreased/low levels SBP in these situations it probably reflected acceptance.

The tail-cuff technique to record blood pressure in rats is a non-invasive and comparatively inexpensive method. It requires restraining and proper habituation of the rat. It might measure the effect of the recording situation more than anything else and measurements in the home cage are not possible. It can be difficult to get a pulse in the tail artery without preheating the rat, but preheating can affect the blood pressure. It also requires practise to analyse the results. The alternative way to measure blood pressure is by using telemetry which can record the pressure on undisturbed animals in their home environment. It does however require expensive equipment and surgical implantation of the transmitters into the rats. The limitation so far has also been that it only can record one animal at a time in the same cage and it requires several receivers per cage to get reliable results in larger cages. Apart from the high price, these factors have prevented the use of telemetry in the studies presented in this thesis. It would otherwise have been the best alternative.

Individual variations (study II-IV and pilot study)

Variations between individuals within rat strains were encountered in different tests in the studies. In the pilot study, the rat with the greatest running wheel distance ran 13.4 km during 24 hours and the rat with the shortest distance ran 1.4 km during 24 hours. This is in line with the earlier findings of short-medium-long distance runners in rats (Afonso & Eikelboom, 2003; Lambert *et al.*, 1996; Narath, Skalicky & Viidik, 2001; Rodnick *et al.*, 1989; Sexton, 1995). It has previously been reported that some rats refuse to run on a treadmill and therefore had to be excluded (Lambert & Noakes, 1990; Norton, Jones & Armstrong, 1990; Sexton & Laughlin, 1994). Only two out of 23 of the rats that were exercised on the treadmill in study III always performed well and ran without hesitation. In the recordings of social interactions in the home cages in study III one rat, kept in a group of four, was never seen displaying any aggressive interactions. Another rat, kept in a group of eight (Q cage), was only seen performing eight social interactions during all observations. The mean total number of interactions for the rest of the rats kept in Q cages was 56 (29-103). As mentioned above, in the EPM test in study III six rats never entered the open arms. Four of them were pair-housed rats, but not cage-mates, and the last two was one from a D cage and one from a Q cage. Two rats from each group size refused to cooperate in the mouth gag cooperation handling test in study III. One of the two rats in S cages was one of the individuals that never entered the open arms in the EPM and the other had a low total activity in the EPM. Three rats in study IV showed the same refusal and they were two SD-rats from the ERC cage and one SH rat from the NOV cage. The SH rat and one of the SD rats had a high total activity in the EPM.

In general, the individual variations between rats in this thesis were not affected by cage type or group size. The environmental and test circumstances were the same for all rats within a study. Thus, it can be concluded that even though inbred animals were used individual behavioural profiles exist. Despite aims to control factors in the animal environment these individuals stand out from the crowd. The group dynamics could possibly explain some of these findings, as the results in study III and the study by Hurst et al. (1999) showed variations between different groups of the same size. In addition, Eskola *et al*. (1999)found effects of litters in variations in parameters in rats five weeks after weaning and suggested that littermates should be evenly allocated into all treatments if possible. Würbel (2001) states that standardisation of the environment could result in animals that are hypersensitive to certain situations and that systematic variation in environmental enrichment would be the best solution to guarantee valid experimental results.

Table 1. The parameters studied in the thesis and their possible implications for health, well-being and welfare of the animals (left) and the effects of the different housing conditions tested (and treadmill exercise in study III) on the parameters (right). SD = Sprague Dawley rats, SH = Spontaneously Hypertensive rats, T = treadmill training, RW= running wheels

Parameter	Health	Well-being	Welfare	Study I dogs	Study II SD rats	Pilot study SD rats	Study III SD rats	Study IV SD & SH rats
Measures of physical activity								
Body weight gain	X	X	X	– –	↓	↓ and ⇆	↓(T) and ⇆	⇆
Food intake	X	X	X	– –	⇆	⇆	⇆	⇆
Muscle oxidative capacity & glycogen content	X			– –	↑	⇆	⇆	– –
Muscle strength	X			– –	↑	↑	– –	⇆
Exercise test performance	X		X	– –	– –	– –	↑	↑
Relative heart weight	X			– –	⇆	⇆	↑(T) and ⇆	⇆
Blood pressure	X			– –	– –	– –	– –	⇆
Insulin	X			– –	– –	– –	↓(T) and ⇆	⇆
Steps (pedometer)	X		X	↑	– –	– –	– –	– –
Active behaviours, home cage	X	X	X	↑	↑	↑ (RW)	↑	↑
Measures of emotional reactivity								
Activity in EPM test		X	X	– –	– –	– –	⇆(T) and ↑	↑
Risk assessment in EPM test		X	X	– –	– –	– –	⇆(T) and ↑	– –
Effects on the animal model								
Clinical parameters	X	X	X	⇆	– –	– –	– –	– –
SH rat model in different cage types			X	– –	– –	– –	– –	⇆
Handling tests		X	X	– –	– –	– –	⇆	⇆
Others								
Corticosterone	X	X	X	– –	⇆	– –	– –	⇆

↑increases ↓ decreases ⇆ not affected – – not tested

General discussion

In all the studies in this thesis, the animals have been offered an increased cage/ pen size and complexity. Both dogs and rats used the space and resources given to them and increased their spontaneous activity. Table 1 is an attempt to summarize all the parameters studied and if they can affect health, well-being and welfare in the animals. Activity and active behaviours of the dogs increased when they had access to an outdoor kennel. In all the rat studies home cage activity affected parameters related to exercise (table 1). The most pronounced effects were found in study II, with young rats housed in the largest cage type tested. In the other three studies factors like age, cage size and length of study differed from study II and resulted in varying training effects. Effects of physical activity are dependent on duration, intensity and frequency of the activity (Henriksson & Sundberg, 2003). The benefit of being able to perform active behaviours was the main effect. However, a secondary effect was the physical activity per se. To further detect effects of physical activity, parameters that are more sensitive than the ones used in this thesis need to be identified. In addition, the increased activity prevented the hypostimulation caused by standard cages, which ought to improve the rats' capacity to meet environmental challenges (Korte, Olivier & Koolhaas, 2007).

When effects of cage type and home cage activity on emotional reactivity were measured in the rats, it was positively affected by increased home cage activity (table 1). A higher home cage activity increased the exploration and level of locomotion in the EPM test and it resulted in more time in the open arms. An increased behavioural repertoire was seen during the observations of home cage behaviour in larger cages (table 1). These cage environments offered choices for the rats regarding where to locate themselves and where to perform certain behaviours. It was for example possible for them to choose to seek protection in a shelter or on top of a shelf instead of having no options as in a standard cage. This gave them an increased control over their situation, which is one of important features of improved well-being and welfare (Baumans, 2005). The physiological hormone corticosterone is often used as an indicator of stress and bad welfare. In this thesis it was measured in study II and IV without any alterations.

Effects of alternative housing systems on the animal model were evaluated in study I, III and IV (table 1). The outdoor housing of the dogs in study I did not affect the clinical parameters to the extent that they deviated from the normal range for dogs. Therefore, the minor differences that were found do probably not have biological relevance. The dogs can thereby be given the opportunity to be outdoors without affecting the scientific results, thus combining Refinement and Reduction. The results in study IV showed that the physiological features typical of the hypertensive rat model were not influenced by an increased home cage activity as expressed by group means and standarderrors. To further analyse variations within groups in the results will add more knowledge about possible effects of housing conditions on the number of animals needed for statistical significance and thereby taking two of the 3R's into consideration (Refine and Reduce). In addition, neither group size (study III) nor cage type (study IV) affected the rats' willingness to be handled in common experimental procedures (table 1). Similar results were found by Augustsson *et al*. (2002) in rats kept in the same type of pen as was used in study II. This indicates that housing rats in alternative cages should not affect the experimental outcome or the accuracy of the animal model used..

Taken together, the results from this thesis show a qualitative benefit from alternative housing.. Both rats and dogs can better control their situation in a bigger cage/pen and they can choose locations and activities. Both species displayed a wider variety of behaviours as a result of environmental and social stimulation and individual variations in the utility of resources were seen. It provides them with a greater capacity to meet environmental challenges, in or out of the home environment. The physical and mental health parts of the physical fitness triangle are stimulated with an increased health, well-being and welfare for the animals.

Recommendations for housing of laboratory rats

The NOV cage, when four cages are connected, resembles the burrow systems of rats in the wild. Small passages opens up into burrows so the rats have to jump down to enter into a cage, have access to food and water etc. Each rat has to pass trough every cage in order to get an overview of the current situation in the entire home cage. This induces a higher activity level with more active behaviours and the rats were observed dashing through all four cages by jumping between the passages and the shelves. The ERC system on the other hand, offers one big area, which the rats easily can overlook by sitting anywhere in the cage. It offers choices of places to rest and possibilities to explore the cage in more dimensions – high and low, deep and shallow. The big shelf is a great shelter in threatening situations and it is large enough to house all rats simultaneously. With the big opening of this cage towards the animal room, it seems like the rats are more perceptive of what is going on in the room. Presumably influences/signals from people etc reach the rats more easily through the large grid front. In the larger cages the rats have a less monotonous daily life. Apart from eating and resting the rats can jump and climb and explore various dimensions of the cage. They can choose whether to sleep in the shelter with the group or alone on the shelf or in the open cage. This will give them totally different views of the surroundings. The idea of connecting cages (as the Q cage in study III and NOV cage in study IV) could be a practicable option for alternative housing of rats. It gives the possibility to adjust cage size after the current size of the group. Each cage in itself must of course provide for the needs of the rats housed in that particular cage. The connection of cages cannot substitute for a bad design of each of theconnected cages. If a small group size would be kept in only one of these cages they will still suffer from bad housing.

Rats do not exercise for the pleasure of exercise itself. They are active for a reason, to explore their surrounding or to reach a resource like food or the company of others, or perhaps to seek solitude. To induce a higher activity level in the home cage it is therefore necessary to make rats work for the resources by making them less easily available. An alternative to the connection of several cages would be to have one larger cage divided into sections which the rats have to work to get access to. This would be possible by taking the idea behind the connected NOV cages and transfer it into a cage similar to the ERC System. Such a large cage could be partitioned into *e.g.* 2/3 and 1/3 of the space by a wall made of Perspex® or a similar see-through material. The wall would be perforated with ventilation holes that also function as "climbing steps" for the rats. On the upper part of this Perspex® wall there are two passages where the rats can move between the two divisions of the cage. The rats have to climb up the wall in order to get access to the entire cage and resources in the different sections. There are two doors to the cage, one opening to 2/3 division and one opening to the 1/3 division. The smaller division of the cage could be used when animal caretakers need to take rats out of the cage. They simply gently shove the rats into the smaller division through another passage in the wall, at floor level, close the passage and then the rats can be fetched from a smaller area, which is easier. In addition, the smaller division could be used for other purposes, *e.g.* post-operative recovery of rats that cannot be put straight back into the group or by mothers who want to get away from their pups.

Conclusions

- Dogs and rats use the increased space and resources offered to them and increase their spontaneous activity in their home environment.

- Physical activity increases animal welfare mainly in a qualitative aspect, by means of a greater frequency and variety of active behaviours affecting physical and mental health. This is the result of a housing environment that stimulates voluntary activity through increased space and volume.

- All alternative housing conditions caused an increase in both physical and social activity and thereby improved the animal welfare.

- The alternative housing conditions did not affect animal models as they were tested in this thesis. These findings should be one step forward on the way towards a greater acceptance of alternative housing systems that put the animals' welfare in focus.

- An alternative cage for rats should be sectioned, allow climbing and have shelters. Most important for animal welfare is group housing.

Future research

- To deduce the effects of deviating individual responses on the within-group variation and what factors that causes these responses in some individuals.

- To asses possibilities for rats to perform adequate risk assessment in improved cage types.

- To study if effects of positive states or emotions can be detected in rats and dogs kept in improved housing systems to learn more about the emotional health of the animals in these systems.

Populärvetenskaplig sammanfattning

Idag används flera miljoner försöksdjur per år i Europa. År 2005 användes i Sverige totalt ca 505 700 djur varav 83 000 råttor och ca 1200 hundar i biomedicinsk forskning, utbildning samt toxikologisk testning. Försöksdjuren spenderar största delen av sina liv i sina hemburar. Traditionell inhysning av försöksdjur har främst utformats för att tillgodose ekonomiska och ergonomiska krav och djurens behov och välfärd har kommit i andra hand. Under de senaste decennierna har försöksdjurens välfärd kommit i fokus och mycket forskning har utförts inom detta område. Vetenskapen om djurens välfärd har traditionellt fokuserat på avsaknaden av negativa effekter som t.ex. dålig tillväxt, stress och stereotypier, snarare än positiva effekter som t.ex. ett mentalt upplevt välbefinnande. Svårigheten med djur är att man inte kan fråga dem och få ett direkt svar på hur de själva upplever sin välfärd.

Syftet med den här avhandlingen har varit att studera effekter av försökshundars- och råttors spontana aktivitet i alternativa inhysningssystem. Dessa alternativa system har haft ökad yta och höjd och varit berikade med olika föremål som givit en ökad komplexitet, jämfört med standardinhysning. Effekter av hembursaktiviteten har mätts i både fysiologiska och beteendeparametrar och utvärderats med avseende på deras betydelse för djurens välfärd. Ytterligare ett syfte med studierna har varit att hitta nyckelfaktorer i hembursmiljön som är viktiga för råttans välfärd och därigenom kunna utforma en ny bättre råttbur. Välfärdsaspekten har fokuserats på vikten av att djuren kan ha kontroll över sin hemmiljö och utföra sina naturliga beteenden. Detta har sin utgångspunkt i den svenska djurskyddslagen. En stor del av de naturliga beteendena för både råttor och hundar är kopplade till social och fysisk aktivitet. Dagens standardinhysning karaktäriseras av att det är en kal, ostimulerande miljö som är helt förutsägbar vilket resulterar i leda och inaktivitet, understimulering, hos djuren. Detta kan orsaka att de system i kroppen som behövs för att anpassa sig till plötsliga förändringar och nya situationer (t.ex. centrala nervsystemet eller immunförsvaret) nedregleras och blir okänsliga. Den kala miljön medför även brist på valmöjligheter och alternativ vilket resulterar i att djuren inte har kontroll över sin situation. Målet är att förbättra djurens välfärd genom att i inhysningen erbjuda en mer varierad miljö med möjlighet till val och kontroll samt ökad aktivitet.

Inom biomedicinsk forskning är det viktigt att kunna kontrollera faktorer i djurens miljö för att vara säker på att det är de experimentella behandlingarna av djuren som givit de resultat man erhållit i en studie, och inte andra okontrollerbara eller oförutsedda faktorer. I djurrummen sker därför en noggrann kontroll av ljus, temperatur, luftfuktighet, ventilation, burmaterial, foder och vatten, strömaterial i burarna etc. noga. En subklinisk infektion kan ha en förödande inverkan på försöksresultaten varför det också är av största vikt med ett bra smittskydd. En kombination av denna rigorösa kontroll, ekonomi, ergonomi och praktisk skötsel har lett till utvecklandet av inhysningssystem för försöksdjur som är lätta att hantera, rengöra och förvara, prisvärda och/eller möjliggör inhysning av många djur på liten yta. Detta har resulterat i att de typer av burar som använts till försöksdjur de senaste decennierna främst tillgodoser djurens basala behov såsom att kunna äta, dricka och vila/sova. Storleken på dagens standardinhysning begränsar möjligheterna till normal fysisk och social aktivitet för djuren. Detta leder till att många försöksdjur är inaktiva och därmed blir överviktiga. Fysisk aktivitet har många kända positiva effekter på välfärd/välbefinnande, hos människor såväl som djur. Positiva effekter av en ökad aktivitetsnivå kan vara lägre kroppsvikt och mindre andel kroppsfett, sänkt blodtryck, ökad uthållighet och styrka i muskler, förbättrade hjärt-kärlfunktioner, ett stärkt immunförsvar samt en depressionshämmande effekt. I djurstudier har man även funnit en ångestdämpande effekt av fysisk aktivitet, en förbättring av inlärning och minne, samt nybildning av neuroner i hjärnan. Summan av dessa effekter gör att en fysiskt aktiv individ får en förbättrad hälsa och välfärd.

Både hundar och råttor är sociala, aktiva och undersökande djur och håller man dem på en begränsad yta leder det till inskränkningar av deras aktiva och sociala beteenden. Nivån av fysisk aktivitet hos hundar och råttor påverkas av interaktioner med artfränder och/eller med miljön. Det krävs dock en inhysningsmiljö som är tillräckligt rymlig och utformad på ett för arten lämpligt sätt, samt att djuren hålls i grupper. Hundar är mycket sociala djur och gruppinhysning är den enskilt viktigaste faktorn när man håller dem som försöksdjur. Möjlighet till fysisk aktivitet utanför hemboxen rekommenderas starkt i föreskrifter om försökshundar. Den aktiviteten är lika viktig för hundars sociala samvaro med andra hundar och med människor som för möjligheten att utföra fler aktiva beteenden. Den traditionella inhysningen av försökshundar har inneburit att de hållits i små boxar och individuell inhysning har förekommit, främst av praktiska skäl. Begränsningar i hundarnas möjlighet att få vistas utomhus är också vanliga. Studien på hundar i denna avhandling syftade till att utvärdera effekter av tillgång till utevistelse på vanliga kliniska parametrar (för kontroll av njur-, lever- och pankreasfunktioner och immunförsvaret) hos hundarna samt deras fysiska aktivitet och utförande av aktiva beteenden. Det var också av intresse att utvärdera hur hundarna nyttjade utemiljön. Totalt 8 hundar (beaglar) hölls antingen enbart inomhus eller med fri tillgång till en uterastgård under dagtid. Inga skillnader hittades i de kliniska parametrarna mellan hundar som enbart hölls inomhus eller med tillgång till en uterastgård. Med möjlighet till utevistelse fördubblade hundarna sin aktivitet, mätt som steg/timme m.h.a. en pedometer och de uppvisade också en högre frekvens av aktiva beteenden. Det fanns stora individuella skillnader i hur hundarna utnyttjade uterastgården, både i antal besök/dag samt i hur lång tid de spenderade ute varje dag.

Försöksråttor är en domesticerad form av brunråttan *(Rattus norvegicus)* och i vilt tillstånd lever brunråttan i stora kolonier i komplexa system av hålor och gångar under marken. De är nattaktiva djur och undersöker sitt närområde regelbundet i jakt på mat mm. Råttor, som är bytesdjur, undviker öppna ytor och håller sig nära väggar och andra strukturer för att inte exponera sig för rovdjur. Det är därför viktigt att deras inhysningsmiljö har en viss strukturell variation och att den kan delas upp i olika avdelningar för olika aktiviteter, t.ex. en bolåda eller liknande som påminner om deras hålor i det vilda. Råttor är skickliga klättrare och en höjd och utformning av buren som möjliggör klättring är därför viktigt. Råttor är ett av de djurslag som används som försöksdjur idag som har sämst burmiljö. De hålls i burar som i förhållande till råttornas storlek är både för små och för låga och därigenom begränsar deras möjlighet att vara fysiskt aktiva och utföra en mängd av sina naturliga beteenden. Små burar begränsar även antalet djur som kan hållas i buren och därigenom antalet sociala interaktioner som råttorna kan utföra.

I tre studier på råttor har effekten av ökad spontan aktivitet i olika inhysningssystem utvärderats i både fysiologiska och beteenderelaterade parametrar. Burtyperna har givit råttorna olika möjligheter till fysisk aktivitet och/eller sociala interaktioner. Några råttor har även tränats på rullmatta för att kunna kvantifiera effekter av måttlig fysisk aktivitet på deras fysiologi och beteende. Den avslutande studien utvärderade två olika typer av kommersiellt tillgängliga alternativa burtyper för råttor. Dessa burar hade en ökad volym och innehöll strukturer som gav råttorna möjlighet att klättra, hoppa, springa samt komma undan högt uppe på hyllor eller i en bolåda. I alla studier har hanråttor av en vanlig förekommande stam, Sprague Dawley, använts och i den avslutande studien kompletterades de med en råttmodell för högt blodtryck, den så kallade spontant hypertensiva råttan (SHR). Eftersom fysisk aktivitet kan ha en blodtryckssänkande effekt så är det relevant att studera om burar som möjliggör en ökad fysisk aktivitet därmed också påverkar denna djurmodell. Burtypers påverkan på djuren skulle kunna vara en av de miljöfaktorerna som kan förändra försöksresultaten. Resultaten i denna avhandling har visat att råttor som hölls i grupp i en stor lösdrift på golvet hade lägre kroppsviktsökning, ökad oxidativ kapacitet och inlagring av glykogen i musklerna, ökad muskelstyrka samt uppvisade fler olika aktiva beteenden jämfört med ensamhållna råttor i standardburar. Den grupp av råttor som tränats på rullmattan hade lägre nivåer av laktat i blodet samt bättre uthållighet i arbetsprov på rullmattan, lägre kroppsvikt och ökad relativ hjärtvikt samt lägre plasmanivåer av insulin jämfört med otränade individer. Råttor som hölls i grupper om fyra eller åtta uppvisade fler sociala interaktioner i hemburen samt en förbättrad prestation i ett arbetsprov utfört på rullmatta, jämfört med dem som hölls i par. Den ökade sociala aktiviteten i hemburen speglades också i ett beteendetest, ”Elevated Plus Maze (EPM)” som mäter råttornas undersökande beteende, riskbedömning av potentiellt farliga sitatuationer samt risktagande. De råttor som hölls i grupper om åtta hade en ökad aktivitet i detta test, uppvisade ett högre risktagande genom att besöka ”farliga” zoner i testarenan oftare/längre samt uppvisade mer riskbedömningbeteenden. För ett bytesdjur som råttan är det viktigt att kunna göra en riskbedömning av en hotfull situation (ett naturligt beteende) för att kunna ta ett beslut om vilken handlingsstrategi som är mest lämplig i den aktuella situationen. Detta leder till en ökad kontroll över situationen vilket är en bidragande faktor till en förbättrad välfärd. Även i en kontrollerad miljö som en försöksdjursanläggning kan situationer som råttorna uppfattar som hotfulla uppstå. I den sista studien uppvisade råttorna, av båda stammarna, dubbelt så mycket aktiva beteenden i kommersiellt tillgängliga alternativa burar jämfört med standardburar. Denna ökade aktivitet avspeglades också i en bättre prestation och ökad uthållighet i ett arbetsprov utfört på en ”klättermatta” och en ökad total aktivitet i EPM testet. En klättermatta är en rullmatta med pinnar fästa på bandet som lutas kraftigt (här 50°) så att råttorna får klättra uppåt i långsam fart istället för att springa framåt på en plan rullmatta. Eftersom råttorna är sådana skickliga klättrare kan detta test passa dem bättre än ett test på rullmatta. Den totala aktiviteten i EPM testet är ett mått på råttornas undersökande och allmänna aktivitetsnivå. Studien visade också att den ökade aktiviteten i de alternativa burarna inte påverkade/fördröjde utvecklingen av högt blodtryck hos den hypertensiva råttstammen.

Den spontana aktiviteten ökade med en större bur- /boxyta och både hundarna och råttorna utnyttjade de resurser som erbjöds dem. Det är viktigt att inte bara öka ytan/volymen utan också se till att inhysningen utformas så att den tillgodoser de beteendemässiga behov som arten i fråga har. Effekterna av hembursaktiviteten på fysiologiska parametrar hos råttorna varierade mellan de olika studierna. Den största effekten hittades hos unga råttor som hölls i lösdriften på golvet. Denna typ av inhysning är inte den mest lämpliga att ha i stor skala ur rent praktisk och skötselmässig synpunkt. De mer ekonomiska och arbetsmässigt realistiska alternativa burtyperna har en miljö som resulterar i måttliga fysiska aktivitetsnivåer hos råttorna. Det har ändå lett till högre frekvens av både aktiva beteenden och sociala interaktioner samt vissa träningseffekter. Både hundarna och råttorna har uppvisat en mer varierad beteenderepertoar. Dessutom finns det individuella variationer i aktivitetsnivå hos både hundar och råttor. De alternativa inhysningsformerna som testats här representerar en varierad miljö där kontroll och valmöjlighet är möjlig. Den ökade aktiviteten visar att understimuleringen i standardburar har motverkats med följd att djuren är bättre förberedda på förändringar i miljön, t.ex. en försökssituation. Det har snarare resulterat i en kvalitativ förbättring än kvantitativa förändringar i fysiologiska hälsoparametrar.

Ett syfte med studierna var att hitta nyckelfaktorer som ökar råttornas välfärd i hemmiljön och med hjälp av dem föreslå hur en förbättrad råttbur skulle kunna utformas. Aktivitet i sig är inget självändamål för råttor utan de är aktiva för att de behöver söka information, mat eller andra resurser. De burtyper som testats som har varit uppdelade i flera sektioner har skapat högst aktivitet hos råttorna. För att få fullständig information om vad som händer i hemmiljön måste hela buren besökas, vilket kräver aktivitet i form av att råttan förflyttar sig från den ena avdelningen till den andra. Ett bra sätt att göra det på är att utnyttja deras fantastiska förmåga att klättra. En stor och hög bur uppdelad i sektioner som råttorna endast når genom passager högst upp i buren skulle kunna stimulera sådan aktivitet. De olika sektionerna kan utrustas med olika resurser t.ex. en bolåda i ena delen och en hög hylla i den andra och råttorna måste då förflytta sig för att använda de olika resurserna.

Sammanfattningsvis visar resultaten i denna avhandling att både hundar och råttor utnyttjar den yta och de resurser som erbjudits dem och det medförde en ökad spontan aktivitet i hemmiljön. Individuella variationer i aktivitet visar att det är värdefullt för djuren att kunna ha kontroll över sin egen situation samt att kunna välja när och var de vill utföra en viss aktivitet. Resultatet blir en mer aktiv vardag för djuren i en varierad miljö som kan tillgodose individens behov och därigenom förbättras deras fysiska och mentala hälsa och välfärd.

References

Adams, N. & Boice, R. 1989. Development of dominance in domestic rats in laboratory and seminatural environments. *Behavioural Processes 19*, 127-142.

Afonso, V.M. & Eikelboom, R. 2003. Relationship between wheel running, feeding, drinking, and body weight in male rats. *Physiol Behav 80*, 19-26.

Andrews, J.S., Jansen, J.H., Linders, S., Princen, A. & Broekkamp, C.L. 1995. Performance of four different rat strains in the autoshaping, two-object discrimination, and swim maze tests of learning and memory. *Physiol Behav 57*, 785-90.

Andrews, N. & File, S.E. 1993. Handling history of rats modifies behavioural effects of drugs in the elevated plus-maze test of anxiety. *Eur J Pharmacol. 235*, 109-12.

Armstrong, R.B. & Laughlin, M.H. 1983. Blood flows within and among rat muscles as a function of time during high speed treadmill exercise. *J Physiol 344*, 189-208.

Armstrong, R.B. & Laughlin, M.H. 1985. Metabolic indicators of fibre recruitment in mammalian muscles during locomotion. *J Exp Biol 115*, 201-13.

Augustsson, H., Lindberg, L., Hoglund, A.U. & Dahlborn, K. 2002. Human-animal interactions and animal welfare in conventionally and pen-housed rats. *Lab Anim 36*, 271-81.

Barnett, S. 1963. *The Rat: A Study in Behaviour. Principles of Ethology and Behavioural Physiology, displayed mainly in the Rat.* 1st edition. Aldine Publishing company. Chicago, USA.

Baumans, V. 2005. Science-based assessment of animal welfare: laboratory animals. *Rev Sci Tech. 24*, 503-13.

Bebak, J. & Beck, A.M. 1993. The Effect of Cage Size on Play and Aggression Between Dogs in Purpose-bred Beagles. *Laboratory Animal Science 43*, 457-459.

Bekoff, M., Daniels, T.J. & Gittleman, J.L. 1984. Life history patterns and the comparative social ecology of carnivores. *Ann Rev Ecol Syst 15*, 191-232.

Berglund, B. 1988. Hjärtats anpassning vid konditionsträning. In: *Konditionsträning i terori och praktik*. Eds. A. Forsberg & B. Saltin). Idrottens Forskningsråd och Svenska Riksidrottsförbundet. pp. 40-46.

Blanchard, D.C. & Blanchard, R.J. 1990. Behavioral correlates of chronic dominance-subordination relationships of male rats in a seminatural situation. *Neurosci Biobehav Rev. 14*, 455-62.

Blanchard, D.C., Blanchard, R.J., Tom, P. & Rodgers, R.J. 1990. Diazepam changes risk assessment in an anxiety/defense test battery. *Psychopharmacology (Berl). 101*, 511-8.

Boice, R. 1977. Burrows of wild and albino rats: effects of domestication, outdoor raising, age, experience, and maternal state. *J Comp Physiol Psychol 91*, 649-61.

Boice, R. 1981. Behavioral comparability of wild and domesticated rats. *Behav Genet 11*, 545-53.

Boissy, A., Manteuffel, G., Jensen, M.B., Moe, R.O., Spruijt, B., Keeling, L.J., Winckler, C., Forkman, B., Dimitrov, I., Langbein, J., Bakken, M., Veissier, I. & Aubert, A. 2007. Assessment of positive emotions in animals to improve their welfare. *Physiol Behav., doi:10.10167/j.physbeh.2006.10.018*.

Boitani, L., Francisci, P., Ciucci, P. & Andreoli, G. 1995. Population biology and ecology of feral dogs in central Italy. In: *The domestic dog, its evolution, behaviour and interactions with people.* (Ed. J. Serpell). Cambridge University Press. Cambridge, United Kingdom. pp. 217-244.

Brain, P. & Benton, D. 1979. The interpretation of physiological correlates of differential housing in laboratory rats. *Life Sci. 24*, 99-115.

Broom, D.M. 1986. Indicators of poor welfare. *Br Vet J. 142*, 524-6.

Calhoun, J.B. 1962 Population density and social pathology. *Sci Am. 206*, 139-48

Campbell, S.A., Hughes, H.C., Griffin, H.E., Landi, M.S. & Mallon, F.M. 1988. Some effects of limited exercise on purpose-bred Beagles. *American Journal of Veterinary Research 49*, 1298-1301.

Clark, J.D., Calpin, J.P. & Armstrong, R.B. 1991. Influence of type of enclosure on exercise fitness of dogs. *American Journal of Veterinary Research 52*, 1024-1028.
Clark, J.D., Rager, D.R. & Calpin, J.P. 1997. Animal well-being. I. General considerations. *Lab Anim Sci. 47*, 564-70.
Clark, J.D., Rager, D.R., Crowell-Davis, S. & Evans, D.L. 1997. Housing and Exercise of Dogs: Effects on Behavior, Immune Function, and Cortisol Concentration. *Laboratory Animal Science 47*, 500-510.
Council of Europe. 2006. Appendix A of the European Convention for the protection of vertebrate animals used for experimental and other scientific purposes (ETS no. 123). Ed. C.o. Europe. pp. 103.
de Jong, J.G., van der Vegt, B.J., Buwalda, B. & Koolhaas, J.M. 2005. Social environment determines the long-term effects of social defeat. *Physiol Behav. 84*, 87-95.
Diaz-Herrera, P., Torres, A., Morcuende, J.A., Garcia-Castellano, J.M., Calbet, J.A. & Sarrat, R. 2001. Effect of endurance running on cardiac and skeletal muscle in rats. *Histol Histopathol 16(1)*, 29-35.
Eskola, S., Lauhikari, M., Voipio, H.-M., Laitinen, M. & Nevalainen, T. 1999. Environmental enrichment may alter the number of rats needed to reach statistical significance. *Scandinavian Journal of Laboratory Animal Science 26*, 134-144.
Essen-Gustavsson, B., Karlstrom, K. & Lindholm, A. 1984. Fibre types, enzyme activities and substrate utilisation in skeletal muscles of horses competing in endurance rides. *Equine Vet J. 16*, 197-202.
Essen, B., Lindholm, A. & Thornton, J. 1980. Histochemical properties of muscle fibres types and enzyme activities in skeletal muscles of Standardbred trotters of different ages. *Equine Vet J. 12*, 175-80.
Evenwel, R. & Struyker-Boudier, H. 1979. Effect of physical training on the development of hypertension in the spontaneously hypertensive rat. *Pflugers Arch. 381*, 19-24.
FAWC, F.A.W.C. Five Freedoms of Farm Animal Welfare Council. http://www.fawc.org.uk/freedoms.htm (accessed 9 September 2007).
Favier, R.J., Constable, S.H., Chen, M. & Holloszy, J.O. 1986 Endurance exercise training reduces lactate production. *J Appl Physiol 61(3)*, 885-9.
Fediuc, S., Campbell, J.E. & Riddell, M.C. 2006. Effect of voluntary wheel running on circadian corticosterone release and on HPA axis responsiveness to restraint stress in Sprague-Dawley rats. *J Appl Physiol. 100*, 1867-75.
Ferguson, S.A. & Cada, A.M. 2004. Spatial learning/memory and social and nonsocial behaviors in the spontaneously hypertensive, Wistar-Kyoto and Sprague-Dawley rat strains. *Pharmacol Biochem Behav. 77*, 583-94.
Fitts, R.H., Booth, F.W., Winder, W.W. & Holloszy, J.O. 1975. Skeletal muscle respiratory capacity, endurance, and glycogen utilization. *Am J Physiol. 228(4)*, 1029-33.
Fuentes, I., Cobos, A.R. & Segade, L.A. 1998. Muscle fibre types and their distribution in the biceps and triceps brachii of the rat and rabbit. *J Anat. 192 (Pt 2)*, 203-10.
Fulk, L.J., Stock, H.S., Lynn, A., Marshall, J., Wilson, M.A. & Hand, G.A. 2004. Chronic physical exercise reduces anxiety-like behavior in rats. *Int J Sports Med 25*, 78-82.
FYSS 2003. *FYSS. Fysisk aktivitet i sjukdomsprevention occh sjukdomsbehandling*. Yrkesföreningen för Fysisk Aktivitet (YFA) och Statens Folkhälsoinstitut. Stockholm, Sweden.
Henriksson, J. & Sundberg, C.J. 2003. Allmänna effekter av fysisk aktivitet. In: *FYSS. Fysisk aktivitet i sjukdomsprevention occh sjukdomsbehandling*. (Ed. A. Ståhle). Yrkesföreningen för Fysisk Aktivitet (YFA) och Statens Folkhälsoinstitut. Stockholm, Sweden. pp. 9-32.
Hetts, S. 1991. Psychologic well-being: conceptual issues, behavioral measures, and implications for dogs. *Vet Clin North Am Small Anim Pract. 21*, 369-87.
Hetts, S., Clark, J.D., Calpin, J.P., Arnold, C.E. & Mateo, J.M. 1992. Influence of housing conditions on beagle behaviour. *Applied Animal Behaviour Science 34*, 137-155.
Hite, M., Manson, H.M., Bohidar, N.R., Conti, P.A. & Mattis, P.A. 1977. Effect of cage size on patterns of activity and health of beagle dogs. *Laboratory Animal Science 27*, 60-64.

Hoffmann, P., Friberg, P., Ely, D. & Thoren, P. 1987. Effect of spontaneous running on blood pressure, heart rate and cardiac dimensions in developing and established spontaneous hypertension in rats. *Acta Physiol Scand 129*, 535-42.
Hubrecht, R.C. 1993. A comparison of social and environmental enrichment methods for laboratory housed dogs. *Applied Animal Behaviour Science 37*, 345-361.
Hughes, H.C., Campbell, S. & Kenney, C. 1989. The Effects of Cage Size and Pair Housing on Exercise of Beagle Dogs. *Laboratory Animal Science 39*, 302-305.
Hurst, J.L., Barnard, C.J., Tolladay, U., Nevision, C.M. & West, C.D. 1999. Housing and welfare in laboratory rats: effects of cage stocking density and behavioural predictors of welfare. *Anim Behav. 58*, 563-586.
James, D.E., Kraegen, E.W. & Chisholm, D.J. 1984. Effect of exercise training on whole-body insulin sensitivity and responsiveness. *J Appl Physiol. 56*, 1217-22.
Joffe, J.M. & Levine, S. 1973. Effects of weaning age and adult handling on avoidance conditioning, open-field behavior, and plasma corticosterone of adult rats. *Behav Biol. 9*, 235-44.
Johansson, B.B. & Ohlsson, A.L. 1996. Environment, social interaction, and physical activity as determinants of functional outcome after cerebral infarction in the rat. *Exp Neurol 139*, 322-7.
Jordbruksdepartementet 1988. Djurskyddslag (1988:534). Ed. S. Riksdag.
Jordbruksverket. http://www.sjv.se/amnesomraden/djurveterinar/djurskydd/statistik/forsoksdjursstatistik.4.b1bed211329040f5080003148.html. (accessed 30 July 2007).
Korte, S.M., Olivier, B. & Koolhaas, J.M. 2007. A new animal welfare concept based on allostasis. *Physiol Behav., doi:10.1016/j.physbeh.2006.10.018*.
Lambert, M.I. & Noakes, T.D. 1989. Dissociation of changes in VO2 max, muscle QO2, and performance with training in rats. *J Appl Physiol. 66*, 1620-5.
Lambert, M.I. & Noakes, T.D. 1990. Spontaneous running increases VO2max and running performance in rats. *J Appl Physiol 68*, 400-3.
Lambert, M.I., Van Zyl, C., Jaunky, R., Lambert, E.V. & Noakes, T.D. 1996. Tests of running performance do not predict subsequent spontaneous running in rats. *Physiol Behav 60*, 171-6.
Larsson, P., Burlin, L., Jakobsson, E. & Henriksson-Larsen, K. 2002. Analysis of performance in orienteering with treadmill tests and physiological field tests using a differential global positioning system. *J Sports Sci. 20*, 529-35.
Leandro, C.G., Levada, A.C., Hirabara, S.M., Manhaes-de-Castro, R., De-Castro, C.B., Curi, R. & Pithon-Curi, T.C. 2007. A program of moderate physical training for Wistar rats based on maximal oxygen consumption. *J Strength Cond Res 21(3)*, 751-6.
Lidfors, L., Wichman, A., Ewaldsson, B. & Lindh, A.-S. 2002. Does larger enriched cages have an effect on behaviour of two strains of laboratory rats? In: *Proceedings of the 36th International Congress of the ISAE, August 6-10, 2002* the Netherlands.
Lowry, O.H. & Passonneau, J.V. 1973. *A Flexible System of Enzymatic Analysis*. Academic Press. New York.
MacDonald, D.W. & Carr, G.M. 1995. Variation in dog society: between resource dipsersion and social flux. In: *The domestic dog, its evolution, behaviour and interactions with people.* (Ed. J. Serpell). Cambridge University Press. Cambridge, United Kingdom. pp. 200-216.
McDougall, S.J., Lawrence, A.J. & Widdop, R.E. 2005. Differential cardiovascular responses to stressors in hypertensive and normotensive rats. *Exp Physiol. 90*, 141-50.
Mering, S., Kaliste-Korhonen, E. & Nevalainen, T. 2001. Estimates of appropriate number of rats: interaction with housing environment. *Lab Anim 35*, 80-90.
Mondon, C.E. & Reaven, G.M. 1988. Evidence of abnormalities of insulin metabolism in rats with spontaneous hypertension. *Metabolism. 37*, 303-5.
Moraska, A., Deak, T., Spencer, R.L., Roth, D. & Fleshner, M. 2000. Treadmill running produces both positive and negative physiological adaptations in Sprague-Dawley rats. *Am J Physiol Regul Integr Comp Physiol. 279*, R1321-9.
Mueller, D.T., Herman, G. & Eikelboom, R. 1999. Effects of short- and long-term wheel deprivation on running. *Physiol Behav 66*, 101-7.

Narath, E., Skalicky, M. & Viidik, A. 2001. Voluntary and forced exercise influence the survival and body composition of ageing male rats differently. *Exp Gerontol 36*, 1699-711.
Newberry, R.C. 1995. Environmental enrichment: Increasing biological relevance of captive environments. *Applied Animal Behaviour Science 44*, 229-243.
Nieman, D.C. 2000. Special feature for the Olympics: effects of exercise on the immune system: exercise effects on systemic immunity. *Immunol Cell Biol. 78*, 496-501.
Norton, K.I., Jones, M.T. & Armstrong, R.B. 1990. Oxygen consumption and distribution of blood flow in rats climbing a laddermill. *J Appl Physiol 68*, 241-7.
Overton, J.M., Tipton, C.M., Matthes, R.D. & Leininger, J.R. 1986. Voluntary exercise and its effects on young SHR and stroke-prone hypertensive rats. *J Appl Physiol 61*, 318-24.
Pellis, S.M. & Pellis, V.C. 1991. Role reversal changes during the ontogeny of play fighting in male rats: attack vs defense. *Aggressive Behavior 17*, 179-189.
Pellow, S., Chopin, P., File, S.E. & Briley, M. 1985. Validation of open:closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. *J Neurosci Methods 14*, 149-67.
Pette, D. & Staron, R.S. 1988. Molecular basis of the phenotypic characteristics of mammalian muscle fibres. *Ciba Found Symp 138*, 22-34.
Pilis, W., Zarzeczny, R., Langfort, J., Kaciuba-Uscilko, H., Nazar, K. & Wojtyna, J. 1993. Anaerobic threshold in rats. *Comp Biochem Physiol Comp Physiol. 106*, 285-9.
Reaven, G.M. & Chang, H. 1991. Relationship between blood pressure, plasma insulin and triglyceride concentration, and insulin action in spontaneous hypertensive and Wistar-Kyoto rats. *Am J Hypertens. 4*, 34-8.
Renner, M.J. & Rosenzweig, M.R. 1986. Social interactions among rats housed in grouped and enriched conditions. *Dev Psychobiol. 19*, 303-13.
Rivlin, A.S. & Tator, C.H. 1977. Objective clinical assessment of motor function after experimental spinal cord injury in the rat. *J Neurosurg. 47*, 577-81.
Rodnick, K.J., Reaven, G.M., Haskell, W.L., Sims, C.R. & Mondon, C.E. 1989. Variations in running activity and enzymatic adaptations in voluntary running rats. *J Appl Physiol 66*, 1250-7.
Rose, R.J. & Hodgson, D.R. 1994. Clinical Exercise Testing. In: *The Athletic Horse. Principals and Practise of Equine Sports Medicine*. Eds. R.J. Rose & D.R. Hodgson). W.B. Saunders Company. Philadelphia, USA.
Ruis, M.A., te Brake, J.H., Buwalda, B., De Boer, S.F., Meerlo, P., Korte, S.M., Blokhuis, H.J. & Koolhaas, J.M. 1999. Housing familiar male wildtype rats together reduces the long-term adverse behavioural and physiological effects of social defeat. *Psychoneuroendocrinology. 24*, 285-300.
Russell, W.M.S. & Burch, R.L. 1959. *The Principles of Humane Experimental Technique*. Methuen & Co, Ltd, UK. London.
Sexton, W.L. 1995. Vascular adaptations in rat hindlimb skeletal muscle after voluntary running-wheel exercise. *J Appl Physiol 79*, 287-96.
Sexton, W.L. & Laughlin, M.H. 1994. Influence of endurance exercise training on distribution of vascular adaptations in rat skeletal muscle. *Am J Physiol 266*, H483-90.
Sharp, J.L., Zammit, T.G., Azar, T.A. & Lawson, D.M. 2002. Stress-like responses to common procedures in male rats housed alone or with other rats. *Contemp Top Lab Anim Sci. 41*, 8-14.
Siu, P.M., Donley, D.A., Bryner, R.W. & Alway, S.E. 2003 Citrate synthase expression and enzyme activity after endurance training in cardiac and skeletal muscles. *J Appl Physiol 94(2)*, 555-60.
Siwak, C.T., Tapp, P.D., Zicker, S.C., Murphey, H.L., Muggenburg, B.A., Head, E., Cotman, C.W. & Milgram, N.W. 2003. Locomotor activity rhythms in dogs vary with age and cognitive status. *Behavioral Neuroscience 117*, 813-24.
Svartberg, K., Tapper, I., Temrin, H., Radesäter, T. & Thorman, S. 2005. Consistency of personality traits in dogs. *Animal Behaviour 69*, 283-291.
Tamashiro, K.L., Nguyen, M.M., Fujikawa, T., Xu, T., Yun Ma, L., Woods, S.C. & Sakai, R.R. 2004. Metabolic and endocrine consequences of social stress in a visible burrow system. *Physiol Behav. 80*, 683-93.

Tang, M., Gandelman, R. & Falk, J.L. 1982. Amelioration of genetic (SHR) hypertension: a consequence of early handling. *Physiol Behav. 28*, 1089-91.
Tipton, C.M., Matthes, R.D., Marcus, K.D., Rowlett, K.A. & Leininger, J.R. 1983. Influences of exercise intensity, age, and medication on resting systolic blood pressure of SHR populations. *J Appl Physiol. 55*, 1305-10.
Van de Weerd, H.A., Aarsen, E.L., Mulder, A., Kruitwagen, C.L., Hendriksen, C.F. & Baumans, V. 2002. Effects of environmental enrichment for mice: variation in experimental results. *J Appl Anim Welf Sci. 5*, 87-109.
Wells, D.L. & Hepper, P.G. 2000. The influence of environmental change on the behaviour of sheltered dogs. *Applied Animal Behaviour Science 68*, 151-162.
Veras-Silva, A.S., Mattos, K.C., Gava, N.S., Brum, P.C., Negrao, C.E. & Krieger, E.M. 1997. Low-intensity exercise training decreases cardiac output and hypertension in spontaneously hypertensive rats. *Am J Physiol 273*, H2627-31.
Wikipedia. http://en.wikipedia.org/wiki/Physical_fitness. (accessed 20 September 2007).
Wisloff, U., Helgerud, J., Kemi, O.J. & Ellingsen, O. 2001. Intensity-controlled treadmill running in rats: VO(2 max) and cardiac hypertrophy. *Am J Physiol Heart Circ Physiol 280*, H1301-10.
Wisloff, U., Loennechen, J.P., Currie, S., Smith, G.L. & Ellingsen, O. 2002. Aerobic exercise reduces cardiomyocyte hypertrophy and increases contractility, Ca2+ sensitivity and SERCA-2 in rat after myocardial infarction. *Cardiovasc Res. 54*, 162-74.
Wurbel, H. 2001. Ideal homes? Housing effects on rodent brain and behaviour. *Trends Neurosci. 24*, 207-11.
Wurbel, H. 2002. Behavioral phenotyping enhanced--beyond (environmental) standardization. *Genes Brain Behav. 1*, 3-8.
Östensson, C.-G. & Henriksson, J. 2003. Diabetes mellitus - typ 2 diabetes. In: *FYSS. Fysisk aktivitet i sjukdomsprevention occh sjukdomsbehandling.* . (Ed. A. Ståhle). Yrkesföreningen för Fysisk Aktivitet (YFA) och Statens Folkhälsoinstitut. Stockholm, Sweden. pp. 147-156.

Acknowledgements

The work of this thesis was only possible thanks to the help and support from number of people (and other creatures). To all of you, thank you! In particular, I would like to express my sincere gratitude to:

My troika of supervising ladies - you truly complement each other and I feel fortunate to have had you as my supervising team:

Kristina Dahlborn– my main supervisor and a very wise lady. You are an experienced supervisor with an amazing feeling for science and research and for the welfare of animals. You always formulate the right and important questions. I have felt very safe and secure and fortunate with you as my main supervisor and you are probably at your best in critical situations. You are always there for me when I really need you and I think you have believed in me more than I have done myself.

Katarina Cvek-Hopkins - my co-supervisor and a very cheerful lady, working on being a very wise lady. You have a fantastic feeling for the handling of animals and you are great support in the work at the animal facility. With your inventive and stubborn mind you can solve any practical problem that arises during the work of the studies. Your fantastic linguistic skills have been extremely valuable and have made my PhD-life a lot easier!

Birgitta Essén-Gustavsson – my co-supervisor and also a very wise lady. My other experienced supervisor, with a great feeling and love for science and research. Your enthusiasm for data and results is very encouraging and stimulating and I am amazed by your deep knowledge in the exciting field of exercise physiology. I hope we have enriched your life with knowledge about the nature of rats during these years!

The animal caretakers at the HVC facility –Åsa, Olga, Karin and Hans for caring about the animals as much as I do and for performing such an important job in such a superb way! Karin, you are a great friend and a great “hundvakt” and I never could have trained the rats on the treadmill without you! Åsa, see you soon at “Fysiologen” and Hans, I truly hope we can have you back with our rodents again!

Former and present PhD-students at the KompFysMed section - Hanna, Anna G, Märit, Robert, Joana and Sarah for sharing ups and downs in the life of a PhD-student. You have been very supporting and stimulating and I wish you all the very best! Robert, thank you for being so patient and helpful with my endless computer questions. Joana, I hope to visit you in Portugal soon!

Miia – for the daily dog walks with discussion about horses, dogs and life. You are a funny Finnish girl with a big heart!

Kristina E – for being so helpful whenever I have stupid small questions about anything. We all love you and we could not cope without you! Kjell-Åke for life-saving efforts in the computer world. You are something of a magician. Kristina K for valuable skills and help in the lab.

The rest of the KM-corridor people – Manne, Kia, Pia, Anna E, Therese, Åsa, Magda, Janne, Annette, Claes and Marie for amusing discussions abut highs and lows in the coffee room. In the end, we are always the ones who know best!

All the people at the animal facilities at Astra-Zeneca in Mölndal for the fantastic support during the dog study, making it so much easier to perform a great study.

Scanbur BK and SVA for kindly letting us borrow cages, Mats Sjöquist for letting us borrow your telemetry system (and Britta!) and for being such a nice travelling company, Björn Ekesten for letting us use the PowerLab, and Åsa Holmberg for kindly participating in the handling tests.

Christina Remes for working so hard with me and the rats in the last study.

All the animals taking part in my studies – you did a great job! I love dogs and rats and I believe that the perfect animal would be a combination of these two species.

Who ever it was that invented duct-tape and post-it notes. My studies would not have been possible to perform without the aid of these marvellous inventions!

All my fellow dog-lovers and great friends at the Uppsala Working Dog Club. I really enjoy the many hours in the forest or at the club sharing our favourite interest – training dogs. It is the best way of getting my mind off work and if it was possible I would do it all day, every day! I look forward to many more hours and many more dogs.

All my dear friends who I met in Uppsala 11 years ago and without whom I would not be the person I am today. Whether you have chosen to move far away or stay in dear old Uppsala (good choice Karin, Anna, Emma, Calle!) you will always mean very much to me. Remember that we have to get together and dance every now and then!

My family:
Pappa and Alona for relaxing visits with great food and interesting discussions. Pappa, thank you for always caring a little bit extra for the welfare of my car!

Daniel and Lisa for being such great, relaxing company whether it is skiing in the mountains or boat-tripping on the sea or just hanging out in Vallentuna. I look forward to many more lovely dinners and thrilling Yatzy-games!

Mamma for always taking care of me and Hallon. You are the greatest of mothers!

Hallon – so much love and joy in such a small dog.