SLU

Effect of metabolic cage housing on rodent welfare

Photograph by Emilia Tarland

Av
Emilia Tarland

Handledare: Katarina Cvek-Hopkins
Inst. för kliniska vetenskaper
Examinator: Kristina Dahlborn

_____

Sammanfattning

Metabolismburar är framtagna för att kunna studera totalintag och utsöndring (metabolism) av olika ämnen, göra totaluppsamlingar av urin och faeces samt kunna ta okontaminerade prov av urin och faeces. Det har däremot gjorts lite forskning för att påvisa eventuella välfärdsproblem hos gnagare placerade i metabolismburar. Gnagare hållna socialt isolerade kan få förhöjda nivåer av kortikosteron och bli mer känsliga för stress jämfört med grupphållna individer. Studier har också funnit förändringar i det centrala nervsystemet och immunsystemet hos individuellt hållna gnagare. Det finns flera dokumenterade negativa effekter av galler golv som t.ex. sår och nervskador i råttors baktassar, förhöjt blodtryck, förhöjd hjärtfrekvens och kroppstemperatur. Speciellt möss visar en stark preferens för bäddmaterial och avsaknad på detta kan vara stressframkallande. Det behövs mer forskning på hur hållning i metabolismburar kan påverka gnagares välfärd så att en metabolismbur som både ger okontaminerade prov av urin och faeces och tillåter djuren att utföra sina naturliga beteenden, kan utvecklas.

Abstract

The metabolic cage is developed to be able to have a control of total intake of feed and water and the excretion with urine and faeces. In addition, one can efficiently collect non contaminated samples of urine and faeces. For the animal, housing in a metabolic cage involves isolation and problems with cage enrichment since that can interfere with the total collection of urine and faeces. However little research has been done to investigate possible welfare problems for rodents placed in metabolic cages. Housing rodents socially isolated may lead to elevated corticosterone levels and more vulnerability to stress compared to group housed individuals. Studies have also found changes in the central nervous system and the immune system in individually housed rodents. Several negative effects of housing on grid floor are documented e.g. lesions and nerve injury in the hind feet of rats, elevated blood pressure, heart rate and body temperature. Especially mice show a strong preference for nesting material and lack of such may be stressful. More research on the effects of metabolism cage housing on rodent welfare are needed to develop a metabolic cage which enables sampling of uncontaminated urine while allowing the animals to perform their natural behaviours.

Introduction

In many scientific studies and in veterinary medicine, collecting urine and faeces for analyses is of great importance. In scientific studies housing in metabolic cages enables examination of e.g. new substances or feed. Urine and faecal analyses can give answers of health and physiological status of an animal. There are several different methods for urine and faeces collection in use and one of the most widespread methods for 24 hour collection is the use of a metabolic cage (Kurien et al., 2004). The metabolic cage exposes the animals to social isolation since the animal is placed by itself without any possibility for social contact. Social isolation is argued to be stressful for mice and rats (Greco et al., 1989; D'Arbe et al., 2002; Nagy et al., 2002). The metabolic cage also exposes the animals for grid floor and lack of nesting material which may influence the animal negatively (Heidbreder et al., 2000; Van Loo et al., 2003).

There are few scientific studies performed on the influence of a metabolic cage on the welfare of an animal. Since many physiological factors are involved when an animal is exposed to a

stressful environment, it may be crucial not only for the wellbeing of the animal, but also for the outcome and reliability of the experiment.

Stress can be defined as factors that alter the internal environment of the body (Sjaastad et al., 2003). The body has some major adaptations to stress in order to maintain homeostasis and prepare for physical activity, such as increased glucocorticoid and catecholamine secretion. Glucocorticoids are secreted from the adrenals as an effect of stimulation from the central nervous system via the hypothalamus and anterior pituitary. In rodents the main glucocorticoid secreted when exposed to stress, is corticosterone (Qiang et al., 2004). Glucocorticoids have immunosuppressive mechanisms and inhibit the expression of multiple inflammatory genes such as cytokines (IL-1, IL-2, IL-3, IL-4, IL-5, IL-6,and IFN-γ), enzymes, receptors and adhesion molecules (Barnes, 1998). The sympathic nervous system is activated under stressful conditions to enhance the physical performances of the body (Sjaastad et al. 2003). Secretions of catecholamines are derived from the adrenal medulla and the main catecholamines involved are adrenaline, noradrenaline and dopamine. Adrenaline and noradrenaline acts as both neurotransmitters and hormones and affects e.g. heart rate, blood pressure and blood glucose levels in order to prepare the body for physical activity.

Enriching the environment for laboratory animals is becoming more and more common and several studies are performed to exhibit the effects of nesting material, cage area, group size and social isolation on the animals wellbeing and physiological features (for review for mice see Olsson & Dahlborn, 2002).

Since rodents are the primary animals used in experimental studies, the aim of this review is to discuss the potential animal welfare issues of keeping rats and mice in metabolic cages. (According to the Swedish Animal Welfare agency, 213 727 mice and 83 321 rats were used in experimental purposes in Sweden 2005). The effects of metabolic cage housing on laboratory rodent's welfare, such as the effect of social isolation, effects of small cage area with minimal possibility to exercise, lack of nesting material and grid flooring, will be reviewed.

Methods for urine & faeces collection

In some studies a total collection of urine and faeces might not be necessary and instead a single sample can be used. Collection of animal urine and faeces is important for analyses of different metabolites and toxic products (Kurien et al., 2004). Urine analysis is one of the most frequently used methods to gain information about the health of an animal. A clinical urine analyse can include e.g. measurement of pH, protein, glucose, bilirubin, haemoglobin and ketone levels. In biochemical as well as nutritional, toxilogical, behavioural and physiological experiments, urine analyses of laboratory animals are an important source of information.

To obtain a non contaminated urine sample, it is important to ensure that the urine does not come in contact with faeces or feed. Furthermore, the collection method should be non expensive and easy to perform. A commonly used method for collecting urine and faeces in biomedical studies is the use of a special metabolic cage and it is debated whether animals in a metabolic cage are exposed to a stressful environment (Eriksson et al., 2004). There is very little published on the potentially negative effects on the animals from being kept in these cages and there is a recent debate concerning the welfare of the animals kept in metabolic cages (Cvek-Hopkins, personal communication).

There are alternative methods that enable single sampling of urine and faeces without any major intervention of the animal but for a 24 hour collection with total control over feed and water intake, metabolic cage utilization may be the only option.

Metabolic cages

Metabolic cages were first developed to examine e.g. digestibility, nutritive substances and nutritional values of different feeds for farm animals in order to compile feed tables. Investigations involving metabolic cages have also provided important information about animals' nutritional requirements (Dahlborn, personal communication).

There are several companies that manufacture commercial metabolism cages for laboratory animals (Techniplast; Harvard apparatus; Braintree scientific). The metabolic cage is designed to avoid contamination of the urine and effectively separate urine and faeces into collection tubes outside of the cage (Kurien et al., 2004). Metabolic cages are used to gain information about metabolic function and how different factors affect the metabolism of the animal (Harvard apparatus). The time the animal is placed in the metabolic cage is normally 24 hours or longer. Metabolic cage housing is assessed to be a moderate degree of difficulty according to the Swedish Animal Welfare agency, which means that housing in these cages may convey some inconvenience for the animals.

A typical metabolic cage is constructed with an upper chamber made of transparent, gnaw-proof polycarbonate (Kurien et al., 2004; Harvard apparatus). A feed chamber is located outside the cage and the size of the feed chamber is designed to prevent rodents to sleep or nest inside. The feed chamber contains of a drawer that is easy to pull out to simplify filling with minimum disturbance of the animal. This drawer is usually not designed to hold ordinary feed pellets but liquids, slurries or powders, to prevent the animal from dragging feed into the cage. The construction of the feeding chamber and drawer prevents urine from getting contaminated with feed. The water bottle is calibrated, located outside the cage and is made of polycarbonate. Under the water bottle there is a calibrated spillage collecting tube which prevents water from entering the cage and contaminating the urine. The spillage collecting tube is calibrated and enables the investigator to calculate the actual water intake of the animal. The cage has grid floor and the urine flows down in the middle of a funnel under the cage to the urine collection tube which is graded in cubic centimetres. Faeces roll down on the side of the funnel into a specific faeces tube that can be removed from outside the cage to prevent disturbance of the animal. See picture 1 for an example of a metabolic cage of older model where the cage is constructed of gratings instead of plastic.

Picture 1. Rat placed in a metabolism cage of older model. Photograph by Emilia Tarland

Alternative methods for occasional urine samples

Kurien and Scofield (1999) describe methods that involved the use of plastic wrap to collect pure urine from mice. They placed clear plastic wrap upon a white paper sheet located outside the animal's home cage. The mouse was then transferred onto the plastic wrap and kept there until it urinated. Using this method urine volumes from 10 to 250 µl could be obtained as fast as in 12 seconds.

In another method for collection of small urine and faeces amounts, the rodent is placed in an empty plastic cage until it urinates. The animal is transferred back into its home cage and the samples are aspirated using a pipette (Dahlborn et al. 1996; Augustsson et al., 2002).

Induced urination

Watts (1971) and Khosho et al. (1985) describe methods that involve pressure on the lower part of the abdomen, over the urinary bladder, of rodents to induce urination. According to

Watts (1971) gentle pressure over the bladder of a mouse could induce urination in volumes from 30 to 100 µl.

Khosho et al. (1985) describes a similar method used on rats. Instead of a petri dish a polystyrene beaker was attached onto the perineal wall with tape that was adherent on both sides. After the beaker was attached the rat was held in its tail and stimulated to urinate. Volumes of 0.1 to 0.8 ml urine was obtained in a few seconds from 80 % of the rats while it took the remaining 20 % approximately 5 to 10 minutes to urinate. Using this method it was found that shaving and cleaning of the beaker attachment area was necessary to avoid contamination of the urine. Holding the beaker by hand was found to be faster but resulted in more spilling and contamination with faeces.

Research on metabolic cage housing of rodents

According to Gil et al. (1999) who compared stress responses of young (three months) and old rats (twelve months) housed in metabolic cages for 7 days, rats of different ages respond differently to isolation stress when housed in a metabolism cage. Locomotion activities in young rats decreased and this may reflect an emotional disturbance. The results in this study indicate that isolation stress through metabolism cage housing is more stressful for young rats.

Eriksson et al. (2004) found that young male rats housed in metabolic cages had reduced weight gains, reduced faecal immunoglobulin A and produced more amounts of faeces after three days of metabolism cage housing. Serum levels of immunoglobulin A decreases when high doses of glucocorticoids is excreted (Griffin & Thomson, 1998) although Eriksson et al. (2004) did not find elevated levels of corticosterone due to the three days stay in metabolism cages. In the performed study the authors argue that the stable corticosterone levels indicated that housing in metabolic cages was not highly stressful to laboratory rats, although the decrease of immunoglobulin A and reduced weight gain might prove the opposite.

Effects of social isolation on rodent welfare

Mice and rats in the wild live in social groups with complex dominance systems (Jensen 1993). Mice show two categories of social behaviour, passive and active social contact (Van der Weerd et al., 1997). Passive social contact is expressed when mice sleep with body contact and provides warmth and security. Both mice and rats sleep together when held in groups and it has been shown in preference tests that mice prefer company over environmental enrichment i.e. supply of nesting material (Van Loo et al., 2004). When mice could choose between a cage without cage mates and an inhabited cage, there was significantly more time spent in the inhabited cage. The results showed that both young and adult mice preferred to share a common sleeping site and usually slept close together irrespective of social status. In the light phase, increasing age was correlated with a significant higher preference for social contact over nesting material. In a study by Van Loo et al. (2001) they found that both dominant and subordinate males preferred to sleep in near contact with other males regardless of their relationships.

 According to Yamada et al. (1999) single housed wild-type mice beyond weaning age, consumed less feed than group housed mice at the first stadium of the experiment. They also found that isolated wild-type mice increased their frequencies of stereotypic behaviours.

Nagy et al. (2002) found that singled-housed C57BL/6J mice had significantly lower weights due to lower soft-lean tissue mass and significantly lower bone mineral content, compared to mice held in groups. In this study hormone levels was not examined, but an increase in corticosterone levels due to stress might lead to a decrease in bone mineral content and soft-lean tissues. High concentrations of glucocorticoids stimulate degradation of fats and proteins in order to increase the plasma concentration of fatty acids and amino acids. Glucocorticoids also increase the rate of bone absorption and reduce the bone formation rate when high levels are secreted over long periods. (Sjaastad et al. 2003; Nagy et al., 2002; Takeshita et al., 2000). Corticosterone is secreted from the adrenal cortex when an animal is exposed to a stressor and can be measured by blood tests or by determination in urine and faeces (Bamberg et al., 2001). Hunt & Hambly (2006) investigated the influence of different housing conditions on stress levels by measuring corticosterone levels in faeces. They found no significant differences in corticosterone levels in single housed mice compared to group housed mice accept on day one and three when corticosterone levels were increased in single housed mice. Hunt and Hambly (2006) propose the need for a minimum 14 days acclimatisation period to ensure that the results of studies that involve individual housing do not get misleading. In contrast to their results, Greco et al. (1989) found a significant increase in corticosterone levels in individually housed rats.

Bartolomucci et al. (2003) examined how individual housing in different extent, influence immuno-endocrine functions in three month old male mice (experiment 1). They also studied the effect of housing conditions on the reaction of the animals when exposed to an acute mild stressor (experiment 2). In the first experiment a male mouse from a sibling group was placed individually in a cage while three of his siblings were randomly selected and placed together. The mice were housed for 1, 7, 14, 21 and 42 days and after the animals were euthanised, corticosteroids, lymphocytes proliferation, β-endorphin concentration, splenocytes and cytokines levels were determined. The result of single-housing versus group-housing revealed no significant differences in corticosterone levels or body weight in any time point. There was a 3 % decrease in body weight of the singled housed mice after one week of isolation. β-endorphin levels were not affected at all, but the individually housed mice showed reduced splenocytes proliferation and IL-2 level at all time points. Other measured white blood cells such as IFN-γ, IL-10 and IL-4 did not significantly differ between the singled-housed and group-housed mice, but there was some effects of time points on IFN-γ and IL-10 among the singled-housed mice. In the second experiment of Bartolomucci et al. (2003), the mice were housed for 1, 7, 14, 21 and 42 days and on the evening before the end of the experiment all animals were subjected to acute stress. The stressor consisted of eight minutes exposure to a novel environment, an open field (OF) arena. The time gap between the stress exposure and the euthanization ought to reveal if the reactivity to the stressor was present for a time afterwards the exposure. The same assays as in experiment 1 were carried out when the animals were euthanised. The results showed a 300 % increase in corticosterone among the individually housed mice compared to their group-housed siblings. The individually housed mice also showed significantly reduced splenocyte proliferation, IL-2 and IL-4 production. The results suggest that individually housed mice do not have a changed immuno competence due to their environment, but that they are more vulnerable when exposed to a stressor.

In 2002 D'Arbe et al. performed a study on social isolation and the release of sympathic neurotransmittor substances in the brains of mice. The neurotransmittor release was indicated by excitatory junction currents (EJCs), which are electrical currents generated when the neurotransmitter is released. After 7-14 days of social isolation there was a significant release of neurotransmittor. The study shows how animal housing can affect the central nervous

system and the importance of reducing stressors in the environment of laboratory animals. Since the reproductive-, cardiovascular-, gastrointestinal- and the immune system are regulated by the sympathic nervous system any change in the central nervous system will have great impact on the animal's physiology.

Effects of cage space on rodent welfare

Würbel (2001) suggests that housing of rodents in cages that restraints their natural behaviours, results in abnormal behaviours, altered brain development and brain functions.

Physical activity is known to induce several positive effects on health and wellbeing (Spangenberg et al., 2005). Comparison between rats held in standard Makrolone type III cages and rats held on large enriched floor pens revealed that rats in standard cages weighed 14 % more than the rats kept in large pens. The rats with spacier pens also showed significantly higher oxidative capacity and 28 % more glycogen content in muscles than rats housed in standard cages. These physical features imply better muscle performance capacity due to increased locomotion.

Hunt & Hambly 2006 found that mice group housed on large floor areas were less stressed than the control groups housed in standard cages, when faecal corticosterone levels were measured. This research group did not find significant differences but a strong trend. They reached significant differences however in time sleeping. Mice housed in groups of three in a small cage slept significantly more than mice housed in groups of three in a large cage and single housed mice. Stress may lead to sleep alterations such as increased sleep time known as "sleep – rebound" (Tiba et al., 2003).

Effects of grid floor on rodent welfare

Rats show a strong preference for solid cage floor compared with grid floor (Manser et al., 1995). 88 % of the rats choose to rest in the solid bottom cage. Since the rats in the study rested 70 to 75 % of the time Manser concluded that housing on solid cage bottoms would improve the welfare of laboratory rats markedly.

Mizisin et al. (1998) compared feet injury in rats held on saw dust and grid floor and found that housing on grid floor induces tactile hypersensitivity and nerve injury in the hind feet.

Heidbreder et al. (2000) found elevated plasma corticosterone levels in rats reared on grid floor compared with rats reared on saw dust.

Housing rats on grid floor lead to elevated blood pressure, heart rate and body temperature (Krohn et al., 2003). The effects of grid floor housing were so strong that blood pressure remained elevated even after the rats had been transferred back to a standard cage with bedding material. Heart rate returned to normal immediately when the transfer was done. This study was performed with telemetry and compared three different housing conditions; grid floor, plastic floor and bedding.

Gordon & Fogelson (1994) examined the impact of cage floor on rats by comparing thermoregulatory patterns of rats placed in acrylic floor cages and metal floor cages. They found significant effects of cage floor on rat's thermoregulatory responses. The metal floor cage and wire-screen cages led to the greatest heat loss in a "phantom rat" test. The test was carried out with a water bottle filled with warm water (42 ºC) placed in the centre of the cage.

The temperature decrease was recorded over 60 minutes. Gordon et al. found that stress on the thermoregulatory system in cold temperatures were minimized by housing in acrylic cages with wood chips bedding while in warm temperatures, grid floor housing minimized stress. As thermoregulatory responses in rats are altered by different housing it is likely that metabolic responses to chemicals and pharmacological agents are also influenced by different cage types.

Effects of nesting material on rodent welfare

Environmental enrichment induces numeral changes in brains of rats such as increased number of neurons, synapses and dendritic branches (Van Praag et al., 2000).

When mice are given nesting material they make nests to sleep in and this seem to affect feed consumption and body weight more than other behaviours (for review, see Olsson & Dahlborn, 2002).

Van Loo et al. (2003) studied the effects of housing conditions on some stress-related parameters in male mice from two different strains. Provision of nesting material for a long time and transferring it during cage cleaning, resulted in lower corticosterone levels in urine, heavier thymuses as well as smaller feed and water consumption compared to mice in standard cages. Mice in enriched cages ate less feed but gained more weight compared to mice in non enriched cages. The weight gain might be due to a stabilized body temperature regulation and less heat loss because of the isolating features of the nesting material (Van der Weerd et al., 1997). Van Loo et al. (2003) came to the conclusion that provision of nesting material and transfer of it when cage cleaning, have stress reducing effects and that it thereby enhances welfare of laboratory mice.

Enrichment with a polycarbonate house and nesting material has no significant effect on stress levels of single housed mice but it significantly decreased stress levels in group housed mice (Hunt & Hambly, 2005). Instead the single housed mice slept and drank significantly more in the enriched cage. Van Loo et al. (2004) showed in a preference test that all of the tested mice choose to sleep in a cage enriched with nesting material instead of an inhabited cage with no nesting material. Nesting material was especially important when sleeping or engaging in sleep-related behaviours.

Discussion

Mice and rats housed in metabolic cages can not perform some of their natural behaviours such as making nests (mice), hide (rats) and interacting socially (both mice and rats). This is in contradiction to the § 4 of the Swedish animal protection law which says that animals shall be housed under such conditions that they are enable to perform natural behaviours.

A majority of the studies reviewed of single housing found effects that indicate an impaired animal welfare (Greco et al., 1989; Yamada et al., 2000; D'Arbe et al., 2002; Nagy et al., 2002; Bartolomucci et al., 2003). Mice and rats wanted to sleep in close body contact with another cage mate in order to get warmth and a feeling of security (Van Loo et al., 2001; Van Loo et al., 2004).

The fact that single housed mice seem to get reduced body weight indicates that they have some physiological disruptions (Nagy et al., 2002). The reduction in bodyweight may be due to increased levels of circulating corticosterone, which may lead to a decrease in e.g. soft lean

tissues and bone mineral content or to an increased heat loss (Nagy et al., 2004; Takeshita et al., 2000). The effect of single housing on corticosterone levels in rodents is debated; Hunt et al. (2006) found no significant differences in corticosterone levels between single and group housed mice while Greco et al. (1989) found significant differences between social isolated rats compared to group housed rats. There may be differences between rats and mice in secreting corticosterone as a reaction to social isolation, but Bartolomucci et al. (2003) who found no significant differences in corticosterone levels in single housed mice and group housed mice, found that single housed mice were significantly more stressed than group housed mice, after exposure to an acute stressor. The results showed a 300 % increase of corticosterone levels compared to animals housed in groups. This result clearly indicate that although the researchers found no differences in corticosterone levels, the animals were not unaffected by the housing conditions.

Social isolation affected the central nervous system of mice and single housed wild type mice showed an increase of stereotypic behaviours which strongly indicate a lack of possibilities to perform natural behaviours in the environment (Yamada et al., 2000)

Housing rodents on grid floors is negative for their welfare. First of all, preference tests have shown that most rats prefer to sleep in solid bottom cages (Manser et al., 1995). Grid floor can cause hypersensitivity and nerve injury in the feet of rats and lead to elevated corticosterone levels as well as elevated blood pressure, heart rate and body temperature (Mizisin et al., 1998; Heidbreder et al., 2000; Krohn et al., 2003). In a metabolic cage the grid floor is a necessity to allow urine and faeces to be collected. The fact that housing on grid floor can have such great impact on the welfare of the animals and by that, the reliability of the experiment, makes it crucial to minimize other factors that affect the animals negatively e.g. social isolation. It would increase the animal's welfare and reduce some of the stress perception, if some kind of nesting material (mice) or a place to hide (rats) were given.

There are many known positive effects of providing nesting material to especially mice. Thus, imply lack of nesting material will affect the mouse negatively. Van Loo et al. (2003) found elevated corticosterone levels and reduced body weights despite bigger feed consumption. In preference tests all mice choose to sleep in a cage enriched with nesting material and it is argued that sleeping in a nest can have thermoregulatory effects (Van der Weerd et al., 1997; Van Loo et al., 2004) The main reason for not providing nesting material to animals in a metabolic cage seem to be to prevent urine and faeces from getting get caught in it and thereby preventing it from falling down the collecting tubes. However I have not found any reports on how frequently rodents excrete urine and faeces in their nests. There need to be more research to find nesting material that can be used in metabolic cages and to exclude that providing nesting material in the metabolic cage does not interact with the reliability of the experiment.

Hunt & Hambly (2006) propose a 14 days acclimatisation period for animals kept isolated, to exclude the possibility for scientific studies to be affected by environmental bias. How this should function when housing rodents in metabolic cages remains unsaid. If 14 days acclimatisation periods are needed to gain unbiased results from the metabolic cage, the function of a 24 hours urine and faeces collection method is surely questioned. The fact that studies reviewed in this paper, performed in an actual metabolic cage, only durated 3 and 7 days (Gil et al., 1999; Eriksson et al., 2004) leaves some questions to investigate. How does long term housing in a metabolic cage affect the animal? Are we willing to expose our laboratory animals to an environment that demands an acclimatisation period of 14 days of

social isolation, in order to gain unbiased results, and are the results really unbiased when the animal has been "acclimatized"? The best solution to this problem may be to develop a metabolic cage that does not expose the animals to complete social isolation.

Social isolation as well as lack of nesting material, housing on grid floor and small cage areas are environmental factors that may expose rodents to stress. The metabolic cage, which combines all these factors, has been technically developed into a very efficient method for collecting faeces and urine. However, the development to reduce stressors has been forgotten. More research on the subject is required and reports of not only potential reliability problems, but actual proved misleading results by altered physiological parameters may speed up the development. A widespread understanding of the impact of stress on animal physiological features, and consequently the reliability of the experimental results, may increase the need for a metabolic cage developed to optimize both the collection and welfare of the animals.

Conclusions

Housing rodents in cages that exposes them to social isolation, grid floor, small area and lack of nesting material may cause physiological changes due to stress responses. Housing rodents in metabolic cages may constitute potential welfare problem for the animals and several unanswered questions need to be evaluated. The traditional metabolic cage needs to be developed into a cage that is efficient for urine and faeces collection while allowing the animals to perform their natural behaviours such as e.g. nesting.

References

Augustsson, H, Lindberg, L. Höglund, A.U. Dahlborn, K. 2002. Human-animal interactions and animal welfare in conventionally and pen-housed rats. *Laboratory Animals* 36, 271-281.

Bamberg, E. Palme, R. Meingassner, J.G. 2001. Excretion of corticosteroid metabolites in urine and faeces of rats. *Laboratory Animals* 35, 307-314.

Barnes, P.J. 1998. Anti-inflammatory actions of glucocorticoids: molecular mechanisms. *Clinical Science* 94, 557–572

Bartolomucci, A. Palanza, P. Sacerdote, P. Ceresini, G. Chirieleison, A. Panerai, A.E. Parmigiani, S. 2003. Individual housing induces altered immuno-endocrine responses to psychological stress in male mice. *Psychoneuroendocrinology* 28, 540-558.

Braintree scientific. April 2007. Products. Metabolic Cages/Stands.
http://www.braintreesci.com/metabolic.htm

Cvek-Hopkins, K. May 2007. *Personligt meddelande.* Husdjursagronom, Sveriges Lantbruks Universitet, Inst. för kliniska vetenskaper.

Dahlborn, K. May 2007. *Personligt meddelande.* Professor, Sveriges Lantbruks Universitet, Inst. för kliniska vetenskaper.

Dahlborn, K. van Gils, B.A.A.. Van de Weerd, H.A. Van Dijk, J.E. Baumans, V. 1996 Evaluation of long-term enrichment in the mouse. *Scandinavian Journal of Laboratory Animal Science* 23, 97-106.

D'Arbe, M. Einstein, R. Lavidis, N.A. 2002. Stressful animal housing conditions and their potential effect on sympathetic neurotransmission in mice. *The American Journal of Physiology. Regulatory, Intergrative and Comparative Physiology* 282, 1422-1428.

Eriksson, E. Royo, F.K, Lyberg. Carlsson, H-E. Hau, J. 2004. Effect of metabolic cage housing on immunoglobulin A and corticosterone excretion in faeces and urine of young male rats. *Experimental Physiology* vol 89, 4, 427-433.

Gil, M.C. Aguirre, J.A. Lemoine, A.P. Segura, E.T. Barontini, M, Armando, I. 1999. Influence of age on stress responses to metabolic cage housing in rats. *Cellular and Molecular Neurobiologi* vol.19, 5, 625-633.

Gordon, C.J. Fogelson, L. 1994. Metabolic and thermoregulatory responses of the rat maintained in acrylic or wire-sxreen cages: implications for pharmacological studies. *Physiology & Behavior* vol. 56, 1, 73-79.

Greco, A.M. Gambardella, P. Sticchi, R. D'Aponte, D. Di Renzo, G. De Franciscis, P. 1989. Effects of individual housing on circadian rhythms of adult rats. *Physiology & Behavior* 45, 363-366.

Griffin, J.F.T, Thomson A.J. 1998. Farmed deer: a large animal model for stress. *Domesticated Animal Endocrinology* 15, 445-456.

Harvard apparatus. April 2007. Metabolic equipment. Metabolic cages for rodents. http://www.harvardapparatus.com/wcsstore/ConsumerDirect/images/site/hai/techdocs/BS4_C_12.pdf

Heidbreder, C.A. Weiss, I.C. Domeney, A.M. Pryce, C. Homberg, J. Hedou, G. Feldon, J. Moran, M.C. Nelson. P. 2000. Behavioral, neurochemical and endocrinological characterization of the early social isolation syndrome. *Neuroscience* vol. 100, 4, 749-768.

Hunt, C. Hambly, C. 2006. Faecal corticosterone concentrations indicate that separately housed male mice are not more stressed than group housed males. *Physiology & Behavior* 87, 519-526.

Jensen, P. 1993. Djurens beteende och orsakerna till det. Stockholm: LTs Förlag

Khosho, F.K. Kaufmann, R.C. Amankwah, K.S. 1985. A simple and efficient method for obtaining urine samples from rats. *Laboratory Animal Science* 35, 513-514.

Kurien, B.T. Everds, N.E. Scofield, R.H. 2004. Experimental animal urine collection: a review. *Laboratory Animals* 38, 333-361.

Krohn, T.C. Hansen, A.K, Dragsted, N. 2003. Telemetry as a method for measuring the impact of housing conditions on rat´s welfare. *Animal Welfare* vol. 12, 1, 53-62.

Manser, C.E. Morris, T.H. Broom, D.M. 1995. An investigation into the effects of solid or grid cage flooring on the welfare of laboratory rats. *Laboratory Animals* vol. 29, 4, 353-363.

Mizisin, A.P. Kalichman, M.W. Garret, R.S. Dines, K.C. 1998. Tactile hyperesthesia, altered epidermal innervation and plantar nerve injury in the hindfeet of rats housed on wire grates. *Brain Research* 788, 13-19.

Nagy, T.R. Krzywanski, D. Li, j. Meleth, S. Desmond, R. 2002. Effect of group vs. single housing on phenotypic variance in C57BL/6J mice. *Obesity Research* 10, 412-415.

Olsson, I.A.S. Dahlborn, K. 2002. Improving housing conditions for laboratory mice: a review of ´environmental enrichment´. *Laboratory Animals* 36, 243-270.

Qiang, D. Salva, A. Sottas, C.M. Niu, E. Holmes, M. Hardy, M.P. 2004. Rapid Glucocorticoid Mediation of Suppressed Testosterone Biosynthesis in Male Mice Subjected to Immobilization Stress. *Journal of Andrology* vol. 25, 6, 973-981.

Sjaastad, Ø.V. Hove, K. Sand, O. 2003. *Physiology of Domestic Animals*, 141, 224, 226-227, 241. First edition. Oslo. Scandinavian Veterinary Press.

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

Swedish Animal Welfare agency. Maj 2007. Etisk prövning av djurförsök, vägledning till ansökan

Swedish Animal Welfare agency. Maj 2007. Djurskyddsfakta. Statistik. Försöksdjursstatistik.

 http://www.djurskyddsmyndigheten.se/Steria/templates/Page.aspx?id=575

Takeshita, N. Yoshino, T. Mutoh, S. 2000. Possible involvement of corticosterone in bone loss of genetically diabetic db/db mice. *Hormone and metabolic research* vol. 32, 4, 147-151.

Tecniplast. April 2007. Metabolic and Diuresis Cage. Metabolic cage for mice. http://www.tecniplast.it/new_improved/metabolic_ing.pdf

Tiba, P.A. Palma, B.D. Tufik, S. Suchecki, D. 2003. Effects of early handling on basal and stress-induced sleep parameters in rats. *Brain research* vol. 975, 1-2, 158-166.

Van der Weerd H.A. Van Loo, P.L.P. Van Zutphen, L.F.M. Koolhaas, J.M. Baumans, V. 1997. Nesting material as environmental enrichment has no adverse effects on behavior and physiology of laboratory mice. *Physiology & Behavior* vol. 62, 5, 1019-1028.

Van Loo, P.L.P. De Groot, A.C. Van Zutphen, L.F.M. Baumans, V. 2001. Do male mice prefer or avoid each other´s company? – Influence of hierarchy, kinship and familiarity. Journal *of Applied Animal Welfare Science* 4, 91-103.

Van Loo, P.L.P. Van der Meer, E. Kruitwagen, C.L.J.J. Koolhaas, J.M. Van Zutphen, L.F.M. Baumans, V. 2003. Long-term effects og husbandry procedures on stress-related parameters in male mice of two strains. *Laboratory Animals*, 38, 169-177.

Van Loo, P.L.P. Van der Weerd, H.A. Van Zutphen, L.F.M. Baumans, V. 2004. Preference for social contact versus environmental enrichment in male laboratory mice. *Laboratory Animals* 38, 178-188.

Van Praag, H. Kempermann, G. Gage, F.H. 2000. Neural consequences of environmental enrichment. *Nature* 1, 191-198.

Watts, R.H. 1971. A simple capillary tube method for the determination of the specific gravity of 25 and 50 µl quantities of urine. *Journal of Clinical Pathology* 24, 667-668.

Würbel, H. 2001. Ideal homes? Housing effects on rodent brain and behaviour. *Trends in Neurosciences* vol. 24, 4, 207-211.

Yamada, K. Ohki-Hamazaki, H. Wada, K. 2000. Differential effects of social isolation upon body weight, food consumtion, and responsiveness to novel and social environment in bombesin receptor subtype-3 (BRS-3) deficient mice. *Physiology & Behavior* 68, 555-561.