ENVIRONMENTAL ENRICHMENT FOR LABORATORY MICE: PREFERENCES AND CONSEQUENCES

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‘a full knowledge of the technology of testing implies an understanding of animal behaviour for its own sake as a pure science, no matter how indirectly it is obtained; but I believe the methods will only work if the experimenter likes animals.’

AP Silverman

Voor pap en mam
Voor de muizen

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INTRODUCTION

Standardisation is an important concept in laboratory animal science. The standardisation of the animals or the animals' environments (e.g. caging, lighting, temperature and air quality) improves the reproducibility of experimental results. The genetic background of the animals used in experiments has been standardised by rigid breeding systems, e.g. by inbreeding, which resulted in inbred strains with minimal genetic variation. Micro-biological standardisation resulted in SPF (specified pathogen free) animals. Experimental procedures are often standardised by following strict rules and regulations (GLP = Good Laboratory Practice). The current housing systems have mainly been developed on the basis of ergonomic and economic factors, providing the basic physiological requirements of animals, such as nutrition, reproduction, good health and sanitation. Husbandry standards have primarily been developed to reduce experimental variables, to ensure reproducibility of experimental results and to meet the convenience of researchers and animal care staff (Benn 1995). The traditional method of housing laboratory animals permits ease in cleaning and capture and also maximises space utilisation within the total room area (Ward & DeMille 1991).

Standard laboratory environments however, only marginally fulfil other needs, such as the performance of natural behaviour or social interactions. It is also recognised that animals have psychological needs, as defined by Poole (1992): 'an animal must be able to acquire experience which enables it to collect information and analyse it, to build up a cognitive picture of the world in which it lives and to act on this knowledge'. The confinement of animals to simplified environments with lack of stimuli may result in animals experiencing psychological distress which may lead to the performance of abnormal behaviours such as stereotypies or passiveness (Chamove 1989a; Ödberg 1987; Poole 1992; Spinelli & Markowitz 1985; Wemelsfelder 1990). When many signs indicate that the current housing conditions affect the well-being of animals negatively, the question arises what kind of animal models are being used in scientific experiments and what the impact is on the reliability of the results and conclusions (Benn 1995).

In recent years the circumstances under which laboratory animals are housed have become a topic of discussion. Several legislative bodies have discussed the care for laboratory animals. This resulted in documents such as the *Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (ETS 123),* by the Council of Europe in 1985 and, based on this Convention, the *Directive for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (86/609/EEC)* by the Council of the European Communities in 1986. More recently, an international workshop in Berlin

reviewed the recommendations of the Convention and the Directive. The proceedings *(The Accommodation of Laboratory Animals in Accordance with Animal Welfare Requirements)* were published in 1993. These publications illustrate that there is a gradual shift from the treatment of an animal as a mere research object to an approach in which more emphasis is put on the well-being of the animal as a biological organism with species-specific capacities and constraints.

The well-being of captive animals is not only dependent on the absence of pain and distress or behavioural abnormalities, but should also impose the least restriction to the physiological and ethological needs of the animals (Benn 1995; Markowitz & Line 1990). This requires environments that can meet these needs as closely as possible. For this purpose standard housing conditions often need some form of 'enrichment'. The principles of environmental enrichment (providing opportunities for naturalistic behaviour to ameliorate behavioural problems), originate from zoos, where the concept emerged during the 1970's. It was then gradually applied in laboratories, as a means to enhance the welfare of experimental animals.

DEFINITIONS OF ENVIRONMENTAL ENRICHMENT

Several definitions of environmental enrichment have been given. Beaver (1989) defines enrichment briefly as additions to an animal's environment with which it can interact, whereas Chamove (1989a) describes it as the improvement of the captive environment with the goal of altering the behaviour of an animal so that it is within the range of the animal's normal behaviour. Newberry (1995) defines environmental enrichment as improvement in the biological functioning of captive animals resulting from modifications to their environment. Successful enrichment should enhance the active character of behaviour, resulting in an increasing frequency of behaviour such as exploration, manipulation, play, and social interaction. At the same time, abnormal patterns of behaviour should largely disappear. (Wemelsfelder 1994). One of the main goals of environmental enrichment as defined by several authors is that by enrichment of the environment animal welfare is enhanced (Beaver 1989; Benn 1995; Chamove 1989a; Hart 1994; Markowitz & Gavazzi 1995; Mench 1994; Poole 1988; Scharmann 1991). In the studies described in this thesis, the assumption that the performance of species-specific behaviour improves the biological functioning of animals in an enriched environment, and that as a consequence their well-being increases, has been used. The welfare concept used has been defined by Broom (1986): the welfare of an individual is its state as regards its attempts to cope with its environment. This refers to how much has to be done in order to cope with the

environment and the extent to which coping attempts are succeeding.

Most authors agree that one of the goals of environmental enrichment is to decrease the incidence of abnormal behaviours and to increase the performance of normal behaviours or behaviours which are within the range of the animal's species-specific behavioural pattern (Beaver 1989; Benn 1995; Chamove 1989a; Hart 1994; Markowitz & Gavazzi 1995; Mench 1994; Poole 1988; Scharmann 1991; Van de Weerd & Baumans 1995). There is, however, no single standard for natural behaviour or a natural environment and when considering behavioural flexibility in the wild, it can be discussed which behaviour is considered to be 'normal' for a certain species (Mench 1994; Newberry 1995). Although the expression of a wild-type behaviour may well correlate with adequate welfare, it may not be the expression per se that results in welfare benefits, but rather the consequences of that expression (Veasey et al 1996). It is therefore important to have well-defined goals of the enrichment programme and to study the desired behaviour and the benefit the animals might experience when exhibiting that behaviour. By identifying which behaviours are important to the animals, it is possible that these behaviours can be satisfied specifically by environmental enrichment (Veasey et al 1996).

Stauffacher (1994) proposes an ethological concept for the development of laboratory housing conditions which meet the basic animal requirements. In near-to-nature environments, crucial stimuli and key features for the normal performance of behaviour are identified, which can later be translated into manageable structures (e.g. group housing system for rabbits, Stauffacher 1992).

The development of a complete new housing system, however, may have major economical and ergonomical consequences and, for that reason, often more simple solutions have to be found to enhance the living conditions of animals. In the environment of an animal a number of aspects can be identified where enrichment can be focused on. These include the social environment (conspecifics, contraspecifics and human beings), and the physical environment, consisting of sensory stimuli (auditory, visual, olfactory and tactile) and nutritional aspects (supply and type of food). Furthermore, there is the psychological appraisal of the environment with aspects such as controllability and predictability (Bloomsmith et al 1991; Van de Weerd & Baumans 1995). When deciding which enrichment techniques are most appropriate, species characteristics, species- and strain-specific behaviours as well as the age and sex of the subjects must be taken into consideration (Bloomsmith et al 1991). Some aspects of the social, physical and psychological environment will be discussed in the next section.

EXAMPLES OF ENVIRONMENTAL ENRICHMENT

Enrichment of the social environment can be performed according to the species-specific social structure. Housing conspecifics in a social group provides an endless variety of meaningful stimulation to the animal (Wemelsfelder 1994)., What is beneficial for a group however, does not necessarily have to be beneficial for every individual in such a group. Not in every social group a stable hierarchy will develop. This depends on the species (and even strain), but also on the characteristics of the individual animals forming the group. Housing female hamsters together may result in high levels of aggression, because the hamster is a solitary living species in nature. In species that develop social hierarchies (such as mice, rats, guinea-pigs and rabbits), group housing of males may lead to high levels of aggression. Group housing male mice in small cages is a highly artificial situation in that defeated subordinates are unable to escape from the territory of the dominant (Brain 1975). Pairing is often performed in larger laboratory animals such as dogs or primates. These pairs however, should be formed with care because while the dominant one benefits from pairing, the less dominant animal may become physiologically depressed (Markowitz & Gavazzi 1995; Reinhardt 1996; Von Holst 1986). For long-term housing of larger animals it would be beneficial to allow the animals to form pairs themselves in order to avoid conflicts as a consequence of incompatible characters.

Cage size is a main aspect of the physical environment and has been a topic in enrichment discussions. Small cages may increase the incidence of stereotyped movements and other non-locomotor abnormal behaviours (Cooper & Nicol 1991; Ödberg 1987). However, this does not mean that large cages ensure the well-being of their residents. Surely, more space to perform behavioural activities such as running, jumping or lying down fully stretched may add to the animal's welfare. On the other hand it has been shown that the number of individuals (and their relationships) in an enclosure is more important than the space available per individual (Chamove 1989a). Another factor is the use of the third spatial dimension (height) within the cage (Ward & DeMille 1991), which can be utilised by inserting shelves or shelters on which animals can sit, e.g. rabbits and dogs will use these types of 'look outs' (Hubrecht 1993; Stauffacher 1994). Furthermore, an environment which is responsive (e.g. provided with objects with which an animal can interact) seems to have more impact on well-being than the amount of space provided per individual (Markowitz & Gavazzi 1995). Ödberg (1987) studied the influence of cage size and enrichment on the development of stereotypies in voles. More voles developed stereotypies in barren cages (large or small), than in enriched cages (large or small). The interior of a cage can be altered by the provision of enrichment objects, which may vary from bedding material to climbing objects or shelters. Different types of cage accessories can be distinguished, e.g. naturalistic objects such as nesting material, gnawing wood

sticks or burrow-like shelters. Or specially designed devices, such as plastic Kong® toys, food delivery mechanisms or puzzle feeders (Markowitz & Line 1990). Dependent on the item introduced, specific behaviours such as exploration, play, activity, foraging or nest building can be stimulated.

Chamove (1989a) stated that increasing psychological space is an important aspect of environmental enrichment. An animal should have control over its environment. It should be allowed to interact with the environment and these interactions should have relevant, predictable consequences (Koolhaas et al 1993). Laboratory animals rarely have control over their environment. Managers of laboratory animal facilities and researchers dictate light cycles, temperatures, husbandry schedules and feeding times (Mench 1994; Spinelli & Markowitz 1985). Confinement restricts the animal's ability to make (behavioural) choices (Stricklin 1995). The inability to have a certain degree of control over their living conditions may result in stress. Joffe et al (1973) reared rats in an environment in which they could control lighting and food and water supply. An open field test showed that these animals were less 'emotional' compared to controls. Animals are likely to develop abnormal behaviour under housing conditions in which they are unable to cope with aversive situations by performing normal behaviour. It is also possible that their behavioural organisation lacks an adaptive behavioural response and therefore their coping behaviour turns out to be unsuccessful (Wechsler 1995). Enrichment objects may provide control over several aspects of the environment. Providing a shelter or a refuge gives laboratory animals the opportunity to withdraw from frightening stimuli outside or inside their cage, or to hide from aggressive cage mates (Van de Weerd & Baumans 1995). Furthermore they can hide from overexposure to light, which may have deleterious effects on the eyes, especially in albino animals (Williams et al 1985). Besides specially constructed shelters (Hubrecht 1993; Stauffacher 1994), many, often simple solutions can be found. Ward & DeMille (1991) provided mice with old drinking bottles, whereas Peters & Festing (1990) used plastic tubes. Tubes were also provided to rabbits (Brooks et al 1993). Nesting material can be provided to mice and rats which can built nests that offer a shelter (Scharmann 1991). One may expect that in this way, the animals are better able to cope in an appropriate way with unpredicted events.

EVALUATION OF ENVIRONMENTAL ENRICHMENT

When introducing enrichment it is important to evaluate the enrichment programme used (Beaver 1989; Bloomsmith et al 1991; Chamove 1989a; Markowitz & Line 1990; Newberry 1995; Van de Weerd & Baumans 1995). This can be done by observations of the animals in their home cages, by submitting animals to preference tests or behavioural tests and by measuring physiological

variables.

Home cage observations

The effects of the introduction of enrichment can be monitored in the home cage of the animals. This effect can be measured quantitatively, by assessing the behavioural pattern before the enrichment was introduced (baseline behaviour) and afterwards, and by quantifying the changes in responses as a consequence of the enrichment programme. Changes that can be seen include an increase in species-typical behaviour and/or a decrease in abnormal behaviour, which are mostly the desired effects. But it may well be that the animals do not respond as expected and they may even hurt themselves while interacting with an enrichment object (Bloomsmith et al 1991). Haemisch et al (1994) found high levels of aggression and unstable dominance relationships after structuring the home cage of groups of male mice. Different strains of animals may respond differently to the enrichment used as has been observed in mice (Van de Weerd et al 1994).

The following studies describe the introduction of enrichment objects and the evaluation of their effects on the behaviour, in *primates:* Bayne et al 1992, 1993, 1994; Line & Morgan 1991; in *dogs:* Hubrecht 1993; in *rabbits:* Brooks et al 1993; Huls et al 1991; in *rats:* Orok-Edem & Key 1994; Chmiel & Noonan 1996; in *mice:* Van Loo et al 1996; Van de Weerd et al 1994. It is also important to assess whether the changes in behaviour are maintained over short or long periods of time and whether this is in accordance with the objectives of the programme. The animals may not be interested or loose interest soon after introduction (Bloomsmith et al 1991; Chamove 1989a; Dahlborn et al 1996; Van de Weerd & Baumans 1995). For larger animals such as primates, enrichment objects are often changed or rotated between cages to avoid boredom.

Preference tests

Another method to evaluate enrichment is the use of preference tests. Preference tests have been used in assessing laboratory animals' choices for environments or for aspects of the environment (Arnold & Estep 1994; Blom et al 1992, 1995; Manser et al 1995; Ottoni & Ades 1991; Van den Broek et al 1995; Van de Weerd et al 1996). Furthermore, the strength of preferences have been established in order to measure the importance that an animal attaches to a preferred option (Dawkins 1983; Sherwin & Nicol 1995). The use and limitations of preference tests have been discussed thoroughly (e.g. Blom 1993; Duncan 1992; Fraser 1996; Van Rooijen 1983/84). These tests have proven to be a helpful tool in enrichment programmes. Allowing animals to choose between several enrichment items may prevent introduction of enrichment items in which the animals show no interest or which may even harm them. Preference tests also show how the animals use the

enrichment, as it is possible that they use enrichment in other ways than how they were intended or envisioned by humans (Bloomsmith et al 1991). By presenting animals various objects and allowing them to choose, some general principles about species-specific properties of enrichment devices can be determined (Mench 1994). Again, it is important to study the animal's long-term response to the preferred objects or environment before implementing these on a large scale (Blom 1993).

Behavioural tests

The effects of environmental enrichment can be evaluated by submitting animals to behavioural tests. The use of behavioural tests to study the influence of rearing in different environments started with Hebb (1947). He found that rats with 'free environment' experience were better problem solvers in his learning test: the Hebb-Williams maze for rat intelligence. Since then many researchers studied the effects of enrichment and found several effects.

The open field test is a classical test which has often been used to study the 'emotionality' of laboratory mice and rats. Animals which show high levels of activity (locomotion) and have low defecation scores in the open field are regarded as being less emotional than animals which show the opposite (Archer 1973, Walsh & Cummins 1976). Most authors found that mice and rats from enriched environments are less emotional than animals from impoverished environments (Denenberg & Morton 1962; Holson 1986; Manosevitz 1970; Manosevitz & Joel 1973; Manosevitz & Montemayor 1972; Prior & Sachser 1994/95). Manosevitz (1970) and Manosevitz & Montemayor (1972) suggest that enrichment produces these effects through interactions between activity and exploration, but that genetic factors, such as differences between inbred and outbred animals, also play an important role. Higher activity levels of enriched housed mice have also been measured with running wheel performance during four days (Manosevitz 1970; Manosevitz & Joel 1973; Manosevitz & Montemayor 1972). Animals from enriched environments have also been reported to be better learners in various tests, such as the radial maze, the elevated Y maze, the Hebb-Williams maze and the Morris water maze. These studies are mainly performed with rats (Escorihuela et al 1995; Forgays & Read 1962; Juraska et al 1984; Kiyono et al 1984). Mice from enriched environments performed better on a food seeking task, but the size of the effect differed per strain and cross, suggesting genetic variability (Henderson 1970b, 1976). Exploration and object interactions in an object-interaction test were also behavioural traits which differed between rats from enriched or standard conditions. Animals from enriched environments showed more diverse behaviours towards the objects, suggesting that they gathered information about the features of their environment differently than animals from

impoverished housing conditions (Renner 1987; Renner & Rosenzweig 1986a; Widman & Rosellini 1990).

Many aspects of the enriched environment play a role in the diverse behavioural effects of enrichment. It is necessary that rats actively interact with enrichment to produce effects in brain and behaviour, only observing other rats interacting with the enrichment did not produce these effects (Ferchmin et al 1975). Perceptual learning as well as visual perception is important, however blind rats with free environment experience performed better than blind rats with restricted experience in the Hebb-Williams maze and elevated Y maze, suggesting that tactile cues also play a role (Forgays & Forgays 1952; Hymovitch 1952). Furthermore, there is a critical period for exposure to enriched conditions, e.g. rats exposed immediately after weaning were better maze problem-solvers than animals exposed earlier or later (Forgays & Read 1962). Henderson (1977) found that increasing cage size or providing extended climbing practice were not sufficient to explain observed enrichment effects in a food searching task, although the availability of space plays a role (Manosevitz & Pryor 1975). Social grouping and interactions (play behaviour) alone appeared to be inadequate to explain environmental enrichment effects (Renner & Rosenzweig 1986b; Rosenzweig & Bennett 1972; Rosenzweig et al 1978).

Many other behavioural tests can be used to study behavioural differences between animals from enriched or standard housing conditions. In the studies described in this thesis, besides the open field test, a cage emergence test (Chamove 1989b; Laininger 1989; Van de Weerd et al 1994), a hole board test (Boissier & Simon 1962; Van de Weerd et al 1994) and an aluminium foil test (Dahlborn et al 1996) have been used. These tests were employed because they deal with different aspects of behaviour and are easy to use.

Measuring physiological variables

Enrichment may not only have consequences for the behaviour of animals, the physiological state of an animal can also be influenced. Therefore physiological variables such as food and water intake, body weight, hormonal levels in plasma or urine, heart rate and the immune status can be monitored (Broom & Johnson 1993; Kingston & Hoffman-Goetz 1996; Markowitz & Line 1990). A loss of body weight in adult animals or an impaired growth in juveniles can be caused by severe housing conditions, such as unstable social groups, or by certain experimental conditions, such as repeated foot shocks or prolonged immobilisation (Broom & Johnson 1993). The susceptibility to disease (suppression of the immune system) can be increased by a variety of biological disturbances (Broom & Johnson 1993).

Measurement of activity in the sympathetic-adrenal medullary system and

in the hypothalamic-pituitary-adrenal cortex system are amongst the most useful in the assessment of the physiological state of an animal (Broom & Johnson 1993). Levels of catecholamines (adrenaline and noradrenaline) and glucocorticoids can be measured. There are different views with respect to the fact whether or not corticosteroids are reliable indicators for chronic stress. Some authors state these can be used for measuring both acute and chronic stress (Quirce & Maickel 1981; Kant et al 1987), while others state that these are only reliable for measuring acute stress (Broom 1988; Broom & Johnson 1993; Carlstead et al 1992, 1993; Manser 1992). Furthermore, there is controversy whether increased levels of adreno-cortical activity are indicative of adverse conditions or stress (Broom 1988; Fraser 1995). Often, the emotional stress of the experimental situation and handling procedures cause an increase in corticosteroid levels (Broom & Johnson 1993; Fraser 1995). It is therefore necessary to collect blood within 3-4 minutes after disturbing an animal (Riley 1981). For catecholamines releasing times are even shorter, within 1-2 seconds after perception of the initiating stimulus (Broom & Johnson 1993). Other methods which do not disturb the animals too much at the time of sampling, are detecting hormonal levels in urine or saliva, or taking samples from animals which have been catheterised. The function of the adrenal cortex can be measured by a stimulation test (CRF or ACTH challenge test) , as repeated exposure to prolonged stress may sensitise the system, so that with a novel disturbing stimulus, a greater response is elicited (Broom & Johnson 1993).

Measurement of heart rate can be a useful parameter for assessing the emotional response of an animal to short-term stressful situations, provided that the measurement in itself does not cause too much disturbance. Other factors (e.g. social position of the animal) must be taken into consideration as well when monitoring heart rate changes (Broom & Johnson 1993).

Reproductive function or success of reproduction may also be measured (Markowitz & Line 1990; Newberry 1995), although it should be realised that laboratory animals have been selected for generations to reproduce well under laboratory conditions. Good health, as suggested by Newberry (1995) may be not a reliable indicator of the success of enrichment programmes either, because in general, standard laboratory environments provide for optimum climates and hygiene which protects the animals from infections. Modern techniques such as biotelemetry systems make it possible to monitor the cardiovascular system, body temperature and activity of an animal while being undisturbed in its own environment (see e.g. Kramer et al 1993), enabling comparisons of different environments without the influence of stressful handling or transport procedures.

The effects of environmental enrichment on brain plasticity have already been described in the 1960’s by Rosenzweig and colleagues. Their main interest

was to study the hereditary and environmental factors that affect learning ability and the effects on the brain. Rats were subjected to two experimental situations. Groups of rats were housed in large enriched complex cages and received training in mazes or they were individually housed under impoverished conditions with minimal stimulation. These treatments resulted in anatomical differences in the brain. Enriched housed rats developed a greater overall weight of the brain, although these differences were rather small. Larger differences were found in the weight of the cerebral cortex, as the cortex became deeper (mainly in the occipital region and dorsal cortex). Histological examination showed that the glia cells increased in number by proliferation (Devenport et al 1992; Katz & Davies 1984; Walsh 1981). Biochemical differences were also found, the total activity of the enzymes acetylcholinesterase (AChE) and cholinesterase (ChE) being increased in the cortex and in the rest of the brain. These enzymes play a role in transmitting messages through the brain (Bennett et al 1964; Rosenzweig 1966; Rosenzweig et al 1960; see also Van Rijzingen 1995). Similar results were found in mice and gerbils (La Torre 1968; Rosenzweig & Bennett 1969). The changes in the brain appeared to be related to learning ability, as groups of rats from enriched conditions performed better on reversal discrimination problems than did controls (Krech et al 1962).

The fact that housing in enriched environments can have profound effects in brain anatomy and chemistry suggested that animals from different environments might also respond differently to some types of brain injury. Several studies have confirmed this, e.g. housing in an enriched environment had positive effects on the functional recovery after cortical and hippocampal lesions (Van Rijzingen 1995), and on the behaviour after septal lesions (Engellenner et al 1982).

When an animal is confronted with environmental changes, it will adapt to this new situation with a range of behavioural and physiological responses. The function of these responses is to maintain homeostasis (Barnett & Hemsworth 1990). Since biological systems are complex, measuring a single behavioural or physiological variable will often not adequately reflect an animal's response to these environmental changes. Therefore it is useful to record a combination of both physiological and behavioural measures. When multiple parameters are effected in a similar way, stronger conclusions about the impact of the environmental change can be made (Markowitz & Line 1990).

The question whether enrichment of the environment can indeed enhance the well-being of laboratory animals can only be answered by interpreting the physiological and behavioural effects that are measured after the animals have been submitted to the enriched environment for some time. According to Broom (1988) in this way these parameters can be used to assess the animal’s welfare.

SCOPE OF THIS THESIS

This thesis describes the study of evaluating environmental enrichment for laboratory mice. The purpose of this study was to find out which of easy to use cage enrichments are appreciated by the animals and what the consequences are of providing such enrichments over a longer period of time.

In the experiments described in Chapter 2 the effect of simple enrichment of standard cages on the response of mice in behavioural tests was investigated.

In the studies presented in Chapters 3 and 4 the preference for different enrichment objects (nesting materials and nest boxes) was investigated, and the question was addressed whether there were differences between strains and (within strains) between sexes. In Chapter 5 the previously found preferences are further elaborated and experiments in which the strength of preference for enrichment was tested, are described.

Preference tests only measure short-term choices of individual animals. Therefore, the effects of housing groups of mice for a longer period of time in cages with previously preferred enrichment was also studied. For this purpose several behavioural and physiological parameters were monitored (Chapter 6).

The effect of enrichment on the behaviour of mice in an open field test is described in Chapter 7. In the experiment described in Chapter 8 the effect of enrichment on 24 h behavioural patterns of mice was investigated. For this study a newly developed automated behavioural registration system was used.

Finally, in Chapter 9 the results of the experiments described in this thesis are evaluated and practical implications for animal welfare and animal experimentation are discussed.

STRAIN SPECIFIC BEHAVIOURAL RESPONSE TO ENVIRONMENTAL ENRICHMENT IN THE MOUSE

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SUMMARY

The influence of environmental enrichment on the behaviour of the mouse has been studied in two inbred strains (C57BL and BALB/c). Male mice of each of the two strains were subjected to behavioural tests after being housed for two months either under standard housing conditions or in an enriched environment. The results of the behavioural tests indicated that the C57BL mice housed in the enriched environment were more reactive and alert compared to mice housed in the standard environment. In the BALB/c mice results may be interpreted as if enriched environments lead to an increased level of anxiety. It is concluded that environmental enrichment has a strain specific effect on the behaviour of mice.

INTRODUCTION

Most environments of laboratory animals have been designed to serve human convenience, with little or no consideration for the animal's nature. Laboratory housing conditions deprive the animal of the possibility of performing its full repertoire of normal behaviour. As a response to this lack of stimulation the animal may show stereotyped behaviour or passiveness (Wemelsfelder 1990).

Environmental enrichment (i.e. additions to an animal's environment with which it can interact) may improve the well-being of captive animals (Beaver 1989). According to Chamove (1989a) the objective of environmental enrichment is to alter behaviour so that it is within the range of the animal's normal behaviour.

A large body of literature has been published on ways to improve the environment of captive animals, e.g. by providing animals with toys or other cage accessories (Bayne et al 1992; Brooks et al 1993; Scharmann 1991). Less attention has been paid to the evaluation of the enrichment program being used (Bloomsmith et al 1991). To obtain a complete picture of the impact of an environmental change on an animal, both frequency and duration of behavioural changes can be determined (Bayne et al 1993). Furthermore, physiological variables, such as heart rate, hormonal levels or reproductive function, can be monitored to assess the responses to changes in laboratory environments (Markowitz & Line 1990). Another method of evaluating environmental enrichment is to observe if the enrichment has effects on the behaviour of animals in behavioural tests (Manosevitz 1970).

In those studies which do evaluate the behavioural effects of enrichment, rats or larger laboratory animals have been monitored (e.g. Holson 1986) but only few authors have studied these effects in mice (Manosevitz 1970; Manosevitz & Montemayor 1972). The aim of our study was to employ behavioural tests to evaluate the behaviour of mice from standard or enriched environments and to investigate if the enrichment had different effects on the behaviour of different strains.

Three tests were used to compare the behaviour of mice of the C57BL and BALB/c strains, housed in standard or enriched environments. The tests were chosen because they deal with different aspects of behaviour. They can also give an indication of the degree of alertness or anxiety, which can possibly alter as a consequence of different housing conditions. The hole board test has first been described by Boissier & Simon (1962) and is designed to test exploratory behaviour, as it takes advantage of the natural tendency of mice to dip their heads into holes. In the cage emergence test a mouse is placed into an unfamiliar cage and the reactivity to escape from this novel environment is measured. An open field test was used to compare the behaviour of the animals in this novel environment. In the second part of the test, the reaction of the animals to a sudden change in the

acoustic environment was studied in order to reveal whether or not the mice are responsive to novel environmental stimuli.

ANIMALS AND METHODS

Animals

Thirty-two male mice from two inbred strains, C57BL/6JIcoU (n=16) and BALB/c AnCrRyCpbRivU (n=16) were used. Animals were reared by individual mothers in an SPF environment. Nesting material (cotton or tissues) was provided to enhance breeding. When the experiment started they were at the age of three weeks. The mice were housed and maintained in a room with conventional hygiene and controlled photo-period (6.00-18.00 h, white light 225 lux), relative humidity (62-66%), temperature (22-23 ^{o}C) and ventilation (15 air changes per h). Tap water and food pellets were provided ad libitum (food pellets RMH-B, Hope Farms, Woerden, The Netherlands).

Housing

Per strain, a group of eight animals was housed in a wire-topped Macrolon type III cage (840 cm^2, UNO roestvaststaal, Zevenaar, The Netherlands) either under enriched conditions or under standard laboratory conditions.

In the enriched environment the cages were provided with 125 g of sawdust (Lignocel 3/4, Rettenmaier & Söhne, Ellwangen-Holzmühle, Germany) and a set of well-defined objects. These objects were: a nest box, consisting of one half of a Macrolon type I cage (204 cm^2) placed on its side, a grid floor (19x10 cm, stainless steel wire, mesh size 10x10 mm^2) placed beneath the food hopper on the bedding, and an opaque plastic tube (∅ 6 cm, length: 16 cm). Additionally nesting material was provided in the form of three tissues (Kleenex, Kimberley-Clark) and 10 g of wood-wool (BMI, Helmond, The Netherlands). The tissues and wood-wool were renewed after cage cleaning once a week. The location of the objects in the enriched cage remained the same during the experiment (Figure 1). In the standard environment the cages were provided only with 125 g of sawdust.

Behavioural testing

During the study, which lasted 2 months, the animals were subjected individually to the three behavioural tests.

Hole board test. A mouse was placed in the centre of a plastic board, measuring 37.5x37.5x3.5 cm, pierced with 16 holes (∅ 3 cm) in 4 rows. The board was fixed at

a distance of 15 cm above a table, light intensity during testing was 270-300 lux (board level). The board was covered with a transparent lid (40x40x20 cm) to prevent the mice from remaining at the edge of the board.

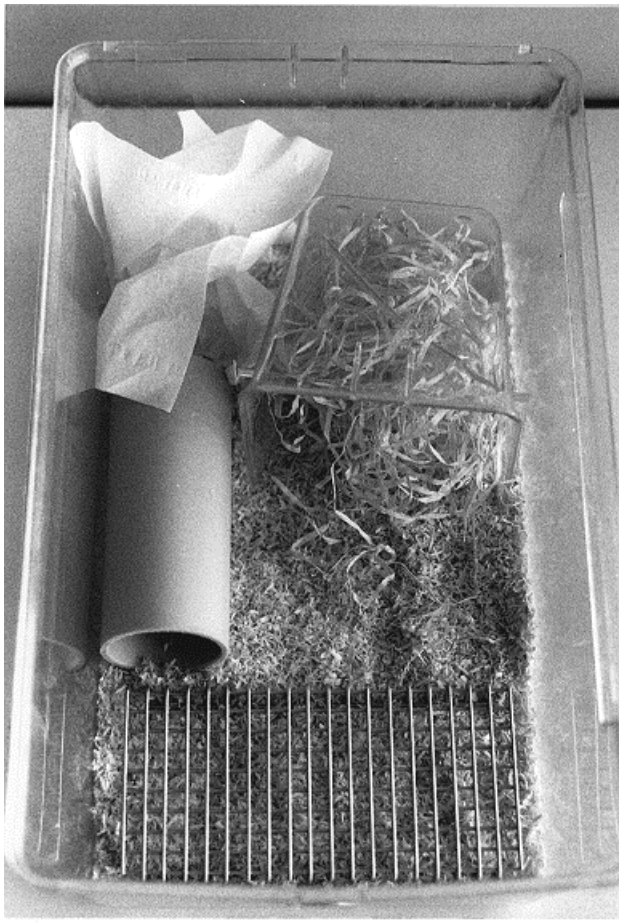

Figure 1 *The location of the objects in the enriched cage.*

The number of holes explored by a mouse during 3 min of testing was counted. A dip was registered if a mouse put its head in a hole at least up to the eye level. Repeated dips into the same hole were not counted unless these were separated by locomotion. Afterwards faeces production was registered. The mice were subjected to this test twice during this study, for the first time on day 29 (enriched) or 30 (standard) and for the second time on day 64 (enriched) or 65 (standard). Testing was performed between 14.00 and 15.00 h.

Cage emergence test. The apparatus consisted of a Macrolon type I cage (204

cm^2) with a hole (∅ 4 cm) in one of the side walls. There was no lid on the cage. Light intensity during testing was 350 lux (floor level). A mouse was placed inside the cage with its back to the opening and the time to escape from the cage (all four feet outside the cage) was registered. The maximum testing time was set at 10 min. Afterwards the number of faecal boli was counted. The mice were subjected to this test between 10.30 and 11.30 h on day 43 (enriched) or at 14.30 h on day 44 (standard).

The open field test consisted of a circular, transparent pvc floor circle (∅ 90 cm) surrounded by an opaque pvc wall (height: 50 cm). Light intensity during testing was 25 lux (floor level). The test was combined with a sudden silence test. During the first part of the open-field test white noise produced by a random-noise generator (General Motor Company) was present (75 dB). After 4 min of behavioural observation this noise was suddenly turned off (remaining background noise: 45 dB) and the behaviour of the mouse was scored for another 4 min.

Each test was recorded with a camera-videosystem, the experimenter not being present in the testing room. Afterwards the behaviour of the animals was scored from the videotape (software used: the Observer v 2.0, Noldus b.v. Wageningen, The Netherlands). The following behavioural elements were recorded during both parts of the open-field test:

Time spent on:
locomotion = movement of the whole body
immobility = non-locomotion, head movements were allowed
grooming = scratching, wiping or licking fur, head or tail
freezing = motionless, no movements of head or body, attentative

Frequency of:
rearing = upright posture standing on hind feet, including leaning against the wall

The number of faecal boli were counted after each test. The mice were subjected to the open-field test between 13.00 and 15.00 h on day 58 (enriched) or 59 (standard). All the tests apparatuses were cleaned with ethanol (70 %) after an animal was being tested.

During the study the groups of mice were observed daily in their home cage (sleeping site, social interactions/fighting, responses to enrichment objects). Food intake per group was measured weekly and body weight was measured at the beginning of the study, after one month and after two months, near the end of the study.

Statistics

Data are given as mean values ± SEM. The results of the tests were analysed by using a SPSS/PC+ statistical computer program (SPSS Inc. Chicago, USA).

The hole board test results were analysed for differences between housing conditions and differences in time by ANOVA followed by t-tests. The results of the cage emergence test were analysed per strain for differences between housing conditions with a Mann Whitney U test. The registered behavioural elements of the open field test were statistically analysed using MANOVA, with the behavioural elements as dependent variables and noise as a within subjects factor, to evaluate differences between the animals of the two housing conditions. Furthermore food intake was analysed by a paired t-test to compare food intake of the groups per date, body weight was analysed by ANOVA. For all the tests the level of statistical significance was pre-set at $P<0.05$

RESULTS

The results of the first hole board test (Figure 2) showed that after one month no significant differences between the animals housed under enriched or standard conditions could be demonstrated in number of holes explored. When the animals were subjected to this test for the second time the C57BL mice housed in the enriched environment explored significantly more holes than the animals housed in a standard cage ($P<0.05$).

When comparing the results of both hole board tests, it appeared that the BALB/c strain showed a significant decrease in number of holes explored in both groups of mice ($P<0.01$). In the C57BL strain mice from the standard environment also showed a significant decrease in number of holes explored ($P<0.01$), but the animals from the enriched environment retained the high exploration level of the first test. In the BALB/c strain there were no significant differences in faeces production between mice from the two housing conditions in both tests, nor in faeces production between the two tests. The C57BL mice did not produce any faeces at all.

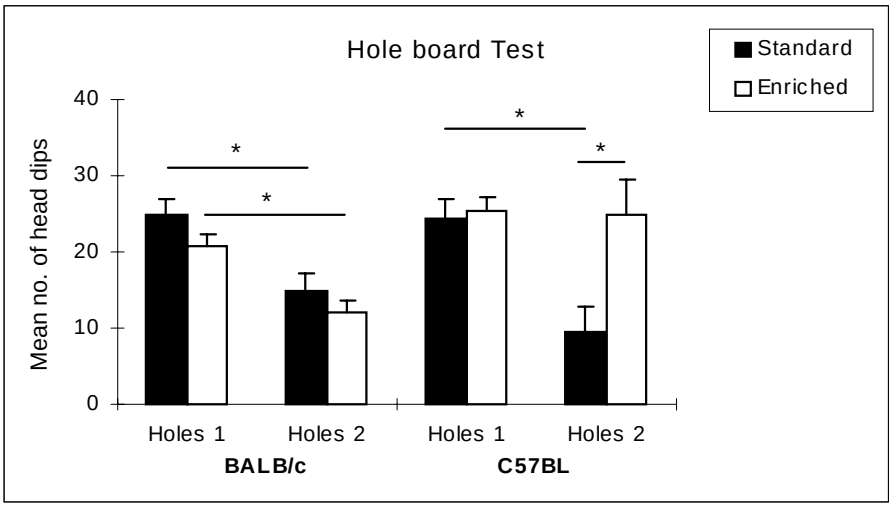

Figure 2 *Effects of housing conditions on the behavioural responses of male mice in the hole board test. Holes 1 = first hole board test, Holes 2 = second hole board test. Data are expressed as mean numbers of head dips ± SEM, N=32. ANOVA and t-tests were used to calculate statistical differences. * P<0.05*

In the cage emergence test (Table 1) the C57BL mice from the enriched environment escaped from the cage significantly faster than the standard housed animals (P<0.01). The BALB/c mice housed under different conditions did not differ significantly from each other. There were no significant differences in faeces production between the two groups of BALB/c mice. The C57BL mice did not produce faeces during the test.

Table 1 *Mean time (s ± SEM) to escape from a cage in male mice from two housing conditions (N=32)*

	Standard	Enriched	P-value
C57BL	16.1 ± 2.3	6.9 ± 1.0	P<0.01
BALB/c	88.1 ± 73.2	223.1 ± 100.2	ns

Differences between housing conditions analysed with Mann Whitney U test.

The results of the open field test are presented graphically in Figure 3. Significant differences between animals from the two housing conditions could be demonstrated in a number of behavioural parameters. In the BALB/c strain the enriched housed animals when compared to the standard housed animals, spent less time on locomotion during the first part of the test with noise on (P<0.01) and during the second part of the test without noise (P<0.01). They showed more freezing behaviour during the first part of the test (P<0.01) and more immobility (P<0.05) and less rearing (P<0.05) during the second part of the test.

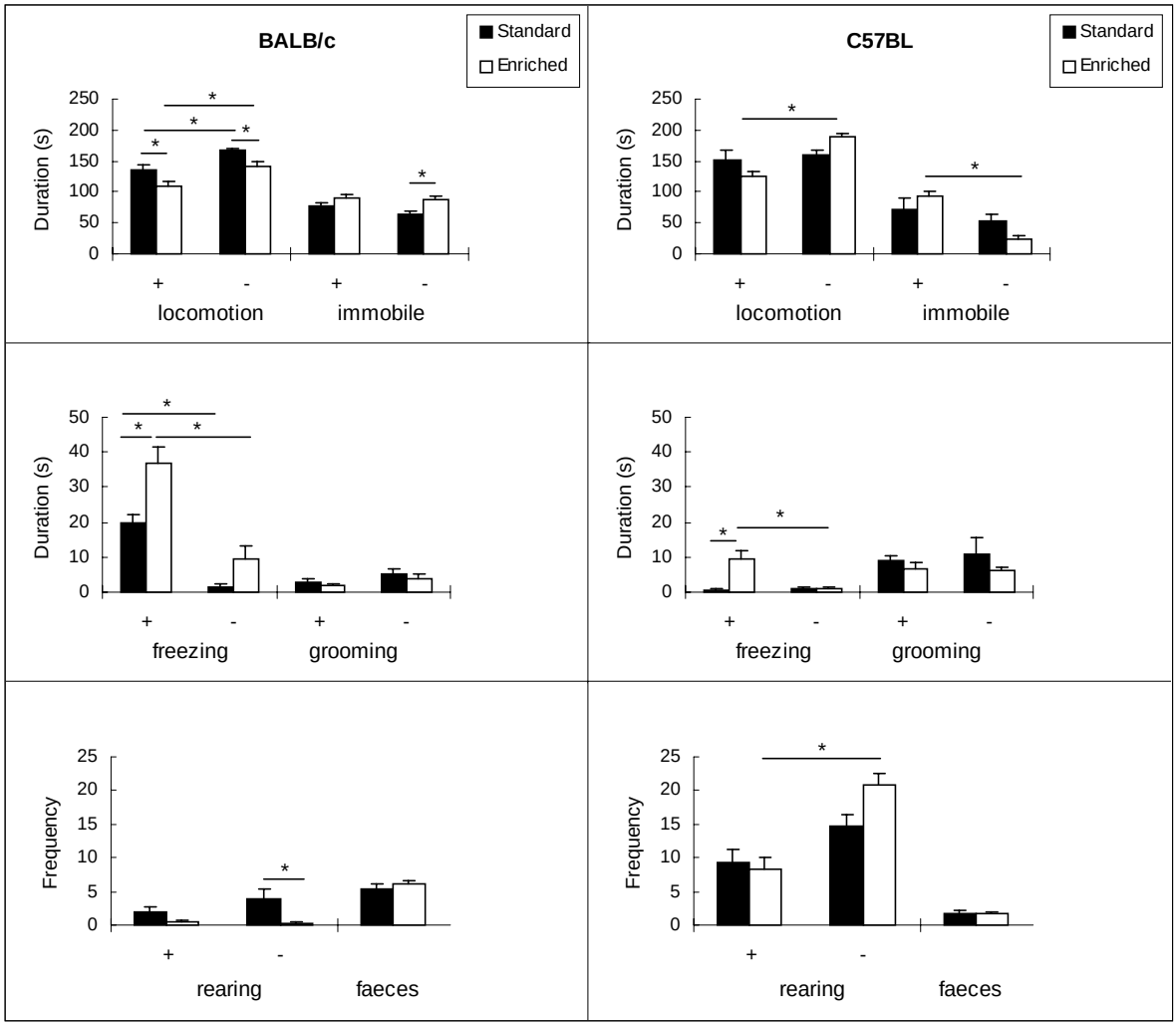

Figure 3 *Effects of housing conditions on the behavioural response of male mice in the open field test combined with a sudden silence test. + indicates that noise is present and - indicates that noise is absent. Data are expressed as mean duration and frequency ± SEM, N=32. MANOVA and t-tests were used to calculate statistical differences. * P<0.05*

In the C57BL strain, mice from the enriched environment showed significantly more freezing behaviour during the first part of the test (P<0.01) compared to standard housed animals. In both strains there were no differences in number of faecal boli produced by animals from both housing conditions.

The behavioural reaction of the animals from both housing conditions to the sudden change in acoustic conditions, was studied by comparing the time spent on a behavioural element in the first part of the test with the time spent on this element in the second part of the test.

In the second part of the test, mice from the BALB/c strain spent significantly less time on locomotion and freezing compared to the first part of the test. This was observed in animals from both housing conditions (for both groups: locomotion

P<0.01, freezing P<0.01). In the second part of the test C57BL mice from the enriched cage spent more time on locomotion (P<0.01) and rearing (P<0.01) and less time on immobility (P<0.01) and freezing (P<0.01). These effects were not seen in animals housed in the standard environment.

When comparing both strains the behavioural tests revealed differences between mice housed in the two environments, but these effects were not the same in the two strains. In the open field test the two groups of BALB/c mice differed in the time spent on several behavioural elements, whereas the two groups of C57BL mice mainly differed in the way their behaviour has changed in the second part of the test compared to the first part. The hole-board test and cage emergence test revealed differences only between the two groups of C57BL mice, the animals from the enriched conditions explored more and escaped from the cage faster.

Differences between the strains were also present in the way they responded to the enrichment objects, according to observations in the animal room. For example the C57BL mice slept mostly in the nest box and underneath the foodhopper in the tissues, whereas the BALB/c mice preferred to sleep in the nest box and in the plastic tube. Furthermore the BALB/c mice soiled the enrichment objects with urine and faeces, whereas the C57BL mice did not.

In both strains a remarkable difference between the two housing conditions was observed in sleeping behaviour. Animals in the standard environment always slept together in one group on top of each other, whereas the animals in the enriched environment slept mostly in two or three smaller groups. Furthermore the enriched housed groups had certain locations in their cage where they urinated mostly. The animals in the standard cages seemed to urinate randomly on the bedding.

Sometimes some fighting occurred, but although this behaviour was not quantified it happened more often in the standard groups than in the enriched groups. Especially in the BALB/c strain, in both environments fighting increased gradually when the animals grew older.

In both strains the mice from the two housing conditions did not differ in body weight. No differences were found in weekly food intake between the two groups of BALB/c mice. The amount of food consumed by the C57BL enriched animals was significantly less (P<0.05) than the amount of food consumed by the standard housed animals.

DISCUSSION

The results of the first hole board test did not indicate differences between mice from the two housing conditions in both strains. In the second hole board test a decrease in number of holes explored was found in all groups except the C57BL enriched animals. Dorr et al (1971) performed a comparable hole board test and repeated this test after one week. They also found a reduction in number of head dips into the holes. The mice were less active and more hesitant, they sniffed more and walked less deliberately. The authors regarded this as a sign of reduced curiosity or habituation. It might indicate that explorative behaviour is diminishing with time. This decrease might be prevented or perhaps postponed with an enriched environment in the C57BL mice but not in the BALB/c mice.

In the emergence test, the C57BL enriched housed animals escaped from the cage significantly faster than did the standard housed animals. This is consistent with the findings of Thiessen et al (1962). They tested C3H mice from a standard group and from a group provided with toys, in a hole-in-wall test which is comparable to our emergence test. They found the mice from the enriched conditions to escape twice as fast as the mice from the standard conditions. Chamove (1989b) housed CLFP mice in cages differing in complexity and performed a box emergence test. The animals from the more complex cages escaped significantly faster than the control animals.

In this study the two groups of the BALB/c strain did not differ significantly from each other in emergence time. Although this might be strain specific it should be mentioned that the lighting during both the hole board and emergence test was bright. Henderson (1972) has stated that albino strains with non-pigmented eyes can be at a disadvantage in behavioural tests with bright background illumination. These mice have a poor visual discrimination and show an increased tendency toward freezing behaviour. We observed that in both groups of the BALB/c strain, some animals did not escape from the cage within 10 min. Different illumination levels during testing had little effect on the behaviour of a pigmented strain like the C57BL (Nagy et al 1970). Thus the different behavioural responses of BALB/c and C57BL mice may (partly) be caused by the differences in sensitivity to the illumination during these two tests.

Manosevitz (1970) and Manosevitz & Montemayor (1972) investigated the behaviour of respectively, random bred and inbred mice, housed under standard and enriched conditions. In both studies they found that animals from the enriched environment were more active than animals from the standard environment in an open field. In contrast Rose et al (1985) found lower activity scores in rats from an enriched environment in an open field test. These results are consistent with our observations during the first part of the open field test. We also found a lower

activity of the enriched housed BALB/c mice compared to the standard housed mice and the same tendency, although not statistically significant between the two groups of C57BL mice. No differences in defecation scores were found between enriched and standard housed animals in both strains. This is in concordance with the results of Manosevitz & Montemayor, but in contrast to previous findings of Manosevitz. According to Manosevitz & Montemayor this discrepancy in findings may be due to the different genotypes used in the studies. Furthermore, in our study mice were housed in the enriched environment after weaning, whereas in both Manosevitz studies mice were housed in enriched cages from birth on.

The C57BL mice housed in the enriched cage showed more locomotion and rearing and less immobility and freezing in the second part of the test. These behavioural changes may partly be a reaction to the sudden change in noise level and partly indicate that the enriched housed mice habituated more easily to the new situation in the second part of the test. Together with their behaviour in the cage emergence test and the hole board test it seemed that these animals were more reactive and alert than the animals from the standard environment. The BALB/c mice housed in the enriched environment differed for a number of behaviours from mice housed in the standard cage. Especially the differences in freezing and locomotion suggest a higher level of alertness or anxiety in these animals. Walsh & Cummins (1976) and Archer (1973) reviewed the validity of the open field test as a test for emotionality or anxiety. The test has been widely used but with various procedures and variables measured. Therefore the results must be interpreted with care. We used the test mainly to compare the behaviour of two differentially housed groups of mice and the results have shown that for a number of behavioural parameters, significant differences between the groups occurred.

With the two other behavioural tests it was also possible to discriminate between the different groups of mice, although an adaptation in lighting conditions when testing albino strains is advisable.

Overall, the results of the tests indicated that mice housed in the enriched environments were more dynamic in their reactions to novel situations than mice from the standard environments. They were more alert and seem to habituate faster as compared to their standard housed counterparts. However, the behavioural effects differed per strain. Manosevitz & Montemayor (1972) also have indicated that genetic factors are of considerable importance in mediating the effects of environmental enrichment.

The C57BL mice from the enriched environments consumed significantly less food than the standard housed animals. However there were no differences in body weight between the two groups. An explanation for this phenomenon might be a difference in thermoregulation of the animals. Chvédoff et al (1980) studied food intake and body weight in groups of mice. They observed a decline in food

consumption with an increased cage density, but hardly any differences in body weight. Their explanation for this was that mice in groups sleep huddled together and by doing so reduce their overall body heat loss, compared to individually housed mice. In our study animals in the enriched cages had nesting material and shelters to sleep in providing them a good insulation. Although this was also the case for the BALB/c mice no difference in food consumption was observed between the two groups of this strain.

The observations in the animal room showed us that the mice in the enriched cage were able to structurise their living environment by manipulating the enrichment objects. The animals were eager to use the enrichment provided and they made nests of the nesting material given. This is in concordance with the observations of Scharmann (1991). Fighting occurred mostly in the BALB/c mice. This strain is known to be more aggressive than the C57BL strain (Mondragón et al 1987).

From the present results it can be concluded that under the conditions of this study relatively simple enrichment of the environment has an influence on the animal's behaviour. The results indicated that C57BL mice housed in the enriched environment became more reactive and alert. In the BALB/c mice results may be interpreted as if the enriched environment lead to an increased level of anxiety.

The results of the present study suggest that the differential, strain specific behavioural response is an important factor to be taken into account when seeking the improvement of the animal's welfare by cage enrichment.

PREFERENCES FOR NESTING MATERIAL AS ENVIRONMENTAL ENRICHMENT FOR LABORATORY MICE

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SUMMARY

Behavioural and psychological needs of laboratory animals generally cannot adequately be met in standard laboratory cages. Environmental enrichment, which provides a more structured environment can enhance the well-being of laboratory animals. They may perform more of their species-specific behaviour and may control their environment in a better way. An easily applicable form of enrichment for laboratory mice is nesting material. Six different types of nesting material were evaluated in a preference test with male and female animals of two strains (C57BL/6J and BALB/c, N=48). No significant differences in preference were found between the strains or between the sexes.

All mice showed a clear preference for cages with tissues or towels as compared to paper strips or no nesting material, and for cages with cotton string or wood-wool as compared to wood shavings or no nesting material. Paper derived materials were preferred over wood derived materials, although the results also suggest that the nature (paper or wood) of the nesting material is less important than its structure, which determines the nestability of the material. Nesting material may be a relatively simple method to contribute to the well-being of laboratory mice.

INTRODUCTION

Standard laboratory cages are designed to fulfil the most essential needs in a laboratory animal's life, such as provision of food, water and a substrate, e.g. bedding or a grid floor, to avoid contact with their excreta. Animals however, also have behavioural and psychological needs most of which cannot be met in these laboratory cages. The animals are able to perform only a part of their complete species-specific behavioural repertoire (Van de Weerd et al 1994), which can result in abnormal behaviour such as stereotypies (Wemelsfelder 1990). Furthermore, animals maintained in unresponsive environments and highly unnatural groupings are less adequate models for extrapolating experimental results to humans (Markowitz & Gavazzi 1995). Environmental enrichment may enhance the well-being of laboratory animals by providing a more structured environment which enables them to perform more of their species-specific behaviour and which gives them more control over their environment (Chamove 1989a; Scharmann 1991). As a consequence, animals from enriched environments may be more physiologically and psychologically stable and better representatives of the species and thus ensure better data collection and scientific results. (Benn 1995; Markowitz & Gavazzi 1995; Scharmann 1991). The introduced enrichment should be interesting for the animals by meeting their behavioural requirements but, from the human point of view, it should be easy to provide, remove and clean (Van de Weerd & Baumans 1995).

Nesting material is an easily applicable form of enrichment for laboratory mice. Both males and females will build a nest when offered nesting materials (Lee 1972, 1973; Lisk et al 1969). Females build nests during pregnancy to bear and raise their young (Broida & Svare 1982; Lisk et al 1969). Hormones such as progesterone play an important role in this maternal nestbuilding (Lisk et al 1969). Between strains both qualitative and quantitative differences in maternal nestbuilding exist (Broida & Svare 1982). In laboratory mice, the use of nesting material reduces the preweaning mortality of pups and enhances the number of litters (Porter & Lane-Petter 1965). Similar results have been reported for rats (Nolen & Alexander 1966). Norris & Adams (1976) found that the type of nesting material used markedly affects the preweaning survival rates in the rat.

Nesting material is also used, both by females and males, as a source of protection e.g. against extreme environmental temperatures (Lisk et al 1969). Behavioural adaptations, such as nestbuilding, must be used for temperature regulation, when the physiological systems alone are inadequate to maintain body temperature (Lynch & Hegmann 1972). Lee & Wong (1970) showed that the amount of cotton used for nestbuilding increased with decreasing temperatures, although significant differences were found between strains of mice. Nests also

offer an opportunity to hide from predators and in the laboratory to avoid aggressive conspecifics, or to provide a shelter from overexposure to light.

When applying enrichment, it is necessary to evaluate the suitability of the enrichment programme, as various species or strains may respond differently to the methods of enrichment (Beaver 1989). Preference tests can be used to determine some general principles about species relevant properties of enrichment devices (Mench 1994). Choice tests have been used to assess the relative preference for or avoidance of several housing conditions in laboratory animals (Blom et al 1992). Blom et al (1996b) and Mulder (1975) offered mice different types of bedding material. Ottoni & Ades (1991) allowed hamsters to choose between nestboxes in relation to food and nesting material and gerbils were offered a choice between partially darkened or transparent cages (Van den Broek et al 1995). Pregnant mice and rats, chose wood derived bedding over beddings with other origins (e.g. clay, corn cob) to build nests before parturition (Mulder 1974, 1975). In Blom's study (1996b), mice showed a preference for shredded filter paper in comparison with smaller particled bedding material.

Nesting material has been studied mainly in relation with pregnancy or cold exposure. The aim of the present study was to investigate whether the nesting materials tested may serve as enrichment and to detect possible differences in preference for these materials between or within strains of mice. For this purpose male and non-pregnant female mice of two inbred strains were tested in a preference test and their choices for different types of nesting material (wood or paper derived) added to otherwise standard environmental conditions were evaluated.

ANIMALS AND METHODS

Animals

Female and male mice of two strains (C57BL/6JIcoU and BALB/c AnCrRyCpbRivU, N=48) were used. They were bred and raised without any nesting material. At the start of the experiment they were 8-10 weeks of age. The experiment was conducted in two cohorts, the first experiment (male mice, n=24) lasted seven weeks, the second (female mice, n=24) lasted four weeks.

The animals were housed (per strain and sex) in groups of six animals in a housing system consisting of two Macrolon type II cages (375 mm^2, UNO Roestvaststaal, Zevenaar, The Netherlands), connected with a passage tube, similar to the tubes used in the preference test system. Both cages were supplied with food-pellets *ad libitum* (RMH-B, Hope Farms, Woerden, The Netherlands), tap water *ad libitum* and sawdust bedding (Lignocel 3/4, Rettenmaier & Söhne,

Ellwangen-Holzmühle, Germany). The animals were kept in conventional rooms with controlled photo period (12:12 L:D, lights on at 07.00 h, approx. 200 lux at 1 m above the floor), temperature (20-22 ^{o}C), relative humidity (50-60%) and ventilation (15 air changes per h). Environmental conditions in the experimental rooms were similar, except for the light intensity which was approximately 300 lux at 1 m above the floor, in order to approach light intensities in standard animal rooms.

Preference test system

The preference test system used in this study has been validated and described in detail by Blom et al (1992). In short, a multiple housing system was used consisting of either two or four test cages (Macrolon type II) connected by non-transparent tubes (PVC, inner dimensions: 2.6x2.6x25 cm) to a central cage (15x15x18 cm, transparent perspex). When testing with a two-cage system the central cage was divided diagonally by a pvc sheet (19x17 cm). A total of six multiple housing systems were used divided over two four-tiered constructions in two similar experimental rooms. Each construction was turned gently during testing to prevent bias due to external influences in the experimental room which could interfere with the choice behaviour of the mouse.

The test cages were supplied with 50 g of sawdust bedding (Lignocel 3/4), a food hopper with equal amounts of food pellets (100 g, RMH-B) and tap water in bottles. The central cage had no food, water or bedding. The movements of the mice between the test cages were detected automatically by means of photo-electrical devices in the passage tubes. The signals were sent to a computer which calculated dwelling times per cage (software: Gate-Watch, Metris System Engineering, Wassenaar, The Netherlands).

Behavioural observations

One of the six multiple housing systems was equipped with a video camera system. Each test cage, including the central one, was provided with a video camera (Panasonic WV-1510). The cameras were connected with the photo-electrical devices, so the movements of the mouse could be followed in the test system. The signals from the video cameras were sent to a time-lapse video recorder (Panasonic AG-6700) which could record 24 h of testing (recording: 1/9 of normal speed). During the night the experimental room with the video equipment had red lights (approx. 5 lux at 1 m) to enable video recordings.

Procedure; Nesting materials

Mice were introduced into the test system between 15.00 and 17.00 h and tested individually during 48 hours. A group of six mice (of one sex and one strain) were

tested simultaneously. The behaviour of one animal was recorded for 12 h during day time (second day of the test) and for 12 h during night time (second night of the test). Food and water of each test cage were weighed before and after the experiment.

Three test series were performed to test six different types of nesting material. In each of the first two series (series 1: paper, series 2: wood), three nesting materials were compared (the fourth cage in each series contained sawdust bedding without nesting material). In the third series, per strain and sex the nesting materials which most animals had chosen in the first two series were compared in a two-cage system. Figure 1 shows the nesting materials and Table 1 describes the materials and gives the amounts provided per series (approximately equal volumes).

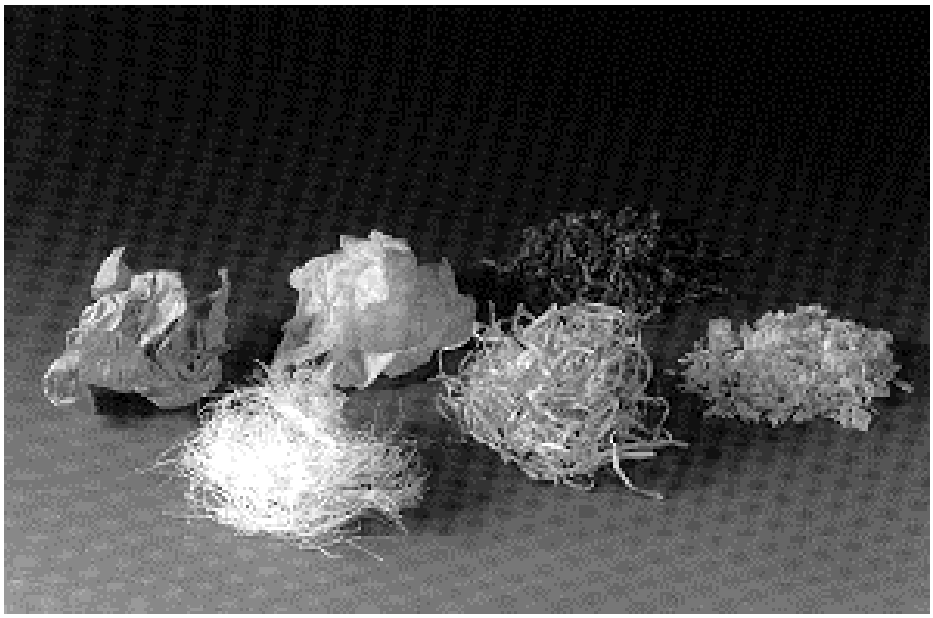

Figure 1 *The six different nesting materials tested in the preference test. Top row (paper series): paper towel (left), tissues (middle), folded paper strips (right). Bottom row (wood series): cotton string (left), wood-wool (middle), wood shavings (right).*

Table 1 *Nesting materials*

Paper		
Trade name	Appearance (size)	Amount
Paper towel[1]	sheet (25x31cm)	1 piece
Kleenex® tissues[2]	sheet (20x21cm)	2 pieces
Enviro-dri[3]	folded strips (11x0.3cm)	5 g
Wood		
Trade name	Appearance (size)	Amount
Sharpy[4]	cotton string (variable)	5 g
Wood-wool[3]	strips (variable)	5 g
Gold Shavings[3]	wood shavings (variable)	5 g

[1] Celtona, Cuijk, The Netherlands
[2] Kimberly-Clark Corporation®, EEC
[3] BMI, Helmond, The Netherlands
[4] VNK, Haarlem, The Netherlands

Statistical analysis

The dwelling data were analysed by distinguishing three time frames: the total of dwelling times during the 48 h of the experiment, the dwelling times during 12 h of day light (second day of the test) and the dwelling times of 12 h of night time (second night of the test). These two latter periods synchronised the periods of collected behavioural data (video tape recordings).

The method of statistical analysis used has been described by Blom et al (1995). Briefly, per test series the dwelling time data (in seconds) were logarithmically transformed as they were not always normally distributed, and to increase the independence of the data. For the same reason, central cage dwelling times were not included in the analysis. Data on food and water intake were not transformed, because they were normally distributed.

The data were analysed using multivariate repeated measures analysis (Wilk's lambda) to evaluate the influence of type of nesting material and interactions on choice behaviour and to detect possible differences between the strains, or sexes of a strain in choice behaviour. Food and water intake were analysed in a similar way as the dwelling times. Statistical significance was preset at $P<0.05$.

Overall significant differences between choice cages in dwelling times and amount of food and water consumed were further analysed using paired t-tests to indicate which of the cages were preferred or avoided. As multiple comparisons were made, the level of statistical significance was preset at $P<0.0083$

(Bonferroni's adaptation).

Behavioural data

The behavioural data on video tape were viewed and analysed using a behavioural observation software package (The Observer v 2.0, Noldus BV, The Netherlands). The tapes were viewed at normal speed, thus behaviour was seen nine times faster than the original behaviour. Every 5 s the behaviour was scored, which corresponds to one sample every 45 s in reality. The following ethogram was used to classify the behaviour (based upon Blom et al 1992):

Sleeping (sl) =
: movements are absent while the animal is in a sitting or lying position. Very short or minor movements during a long resting period (e.g. turning) are not considered as an interruption.

Manipulation (man) =
: manipulation of the nesting material, includes shredding, fraying, dragging and nestbuilding behaviour.

Grooming in the nest (gr-i) =
: while sitting or standing in its nest, the mouse is shaking, scratching, wiping or licking its fur, snout, ears, tail or genitals.

Grooming outside the nest (gr-o) =
: same as gr-i, but outside the nest

Ingestive behaviour (ing) =
: includes eating and drinking behaviour. Eating: gnawing on food particles from the food hopper or from the sawdust, coprophagy is included as well. Drinking: licking the nipple of the drinking bottle.

Locomotion (loco) =
: all other movements (e.g. walking, running, jumping).

Climbing (cli) =
: climbing on or hanging from the bars of the wire cage lid or food hopper, or standing on the passage tube or drinking nipple. While climbing or hanging the hind legs or tail may touch the cage walls.

Rearing (rear) =
: standing position with the forepaws not touching the cage floor. The animal is standing on its hind feet or toes, usually supporting itself with the tail. The fore-paws may lean against the passage tube, cage wall or food hopper.

Digging (dig) =
: bedding material is pushed forwards or backwards with nose, fore paws or hind legs. Mouse moves around or is sitting in one place.

Descriptive statistics were used to analyse the behavioural data, because only two animals from each sex and strain group (N=12) were observed per test series. The results were used to describe the behaviour of the mice in the different

test cages during a test series.The distribution of behaviour in each test cage was analysed for the night and day time period separately.

RESULTS

Figure 2 illustrates the mean relative dwelling times per cage for the paper series. Figure 3 and 4 show the same for the wood series and the paper vs wood series, respectively. Figure 5 (active behaviour) and Figure 6 (sleeping) give examples of the distribution of behaviour for the four cages in the paper and wood series.

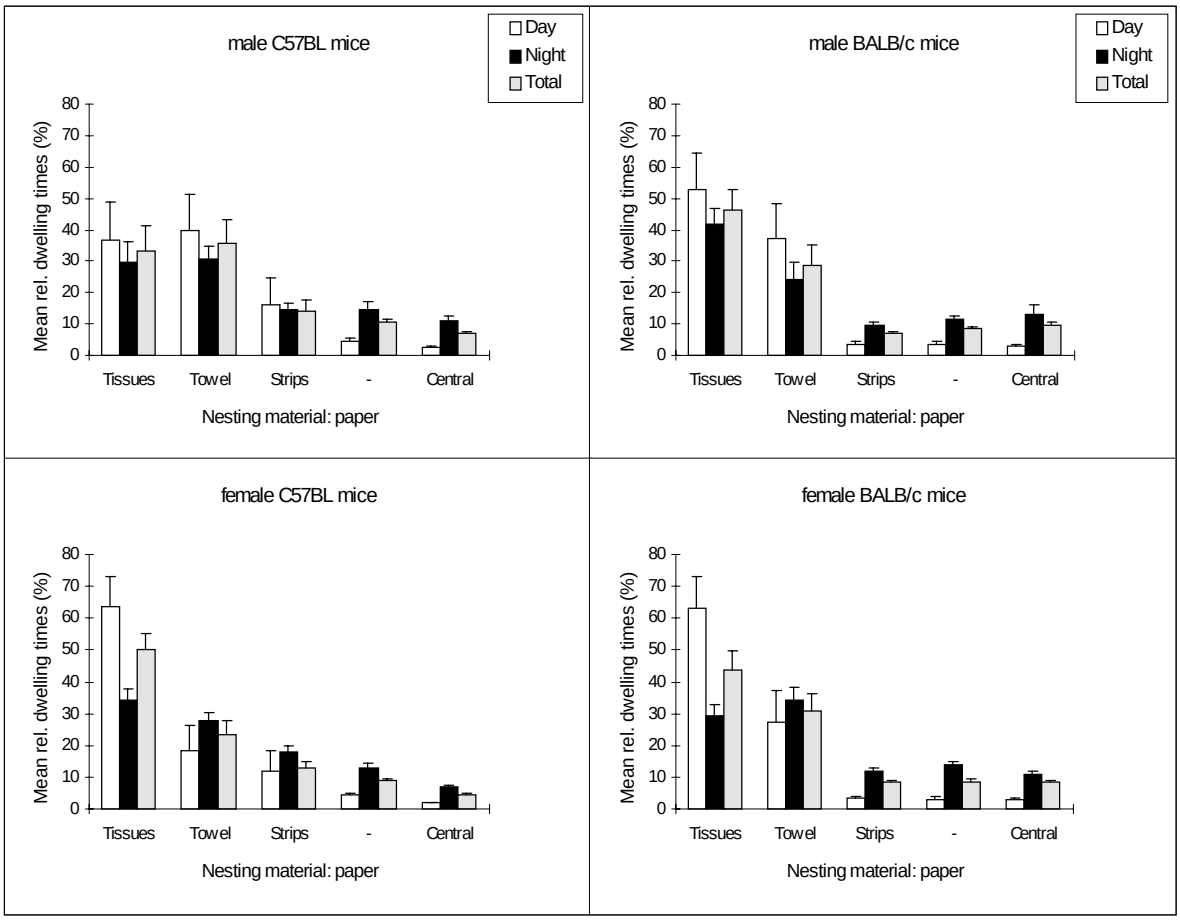

Figure 2 *Results of the preference test with three types of paper derived nesting material. Mean relative dwelling times per cage for day (= 12 h), night (=12 h) and total (=48 h) period, for mice of two strains (N=48).*

Cage choice

No significant differences in cage preferences (dwelling times) were found between strains in the paper series and the wood series, except for the total data of the paper series (MANOVA, $P<0.05$). The cause for this difference is not very clear. Also, no significant differences between the sexes of a strain were found, in all three test series and during all three time periods. Therefore, per strain both sexes were analysed together in the paired t-test analysis.

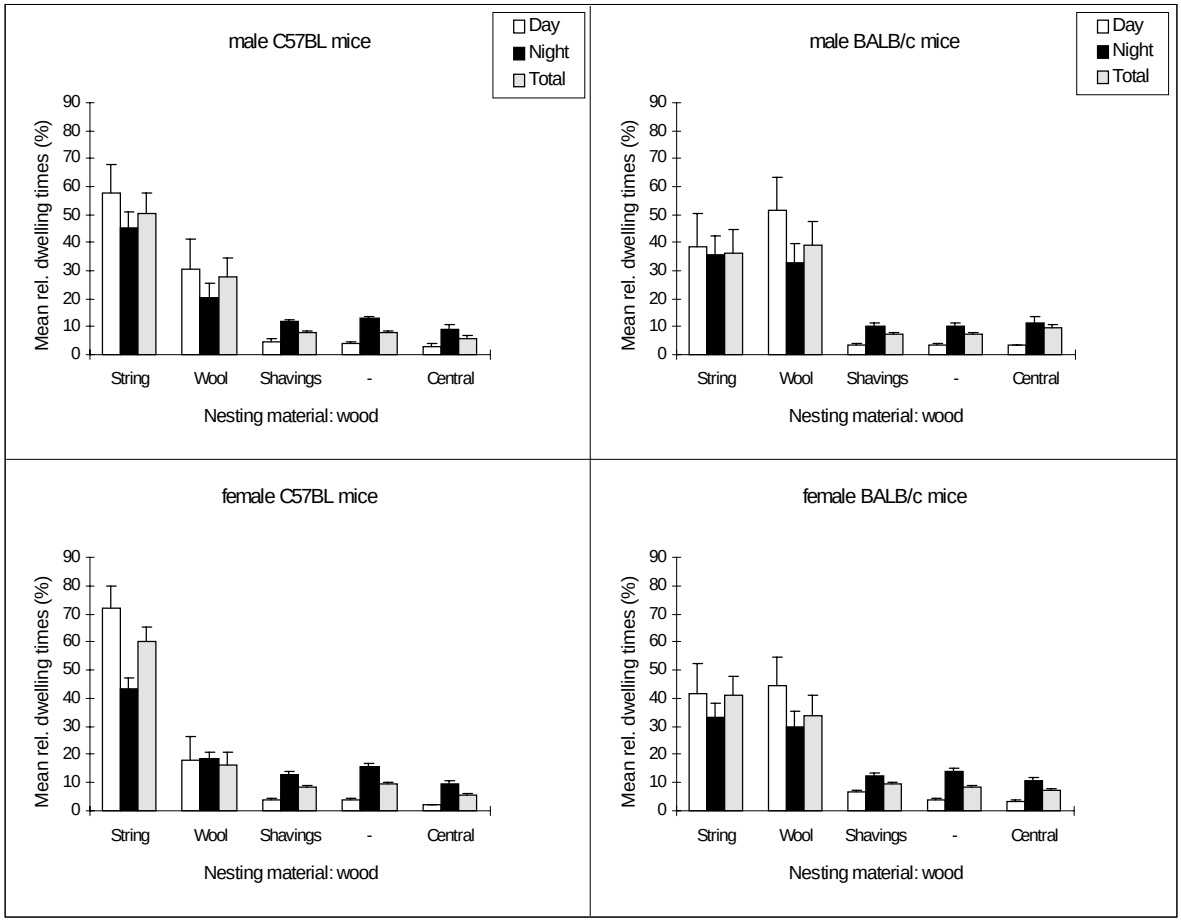

Figure 3 *Results of the preference test with three types of wood derived nesting material. Mean relative dwelling times per cage for day (= 12 h), night (=12 h) and total (=48 h) period, for mice of two strains (N=48).*

Mice of both strains chose significantly for a particular cage during all three time periods of both the paper and wood series (MANOVA, for all: $P<0.001$). Figure 2 illustrates that in the paper series the cages with the tissues and paper towels were preferred, whereas from Figure 3 it can be concluded that in the wood series the cages with the cotton string and wood-wool were preferred. In the paper series the biggest contrasts between cages were found to exist between the cages with tissues and towel on the one hand and the cage with paper strips and the cage without nesting material on the other. This was most clear for mice of the

BALB/c strain (paired t-test, all P<0.001). For the C57BL mice it was less obvious. The differences between the cages with either tissues or towel and the cage without nesting material were significant (paired t-test, all P<0.01), just as the contrast between the cages with the towel and paper strips (paired t-test, all P<0.005).

In the wood series mice of both strains spent significantly more time in the cages with cotton string and wood-wool in comparison to the cage with wood shavings and the cage without nesting material (both strains: paired t-test, all P<0.005). Only during night periods the contrast between the cage with the wood-wool and the cages with wood shavings or without nesting material were not significant for the C57BL. For the C57BL strain significant differences between the cages with the cotton string and wood-wool were found (paired t-test, all P<0.005).

In the paper vs wood series (Figure 4) the materials which most mice had chosen in the paper and in the wood series were compared; namely the paper towels vs cotton string for the C57BL males, and the tissues vs wood-wool for the BALB/c males. Females of both strains preferred the tissues and cotton string. Data were analysed per strain. The C57BL mice chose significantly for the cage with the paper nesting material (males: towels, females: tissues), but only during the total and day period (MANOVA, both: P<0.005). BALB/c mice did not make significant cage choices during all three time periods of this series.

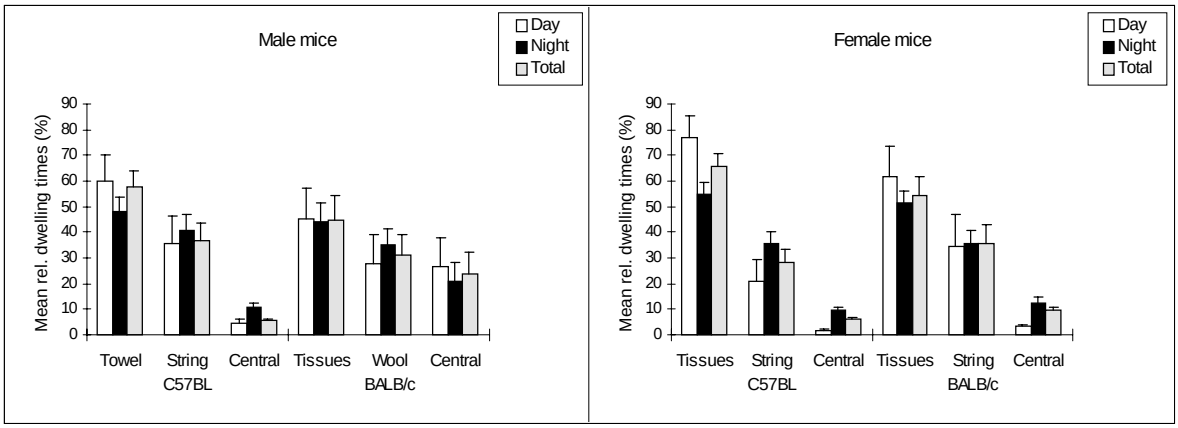

Figure 4 *Results of the preference test with the two most preferred nesting materials from the paper and wood series (as indicated). Mean relative dwelling times per cage for day (= 12 h), night (=12 h) and total (=48 h) period, for mice of two strains (N=48).*

Food and water intake

Significant differences between the strains in food and water intake were found in the wood series only (MANOVA, both: P<0.05). BALB/c mice consumed overall more food than the C57BL mice. In the paper and wood series no significant differences between the sexes of the strains were found in cage choice for food or water intake.

In the paper series mice of both strains made significant cage choices for water consumption, but not for food consumption. C57BL mice drank most in the cages with tissues and no nesting material (MANOVA, P<0.001); BALB/c mice in the cages with tissues and towels (MANOVA, P<0.005). Significant contrasts were found between the cage with paper strips and the cage without nesting material (C57BL: paired t-test, P<0.01) and the cages with paper strips and tissues (paired t-test, C57BL: p<0.01; BALB/c: P<0.001). In the wood series significant cage choices for food intake were made by the BALB/c mice, they ate most in the cages with wood shavings and no nesting material (MANOVA, P<0.005). The main contrasts were found between the cages with wood shavings and either wood-wool or cotton string (BALB/c: paired t-test, P<0.01). Significant cage choices for water consumption were made by the C57BL mice, they drank most in the cages with cotton string and no nesting material (MANOVA, P<0.05).

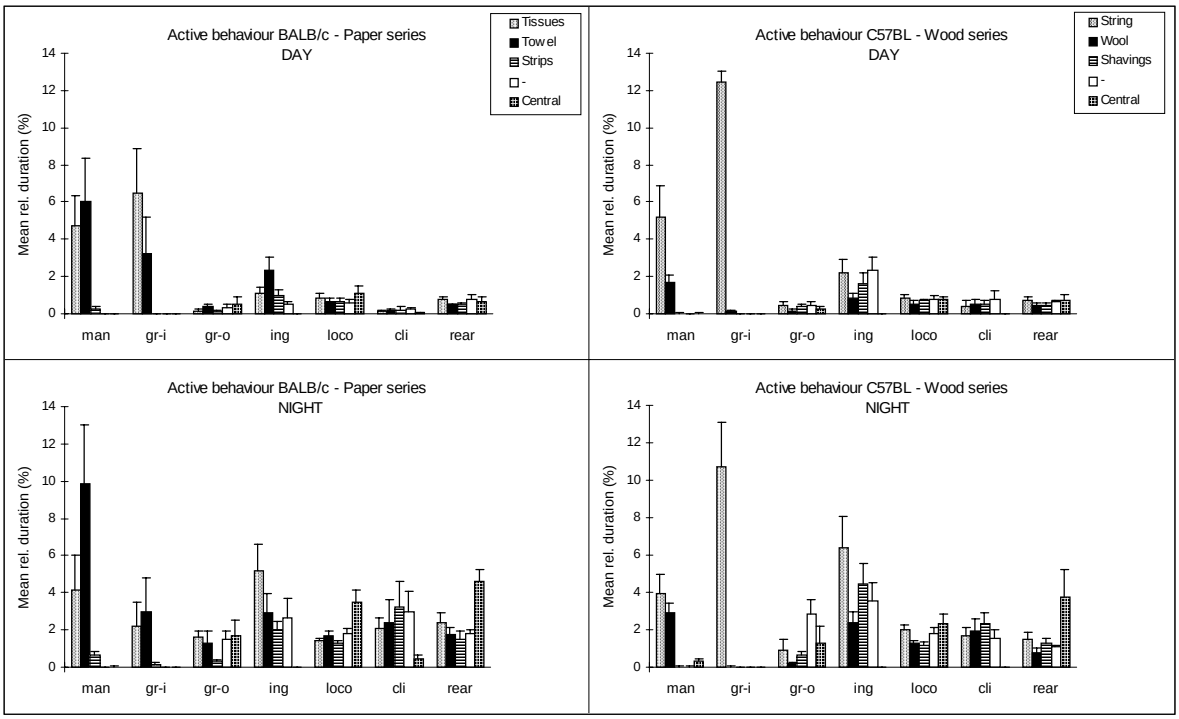

Figure 5 *Some results of the behavioural observations from preference tests for nesting material with mice of two strains (N=8). Left: paper series. Right: wood series. Mean relative time spent on indicated active behaviour during day (=12 h) and night (=12 h) period. (See Animals and Methods for explanation of abbreviations).*

In the paper vs wood series, food and water intake of the BALB/c mice did not differ significantly between the cages. The C57BL mice did not make a significant cage choice for water consumption, but the male mice ate significantly more in the cage with the paper towel than in the other cage (MANOVA, P<0.05), whereas the females did not make a significant cage choice.

Behavioural data

A striking behaviour performed by approximately half the number of animals, is the combining of nesting materials by dragging material from one cage to another. In the paper series the animals that dragged, always made a combination of tissues and towel. In the wood series the cotton string was combined with wood-wool and sometimes a few wood shavings were added. In general, the preferred materials were combined.

Figure 5 illustrates the distribution of active behaviour and Figure 6 sleeping, during day and night for the BALB/c mice (paper series) and the C57BL mice (wood series). During the day time the mice mostly slept in their preferred cage, where they also showed manipulation of the nesting material and grooming in the nest. During the night the mice were more active and they performed active behaviours (ingestive behaviour, locomotion, climbing, rearing, digging) in all cages of the test system, although a fair amount of sleeping was performed in the preferred cage.

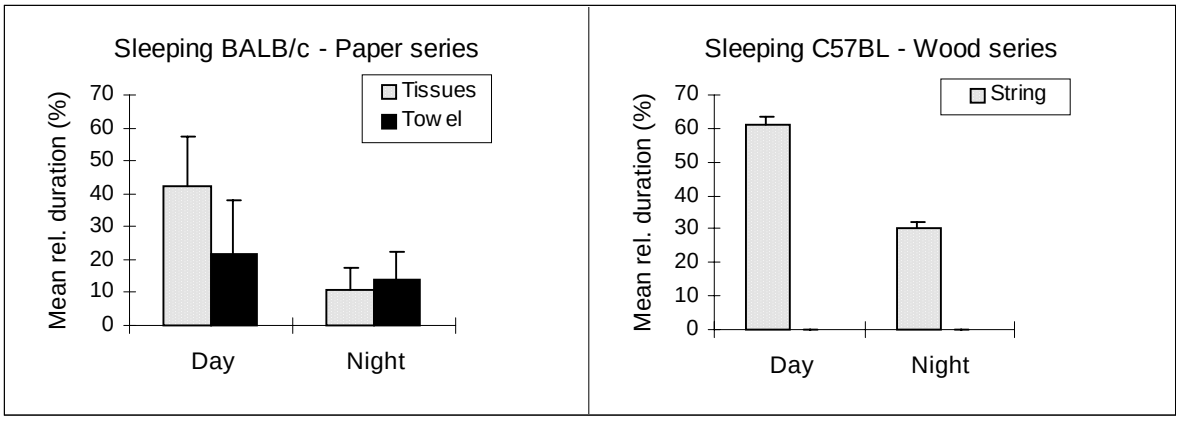

Figure 6 *Some results of the behavioural observations from preference tests for nesting material with mice of two strains (N=8). Left: paper series. Right: wood series. Mean relative time spent on sleeping behaviour during day (=12 h) and night (=12 h) period. (See Animals and Methods for explanation of abbreviations).*

DISCUSSION

The results indicate that mice preferred cages with nesting material and that they discriminate between different nesting materials and make consistent choices when submitted to a preference test. Cages with tissues or towels are preferred over cages with paper strips, whereas cages with cotton string or wood-wool are preferred over wood shavings as nesting material. C57BL mice preferred paper derived nesting materials over wood derived materials. BALB/c mice did not make

a significant choice between the two materials offered, but in most cases combined them. In the third series three male BALB/c spent most time in the central cage, where they combined all nesting materials. An explanation for this behaviour might be that when testing with only two cages, a piece of pvc is dividing the central cage in two parts, making this area smaller and relatively dark.

The fact that some animals combine nesting material might suggest that there is not a clear preference for the nature of nesting material (e.g. paper or wood) but that other features of the nesting material such as the structure (e.g. shredded or as a sheet), also play a role. In a choice test with mice, Mulder (1975) found a significant preference for bedding materials from a wood origin (aspen and cedar), but he did not test paper products. Both cellulose wadding and shredded paper as well as wood chips yielded low to normal preweaning mortality in mouse litters in the study by Porter & Lane-Petter (1965). In the study by Blom et al (1996b), both C57BL and BALB/c mice showed a preference for shredded paper bedding instead of sawdust or wood chips. Behavioural observations indicated that manipulation of the bedding and resting in nests were performed mostly on this type of bedding. In the study by Nolen & Alexander (1966) best breeding results were found when providing the rats with shredded paper as nesting material, whereas in the study by Norris & Adams (1976) better breeding results were obtained with wood-wool instead of paper tissues. These results also suggest that the nature of the nesting material might be less important than the structure (e.g. shredded or as a sheet). The structure may be important because it determines the nestability of the material. In the present study, the characteristic feature which the preferred nesting materials have in common is that the mice can manipulate them to build nests, and by doing this, they are able to structure their environment.Towel and tissues were shredded to build nests and the wood-wool and cotton string were shaped into the desired form. When nesting material is put into the home cages of mice they start building nests within minutes after introduction (Schneider & Chenoweth 1970; Watson 1993). With cage cleaning, these nests can be transported completely to the clean cage. In the present study several animals, especially of the BALB/c strain also combined the two preferred nesting materials to make more complicated nests. Pennnycuik (1973) also observed that mice moved nesting material (wood-wool) to the nestbox selected as nestsite. The behavioural observations in the present study showed that 10-20% of the timebudget was spent on manipulation of the nesting material during day or night.

Another aspect of the nesting material which could be an important criterion for selection by the mice is the degree of light absorption. Mice are nocturnal animals who often prefer to hide and sleep at dark places during day time. Exposure to light can cause damage to the eyes (Clough 1987). However, most of

the nesting materials preferred in the present study allowed some penetration of light (e.g. the tissues and cotton string). Only the paper towels could provide a shelter for light, but only if the mice were completely covered by the materials of their nest, which was mostly not the case.

In general, there were no differences in cage choice between the day and night periods. During darkness the mice spent most of their time in the same cage as during light periods, which was illustrated by the behavioural observations. However, during the night period the mice also visited the other cages of the testsystem. In general, the results showed that the mice visited all the cages of the housing system with approximately the same frequency, however the preference is based on the duration of their stay. Since sleeping requires most time, the cage selected for resting is the preferred cage by definition (Blom et al 1992). Figure 5 illustrates the increased activity of the mice during the night. In contrast with the present results, experiments with rats showed that different cages are preferred during day and night, suggesting that various behavioural activities require different cage floor covering (Van de Weerd et al 1996).

The study by Blom et al (1992) showed that from the recorded behaviour, 65% of the time is spent on sleeping, grooming and digging behaviour. In the present study, the same amount of time is spent on sleeping, grooming in the nest and manipulation of the nesting material. In the study by Blom et al mice did not have nesting material, so it seemed that they performed digging as a kind of redirected behaviour for nestbuilding activities. In the present study nestbuilding behaviour could be performed with the nesting material and digging was less frequently observed.

Mice of both strains consumed equal amounts of food in all four cages of the paper series. However, most water was consumed in the cage provided with tissues. This was also the preferred cage for all mice, except male C57BL. In the wood series the BALB/c mice ate most in the cage without nesting material (males) and the cage with the wood shavings (females) and the C57BL mice drank mostly in the cage with the cotton string. In the third series only male C57BL mice had a preference for the cage with the paper towel to consume food.

These results are not very consistent and are only partly in concordance with the results of Blom et al (1996b), who found that eating and drinking behaviour of the mice was similar for the four test cages with different types of floor covering. Hamsters, on the contrary, consumed most food close to their nests, because they preferred nestboxes nearest to the food source (Ottoni & Ades 1991). Food intake differed significantly between the strains, with BALB/c mice eating more than C57BL mice. This can be explained by a difference in weight; BALB/c mice are in general heavier than C57BL mice. Water intake did not differ between the strains.

Although no differences between the strains were observed regarding the choice of nesting material, there was strain-specific behaviour towards the nesting material. Tissues and towels were shredded more thoroughly by BALB/c mice than by C57BL mice. Also the shape of the nests differed. The BALB/c mice build dome-shaped nests (see Figure 7), whereas the C57BL mice made bowl-shaped nests. This has also been described by Lee & Wong (1970). Other studies have also reported differences in the shapes of sleeping nests between mice of different strains (Lee 1972, 1973; Lynch & Hegmann 1972, 1973). These differences in nestbuilding between strains are probably genetically determined (Lynch & Hegmann 1972).

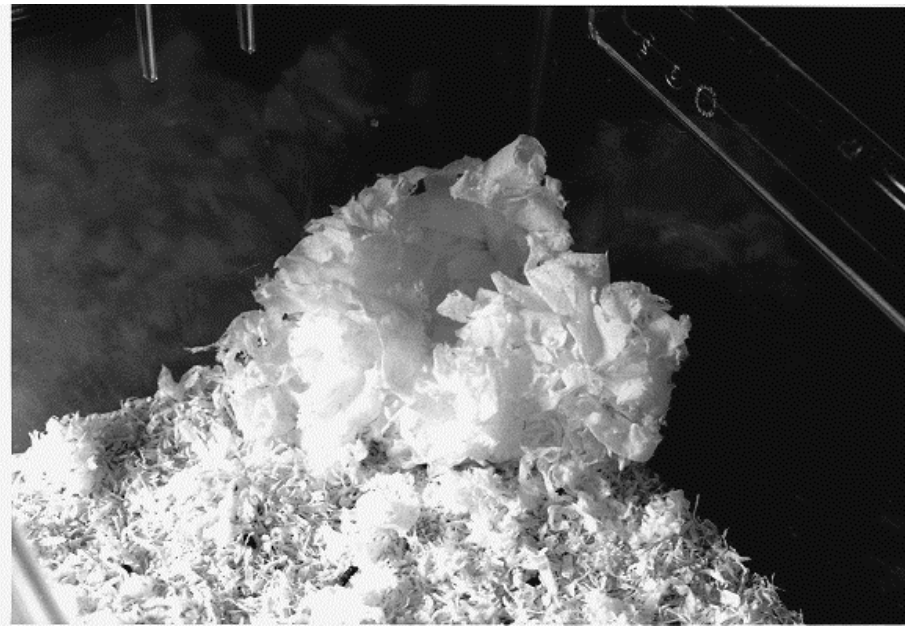

Figure 7 *Dome-shaped nest made of shredded tissues by a male BALB/c mouse.*

The results of this study show that laboratory mice prefer nesting material which they can use for nestbuilding. By providing them with nesting material the animals are able to use an active strategy to manipulate and control more aspects of their environment, which is important for the effectiveness of enrichment (Mench 1994; Sluyter et al 1995). Nesting material is easily applicable in standard cages and thus may be a relatively simple method to contribute to the well-being of laboratory mice.

Acknowledgements
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PREFERENCES FOR NEST BOXES AS ENVIRONMENTAL ENRICHMENT FOR LABORATORY MICE

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SUMMARY

In nature mice live in burrows with nest chambers, where they breed and may hide for predators. In the laboratory a shelter or refuge is an easily applicable form of enrichment, which may enhance the welfare of laboratory mice by giving them more control over their environment. A nest box provides an opportunity to withdraw actively from frightening stimuli outside or inside the cage.

Six nest boxes made of different materials were evaluated in a preference test with male and female mice of two strains (C57BL/6J and BALB/c). In general mice showed a preference for cages with a nest box made of grid metal as compared to clear or white perspex nest boxes, or no nest box and a preference for a cage with a nest box of perforated metal as compared to nest boxes made of grey PVC or sheet metal, or no nest box. When testing a nest box with one open side against a nest box with two open sides, most mice preferred the nest box with one open side and were observed to lie with their heads directed towards the opening.

The results of this study show that nest boxes may be used for enrichment purposes, although it is not entirely clear yet what the main determining features for the animal's choice are. When providing nest boxes as shelters, the structure and design of this type of enrichment should be taken into account, because these may have an effect on the social structure of groups of mice.

INTRODUCTION

Habitat selection or nest-site choice by mice is an important form of behaviour oriented towards the individual's maintenance and survival and its reproductive success (the nest is the most suitable place for breeding), and as a consequence, this choice behaviour is vital to the survival of the species (Buhot-Averseng 1981). In nature, wild rodents live in complex burrows, consisting of tunnels and nest chambers in which they establish their nests (Adams & Boice 1981). When nest boxes were placed in the ground in woods, within 24h, at least 75% of them were visited by rodents, using the nest boxes for different activities: as a feeding post, for storage of food, for the construction of a nest or for bearing and raising offspring (Ryszkowski & Truszkowski 1970; Truszkowski 1974). When mice are startled they will flee to the nearest cover or to their nest if it is not too far away (Schleidt 1951). Laboratory mice explored a novel arena more when a burrow-like shelter was present and they used the burrow for hiding when a predator model was introduced (Birke et al 1985).

Most environments of laboratory animals have been designed to serve human convenience, with little consideration for the animal's nature (Van de Weerd et al 1994). Environmental enrichment (i.e. additions to an animal's environment with which it can interact), provides a more structured environment which enables laboratory animals to express more of their species-specific behavioural patterns and thus may enhance their well-being (Beaver 1989; Scharmann 1991). Providing a shelter or refuge gives laboratory animals more control over their environment because it gives them the opportunity to actively withdraw from frightening stimuli outside or inside their cage (e.g. aggressive conspecifics), as well as hide from too much light (Van de Weerd & Baumans 1995). Different types of shelters have been provided in the home cages of laboratory mice for enrichment purposes, eg old drinking bottles (Ward & DeMille 1991), plastic tubes (Peters & Festing 1990), a perspex nest box (Van Loo et al 1996) or a PVC labyrinth (Haemisch et al 1994). Most of those shelters, however, are not very practical in the daily care of laboratory animals. Moreover, there is very little systematic research on the type of shelter required. The utilisation by the animal of a provided object can be measured with the use of behavioural observations or automatic measurements of behaviour (Büttner 1993).

Measuring the preferences of animals is a method of assessing what the animal regards as being better for its welfare and it leads to possibilities for designing better housing conditions (Broom 1988). In this context choice tests have been used in various ways, Blom et al (1992, 1995) studied the relative preference or avoidance for bedding and other housing conditions in mice and rats, and Ottoni & Ades (1991) studied preferences for nest boxes in relation to

food and nesting material in hamsters. In an earlier study, Van de Weerd et al (accepted/a) assessed the preference of mice for nesting material to be used as enrichment. Buhot (1981, 1986, 1987, 1989) studied choice behaviour of mice in order to get information about the perceptual abilities of mice to use the spatial properties of their environment for nest-establishment. In a number of studies individual mice or mice in groups were offered nest boxes differing in size, shape and material.

In the present study the preference of laboratory mice for different types of nest boxes was studied in order to test which type of nest box could be used as enrichment for laboratory mice. Mice were offered a free choice for either a cage with or without a nest box, by offering four cages similar in dimensions and contents (food, water and bedding), except for the presence of a nest box.

The experiments of Buhot (1981, 1986) using nest boxes with different shapes revealed a strong preference of mice for nest boxes with a rectangular shape. Adams & Boice (1981) allowed mice to dig burrows in burrow boxes, where it appeared that laboratory mice made almost identical complex burrows as wild mice. Individual mice of the two inbred strains used, made nest chambers which all had the typical dimensions of 8x10x6 cm. Therefore, in the present study rectangular nest boxes with 8x10x6 cm dimensions, but made of different materials, were tested as potential sources of enrichment for laboratory mice in a preference test. The preference for open (two open sides) or closed (one open side) nest boxes was also studied, as natural burrows of mice contain more than one opening (Adams & Boice 1981; Dudek et al 1983).

ANIMALS AND METHODS

Animals

Female and male mice of two strains (C57BL/6JIcoU and BALB/c AnCrRyCpbRivU, N=47) were used. The animals had previously been used in tests studying preferences for nesting material, the time span between those tests and the ones described here was a period of 18 weeks. At the start of the experiment the mice were 26-28 weeks of age. The experiment was conducted in two cohorts, the first experiment (female mice, n=24) lasted seven weeks, the second (male mice, n=23) lasted eight weeks. One male BALB/c mice died before the experiments started.

The animals were housed (per strain and sex) in groups of six animals (and one group of five) in a housing system consisting of two Macrolon type II cages (375 mm^2, UNO Roestvaststaal, Zevenaar, The Netherlands), connected with a passage tube, similar to the tubes used in the preference test system. Both cages

were supplied with food-pellets *ad lib* (RMH-B, Hope Farms, Woerden, The Netherlands), tap water *ad lib* and sawdust bedding (Lignocel 3/4, Rettenmaier & Söhne, Ellwangen-Holzmühle). The animals were kept in conventional rooms with controlled photoperiod (12:12 L:D, lights on at 07.00 h, approx. 200 lux at 1 m above the floor), temperature (20-22 ^{o}C), relative humidity (50-60 %) and ventilation (15 air changes h^{-1}). Environmental conditions in the experimental rooms were similar, except for the light intensity which was approximately 300 lux at 1 m above the floor, in order to approach light intensities in standard animal rooms.

Preference test system

The preference test system used in this study has been validated and described in detail by Blom et al (1992). In short, a multiple housing system was used consisting of either two or four test cages (Macrolon type II) connected by non-transparent tubes (PVC, inner dimensions: 2.6x2.6x25 cm) to a central cage (15x15x18 cm, transparent perspex). When testing with a two-cage system the central cage was divided diagonally by a PVC sheet (19x17 cm). A total of six multiple housing systems were used divided over two four-tiered constructions in two similar experimental rooms. Each construction was turned gently during testing to prevent bias due to external influences in the experimental room which could interfere with the choice behaviour of the mouse.

The test cages were supplied with 50 g of sawdust bedding (Lignocel 3/4), a food hopper with equal amounts of food pellets (100 g, RMH-B) and tap water in bottles. The central cage had no food, water or bedding. The movements of the mice between the test cages were detected automatically by means of photo-electrical devices in the passage tubes and sent to a computer which calculated the dwelling times per cage (software: Gate-Watch, Metris System Engineering, Wassenaar, The Netherlands).

Behavioural observations

One of the six multiple housing systems was equipped with a video camera system. Each test cage, including the central one, was provided with a video camera (Panasonic WV-1510). The cameras were connected with the photo-electrical devices, so the movements of the mouse could be followed in the test system. The signals from the video cameras were sent to a time-lapse video recorder (Panasonic AG-6700) which could record 24 h of testing (recording: 1/9 of normal speed). During the night the experimental room with the video equipment was illuminated with red light (approx. 5 lux at 1 m) to enable video recordings.

Procedure and nest boxes

Mice were introduced into the test system between 15.00 and 17.00 h and tested individually during 48 h. A group of six mice (of one sex and one strain) was tested simultaneously. Food and water of each test cage were weighed before and after the experiment.

Three test series were performed to test six nest boxes made of different materials, with the following dimensions: 8x10x6 cm (see Figure 1).

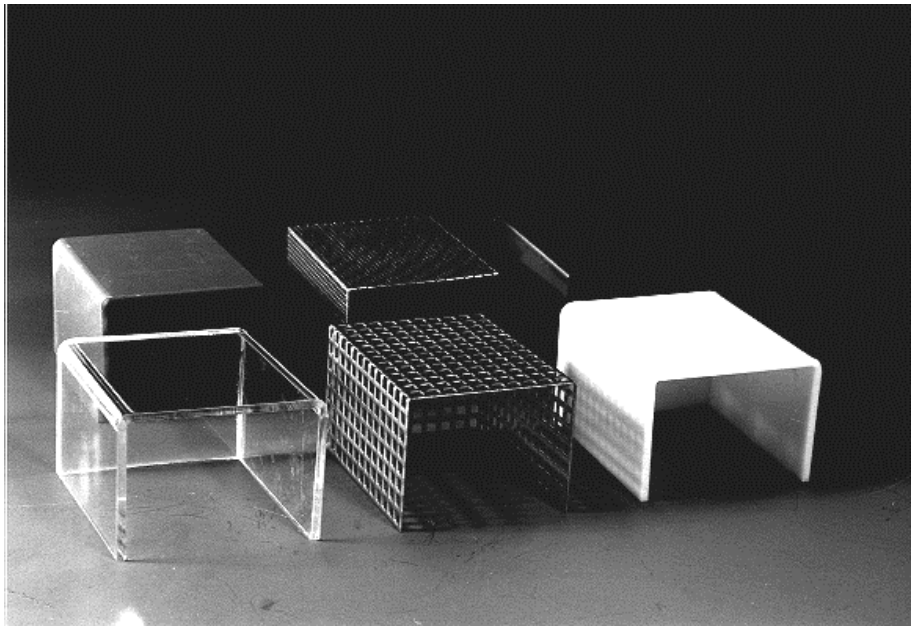

Figure 1 *The six different nest boxes tested in the preference test. Bottom row (light series): clear perspex (left), metal grid (middle), white perspex with holes (right). Top row (dark series): grey PVC (left), perforated sheet metal (middle), sheet metal (right).*

In the first series, three nest boxes were tested, made of materials which where rather translucent (light series). They were made of clear perspex, metal grid and white perspex with three holes in each side wall, two in the back wall and nine holes in the roof (diameter of holes: 5 mm). In the second series three nest boxes were tested, made of more opaque materials (dark series). They were made of grey PVC, perforated sheet metal and sheet metal. In both series, the fourth cage contained only sawdust bedding. In the third series, per strain and sex the nest boxes which most animals had chosen in the first two series were tested in a two-cage system.

In a fourth test series the perforated metal nest box (closed) was tested

against a similar nest box which had the rear wall removed (open), to test whether the mice preferred an open or closed nest box. For this purpose a two-cage test system was used.

During the series with the light nest boxes the behaviour of one animal was recorded for 12 h during day time (second day of the test) and for 12 h during night time (second night of the test). The behaviour of the mice during a series with dark nest boxes was not recorded, because it was not possible to observe a mouse when it was in a dark nest box.

Statistical analysis

The dwelling time data were analysed by distinguishing three time frames: the total of dwelling times during the 48 h of the experiment, the dwelling times during 12 h of day light (second day of the test) and the dwelling times of 12 h of night time (second night of the test). These two latter periods synchronised the periods of collected behavioural data (video tape recordings).

The method of statistical analysis used has been described by Blom et al (1995). Briefly, per test series the dwelling time data (in seconds) were logarithmically transformed as they were not always normally distributed, and to increase the independence of the data. For the same reason, central cage dwelling times were not included in the analysis. Food and water intake data were not transformed, because they were normally distributed.

The data were analysed using multivariate repeated measures analysis (Wilk's lambda) to evaluate the influence of type of nest box and interactions on choice behaviour and to detect possible differences between the strains or the sexes of a strain in choice behaviour. Food and water intake were analysed in a similar way as the dwelling times. Statistical significance was pre-set at $P<0.05$.

Overall significant differences in dwelling times and amount of food and water consumed between choice cages were further analysed using paired t-tests to indicate which of the cages were preferred or avoided. As multiple comparisons were made, the level of statistical significance was pre-set at $P<0.0083$ (Bonferroni's adaptation).

Behavioural data

The behavioural data on video tape were viewed and analysed using a behavioural observation software package (The Observer v 2.0, Noldus BV, The Netherlands). The tapes were viewed at normal speed, thus behaviour was seen nine times faster than the original behaviour. Every 5 s the behaviour was scored, which corresponds to one sample every 45 s in reality. The following ethogram was used to classify the behaviour (based upon Blom et al 1992):

Sleeping in a nest box (sl-in) =

movements are absent while the animal is in a sitting or lying position. Very short or minor movements during a long resting period (eg turning) are not considered as an interruption.

Sleeping outside a nest box (sl-out) =

same as previous, except that the behaviour is performed outside a nest box.

Grooming in nest box (gr-in) =

while sitting or standing, the mouse is shaking, scratching, wiping or licking its fur, snout, ears, tail or genitals.

Grooming outside a nest box (gr-out) =

same as grooming in a nest box, except performed outside a nest box.

Ingestive behaviour (ing) =

includes eating and drinking behaviour. Eating: gnawing on food particles from the food hopper or from the sawdust, coprophagy is included as well. Drinking: licking the nipple of the drinking bottle.

Exploration in a nest box (ex-in) =

this includes all locomotion (movements) and digging (pushing bedding material forwards or backwards with nose, fore paws or hind legs) performed in a nest box.

Exploration outside a nest box (ex-out) =

locomotion, rearing (standing on hind feet, fore paws not touching the floor) and digging performed outside a nest box.

Exploration on a nest box (ex-on) =

locomotion and rearing on a nest box

Climbing (clim) =

climbing on or hanging from the bars of the wire cage lid or food hopper, or standing on the passage tube or drinking nipple. While climbing or hanging the hind legs or tail may touch the cage walls.

Descriptive statistics were used to analyse the behavioural data, because only two animals from each sex and strain group (N=12) were observed in the test series with the light nest boxes. The distribution of behaviour in each test cage was analysed for the night and day time period separately.

RESULTS

Cage choice

In the series with light and dark nest boxes, significant cage choices were made by mice of both strains during all three time periods (all $P<0.05$), except for the BALB/c mice during the night period in the dark nest boxes series.

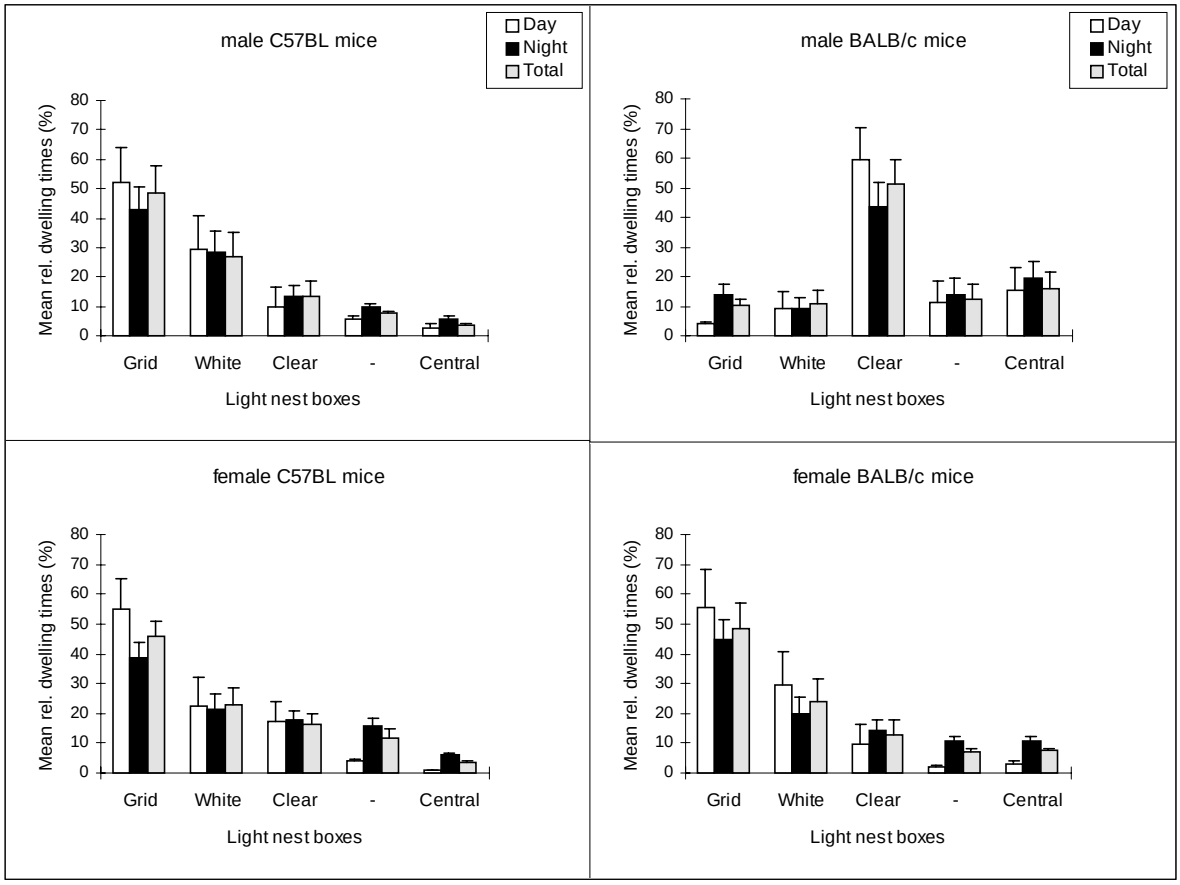

Figure 2 *Results of the preference test with three nest boxes made of light materials. Mean relative dwelling times (and SEM) per cage for day (= 12 h), night (=12 h) and total (=48 h) period, for mice of two strains (N=47).*

Figure 2 shows the mean relative dwelling times (and SEM) per cage for the series with the light nest boxes. C57BL mice preferred the metal grid nest box (no significant difference between the sexes). Main contrasts found for the C57BL mice were between the cage with the metal grid nest box on the one hand and the clear perspex nest box or cage without a nest box on the other (paired t-tests, all $P<0.005$, all three time periods). BALB/c males preferred the clear perspex nest box, BALB/c females the metal grid nest box (significant difference between the sexes in all three time periods, MANOVA, all $P<0.001$). Main contrasts for both sexes were found to exist between the cages with the metal grid nest box and the

clear perspex nest box (paired t-tests, all P<0.01, all three time periods). For the BALB/c females significant contrasts were also found between the cage without a nest box and the cage with the metal grid nest box (paired t-tests, all P<0.001, all time periods), and for the BALB/c males between the cage with the clear perspex nest box on the one hand and the cage without a nest box or the cage with the white perspex nest box on the other (paired t-test, all P<0.01, all time periods). In this series the difference between the strains appeared to be significant during total and night time period (MANOVA, both P<0.05).

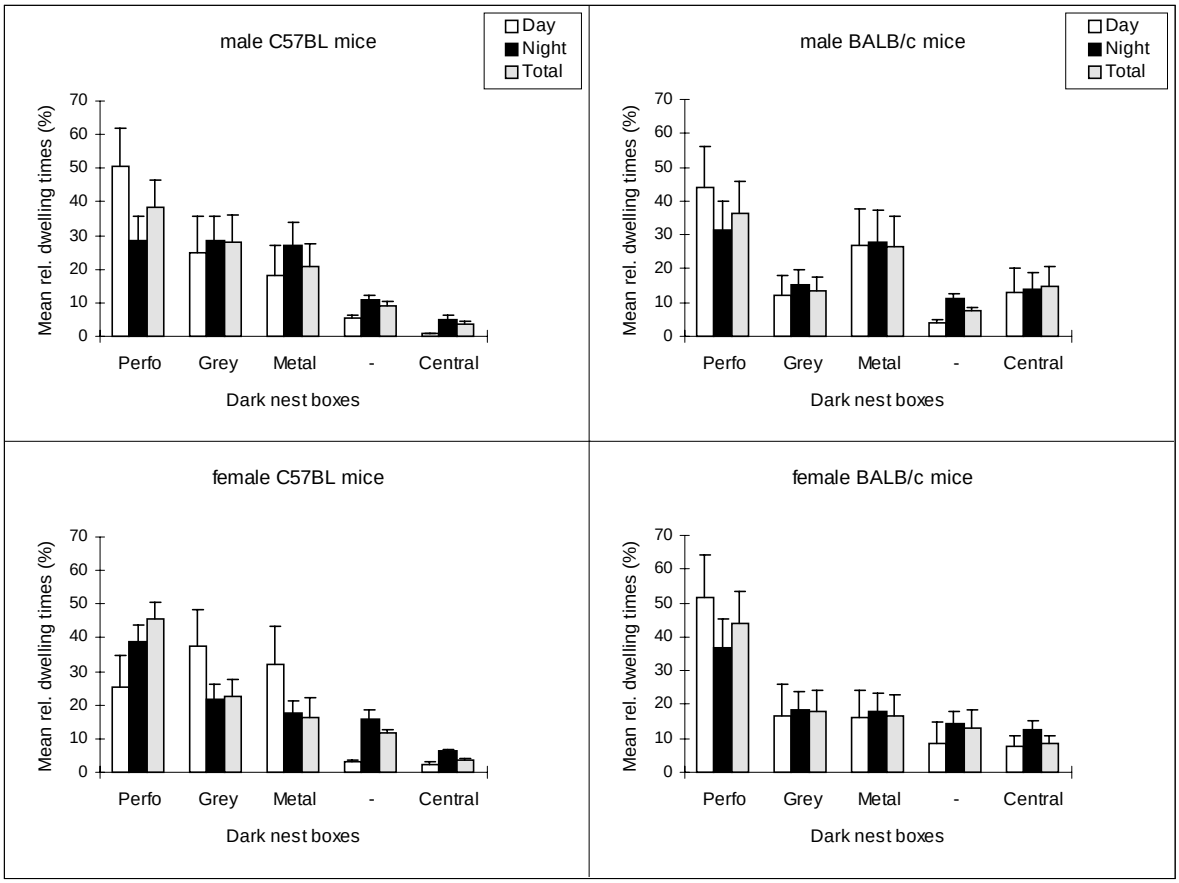

Figure 3 *Results of the preference test with three nest boxes made of dark materials. Mean relative dwelling times (and SEM) per cage for day (= 12 h), night (=12 h) and total (=48 h) period, for mice of two strains (N=47).*

Figure 3 shows the mean relative dwelling times (and SEM) per cage for the series with the dark nest boxes. C57BL mice chose the perforated metal nest box (no significant difference between the sexes). Significant contrasts were found for almost all time periods between the cage without a nest box on the one hand and the cages with the perforated metal, grey PVC or sheet metal nest box on the other (paired t-tests, all P<0.005). BALB/c mice preferred the perforated metal nest box (no significant difference between the sexes). The only significant

contrast here was found between the cage with the perforated metal nest box and the cage without a nest box, but only for the total time period (paired t-test, P<0.001). In this series there was no significant difference between the strains in cage preference.

In the light vs dark series the nest boxes which most mice had chosen in the previous two series were tested against each other, being the perforated metal nest box vs the metal grid nest box for the C57BL mice and BALB/c female mice, and the perforated metal nest box vs the clear perspex one for the BALB/c male mice. No significant cage choices were made by both strains during all time periods (results not shown).

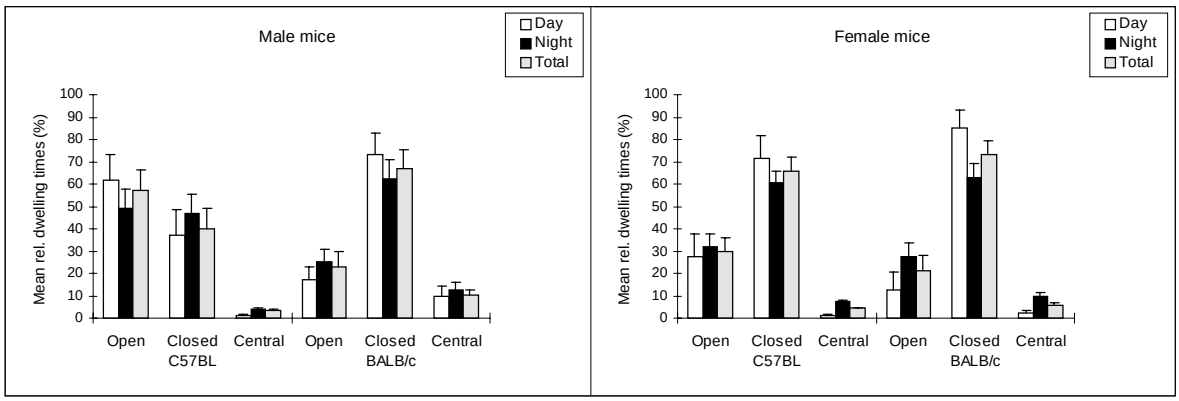

Figure 4 *Results of the preference test with the open (two open sides) vs closed (one open side) nest box. Mean relative dwelling times (and SEM) per cage for day (= 12 h), night (=12 h) and total (=48 h) period, for mice of two strains (N=47).*

In the open vs closed nest box series (Figure 4) the BALB/c mice significantly chose for the closed nest box during all three time periods (MANOVA, all P<0.005). In the C57BL strain only the females made a significant choice for the closed nest box during the total time period (MANOVA, P<0.05, difference between the sexes, MANOVA, P<0.05). There was a significant difference between the strains in this series during the total and day time period (MANOVA, all P<0.05).

Food and water intake

In all four test series no significant cage choices for food intake were made by either strain. Significant cage choices for water consumption were made in the series with light nest boxes. C57BL mice drank most in the cages with the white perspex nest box and the cage without a nest box. (MANOVA, P<0.05). BALB/c mice drank most in the cage without a nest box (MANOVA, P<0.005). There were no differences between the sexes in both strains. However, the difference between the strains was significant (MANOVA, P<0.05). Significant contrasts for

water intake were found for the BALB/c mice between the cage without a nest box on the one hand and the cages with the white or clear perspex nest box on the other hand (paired t-tests, all P<0.005).

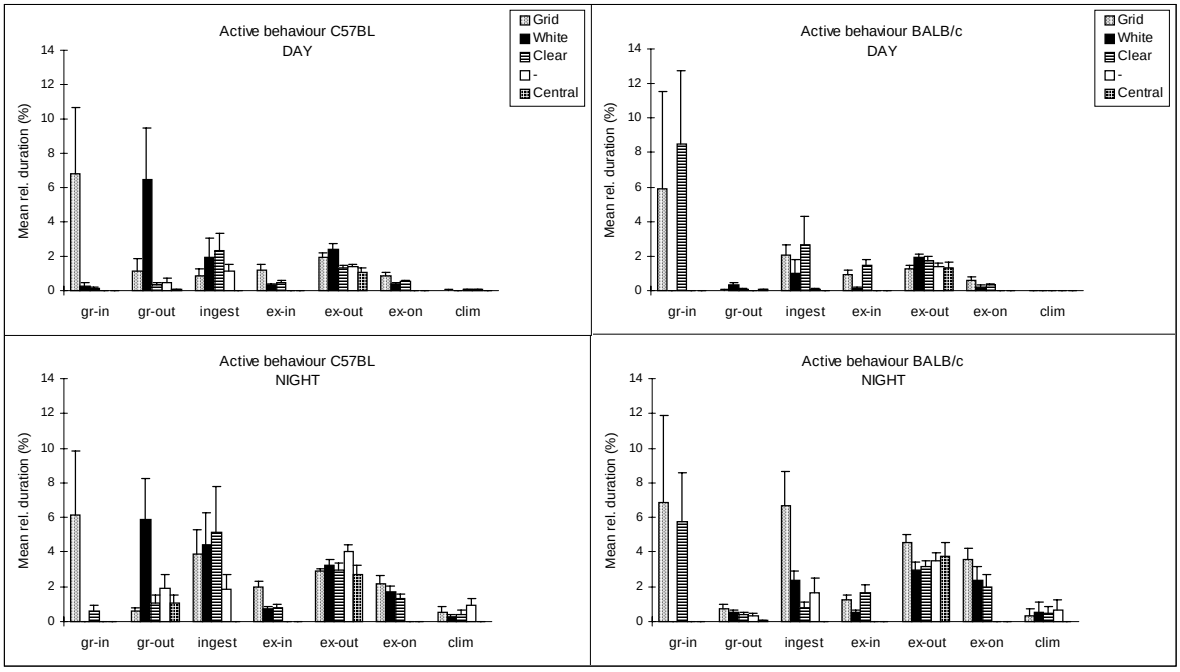

Figure 5 *Results of behavioural observations from preference tests with light nest boxes with mice of two strains (N=7). Mean relative time (and SEM) spent on indicated active behaviour during day (=12 h) and night (=12 h) period (See Animals and Methods for explanation of abbreviations).*

In the series with dark nest boxes BALB/c mice (no significant difference between the sexes) drank most in the cage without a nest box (MANOVA, P<0.05), the contrasts between the cage without a nest box and the cages with the grey PVC or sheet metal nest box were significant (paired t-test, both P<0.005). In the light vs dark nest box series and in the open vs closed series no significant cage choices for water consumption were made by either strain.

Behavioural data

Figure 5 illustrates the distribution of active behaviour during day and night periods for BALB/c and C57BL mice in the series with the light nest boxes. Figure 6 shows sleeping behaviour during day and night periods. Behavioural categories are indicated as relative durations of the total amount of behaviour performed. Behavioural data of one BALB/c male mouse is left out, because it preferred the central cage for sleeping. As this was the only mouse showing this preference its behaviour was not considered representative for the behaviour of the other mice in this test series.

During day time the mice mostly slept in their preferred cage in the nest box, but some C57BL mice sometimes slept in front of the nest box (sl-out). During the night the mice were active and showed exploration, climbing and ingestive behaviour in all four cages of the test system, although also a fair amount of time was spent on sleeping.

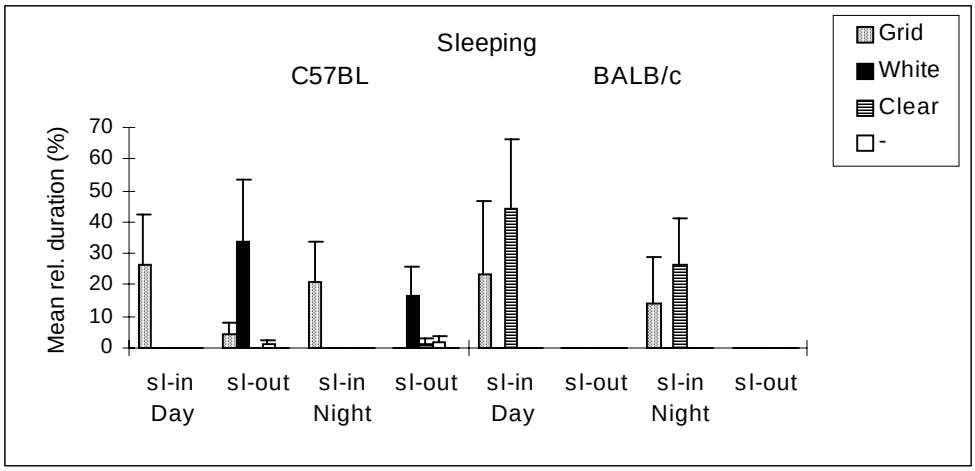

Figure 6 *Results of behavioural observations from preference tests with light nest boxes with mice of two strains (N=7). Mean relative time (and SEM) spent on sleeping behaviour during day (=12 h) and night (=12 h) period (See Animals and Methods for explanation of abbreviations).*

DISCUSSION

The results of this study show that the mice do make choices between different cages and that they prefer a cage containing a nest box. In general, the preferred nest boxes were those consisting of perforated metal or grid metal. These results are comparable with the results obtained by Buhot (1981, 1986, 1987, 1989) who used a different approach. In her study mice had to choose between six nest boxes in six successive trials. Within a trial the nest boxes were identical in design but different in material, whereas between trials different designs were used (each nest box had only walls but no roof). Mice appeared to make their choices on the basis of the materials and not on the basis of the design. The preferred materials in Buhot's study were the darker ones: close grid and perforated metal. These materials are comparable with the perforated metal and grid metal in our study. Both metal structures allow olfactory cues to pass, but are difficult to look through; however, some light may penetrate. Buhot concluded that visual cues may have been of most importance in nest box choice and that the mice used the same criterion (darkness) to make a choice. Hamsters also chose a dark nest box as nest site, the degree of illumination of the nest box was the criterion that prevailed

over the size of the nest box and the distance to a nesting material dispenser, but only two types of nest boxes - clear or black - were offered (Ottoni & Ades 1991).

In our test series with the dark nest boxes, however, the mice did not prefer the darkest nest boxes (sheet metal or grey PVC), but chose the perforated metal nest box. In the series with light nest boxes the BALB/c male mice preferred the clear perspex nest box, which let most light through, compared with the others. This choice is difficult to explain if one expects mice to choose a dark nest box as a shelter against too much light. BALB/c mice are albinos and therefore their eyes are sensitive to retinal damage caused by high light intensities (Clough 1987). We also observed that in the series with the light nest boxes some C57BL mice (two males, seven females) did not always occupy the nest box, but sometimes slept in front of it. The behavioural graph (Figure 6) illustrates this behaviour for the C57BL strain. Apparently, these mice did not use the nest box to hide for light either, but C57BL mice have pigmented eyes and are therefore less sensitive than BALB/c mice for high light intensities. But when the mice were disturbed they always ran into the nest box for hiding. Schleidt (1951) described similar behaviour, he observed mice sleeping in front of glass tubes which were too small to sleep in, but when they were alarmed they fled into the tubes.

The results suggest that not only visual cues determine the choice of the mice for a nest box, but perhaps also olfactory cues play a role. Most mice chose nest boxes which were not made of completely closed material, but contained holes in the walls (perforated metal and grid metal), which allow olfactory cues to pass. Odours play an important role in social behaviour of mice, especially in identifying individuals and their dominance status (Rawleigh et al 1993). Schleidt (1951) describes that wild house mice and field mice use different cues when selecting an object, depending on whether they choose a shelter or a nesting site. For the choice of nest site tactile cues are most important, whereas for a shelter light intensity cues are most relevant. Mackintosh (1973) has shown that in large enclosures male mice appear to use visual cues for the detection of territorial boundaries. More research is needed to find out what under various environmental conditions, the most important criteria for nest box choices are.

In the experiments by Buhot-Averseng (1981) the animals showed a high agreement in nest box choice. However, in our study, although overall choices were significant for the groups, individual mice did not always made a clear choice for one nest box, but spent time in two nest boxes (0-5 animals per group). The incidence of this behaviour was highest in the group of female C57BL mice. In a field study on the utilisation of nest boxes by rodents Truszkowski (1974) observed that adult rodents (except females rearing young) usually use a large number of shelters simultaneously. The experimental set-up of Buhot-Averseng did not allow the mice to choose more than one nest box, because after two hours

of time spent in one nest box it was removed.

The behavioural diagram in Figure 5 shows that climbing on the cage lid does not occur very often during day or night, although in standard cages it is a major and regularly occurring component of locomotor activity (Büttner 1991). When comparing Figure 5 with similar patterns obtained from observations of mice in preference tests with nesting material (Van de Weerd et al accepted/a), it can be seen that climbing has a much higher frequency. It seems that in the present study sitting on the nest box (in Figure 5: exploration on a nest box) partly substitutes climbing on the cage lid, so the nest box also serves as a climbing object.

The results of test series with the open vs closed nest box show that most mice preferred the closed nest box with only one open side, which seems to contradict the observation that they normally build burrows with more openings (Adams & Boice 1981; Dudek et al 1983). The structure of the nest boxes, however, did not allow the mice to control both openings when lying inside, so this may have been the reason that they preferred the nest box with only one open side. The animals were also observed to lie at the rear end of the nest box with their heads directed towards the opening. Mice provided with bottles as refuges showed similar behaviour, they tended to sleep with their heads toward the bottle opening (Ward & DeMille 1991).

Although the results of this study do not clearly indicate which criteria mice use to choose a nest box, it is clear that individual mice prefer a cage with a nest box and avoid cages without one. It is important, however, to consider the structure of the nest boxes when using them as enrichment. Slight modifications in the enriched environment can lead to unpredictable effects on mice (Bergmann et al 1994/1995). Several authors have reported increased aggression when applying a structure offering shelter in the home cage of group housed mice. Haemisch & Gärtner (1994) and Haemisch et al (1994) used a horizontal labyrinth consisting of vertical PVC dividers in a standard cage. In these enriched cages they observed an increase in intermale aggression when introducing unfamiliar males and a less stable social structure. The authors suggested that the available structures enhanced the territorial tendencies of the male mice. Similar effects on aggression were reported by Bergmann et al (1994/1995) who used a cage insert consisting of a passage-way, comparable with the structure used by Haemisch & Gärtner (1994) and Haemisch et al (1994). This structure led to an increase in aggression, measured as number of bite wounds.

Other authors have used shelter-like structures which did not elicit a higher incidence of aggression. Chamove (1989b) used an insert which created corridors and Ward & DeMille (1991) used plastic bottles as shelters. In a study by Rawleigh & Kemble (1992) the provision of a nest box decreased offensive

behaviour in a resident-intruder test. Differences between these studies may be partly explained by the different strains of mice used, as some strains are reported to be more aggressive (Mondragón et al 1987) and it is likely that these strains are more sensitive for effects of enrichment. Haemisch & Gärtner (1994) also reported differences in the level of aggression between the two strains used (DBA/2J and CBA/J).

The results of the above mentioned studies, also suggest that the structure of the insert might be a determining factor in stimulating aggression. Inserts in the cage structure the living environment and this may have an influence on the hierarchical system which normally develops when male mice are kept in a group (Haemisch et al 1994; Mackintosh 1981). Structures with only a few openings do not resemble natural settings. When given wild mice and laboratory mice the opportunity to burrow, their burrows contain al lot of openings to the surface, so there always are several routes for fleeing (Adams & Boice 1981; Dudek et al 1983). This was impossible in the designs used by Haemisch et al (1994), Haemisch & Gärtner (1994), and Bergmann et al (1994/1995), which may have given dominant males the opportunity to defend the few entrances into the corridors and this may have led to social instability. Bergmann et al (1994/1995) used a second passage way with more openings as well, which did not increase the number of bite wounds as much as the structure with only two openings. It is therefore important to study the effects of the application of a shelter for enrichment purposes (taking into account the structure and design), before applying it on a larger scale (Van de Weerd & Baumans 1995). All mice in this study preferred a cage with a nest box, which implies that nest boxes may be used for enrichment purposes.

Acknowledgements

The authors like to thank NA Miltenburg for construction of the nest boxes.

STRENGTH OF PREFERENCE FOR NESTING MATERIAL AS ENVIRONMENTAL ENRICHMENT FOR LABORATORY MICE

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SUMMARY

The present paper describes two experiments in which preferences of laboratory mice for materials which could serve as cage enrichment are investigated. Previous research revealed clear preferences for a cage with nesting material or a nest box instead of a cage with only bedding material. Both enrichment items may offer a hiding place e.g. as a means to avoid aggressive cage mates or over-exposure to light.

In the first experiment, the most preferred nesting material and the most preferred nest box (results from previous studies) were tested against each other. A strong preference of all mice was found for the nesting material. In the second experiment, the preferred nesting material was combined with a grid floor (previously found to be avoided) and the nest box was combined with bedding material. All mice preferred the cage with the nesting material. The mice were highly motivated to use nesting material, despite the presence of a grid floor. Thus it is concluded that providing a cage with nesting material may have a positive effect on the well-being of laboratory mice.

INTRODUCTION

Environments of laboratory animals have often been designed on the basis of economic and ergonomic aspects, with little or no consideration for animal welfare. Laboratory housing conditions can deprive animals of the possibility to perform a full repertoire of normal behaviour (Van de Weerd & Baumans 1995). The inability to engage in species-specific behaviour may cause signs of suffering such as abnormal behaviour or pathology (Jensen & Toates 1993). Environmental enrichment alters the environment by introducing materials or objects which are stimulating for the animals and which allow them to express more of their natural behavioural repertoire, thereby enhancing their well-being. Different animal species may have different enrichment requirements and when introducing enrichment to an animal's environment it is very important to evaluate whether or not the animal responds to the enrichment. Preference tests can be used to determine some general principles about species-relevant properties of enrichment devices (Mench 1994).

Previous studies on the preferences of laboratory mice for items which could serve as enrichment, revealed clear preferences for a cage with nesting material or a nest box instead of a cage with only bedding material (Van de Weerd et al submitted/a; accepted/a). Nesting material may have several functions. By building a nest mice can regulate their temperature and avoid too much light or hide for aggressive cage mates. Nest boxes may provide a shelter or refuge because they give mice the opportunity to actively withdraw from frightening stimuli inside or outside their cage (Van de Weerd and Baumans 1995).

The present paper describes two experiments in which these preferences are further investigated. The first experiment investigates if mice prefer a nest box over nesting material or vice versa by testing the most preferred nesting material and the most preferred nest box from both previous studies against each other. This experiment showed a strong preference of all mice for the nesting material. Therefore, in a second experiment the importance or strength of the preference for nesting material was studied.

One general criticism on preference tests is that they only give information about the relative properties of the choices given, but do not indicate the importance an animal attaches to a preferred option. In order to interpret the results of preference tests and to be able to apply them to practical situations where an improvement in welfare is sought, the strength of the preferences should be established (Broom 1988; Broom & Johnson 1993; Dawkins 1983; Duncan 1992; Fraser 1996). Where animals show that they are willing to work hard for the choices offered it is reasonable to conclude that their welfare is improved by achieving that objective (Broom 1988).

Several methods have been developed to measure the strength of preferences (see also Sherwin & Nicol 1995), e.g. the instrumental or operant technique approach, where an animal has to learn to activate some mechanisms such as lever pressing or lifting a weighted door (Collier et al 1990; Duncan 1992; Manser et al 1996; Roper 1973) or the natural obstacle or obstructive techniques approach where an animal has to overcome a natural barrier such as a narrow gap or water (Duncan 1992; Sherwin & Nicol 1995). An animal may however, not always be able to learn an operant response (Duncan 1992), it is therefore important that they associate the required activities with the goals to be reached and that the behaviour required for expressing the preference is reasonably natural for the type of reward (Fraser 1996). Behaviours such as lever pressing or lifting a weight are not very natural for most animals.

In experiment 2 of this study we have adopted the method of balancing one preference against another, as previously used by e.g. Van Rooijen with gilts (1980), Dawkins with hens (1981, 1983) and Blom et al with mice (1993). The testing variables (nesting material and nest box) were balanced against cage floor covering. Previous preference tests with the same strains of mice, showed that mice preferred bedding material and avoided wire mesh as floor covering (Blom et al 1996b). Thus the preferred nesting material (Van de Weerd et al accepted/a) was combined with the previously avoided grid floor and the preferred nest box (Van de Weerd et al submitted/a) was combined with previously preferred bedding material. This approach will give an indication whether the mice are willing to accept a grid floor in order to use the nesting material or whether the combination of sawdust with the nest box is more attractive.

ANIMALS AND METHODS

EXPERIMENT 1

Animals

Female and male mice of two strains (C57BL/6JIcoU and BALB/c AnCrRyCpbRivU, N=47) were used. The experiment was conducted in two cohorts. At the start of the first cohort (females, n=24) the mice were 13-14 weeks of age. At the start of the second cohort (males, n=23) the mice were 30-31 weeks of age. One male BALB/c mouse died before the experiments started. Both groups of mice were familiar with the nesting material and nest box offered in the test series (either in previous preference test series or in their home cages).

Housing

The animals were housed (per strain and sex) in groups of six animals in a housing system consisting of two Macrolon type II cages (375 mm^2, UNO Roestvaststaal, Zevenaar, The Netherlands), connected with a passage tube, similar to the tubes used in the preference test system to allow the mice to get used to them. Both cages were supplied with food pellets *ad libitum* (RMH-B, Hope Farms, Woerden, The Netherlands), tap water *ad libitum* and sawdust bedding (Lignocel 3/4, Rettenmaier & Söhne, Ellwangen Holzmühle, Germany). The animals were kept in conventional rooms with controlled photo period (12:12 L:D, lights on at 07.00 h, approx. 200 lux at 1 m above the floor), temperature (20-22 ^oC), relative humidity (50-60 %) and ventilation (15 air changes h^{-1}). Environmental conditions in the experimental rooms were similar, except for the light intensity which was approximately 300 lux at 1 m above the floor, in order to approach light intensities in standard animal rooms.

Preference test system

The preference test system used in this study has been validated and described in detail by Blom et al (1992). In short, a multiple housing system was used consisting of two test cages (Macrolon type II) connected by non-transparent tubes (PVC, inner dimensions: 2.6x2.6x25 cm) to a central cage (15x15x18 cm, transparent perspex). The central cage was divided diagonally by a PVC sheet (19x17 cm). A total of six multiple housing systems were used divided over two four-tiered constructions in two similar experimental rooms. Each construction was turned gently during testing to prevent bias due to external influences in the experimental room which could interfere with the choice behaviour of the mice.

The test cages were supplied with a food hopper with equal amounts of food pellets (100 g, RMH-B) and tap water in bottles. The central cage had no food, water or bedding. The movements of the mice between the test cages were detected automatically by means of photo-electrical devices in the passage tubes. The signals were send to a computer which calculated dwelling times per cage (software: Gate-Watch, Metris System Engineering, Wassenaar, The Netherlands).

Behavioural observations

One of the six multiple housing systems was equipped with a video camera system. Both test cages and the central one, were provided with a video camera (Panasonic WV-1510). The cameras were connected with the photo-electrical devices, so the movements of the mouse could be followed in the test system. The signals from the video cameras were sent to a time-lapse video recorder (Panasonic AG-6700) which could record 24 h of testing (recording: 1/9 of normal speed). During the night the experimental room with the video equipment had red lights (approx. 5 lux at 1 m) to enable video recordings.

Procedure

Mice were introduced into the test system between 15.00 and 17.00 h and tested individually during 48 h. A group of six mice (of one sex and one strain) was tested simultaneously. The behaviour of one animal was recorded for 12 h during day time (second day of the test) and for 12 h during night time (second night of the test). Food and water of each test cage were weighed before and after the experiment.

Per strain and sex group the most preferred nesting material (Van de Weerd et al accepted/a) was tested versus the most preferred nest box (Van de Weerd et al submitted/a). Both items were offered in a test cage supplied with 50 g of sawdust bedding (Lignocel 3/4). Table 1 shows the materials tested.

Table 1 *Materials tested in experiment 1*

Animals	Nesting material (amount)	Nest box (8x10x6 cm)
C57BL		
males	Paper towel[1] (1 piece)	perforated metal
females	Kleenex® tissues[2] (2 pieces)	perforated metal
BALB/c		
males	Kleenex® tissues[2] (2 pieces)	clear perspex
females	Kleenex® tissues[2] (2 pieces)	perforated metal

[1] Celtona, Cuijk, The Netherlands
[2] Kimberly-Clark Corporation®, EEC

EXPERIMENT 2

Animals

Previous experiments with preferences for nesting materials and nest boxes revealed no major differences in preferences between the sexes of a strain, therefore in experiment 2 only female mice were used. The same female mice of experiment 1 were used in experiment 2 (C57BL/6JIcoU and BALB/c AnCrRyCpbRivU, N=24). At the start of experiment they were 16-17 weeks of age.

Housing conditions, test system and behavioural observations were similar as described for experiment 1.

Procedure

Mice were introduced into the test system between 15.00 and 17.00 h and tested individually during 48 hours. A group of six mice (one strain) was tested simultaneously. The behaviour of one animal was recorded for 12 h during day time (second day of the test) and for 12 h during night time (second night of the test). Food and water of each test cage were weighed before and after the experiment.

The perforated metal nest box of experiment 1 was offered in a test cage with 50 g of sawdust bedding (Lignocel 3/4) and was tested against nesting material (Kleenex tissues, 2 pieces). The nesting material was offered in a cage with an inserted wire grid floor (stainless steel wire, rod diameter 2 mm, mesh size 10x10 mm^2). See Figure 1.

Statistical analysis (experiment 1 and 2)

The dwelling data were analysed by distinguishing three time frames: the total of dwelling times during the 48 h of the experiment, the dwelling times during 12 h of day light (second day of the test) and the dwelling times of 12 h of night time (second night of the test). These two latter periods synchronised with the periods of collected behavioural data (video tape recordings).

The method of statistical analysis used has been described by Blom et al (1995). Briefly, per test series the dwelling time data (in seconds) were logarithmically transformed as they were not always normally distributed, and to increase the independence of the data. For the same reason, central cage dwelling times were not included in the analysis. Data on food and water intake were not transformed, because they were normally distributed.

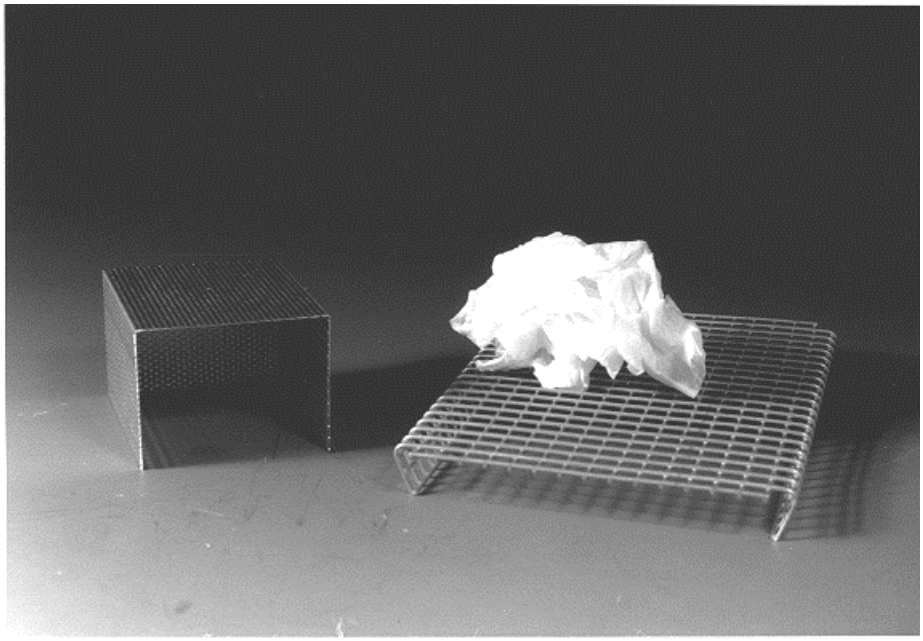

Figure 1 *Materials tested in experiment 2. Left: perforated metal nest box. Right: grid floor with two Kleenex tissues.*

The data were analysed using paired t-tests to evaluate the influence of cage contents on choice behaviour and to detect possible differences in choice behaviour. Food and water intake were analysed in a similar way as the dwelling times. Statistical significance was pre-set at $P<0.05$.

Behavioural data

The behavioural data on video tape were viewed and analysed using a behavioural observation software package (The Observer v 2.0, Noldus BV, The Netherlands). The tapes were viewed at normal speed, thus behaviour was seen nine times faster than the original behaviour. Every 5 s the behaviour was scored, which corresponds to one sample every 45 s in reality. The following ethogram was used to classify the behaviour (based upon Blom et al 1992):

Sleeping in nest box or nesting material (sl-in) =

movements are absent while the animal is in a sitting or lying position. Very short or minor movements during a long resting period (e.g. turning) are not considered as an interruption.

Sleeping outside nest box or nesting material (sl-out) =

same as sleeping in, except the behaviour is performed outside nest box or nesting material.

Grooming in nest box or nesting material (gr-in) =

while sitting or standing, the mouse is shaking, scratching, wiping or licking its fur, snout, ears, tail or genitals.

Grooming outside nest box or nesting material (gr-out) =

same as grooming in, except performed outside nest box or nesting material.

Manipulation (man) =

manipulation of the nesting material (shredding, fraying, dragging and nest building behaviour) or nest box (pushing, pulling, gnawing).

Ingestive behaviour (ing) =

includes eating and drinking behaviour. Eating: gnawing on food particles from the food hopper or from the sawdust, coprophagy is included as well. Drinking: licking the nipple of the drinking bottle.

Exploration in nest box or nesting material (ex-in) =

this includes all locomotion (movements), rearing (standing on hind feet, fore paws not touching the floor) and digging (pushing bedding material forwards or backwards with nose, fore paws or hind legs) performed in nest box or nesting material.

Exploration outside nest box or nesting material (ex-out) =

locomotion, rearing and digging performed outside nest box or nesting material.

Exploration on nest box (ex-on) =

locomotion and rearing on a nest box

Climbing (clim) =

climbing on or hanging from the bars of the wire cage lid or food hopper, or standing on the passage tube or drinking nipple. While climbing or hanging the hind legs or tail may touch the cage walls.

Descriptive statistics were used to analyse the behavioural data, because only two animals from each sex and strain group (N=12) were observed per test series. The results were used to describe the behaviour of the mice in the different test cages during a test series.

RESULTS

Experiment 1

Figure 2 illustrates the mean relative dwelling times per cage for experiment 1.

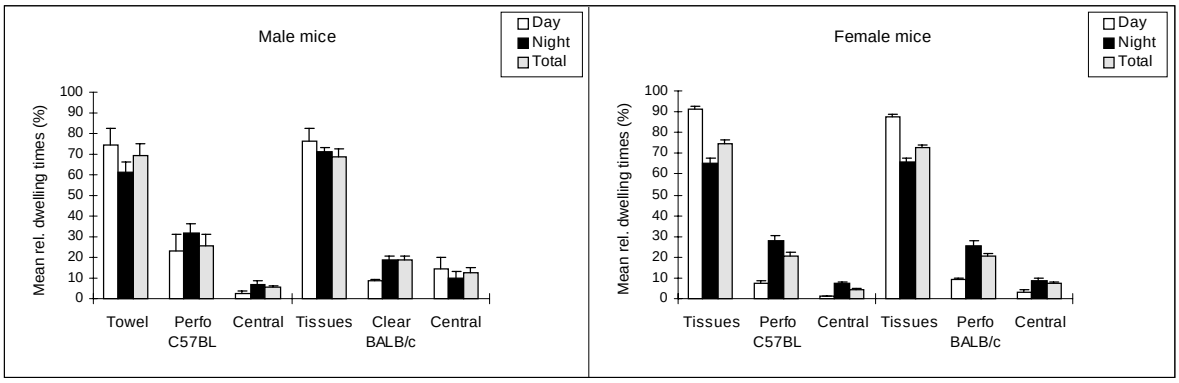

Figure 2 *Results of the preference test with nesting materials and nest boxes. Mean relative dwelling times (and SEM) per cage for day (= 12 h), night (=12 h) and total (=48 h) period, for male and female mice of two strains (N=47).*

All mice spent significantly more time in the cage with the nesting material during all three time periods (C57BL male mice: paper towel, all $P<0.05$, other groups: tissues, all $P<0.001$). BALB/c males spent significantly more time in the cage with the (clear perspex) nest box to eat ($P<0.05$), the other groups did not have a preferred cage for food intake. Female mice of both strains preferred the cage with the nesting material (tissues) for drinking (both $P<0.05$).

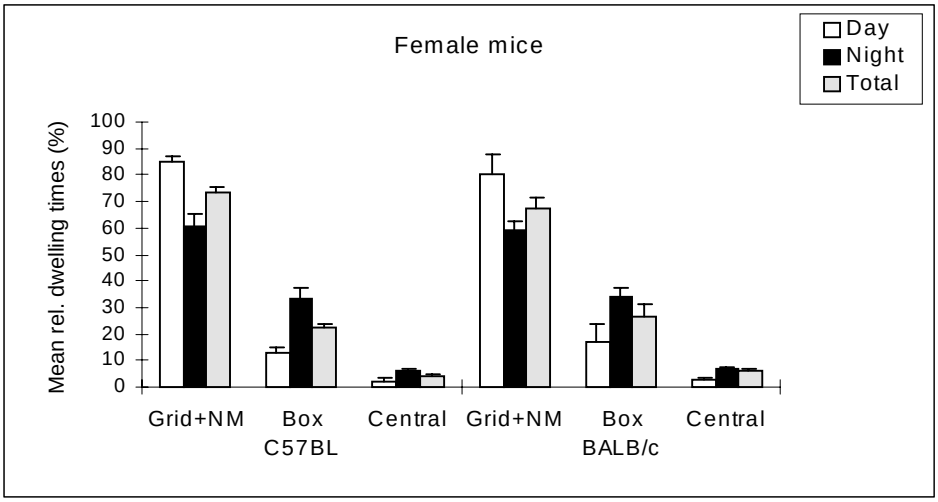

Figure 3 *Results of the preference test with nesting material provided on a grid floor and a nest box on bedding material. Mean relative dwelling times (and SEM) per cage for day (= 12 h), night (=12 h) and total (=48 h) period, for female mice of two strains (N=24).*

Experiment 2

Figure 3 illustrates the mean relative dwelling times per cage for experiment 2. Female mice of both strains preferred the cage with the nesting material on the grid floor during all three time periods (all $P<0.01$). They preferred the cage with the nesting material (tissues) on the grid floor to drink (both $P<0.05$), but had no preferred cage for eating.

Behavioural data

Figure 4 illustrates the distribution of active behaviour (no sleeping) for BALB/c and C57BL mice in experiment 1 and 2. Only the night data are shown, because day time data had similar patterns in both series. A lot of grooming outside the nest box (gr-out) is performed in both experiments, but grooming outside the nesting material is mostly seen in experiment 1 and not in experiment 2 where the nesting material is provided on the grid floor.

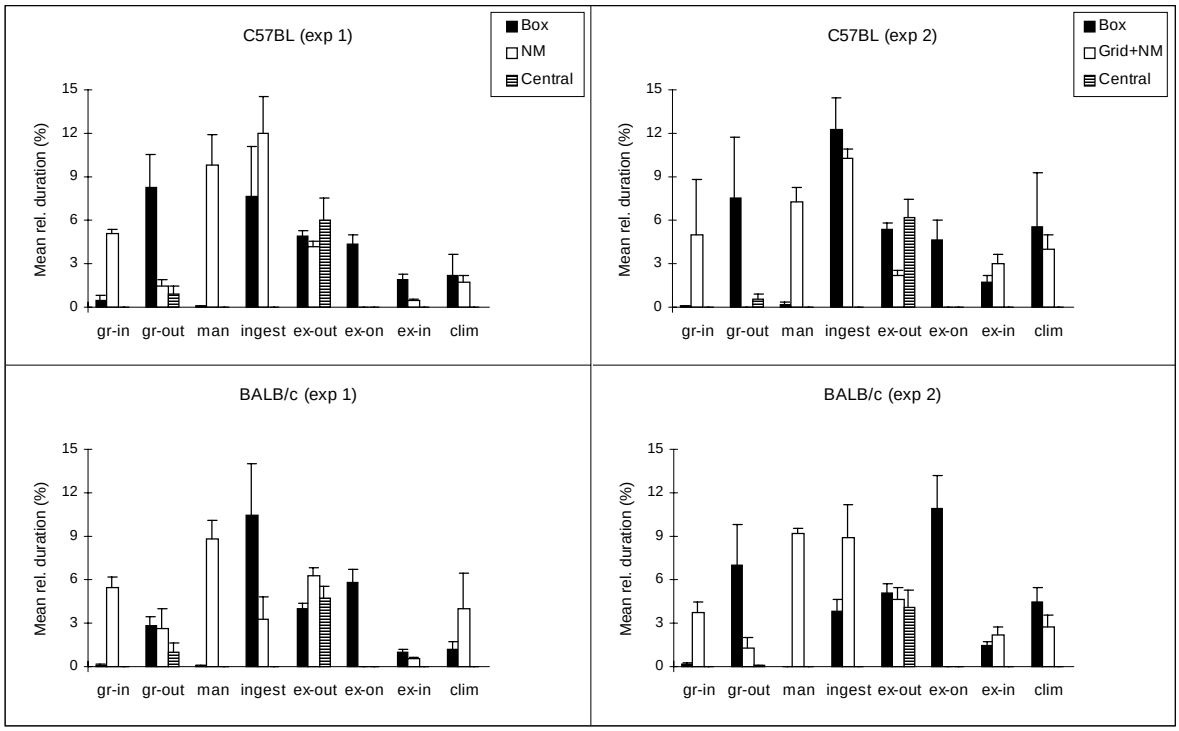

Figure 4 *Mean relative time (and SEM) spent on indicated active behaviour during the night time period (12 h). Left: experiment 1, preference tests with nesting material vs a nest box (male and female mice of two strains, N=8). Right: experiment 2, preference tests with nesting material on a grid floor vs a nest box on bedding material (female mice of two strains, N=4). (See Animals and Methods for explanation of abbreviations).*

Figure 5 shows the distribution of sleeping behaviour during the night in experiment 1 and 2. Most sleeping was performed in the nesting material (sl-in). When comparing the night time behavioural patterns from both series, it can be seen that in experiment 2 the mice show more sleeping in the cage with the nest box, although they do not lie inside the nest box (sl-out), this is observed to a much lesser extent in experiment 1, with the nesting material provided on bedding.

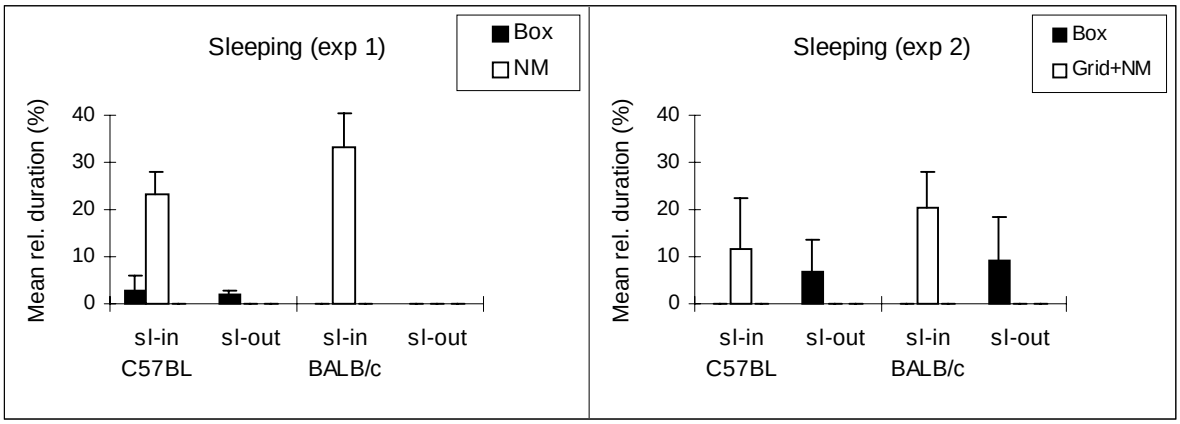

Figure 5 *Mean relative time (and SEM) spent on sleeping behaviour during the night time period (12 h). Left: experiment 1, preference tests with nesting material vs a nest box (male and female mice of two strains, N=8). Right: experiment 2, preference tests with nesting material on a grid floor vs a nest box on bedding material (female mice of two strains, N=4). (See Animals and Methods for explanation of abbreviations).*

DISCUSSION

All groups of mice showed a clear preference for the nesting material in experiment 1, with 60-90 % of the time spent in the cage with the nesting material. Cages with nest boxes were mainly visited during the night, when the mice were active and explored the test system (see Figure 4). In previous preference tests with different types of nest boxes, mice did prefer a cage with a nest box to one with no nest box (Van de Weerd et al submitted/a), but in the present experiment the nesting material appeared to be more attractive. This was also shown clearly in experiment 2, where again, all mice had a strong preference for the cage with the nesting material although a grid floor was present. Previous studies have shown that rodents avoid grid floors when alternatives are offered (Arnold & Estep 1994; Blom et al 1996a, 1996b; Manser et al 1995, 1996; Van de Weerd et al 1996).

In previous preference tests with nesting material, approximately half the number of (naive) mice made a combination of the most preferred nesting materials by dragging them from one cage to another (Van de Weerd et al accepted/a). In the present study this behaviour was not seen. In both experiments all mice spent most time in the cage with the nesting material and even in experiment 2 the mice did not drag the nesting material to the cage with the nest box to combine both commodities or at least lie in the bedding. It can be argued that they accepted the grid floor in order to rest in the nesting material, as the nesting material masks the structure of a grid floor. Figure 6 shows the type of

nests the mice made on the grid floors, which have the same shape as nests constructed on bedding.

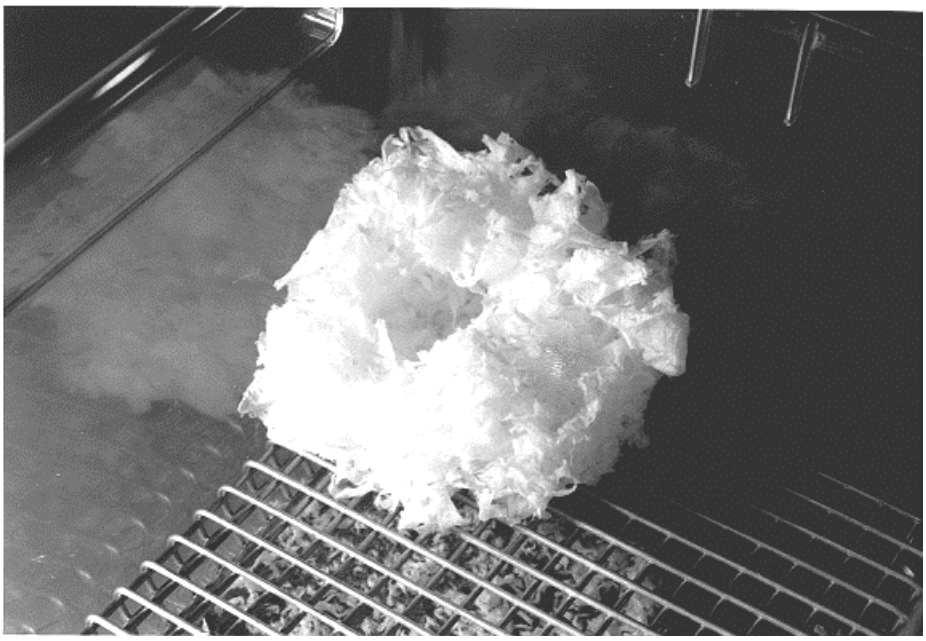

Figure 6 *Example of a nest built on the grid floor (experiment 2).*

These results imply that nesting materials are much more attractive for mice than nest boxes. In natural settings nest boxes may be used by rodents as a feeding post, as a storage for food, for the construction of a nest or for the bearing and raising of offspring as described by Ryszkowski & Truszkowski (1970). They may also offer an opportunity to hide from predators. In the laboratory the function of a nest box is more restricted, the main function probably is to offer a shelter for overexposure to light or to avoid aggressive cage mates (Van de Weerd & Baumans 1995). Nesting material has similar functions, but differs from nest boxes in that it can be manipulated to build a nest and by doing this, the mice are able to structure their environment (Van de Weerd et al accepted/a). Another main function of a nest is to shelter animals from variations in environmental temperatures (Brain & Rajendram 1986). Both males and females will build a nest when offered nesting materials and there is a strong genetic influence on nest building behaviour (Brain & Rajendram 1986; Lee 1972, 1973; Lisk et al 1969; Lynch & Hegmann 1972).

Several studies have shown that mice are willing to work in order to get nesting material, e.g. when nesting material is put on the cage lid, they start pulling it into the cage (Lisk et al 1969; Lynch & Hegmann 1972; Wolfe & Barnett 1977). Roper (1973) showed that mice can be trained to press a key in order to

obtain nesting material (paper strips). The paper acted as a reinforcer for this response. Collier et al (1990) described a similar experiment with rats, which were motivated to press a response bar often in order to reach a nest, although they were willing to press more in order to reach food or water. This phenomenon has also been described in other studies, which compared the demand for certain behavioural activities with the demand for food or water (Dawkins 1983; Matthews 1994; see also discussion in Roper 1973). In general, animals are willing to work harder for food or water than for other commodities. This is not surprising, because animals will almost always be highly motivated to gain access to food and water because this is a an essential need for survival (Matthews 1994). Measurement of the motivation of an animal to obtain a resource will be dependent on the alternatives offered, the elasticity for the demand will be greater when it is more substitutable for a commodity concurrently available (Barnett & Hocking 1981; Lea & Roper 1977). Sherwin & Nicol (1995) combined food searching behaviour with the occurrence of natural obstacles (air stream, water or a narrow gap), thus the willingness to overcome these obstacles to obtain food was used as a yardstick. Dawkins (1981) examined the priorities hens gave to two features of their environment, namely size and flooring of a cage. A comparable approach was used in experiment 2 of the present study, in which commodities addressing related behavioural activities were compared i.e. type of cage flooring (bedding or grid) combined with materials offering shelter (nest box or nesting material). Mice preferred the nesting material although during the night some mice slept in the bedding of the cage with the nest box, but not inside the nest box (see Figure 5). This practical approach allows for the comparison of various environmental aspects and may directly lead to designs for better housing conditions.

Laboratory environments are barren and often poorly structured and contain few features that can be manipulated or changed by the animal's behaviour. This makes it difficult for animals to adopt a behavioural response that reduces the effect of aversive stimuli (coping) in stressful situations (Wechsler 1995). By providing nesting material mice are able to structure their environment by manipulation of the nesting material and this gives them more control over their living conditions. More control may enhance their well-being (Beaver 1989; Chamove 1989a; Van de Weerd & Baumans 1995). Nesting material also allows mice to perform species-specific nest building behaviour. The inability to engage in species-specific behaviour may cause signs of suffering and the mere possibility to perform certain behaviours may decrease the physiological effect of stressful situations (Jensen & Toates 1993). Species-specific behaviour has evolved from continuous adaptations to the natural environment. Despite generations of domestication of mice in the laboratory, adaptive behavioural

strategies such as burrowing are still present in laboratory strains and do not appear to be different from wild mice (Adams & Boice 1981). Nest building is related to burrowing activities (Brain & Rajendram 1986) and can be seen as an active strategy of a mouse to control its environment (Sluyter et al 1995).

Housing systems should be designed to allow animals to perform effective coping behaviour when confronted with aversive stimuli, in order to prevent poor welfare (Wechsler 1995). When housing systems cannot be altered immediately, the provision of environmental enrichment such as nesting materials may be a relatively easy, short term solution to enhance well-being. Natural selection, domestication and experience have shaped decision making in animals in such a way that the resultant behaviour is optimally adapted to the current environmental circumstances. In general, this will enhance biological fitness and promote welfare (Fraser 1996; McFarland 1977). It is therefore reasonable to conclude that animal welfare is improved by achieving the objective the animal is willing to work for, and that reaching this objective is experienced as positive (Broom 1988; Van Rooijen 1983/1984). Mice in this study were highly motivated to lie in nesting material, even when presented on a grid floor. Thus we may conclude that nesting material has a positive effect on their well-being.

LONG-TERM BEHAVIOURAL AND PHYSIOLOGICAL EFFECTS OF NESTING MATERIAL AS ENVIRONMENTAL ENRICHMENT FOR LABORATORY MICE

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SUMMARY

Environmental enrichment may improve the quality of life of captive animals by altering the environment of animals so that they are able to perform more natural behaviour. When enrichment is introduced into an animal's environment, it is important to evaluate the effect of the enrichment programme and to assess whether the animal continues to use the enrichment in the long term.

Groups of mice were housed under either standard or enriched conditions for several weeks. Nesting material which was highly preferred in previous studies was used as enrichment. During the period of differential housing several behavioural parameters (behavioural tests and handling) and physiological parameters (urine and plasma corticosterone, food- and water intake, body- and adrenal weight) were monitored to determine the impact of environmental enrichment. Observations were made to determine whether or not the mice continued to use the enrichment.

The results indicated that throughout the study all mice used the nesting material to build nests and that mice from enriched conditions weighed more than mice housed under standard conditions, although the latter consumed more food. No major differences for behavioural and physiological parameters were found between the groups of mice housed under different conditions. Therefore it is not likely that supply of nesting material will jeopardise the outcome of experiments.

INTRODUCTION

Improvement of the quality of life for laboratory animals has received a lot of attention in recent years. Environmental enrichment may ameliorate some of the problems caused by containment (such as the occurrence of abnormal behaviour) by altering the environment of animals in such a way that they are able to perform more of the behaviour which is within the range of the animal's species-specific repertoire (Beaver 1989; Chamove 1989a; Scharmann 1991). Captive environments may be chronically stressful to animals if there is no or only limited opportunity for active behavioural responses as a means of coping with aversive stimulation (Carlstead et al 1993). Well designed housing systems allow for effective coping behaviour, which may enhance welfare (Wechsler 1995).

When enrichment is introduced into an animal's environment, it its important to evaluate the enrichment programme used. It should be assessed whether or not the animal uses the enrichment, and whether the objectives are achieved. It is also important to assess whether the animal continues to use the enrichment in the long-term (Bloomsmith et al 1991; Chamove 1989a; Van de Weerd & Baumans 1995).

Several effects of enrichment on the behaviour of laboratory rats (e.g. Renner & Rosenzweig 1986b; Rose et al 1985; Widman & Rosellini 1990) and mice (e.g. Manosevitz 1970; Manosevitz & Joel 1973; Manosevitz & Montemayor 1972; Manosevitz et al 1968) have been described. In an earlier study, Van de Weerd et al (1994) showed that mice from enriched housing conditions differed from mice kept in standard environments when submitted to several behavioural tests.

Enrichment may not only influence behaviour of the animals but can also have an effect on physiological parameters (Haemisch et al 1994; Kingston & Hoffman-Goetz 1996; Whary et al 1993). A variety of behavioural and physiological parameters can be monitored to determine the impact of environmental enrichment (Markowitz & Line 1990). A widely recognised indicator of stress or anxiety is the measurement of the level of adrenal activity. Altered hormonal levels may indicate that an animal is experiencing aversive stimuli or is having difficulties to cope with a specific situation (Carlstead et al 1993; Broom 1988; Dahlborn et al 1996; Wechsler 1995). Prolonged elevated levels of plasma corticosteroids have adverse effects on the immune system (Riley 1981).

Enrichment may indeed influence corticosteroid levels as was found by Hull et al (1976): gerbils from enriched environments had lower cortisol levels than gerbils from non-enriched groups. Methods to obtain the blood samples needed for the assessment of plasma corticosteroid levels, are regarded as invasive and therefore stressful for the animal. An alternative method for mice which often have

to be killed in order to get enough blood, may be the measurement of corticosterone in urine as described by Dahlborn et al (1996). The use of urinary cortisol as a non invasive, stress free method for assessing changes in adrenal activity has been used in humans (Brantley et al 1988; Kiecolt-Glaser et al 1984). Carlstead et al (1992, 1993) validated the method for felids and used it for the assessment of stress in laboratory cats. Dahlborn et al (1996) found differences in corticosterone levels between mice from three different housing conditions by using the urine method.

In the present study groups of mice were housed under either standard or enriched conditions for several weeks and during this period behavioural and physiological parameters were monitored based on the study by Dahlborn et al (1996). The behavioural tests used were an open field test with objects and an aluminium foil test (a combination of an emergence test and exploration test) to study the reactions of the mice in these novel environments with unfamiliar objects. Behavioural differences between mice from enriched and standard conditions were detected with these tests in previous studies (Dahlborn et al 1996; Van de Weerd et al 1994). The physiological parameters monitored in this study were, besides corticosterone measurements in urine and plasma, food- and water intake, body weight and adrenal weight. These variables have been reported to differ between animals from enriched and standard conditions (Hull et al 1976; Thiessen et al 1962; Van de Weerd et al 1994) and also between strains (Van de Weerd et al 1994).

Handling is a common procedure in laboratory animal husbandry and effects of handling on rodent behaviour have been described. In general, handled animals became less emotional or fearful (Denenberg & Morton 1962; Escorihuela et al 1995; Mazurski 1994). Little is known about the influence of enrichment on the reaction to handling of mice. Therefore, a score was given during weekly cage cleaning to observe if mice from enriched or standard housing conditions show different behavioural responses to handling.

The enrichment used in this study was based on the outcome of several previous experiments investigating the preferences of mice for different enrichment objects and materials (Van de Weerd et al submitted/a; accepted/a/b). In the present experiment the highly preferred nesting material from these previous studies was used as enrichment. Two different strains of mice were used to detect possible strain differences.

Choice tests mostly establish preferences of individual animals over short time periods and it is thus not known if these preferences are beneficial to an animal's welfare in the longer term (Fraser 1996), or what the effects are in groups of animals. In the present study the enrichment was given to mice in groups for a longer period of time to study effects on behaviour and physiology and to observe

if mice continue to use the enrichment.

ANIMALS AND METHODS

Animals

Thirty-six male and 36 female mice of two inbred strains (C57BL/6NCrlBR and BALB/cAnNCrlBR) were used. At the start of the experiment (week 0) they were three weeks of age. The whole experiment lasted 11 weeks. The mice were housed in a room with conventional hygiene and controlled photo-period (lights on 6.00-18.00 h, white light 225 lux), relative humidity (55-75 %) and temperature (22-24 ^{o}C). The mice were housed in groups of three (same sex and strain) in wire topped Macrolon type II cages (375 cm^2, UNO Roestvaststaal, Zevenaar, The Netherlands) provided with 50 g of sawdust (Lignocel 3/4, Rettenmaier & Söhne, Ellwangen-Holzmühle, Germany). Tap water and food-pellets (RMH-B, Hope Farms, Woerden, The Netherlands) were provided ad libitum.

Groups of three mice were composed on the basis of body weight of the mice at three weeks of age, so that groups had comparable mean body weights. The groups were then randomly allocated to either standard housing conditions (36 mice, 12 groups), the cages only provided with sawdust bedding (Lignocel 3/4) or enriched housing conditions. Enriched cages were provided with sawdust bedding as well as nesting material (3 Kleenex tissues, Kimberly-Clark Corporation®, EC). The tissues were renewed weekly with cage cleaning. The animals were individually marked on the tail by a colored water proof marker.

PHYSIOLOGICAL PARAMETERS

Body weight, food and water intake

Each week throughout the study, the mice were weighed individually and food and water intake were measured per group.

Urine sampling

Every two weeks urine samples for corticosterone and creatinine analysis were collected (method described by Dahlborn et al 1996). In the morning (between 9.00 and 10.00 h) the mice were placed individually in an empty Macrolon type I cage (204 cm^2) until they urinated, but no longer than 50 minutes. Urine was then collected with a syringe and stored in polypropylene tubes at -20 ^{o}C. Urine of males and females was collected alternating weekly, thus urine of males was collected in weeks 2, 4, 6, 8 and 10 of the experiment (at the ages of 5, 7, 9, 11 and 13 weeks) and of females in weeks 3, 5, 7, 9 and 11 of the experiment (at the

ages of 6, 8, 10, 12 and 14 weeks).

BEHAVIOURAL PARAMETERS

Handling

During weekly cage cleaning the mark on the tail of the mice had to be renewed. This was done by putting the mouse on the table and holding it by its tail with one hand, while with the other hand the mark was redone. A score was given for the behaviour during this handling procedure. Because individual housing during urine collection might influence behaviour during tail marking afterwards, two handling scores were given: one week a score for the behaviour during normal handling (no urine collected) and the next week a score for the behaviour during handling after urine was collected.

The following scores were used:
1 = mouse is sitting tranquil, does not move.
2 = mouse moves a little with head or body.
3 = mouse is 'walking' with front paws.
4 = mouse is 'running' with front paws, sometimes turns around, pulling to get away.
5 = same as 4 but the mouse is also squeaking.
6 = same as 4 but mouse is also turning around and wrestling frantic to get free.
7 = same as 6 but the mouse is also biting or tries to bite.

Open field test with objects

In the 6th week of the experiment (age of the mice: 9 weeks) the mice were subjected to an open field test with two objects (see also Dahlborn et al 1996). These objects were added to the open field to observe if mice from enriched or standard housing conditions would react differently towards these objects. The circular open field (∅ 90 cm) was surrounded by a gray PVC wall (height 50 cm). The floor was divided into one inner and one outer circle and in total the area was divided into 12 more or less equal areas. Two clear perspex, V-shaped objects with holes (cheese slice, IMS, Cheshire, UK), were placed (upside down) on the radius were it crossed the inner circle. During testing light intensity was 60 lux (floor level). At the start of a test each mouse was placed in the same area (near an object) and observed during 10 min. Testing (per strain) was conducted on two consecutive days. The first day mice from standard conditions were tested, the next day mice from enriched conditions. Testing started at 9.30 h. Between two tests, the apparatus was cleaned with alcohol. A video system was used to record each test, so that the experimenter did not need to be present in the testing room. Afterwards the behaviour of the animals was scored from video tape using a

behavioural observation software package (The Observer v 2.0, Noldus bv Wageningen, The Netherlands).

Based on the study by Dahlborn et al (1996), only behavioural elements which previously indicated differences between animals from different housing conditions were scored. The following behavioural elements were scored.

Duration (in s) of:

locomotion	=	walking.
immobility	=	sitting idle, not walking, includes freezing.
interaction with object	=	sniffing, gnawing object, walking through a hole in object, sitting under or rearing against object.
sitting on object	=	climbing on and sitting on object or walking over it (four feet from the ground).

After the test the fecal boli were counted.

Aluminium foil test

In the 9th week of the experiment (age of the mice: 12 weeks) the mice were subjected to an aluminium foil test (see also Dahlborn et al 1996). The test consisted of a Macrolon type I cage with an opening (∅ 4 cm) in one of the narrow sides. The floor was covered with 25 g of sawdust (Lignocel 3/4). A piece of aluminium foil (10.5 x 14 cm) was placed opposite to the opening in the cage. The small cage was placed in a Macrolon type III cage (840 cm^2) containing 75 g of sawdust (Lignocel 3/4). The mouse was placed in the small cage between the opening and the aluminium foil, with its head towards one of the long sides. During testing light intensity was 80 lux (cage level). The observation time was 10 min. Testing (per strain) was conducted on two consecutive days, the first day mice from standard conditions were tested, the next day mice from enriched conditions. Testing started at 9.30 h. Between two tests sawdust and aluminium foil were renewed and the cages were cleaned with alcohol. This test was also recorded on video tape, so that the experimenter did not need to be present in the testing room. Afterwards the behaviour of the animals was scored from video tape using a behavioural observation software package (The Observer v 2.0).

Based on the study by Dahlborn et al (1996), only behavioural elements which previously indicated differences between animals from different housing conditions were scored. The following behavioural elements were scored.

Duration (in s) of:

latency out	=	time to leave the small cage.
latency in	=	time to return for the first time into the small cage.
latency digging	=	time to start digging for the first time.
interaction foil	=	all interactions with the foil, including gnawing, on or under

		foil, sniffing foil.
digging	=	digging in the sawdust of the small or large cage.
grooming	=	licking fur, snout, genitals or tail.
climbing	=	climbing on the walls of the small cage with four feet from the ground

EUTHANASIA

Collection of blood and adrenals.

At the end of the experiment mice of one strain and housing condition were euthanised on the same day between 10.00 and 11.30 h. A cage with a group of mice was transported to a room next to the animal room, where all mice of this group were killed at the same time by 3 technicians, in order to rule out order effects on plasma corticosterone concentrations. Mice were decapitated (using scissors) within a few seconds after fixation, blood was collected in ice-cooled 2 ml tubes containing K2-EDTA (2.0 mg/ml, Greiner, Alphen a/d Rijn, The Netherlands). Adrenals of male mice were removed, as soon as the blood was collected. Adrenals were put into pre-weighed sheets of aluminium foil and weighed afterwards.

ANALYSES

Urine corticosterone and creatinine

Urinary creatinine concentrations were determined with the use of a commercial test combination (Creatinine, MA-KIT 10 ROCHE, Roche Diagnostics) on a COBAS-BIO auto-analyser (Hoffmann-La Roche BV, Mijdrecht, The Netherlands).

For corticosterone analyses 100 µl of urine was extracted with 200 µl of dichlormethan. Then, 5 µl of the dichlormethan phase was transferred in duplicates and analysed according to the method described by Westerhof et al (1992).

Plasma corticosterone

Blood was separated by centrifugation (3000 rpm for 10 min and 10000 rpm for 2 min, at 20 ^{o}C) and the plasma stored at -20 ^{o}C until assayed. Total corticosterone plasma concentration was measured in duplicate using a double antibody 125I Rats & Mice RIA-kit (ICN Biomedicals, Zoetermeer, The Netherlands).

Statistical analyses

The results of the tests were analysed using SPSS/Windows 6.0 statistical computer programme (SPSS Inc. Chicago, USA).

Food and water intake were analysed for possible differences between

strains, sexes and housing conditions and also time effects using repeated measures MANOVA. Body weight was analysed in a similar way, taking possible group effects into account as well.

Urine corticosterone, the ratio of corticosterone and creatinine and plasma corticosterone appeared to be not normally distributed, therefore they were logarithmically transformed. Urine corticosterone, the ratio (with time before urination as covariable) and plasma corticosterone were analysed for differences between strains, sexes and housing conditions using ANOVA, taking into account possible group effects. Data of two-weekly urine samples were analysed separately, so five analyses on urine corticosterone and ratio were performed.

Adrenal weights of the males were analysed for strain and housing condition effects using ANOVA. The data from both behavioural tests were analysed with MANOVA, for differences between strains, sexes and housing conditions. Fecal boli produced in the open field were analysed with a Mann-Whitney U test. Weekly handling scores (normal and after urine sampling) were analysed using a non-parametric test (Mann-Whitney U) for housing effects.

When multiple comparisons were made, a correction of the P-value with Bonferroni's adaptation was made. The level of statistical significance was pre-set a $P<0.05$.

RESULTS

Body weight, food and water intake

Figure 1 shows the body weights of the mice during the experiment. There was an overall significant difference between housing conditions. Mice from enriched housing conditions weighed more than mice from standard housing conditions ($P<0.05$). Overall, BALB/c mice were heavier than C57BL mice ($P<0.01$) and males weighed significantly more than females ($P<0.01$). A significant time effect was found as well ($P<0.01$), reflecting an increasing weight gain in the course of time.

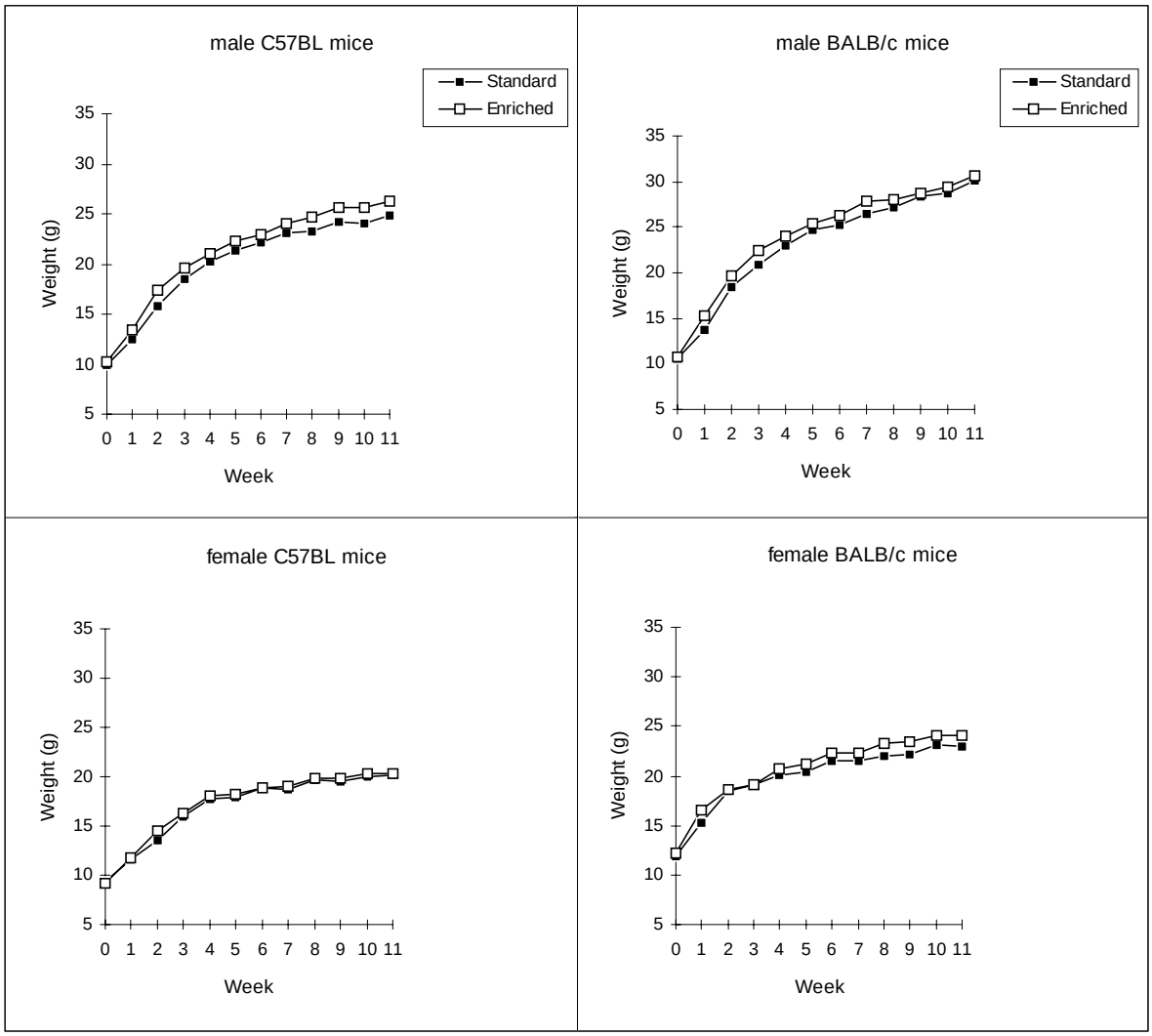

Figure 1 *Mean body weight of female and male mice from two strains (N=72) during 11 weeks of housing in either standard cages or cages enriched with tissues as nesting material.*

Figure 2 shows food intake of the mice during the experiment. There was an overall significant difference between housing conditions, mice from enriched housing conditions consuming less food than mice from standard housing conditions ($P<0.01$). Furthermore, BALB/c mice ate more than C57BL mice ($P<0.01$) and males consumed more food than females ($P<0.01$). Water intake did not differ between the housing conditions (data not shown). Differences between the sexes however, were found ($P<0.01$), males consumed more water than females.

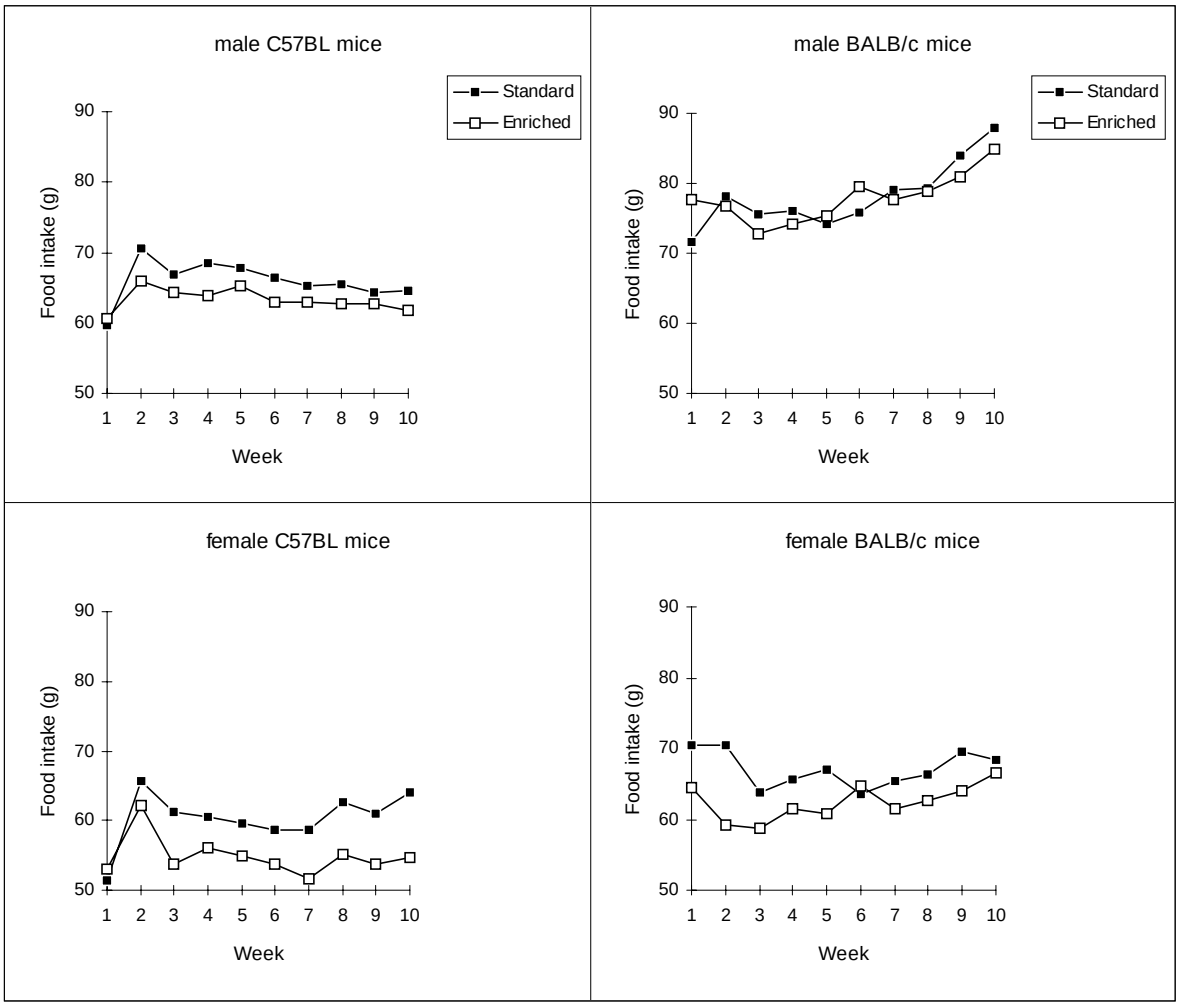

Figure 2 *Mean food intake of groups female and male mice from two strains (N=72) during 11 weeks of housing in either standard cages or cages enriched with tissues as nesting material.*

Urine and plasma corticosterone

Not all urine samples could be analysed, because mice did not always produce enough urine, but in general, 5-9 samples per group of mice could be used each week.

Differences in urine corticosterone concentrations (Table 1) between housing conditions were found for BALB/c mice only in the fifth sample, BALB/c mice from the enriched conditions had a higher corticosterone level ($P<0.05$). Further differences between housing conditions were not found.

Significant differences between the strains were found in the first, third and fifth sample (all $P<0.05$), in general BALB/c mice had higher levels. Significant differences between the sexes were found in all samples (all $P<0.01$), except for the first sample of the C57BL mice. Females had much higher levels as males.

Table 1 *Urine corticosterone values (mean and SD)*

			Urine cortico (nmol/l)		Ratio cr/co (nmol/mmol)	
	Sample	Wk	Standard	Enriched	Standard	Enriched
BALB/c	1	2	42.4 (28.4)	36.0 (22.3)	7.7 (4.6)	7.8 (4.8)
males	2	4	46.0 (30.8)	52.6 (31.4)	8.0 (5.8)	9.1 (4.4)
(n=18)	3	6	21.8 (0.8)	40.4 (14.6)	4.0 (0.3)	6.5 (2.3)
	4	8	33.2 (4.4)	59.6 (31.3)	5.1 (0.6)	11.5 (8.1)
	5	10	54.5 (13.5) a	76.6 (17.6) b	10.3 (3.0)	11.2 (3.7)
BALB/c	1	3	163.3 (105.0)	287.9 (109.2)	25.0 (12.6)	50.8 (16.4)
females	2	5	191.6 (86.5)	185.6 (73.8)	35.8 (18.0)	31.1 (10.5)
(n=18)	3	7	277.9 (110.2)	252.4 (97.7)	44.5 (18.2)	44.2 (14.6)
	4	9	213.7 (108.5)	193.4 (90.4)	37.1 (17.2)	38.8 (13.9)
	5	11	198.0 (60.5) a	236.9 (70.3) b	34.6 (11.8)	43.2 (15.5)
C57BL	1	2	133.9 (100.9)	122.4 (56.0)	17.6 (12.1) a	19.4 (6.6) b
males	2	4	34.7 (30.3)	37.0 (28.9)	6.4 (5.4)	7.0 (3.3)
(n=18)	3	6	47.8 (34.4)	24.2 (14.1)	12.0 (11.0)	5.1 (2.1)
	4	8	70.3 (72.6)	24.9 (11.1)	17.3 (19.2)	4.5 (1.6)
	5	10	43.0 (28.1)	28.2 (19.8)	8.2 (5.0)	5.0 (3.2)
C57BL	1	3	141.6 (64.6)	176.2 (84.4)	32.7 (18.3) a	51.6 (42.9) b
females	2	5	220.9 (124.8)	120.4 (39.4)	64.9 (18.9)	30.7 (18.1)
(n=18)	3	7	143.6 (67.9)	146.3 (82.9)	42.8 (35.5)	42.9 (31.7)
	4	9	181.1 (64.0)	153.0 (73.6)	39.1 (9.3)	40.8 (20.5)
	5	11	190.9 (93.8)	198.5 (116.6)	56.2 (42.4)	65.4 (39.9)

a,b indicate a significant difference between the two housing conditions ($P<0.05$)

Differences between housing conditions in the ratio of urine corticosterone and creatinine (with time to urination as covariable, Table 1) were found for the C57BL mice only in the first sample, C57BL mice from the enriched conditions had a higher corticosterone ratio ($P<0.05$). Further differences between housing conditions were not found. Significant differences between the strains ($P<0.05$) were found only in the fifth sample: BALB/c males had higher ratio's than C57BL males and BALB/c females had lower ratio's than C57BL females. Significant differences between the sexes were found in all samples (all $P<0.01$), except for the first sample of the C57BL mice, and the third sample of the BALB/c mice. Females had higher ratio's as males.

No significant differences between strains, sexes or housing conditions were found for plasma corticosterone. A high degree of between group variation was observed (Table 2).

Table 2 *Plasma corticosterone values (ng/ml) in week 11 of the experiment*

	Standard	Enriched

BALB/c	males	17.9 (14.5)	22.5 (24.9)
	females	35.8 (46.0)	47.4 (35.9)
C57BL	males	12.1 (7.0)	13.4 (15.7)
	females	14.1 (15.8)	51.3 (39.3)

Data given as mean and (SD), N=72

Adrenal weight

No housing effect was found in adrenal weights, but a significant difference between the strains was found ($P<0.01$). BALB/c mice had larger adrenals (standard 5.9 ± 1.0 mg, enriched 4.9 ± 0.8 mg) than C57BL mice (standard 3.1 ±.1.0, enriched 3.2 ± 0.6 mg). Because there were significant differences in body weight between mice from standard or enriched housing conditions, the adrenal weights were also analysed with body weight as covariable, but again no differences between the housing conditions were found.

Table 3 *Open field test behaviour in week 6 of the experiment*

	BALB/c males		BALB/c Females	
	Standard	Enriched	Standard	Enriched
locomotion	74.6 (64.3)	106.0 (72.9)	155.7 (83.3)	55.2 (44.7)
interaction with object	183.6 (173.6)	389.7 (107.8)	254.5 (150.2)	292.5 (232.2)
sitting on object	137.2 (249.5)	10.2 (14.4)	17.8 (28.7)	61.6 (151.0)
immobility	32.8 (51.8)	3.7 (3.7)	19.6 (37.6)	23.0 (25.2)
faeces (no. of boli)	6.7 (1.8)	9.4 (2.9)	4.7 (2.4)	8.0 (2.1)
	C57BL males		C57BL females	
	Standard	Enriched	Standard	Enriched
locomotion	359.7 (24.4)	331.3 (48.3)	346.0 (48.3)	329.3 (33.0)
interaction with object	97.4 (21.5)	115.2 (43.3)	98.3 (16.7)	106.8 (31.2)
sitting on object	20.0 (15.0)	13.3 (11.1)	15.8 (13.7)	11.4 (12.7)
immobility	2.7 (2.0)	7.5 (9.9)	3.5 (2.8)	2.4 (2.2)
faeces (no. of boli)	1.9 (2.1)	0.8 (1.4)	0.4 (0.7)	0.3 (0.7)

Data given as mean duration and (SD), N=72

Behavioural tests

Results of the open field test (Table 3) showed a small, but significant housing effect ($P<0.05$) in the C57BL strain, when all behavioural elements were analysed together. BALB/c mice from enriched conditions defecated more than BALB/c

mice from the standard conditions (P<0.01). Strain differences were found, BALB/c mice showed more immobility and more interaction with objects, but less locomotion than C57BL mice (P<0.01).

Table 4 *Aluminium foil test behaviour in week 9 of the experiment*

	BALB/c males		BALB/c Females	
	Standard	Enriched	Standard	Enriched
Mean duration (s):				
interaction with foil	19.1 (25.9)	27.7 (16.4)	30.1 (29.7)	21.8 (19.2)
digging	47.7 (25.9)	28.2 (32.5)	27.5 (21.1)	34.1 (28.1)
grooming	5.8 (4.2)	8.2 (4.8)	12.7 (9.4)	17.8 (9.8)
Latency (s):				
latency out	18.9 (11.6)	40.1 (31.1)	27.8 (15.7)	38.9 (18.8)
latency in	165.1 (135.5)	168.0 (159.6)	163.1 (101.8)	275.8 (185.0)
latency dig	192.4 (46.4)	270.7 (129.5)	228.0 (123.4)	247.4 (136.4)
	C57BL males		C57BL females	
	Standard	Enriched	Standard	Enriched
Mean duration of (s)				
interaction with foil	51.1 (31.9)	57.1 (36.8)	44.1 (25.8)	35.3 (24.8)
digging	47.1 (20.7)	41.4 (20.6)	23.4 (9.2)	34.2 (13.9)
grooming	11.7 (3.5)	17.2 (7.6)	8.4 (6.2)	11.1 (4.2)
Latency (s):				
latency out	13.9 (7.7)	22.4 (17.6)	13.6 (7.9)	12.5 (7.7)
latency in	178.3 (85.2)	240.8 (212.5)	200.5 (88.5)	161.6 (70.5)
latency dig	143.8 (41.9)	178.1 (84.7)	197.7 (79.7)	123.0 (63.5)

Data given as mean and (SD), N=72

The aluminium foil test did not show housing effects (Table 4). Climbing behaviour was not analysed because it occurred only infrequently. Overall, C57BL mice were more active than BALB/c mice (P<0.01). C57BL mice left the small cage sooner than BALB/c mice (latency out, P<0.01) and also started digging sooner (latency to dig, P<0.01). Differences between the sexes were found, in the BALB/c strain, females groomed more than males (P<0.01), C57BL males dug more than females (P<0.01).

Handling

The scoring of the influence of housing conditions on the response to handling did not reveal consistent results. When the first score for normal handling (no urine collection) was given, male mice from both strains housed under enriched conditions had a higher score (both $P<0.05$), meaning they were more responsive to handling. However, when the second score for the BALB/c females ($P<0.01$) and the fifth and sixth scores for the C57BL males were given ($P<0.01$ and 0.05 respectively), the highest responses were for mice from the standard conditions.

Handling after urine collection also yielded inconsistent differences. When the second, third and fifth scores of C57BL males were given, standard housed mice had higher scores (all $P<0.05$) than enriched housed animals. Similar results were found for the fourth and fifth scores of C57BL females (both $P<0.01$). The fifth score of the BALB/c males, however, showed an opposite effect ($P<0.05$).

DISCUSSION

The largest and most consistent effects between mice from enriched or standard housing conditions were found in body weight and food intake. Mice from enriched conditions weighed more, although they consumed less food. An explanation for this effect may be that the nesting material allows the mice to regulate their body temperature. Throughout the study the mice manipulated the nesting material to form nests in which they slept. The nesting material probably provided insulation, which could reduce body heat loss. As a consequence they might have needed less food. Watson (1993) also found a reduced food intake in individually housed mice provided with gauze pads as nesting material, although she did not found differences in body weight. She gave two possible explanations for her findings: mice with nesting material may have used less energy for stereotypic behaviours and/or they were better able to conserve body heat. This latter effect was described by Chvédoff et al (1980) for groups of mice in comparison with mice housed individually or in pairs. Food consumption was declined in mice kept in groups because they slept huddled together. Several other authors have also found that mice from enriched conditions weighed more than mice from standard housing conditions (Dahlborn et al 1996; Henderson 1970a; Manosevitz & Joel 1973; Van de Weerd et al 1994). However, no differences in body weight have also been reported by other authors (Manosevitz 1970; Thiessen et al 1962).

No differences in corticosterone levels in blood plasma were found, suggesting that corticosterone levels were not altered as a consequence of the different housing conditions. These results are in concordance with Dahlborn et al (1996). Haemisch & Gärtner (1994) and Haemisch et al (1994) found elevated plasma corticosterone levels in male mice from enriched conditions. The main

cause for these findings, however, were the high levels of aggression and the changes in social organisation in the mice housed under the enriched conditions. The development of a social hierarchy plays an important role in animal stress (Peng et al 1989). Plasma corticosterone levels of enriched housed mice did show a greater variability than of standard housed mice (see SD in Table 2). This might indicate that enrichment influences social relationships. However, no major differences in levels of aggression were observed between standard and enriched groups of mice.

In concordance with the plasma measurements, corticosterone measurements in the urine showed no consistent housing effects either. Only the fifth urine corticosterone sample of the BALB/c strain and the first corticosterone/creatinine ratio of the C57BL mice revealed a housing effect. Corticosterone was measured on two levels, neither of which showed major housing effects. Plasma corticosterone (free and protein-bound) reflects levels from one time point, whereas (free) corticosterone in the urine reflects levels from a period of time, assembled in the urine. These differences might explain the effects - although small - found in the urine but not in the plasma samples.

No conclusions can be drawn from the differences in the behaviour scores during handling. The handling scores after urine collection were most consistent. They show a trend that, towards the end of experiment, the C57BL mice from enriched cages are more tranquil than standard animals. However, the last score of the BALB/c males showed an opposite effect. Rats which were handled and had enriched housing experience, were better learners and had lower emotionality scores in an open field test (Denenberg & Morton 1962; Escorihuela et al 1995). Thus it can be hypothesised that animals from enriched environments will react less emotional (more tranquil) and get used sooner to handling procedures as performed in this study. The present results however, are not consistent enough to support this assumption.

The behavioural tests did not indicate major differences between mice from the two housing conditions. In the open field test, the enriched BALB/c mice had higher defecation scores than standard housed animals. This finding is difficult to explain and is not in agreement with the results of other authors (Chamove 1989b; Manosevitz 1970; Manosevitz & Joel 1973). Dahlborn et al (1996) performed a similar open field test. The only differences (from 14 behavioural elements measured) they found between mice from standard environments and from cages enriched with only nesting material, was that C57BL mice from environments enriched with nesting material interacted significantly more with the objects than standard housed animals. But most behavioural differences were found in mice from cages, not only enriched with nesting material but also with objects. Dahlborn et al (1996) also performed the aluminium foil test, and did not find differences

between groups enriched with only nesting material and standard groups. This is in concordance with the present results. Other authors, however, did find differences between mice from standard and enriched housing conditions in behavioural tests (Chamove 1989b; Manosevitz 1970; Manosevitz & Montemayor 1972; Manosevitz & Joel 1973; Prior & Sachser 1994/95; Van de Weerd et al 1994), but they always used cages with many enrichments, comparable with the enriched cage with objects Dahlborn et al (1996) described.

In this study, nesting material which was highly preferred in previous studies, was the only source of (continuous) enrichment. Significant differences in physiological and behavioural parameters were found between strains, but not between mice from standard and enriched housing conditions. The only consistent housing effect was reflected by differences in body weight and food intake. In other studies where major effects have been observed, usually different objects were used and often these objects were changed daily, which may cause arousal and even provoke a stressful situation for the animals. In the study of Dahlborn et al (1996), mice enriched with only nesting material, did not differ in behavioural and physiological parameters from mice from standard conditions, but differences were found when mice from environments enriched with nesting material and objects were compared with mice from standard environments.

No detrimental effects of the use of the tissues as nesting material have so far been reported. A screening for contaminants (similar to screenings of bedding materials) did not reveal any toxic substances. Mice provided with nesting material (gauze pads) have been monitored using a battery of hematological and biochemical values (Watson 1993). No differences between test and control animals were found in all these parameters. Also, no lesions in the gastrointestinal tract were found.

In previous studies mice had a very strong preference for nesting material, probably because it can be manipulated for nest building. The provision of nesting material as enrichment might therefore enhance the welfare of mice (Van de Weerd et al accepted/b). Since nesting material does not influence behaviour and physiology of mice in groups to a large extent, it is not likely that supply of nesting material will jeopardise the outcome of experiments. Thus, there seems to be no good reason to deprive laboratory mice from this form of enrichment.

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EFFECTS OF ENVIRONMENTAL ENRICHMENT ON THE BEHAVIOUR OF MICE IN OPEN FIELD TESTS

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SUMMARY

Results of open field tests with mice housed under enriched or standard conditions revealed that the locomotor activity of animals from enriched housing conditions is lower than that of animals from standard housing conditions. However, when only the first 3 min of the total test period of 15 min were considered, reversed results were found. The present results seem to indicate that differences in test duration are responsible for the contradicting results in locomotor activity of mice as reported in previously published papers.

It may be concluded that animals from enriched housing conditions habituate faster to the test situation, reflected in a (rapid) decline in exploratory behaviour, whereas control animals longer continued to explore the new environment.

INTRODUCTION

Enrichment of the environment can influence the behaviour of animals. This was first described by Hebb (1947), who reared rats in a free environment (as pets at home). He tested these rats in a learning maze and compared their behaviour with rats with restricted experience. The rats with enriched experience were better at solving the problems in the maze. Since then several different behavioural tests have been used to evaluate effects of environmental enrichment.

The open field test is a classical behavioural test which has been widely used to study the 'emotionality' of laboratory mice and rats. Animals that show high levels of activity (locomotion) and have low defecation scores in this novel environment are regarded as being less emotional than animals that show the opposite (Archer 1973, Walsh & Cummins 1976). Differences between animals from enriched and standard environments in open field test behaviour have been found in both mice and rats. Several authors found that animals from enriched environments had higher activity levels than animals from control environments (Ardila et al 1977; Holson 1986; Manosevitz 1970; Manosevitz & Joel 1973; Manosevitz & Montemayor 1972; Prior & Sachser 1994/95). Other authors, however, found the opposite: mice and rats from enriched housing conditions were less active in the open field test or their activity did not differ from standard housed animals (Denenberg & Morton 1962; Rose et al 1985; Van de Weerd 1994; Van Rijzingen et al submitted).

Among the authors who perform open field tests, a wide variety exists in the physical characteristics of the apparatus used (size, shape and colour of the arena), the procedures (single or repeated testing) and testing environment (amount of lighting and noise levels) (see Archer 1973 and Walsh & Cummins 1976 for reviews). Another factor which differs between studies is the duration of testing, ranging from short tests (2-3 min) to longer tests (8-15 min). Van Rijzingen et al (submitted) found no overall difference in locomotor activity of rats when a time frame of 10 min of open field testing was analysed. However, during the first 2.5 min of the test, rats from the enriched housing conditions showed significantly more locomotion than rats from standard conditions. This suggests that the duration of testing might be an important factor in explaining the differences in locomotor activity found between animals from standard or enriched housing conditions.

In the present study an open field test was performed with mice of two strains, in order to test whether the contradicting findings as reported for the mouse can be attributed to differences in the duration of the test.

ANIMALS AND METHODS

Animals and housing conditions

A total of 64 naive male mice of an inbred strain (BALB/cAaNCrlBR, n=32) and an outbred strain (Crl:NMRI BR, n=32) was used. They were bred without nesting material. At the start of the experiment they were three weeks of age. The mice were housed in a room with controlled photo-period (lights on 7.00-19.00 h, white light 355 lux (at 1 m above the floor), relative humidity (55 ± 5 %) and temperature (22 ± 1 ^{o}C), a radio played 24 h a day. The mice were housed per strain in groups of four animals in wire topped Macrolon type III cages (840 cm^2, UNO Roestvaststaal, Zevenaar, The Netherlands) provided with sawdust (Lignocel S 8/15, Rettenmaier & Söhne, Ellwangen-Holzmühle, Germany). Tap water and food-pellets (RMH-TM, Hope Farms, Woerden, The Netherlands) were provided ad libitum.

Per strain half the number of animals (four groups) were randomly allocated to either standard or enriched housing conditions for a period of six weeks. Standard cages contained only sawdust bedding (Lignocel S 8/15). Enriched cages were provided with sawdust bedding and the following objects: a nest box, consisting of a plastic PVC tube (length: 11 cm, ∅ 7 cm) big enough to give shelter to all animals of a group, nesting material (1 tissue, 25x44 cm, Kim wipers, Kimberly-Clark Corporation®, Veenendaal, The Netherlands) and a metal climbing grid, vertically attached to the cage lid (size: 16x10 cm, mesh size: 5x5 mm). The location of the objects remained the same throughout the experimental period. The tissues were renewed with weekly cage cleaning. Four days before the start of an experiment the mice were brought in their cages to the test room to acclimatise.

Test system

The animals were introduced individually into a novel environment consisting of a 50x50x50 cm black arena. Light intensity at test cage level was 7.5 lux. Locomotor activity was measured with a Video-track system (type 512, Electronique Lyonnaise, Lyon, France).

Procedure

Mice were tested three times on three consecutive days. Tests were performed between 9.00 and 15.00 h. Duration of a test was 15 min. Groups were tested in random order, the four mice of a group were tested at the same time (in four similar test systems). Between mice, the test systems were cleaned with 1% TEGO 51\15 DL (amfotensiden 90 g/l, TH Goldschmidt NV, Amsterdam, The Netherlands).

The duration (in s) of three levels of activity were measured (based on the speed of movement of the mouse):

high activity = walking and running.
low activity = sitting in one place, including small movements with head or paws.
inactivity = no movements (freezing and resting).

Statistical analyses

Data were analysed per test day with a MANOVA, to detect possible differences between strains and housing conditions. The level of statistical significance was pre-set at $P<0.05$.

RESULTS

Three weeks before the test one BALB/c mouse (standard housing) was removed from its group, because it showed high levels of aggression, thereby wounding its cage mates badly. The remaining mice were included in the experiment and their performance did not differ from the other standard housed BALB/c mice. Figure 1 depicts the results of total open field activity (top) and activity per 3 min intervals (bottom).

On DAY 1, no differences were found in time spent on the three behavioural categories between mice from the two housing conditions. Significant differences between the strains were found. NMRI mice were more active than BALB/c mice (high activity $P<0.01$) and showed less inactivity ($P<0.01$).

On DAY 2 significant differences between mice from the two housing conditions were found in both strains. Mice from enriched environments were less active than mice from standard environments (high activity $P<0.05$). Strain differences on this day were similar as differences on day 1 (high activity $P<0.01$, inactivity $P<0.01$).

On DAY 3 similar differences between mice from the two housing conditions were found in both strains. Mice from enriched environments were less active than mice from standard environments (high activity $P<0.05$). Significant differences between the strains were found for all three behavioural parameters. NMRI mice were more active than BALB/c mice (high activity $P<0.01$, low activity $P<0.01$) and showed less inactivity ($P<0.01$).

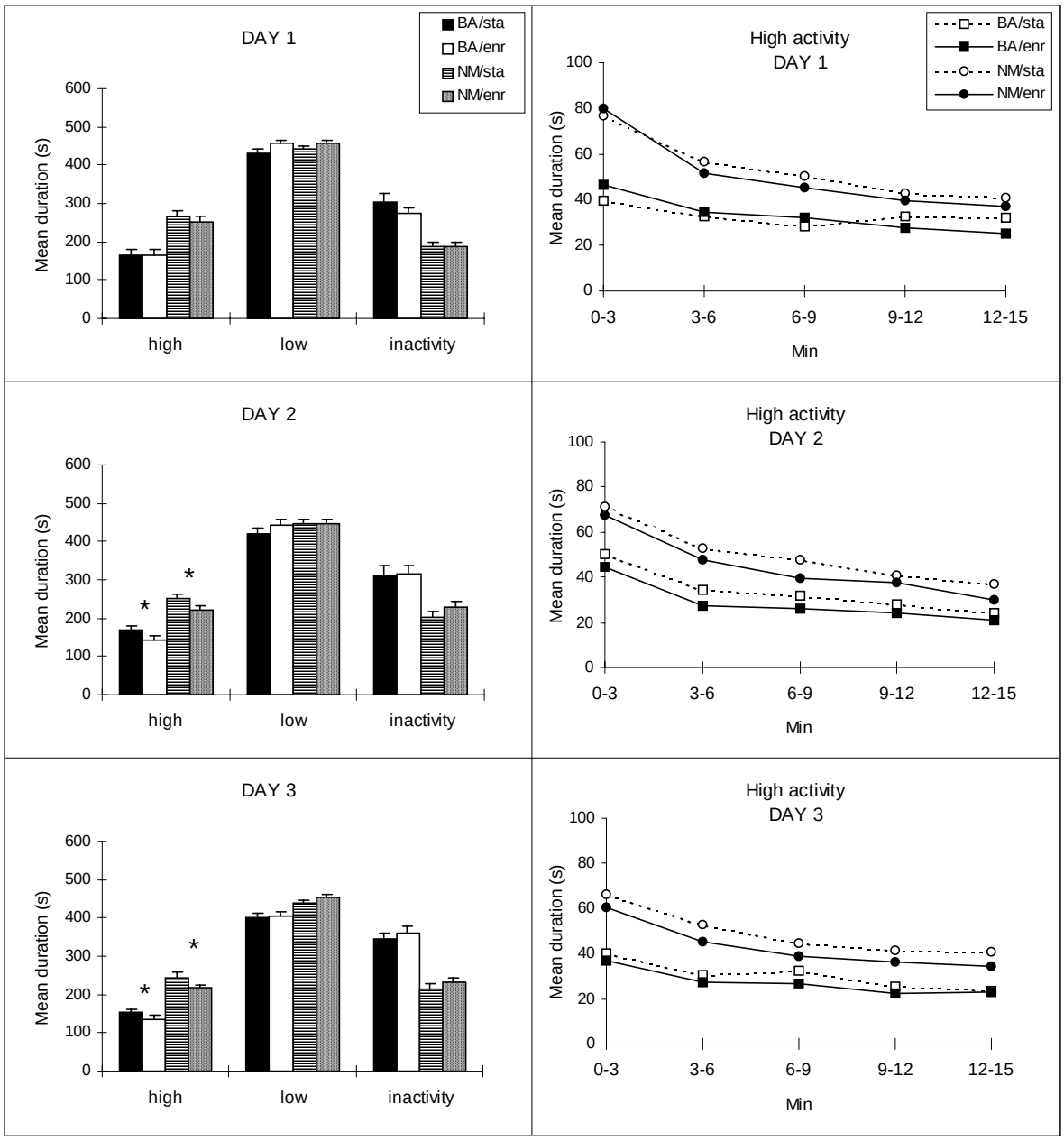

Figure 1 *Open field test results of male mice of two strains and two housing conditions (N=63). Left: Mean time (and SEM) spent on indicated behaviour during 15 min of testing on three consecutive days. Right: mean time spent on high activity per 3 min intervals. * p<0.05, significant difference between housing conditions.*

DISCUSSION

The results indicated a difference in both strains between mice from the enriched housing conditions and the standard conditions. The enriched housed mice were less active on day 2 and 3 of the test. This difference was only found in high

activity (locomotion).

These results are comparable with an earlier study employing the open field test by Van de Weerd et al (1994), in which we found that BALB/c mice from enriched conditions showed less locomotion than standard housed mice during 8 min of open field testing. Similar results were also found in an exploration test (comparable with an open field test) used by Manosevitz & Montemayor (1972) and Manosevitz & Joel (1973). They found that over 5 consecutive daily trials mice reared in enriched conditions explored less (measured as locomotor activity) than mice from impoverished conditions. In other studies, however, contradicting results have been found.

The main difference between studies with opposite results in open field activity scores are the differences in test duration. When comparing the test durations it appeared that in short open field tests (2-3 min trials on consecutive days) mice from enriched conditions were more active than mice from standard or impoverished conditions (Manosevitz 1970; Manosevitz & Joel 1973; Manosevitz & Montemayor 1972; Prior & Sachser 1994/95), whereas in longer open field tests (1 trial for 8-15 min) it was found that mice from standard conditions were overall more active than mice from enriched conditions (Van de Weerd et al 1994; and the exploration test of Manosevitz & Montemayor 1972; Manosevitz & Joel 1973).

The observation that the duration of testing can explain the contradicting results, is supported by the data of the present experiment. When the data are split into 3 min periods, the first 3 min period on day 1 shows that in both strains the mice from enriched conditions were more active than mice from standard conditions. In the following periods the reverse was found (for the NMRI mice in the 3-6 min period, for the BALB/c mice in the 9-12 min period). On day 2 and 3, mice from enriched environments were overall less active. In the study on rats by Van Rijzingen et al (submitted), after the first 2.5 min of the open field test the activity of the enriched housed rats declined fast to levels below those of rats from standard conditions. A decrease in activity over trials on consecutive days has also been reported. Mice from both housing conditions in the exploration test of Manosevitz & Montemayor (1972) and Manosevitz & Joel (1973) showed a reduction in activity over trials, but this decrease was larger in mice from enriched conditions. A similar finding has been reported for rats (Holson 1986).

Overall, the results suggest that animals from enriched conditions habituate faster to the test situation, reflected in a faster decline in exploration activity as compared to control animals, who continue to explore (Manosevitz & Joel 1973). When open field tests are short (3 min or less) this habituation may not be detected. Animals from enriched housing conditions are more competent to control their environment and therefore learn to cope with novel and unexpected changes in their environment (Chamove 1989a; Rose 1994; Wemelsfelder 1994).

As a consequence they might be better able to cope with circumstances during experiments as compared with animals from standard environments. The results of the present experiment, as well as results from other studies, indicate that this might be the case. Animals from enriched conditions can adapt faster to novel environments and thus may be less aroused by the novelty or unfamiliarity of the testing apparatus (Manosevitz 1970, Van Rijzingen et al submitted).

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BEHAVIOURAL PATTERNS OF LABORATORY MICE HOUSED UNDER ENRICHED AND STANDARD HOUSING CONDITIONS

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SUMMARY

A newly developed behaviour registration system LABORAS was used to register automatically the behavioural patterns of mice during 24 h. Prior to this test the mice were housed for six weeks under either enriched or standard conditions. Housing conditions during the test were similar to those before the test. We have tested whether the behavioural patterns of animals in enriched housing conditions differ from their counterparts in non-enriched environments, in particular whether a difference in time budget or in the circadian rhythm of behaviour exists.

With the LABORAS behaviour registration system no effects of environmental enrichment on the circadian rhythm of behaviour of the mice were detected. However, differences in time budget of mice housed in the two different environments were established as well as differences in behaviour between strains.

INTRODUCTION

Environmental enrichment is the process of improving the living conditions of laboratory animals with the goal to enhance their welfare. By introducing enrichment into the animals' environments they are able to express more of their species-specific behavioural repertoire (Chamove 1989a; Van de Weerd & Baumans 1995). As a consequence, the behavioural patterns of animals housed under enriched housing conditions will differ from their counterparts in non-enriched environments which are only able to perform a smaller part of their behavioural repertoire. Several studies have described differences in behaviour between animals housed in enriched or standard environments. Rabbits in cages enriched with objects showed an increase in active behaviour (consisting mainly of interactions with the enrichment objects) as compared to rabbits housed without enrichment who spent 80% of their time being inactive (Brooks et al 1993; Huls et al 1991). Rats provided with gnawing objects also showed an increase in active behaviour and a decrease in fighting (Orok-Edem & Key 1994). A nest box provided to mice increased explorative behaviour for a short period of time and changed space usage for a longer period of time: the mice slept in the nest box (Van Loo et al 1996).

Thus, environmental enrichment may change the time spent on different behavioural categories (time budget). It is also possible that the distribution of behaviour in time (circadian rhythm) changes as a consequence of environmental enrichment. Mice have clear circadian rhythms in their behaviour. They are most active during the night, with an activity peak shortly after the onset of the dark period. Thereafter periods of rest and activity alternate. Before dawn they show another, less prominent activity peak. During the day they mostly sleep (Schlingmann et al submitted; Van Oortmerssen 1971; Weinert 1994-96).

Evaluation of the effects of environmental enrichment on the behavioural patterns of laboratory mice requires behavioural observations during prolonged periods of time, using e.g. time lapse video recording techniques. Acquiring and analysing this information is rather time consuming and this can be reduced with the use of automated behaviour registration systems. Most behaviour registration systems only measure overall activity (e.g. Barclay et al 1988; Minematsu et al 1991; Young et al 1993). Whereas it might be important to register more behaviours at the same time. Some behaviours might increase while at the same time others might decrease, in which case overall activity remains the same and differences are not detected (Baumans et al in press).

In the present study the newly developed system, LABORAS (Bulthuis et al in press; Van de Weerd et al in preparation) was used for the automated registration of behaviour in six different categories during prolonged periods of

time. Mice of an outbred and an inbred strain were used in order to study possible influences of environmental enrichment on circadian rhythms and time budgets.

ANIMALS AND METHODS

Animals and housing conditions

A total of 48 naive male mice of an inbred strain (BALB/cAaNCrlBR, n=24) and an outbred strain (Crl:NMRI BR, n=24) was used. They were bred without nesting material. At the start of the experiment they were three weeks of age. The mice were housed in a room with controlled photo-period (lights on 7.00-19.00 h, white light 355 lux (at 1 m above the floor), relative humidity (55 ± 5 %) and temperature (22 ± 1 ^{o}C), a radio played 24 h a day. The mice were housed per strain in groups of four animals in wire topped Macrolon type III cages (840 cm^2, UNO Roestvaststaal, Zevenaar, The Netherlands) provided with sawdust (Lignocel S 8/15, Rettenmaier & Söhne, Ellwangen-Holzmühle, Germany). Tap water and food-pellets (RMH-TM, Hope Farms, Woerden, The Netherlands) were provided ad libitum.

Per strain half the number of mice (three groups) were randomly allocated to either standard or enriched housing conditions for a period of six weeks. Standard cages contained only sawdust bedding (Lignocel S 8/15). Enriched cages were provided with sawdust bedding and the following objects: a nest box, consisting of a plastic PVC tube (length: 11 cm, ∅ 7 cm), big enough to give shelter to all animals of a group, nesting material (1 tissue, 25x44 cm, Kim wipers, Kimberly-Clark Corporation®, Veenendaal, The Netherlands) and a metal climbing grid, vertically attached to the cage lid (size: 16x10 cm, mesh size: 5x5 mm). The location of the objects remained the same throughout the experimental period. The tissues were renewed with weekly cage cleaning. Four days before the start of a test the mice were brought in their cages to the test room to acclimatise.

The LABORAS test system

The LABORAS system is a fully automated device for the recording of behaviour of individually housed mice or rats. The system consists of a sensing platform (Figure 1) which is positioned on two orthogonally placed sensors and a third fixed point. A Macrolon cage (type II or III) is placed on this sensing platform. Each sensor transforms the mechanical vibrations caused by the movements of the animal into electrical signals, which are stored on a computer. Each movement has its own unique frequency and amplitude pattern and thus separate behavioural categories can be distinguished and classified by the computer. The

upper part of the cage (with the cage lid) is separated from the lower part of the sensing platform to detect climbing behaviour, because in this way a signal is not evoked by climbing behaviour (either on the cage lid or climbing grid).

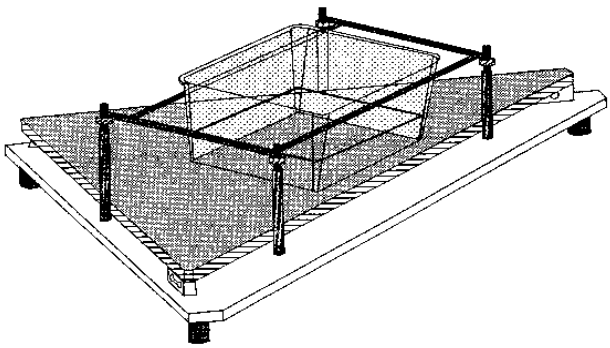

Figure 1 *The LABORAS behaviour registration system. Sensing platform with cage.*

Procedure

A mouse was placed in a (clean) Macrolon type III cage (840 cm^2) similar to its home cage (either enriched or standard). Enrichment was provided during the test, because otherwise the absence of enrichment might evoke behaviours (such as increased exploration) and the behavioural patterns will not be representative for a mouse in an enriched cage. During 24 h the behaviour of a mouse was

recorded. Each test started between 14.00 and 16.00 h. Testing lasted for three weeks. Per week four groups of mice were tested in random order, the four mice of a group were tested at the same time, each on one sensing platform.
The LABORAS system distinguishes the following behavioural categories (Based on the ethogram in Schlingmann et al submitted).

resting =
: movements are absent while the animal is in a sitting or lying position. Very short movements (e.g. turning over while sleeping) are not considered as an interruption.

locomotion =
: activities such as walking, running, or jumping.

climbing =
: climbing and hanging on the bars of the wire cage lid or food hopper or on the climbing grid in the enriched cages. While the animal is climbing or hanging, the hind legs or tail may touch the floor or side wall of the cage.

grooming =
: the mouse is shaking, scratching, wiping or licking its fur, snout, ears, tail or genitals.

eating =
: the animal eats food pellets while standing upright, gripping the bars of the food hopper with its front paws, and gnawing the food between the bars. It also includes gnawing a particle of food clasped between the front paws.

drinking =
: the animal stands upright to lick water from the water bottle.

undefined =
: all behaviour which is not classified in one of the previous categories.

Statistical analyses

Data were analysed using an ANOVA, to detect possible differences between the strains and housing conditions. The 24 h of the experiment were divided in six time periods: 1-3 , 4-6, 7-9, 10-12, 13-18, 19-24 h after the start of the experiment. Per time period the relative time spent on each behavioural category was calculated and analysed. A group of mice is treated as an experimental unit, to rule out influences of home cage and testing time. The level of statistical significance was pre-set at $P<0.05$

RESULTS

Figure 2 shows the results of the 24 h behaviour registration. Per time period, the

mean time spent on each of six behavioural categories are shown per strain and housing system. The data of the category 'undefined' are not shown (overall less than 10%).

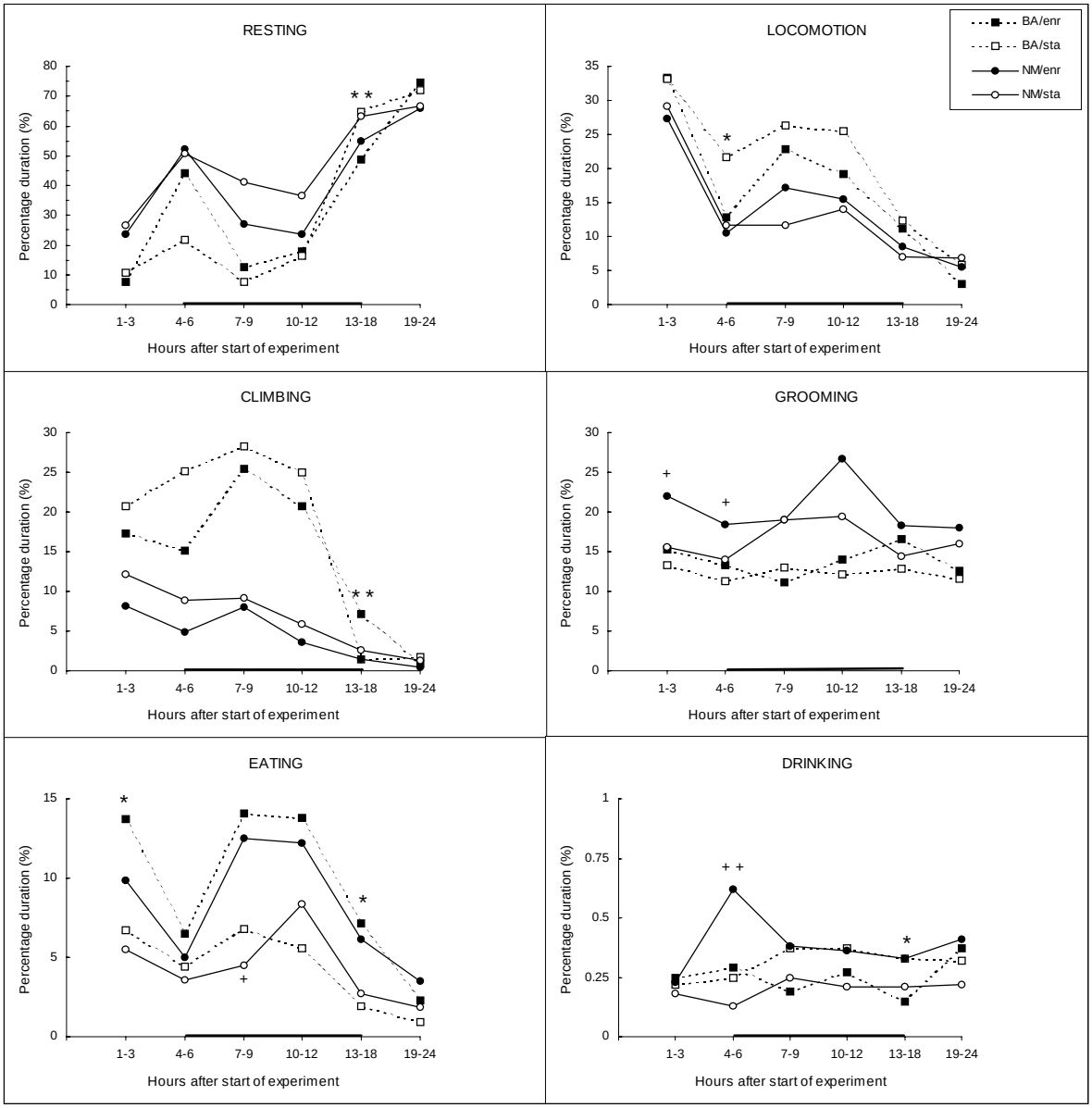

Figure 2 *Results of LABORAS behaviour registration system. Behavioural patterns of male mice of two strains (BALB/c and NMRI, N=48) housed under enriched or standard conditions are shown. Relative mean time spent on behaviour in six different categories during 24 h of testing. * p<0.05, ** P<0.01, significant difference between housing conditions in BALB/c mice. + p<0.05, ++ P<0.01, significant difference between housing conditions in NMRI mice. Lights went out in the 4-6 h period and on again in the 13-18 h period.*

Overall, BALB/c mice from enriched conditions spent more time on eating (1-24 h, P<0.05, also significant in 1-3 h period, P<0.05 and 13-18 h period, P<0.05). In the 4-6 h period standard housed mice spent more time on locomotion

(P<0.05). The main differences between mice from the two housing conditions in this strain were found in the 13-18 h time period (enriched housed mice: more climbing, P<0.01 and eating, P<0.05 and standard housed mice, more sleeping, P<0.01 and drinking, P<0.05).

Overall, NMRI mice from enriched conditions showed more grooming (1-24 h, P<0.05, also significant in the 1-3 and 4-6 period, both P<0.05) and drinking (1-24 h, P<0.05, also significant in the 4-6 h period, P<0.01). Enriched housed mice also spent more time on eating in the 7-9 h period (P<0.05). NMRI mice from standard housing conditions spent overall more time on climbing (1-24 h, P<0.05) and on sleeping (1-24 h, P<0.05).

Overall significant differences between the strains were found, BALB/c mice spent more time on climbing (1-24 h, P<0.001) and locomotion (1-24 h, P<0.01) than NMRI mice, whereas NMRI mice spent more time on grooming (1-24 h, P<0.001) and resting (1-24 h, P<0.01) compared to BALB/c mice.

DISCUSSION

The results of the 24 h behaviour observations showed similar overall behavioural patterns in both strains. High levels of activity were observed in the first 3 h (high levels of locomotion and climbing). After the introduction of mice in new cages (e.g. after cage cleaning) high levels of activity associated with exploration are often seen (Saibaba et al 1995). During the dark period, the mice were also active and showed high levels of eating behaviour. When the lights turned on again (13-18 h period), resting increased. Grooming was fairly constant during the whole 24 h period. These behavioural patterns are consistent with circadian rhythms of mice as found by other authors (Büttner 1991; Minematsu et al 1991; Schlingmann et al submitted; Weinert 1994-96). Overall, the results showed that the circadian rhythms in behaviour were not influenced by environmental enrichment. However, housing effects were found in both strains indicating that mice housed in enriched environments had different time budgets than mice housed under standard conditions.

The most consistent housing effects were found in the NMRI strain. During the whole 24 h period enriched housed mice spent more time on grooming and drinking, whereas standard housed animals spent more time on climbing and sleeping. In the BALB/c strain evident housing effects were found 13-18 h after the start of the experiment when the lights turned on again. However, the differences were not consistent with the rest of the period, e.g. enriched housed animals showed more climbing, but in the period from 1-13 h, standard housed animals showed more climbing.

In both strains enriched housed mice spent more time on eating (but only significant for the BALB/c mice). This finding seems to differ from previous studies (Van de Weerd et al 1994; Van de Weerd et al submitted/b), in which enriched housed mice were found to eat less than standard housed mice. The LABORAS system, however, measures eating behaviour (eating from the food hopper and eating a food particle clasped between the paws), in contrast with other studies which measure the amount of food which disappears from the food hopper. Similar observations apply for drinking behaviour.

In both strains climbing behaviour was found to be higher in the standard housed animals than in the enriched housed animals. These differences are mainly present in the first time period, but practically nil in the last time period. This is somewhat surprising, since the enriched housed mice have a climbing object in their cage (attached to the food hopper). Thus, higher climbing frequencies were expected in these groups, but this was only the case in the 13-18 h period of BALB/c mice. This suggests that standard housed animals were more active than enriched housed mice. Climbing is a part of exploration but excessive explorative climbing may lead to the development of stereotypies as was shown in ICR mice (Würbel et al 1996). Prior to the experiment the mice were housed in groups, but during the experiment they were individually housed. Isolation may have effects on the behaviour and physiology of an animal (Brain 1975). Mice in enriched environments may experience this isolation differently from mice in standard cages, which may be expressed in their behaviour. An indication that this could be the case are the lower levels of climbing in the enriched housed animals as compared with standard housed animals. A closer analysis of the first hours of the experiment, when the mice are exploring their cages may yield more information.

Significant differences between the strains were found. NMRI were less active (more grooming and sleeping), than BALB/c mice (more locomotion and climbing). The mice were housed in familiar environments, except that the cages were clean and they were housed individually during the test. It seemed that NMRI adapted faster to these new conditions by spending more time grooming and sleeping than the BALB/c mice, which showed more explorative behaviours (locomotion and climbing). BALB/c mice are known to be emotional and neophobic, they fear novel environments (Griebel et al 1993; Thiessen 1961), whereas NMRI mice are more tranquil. These different strain characteristics seem to be reflected in the behaviour of the mice.

The reactions of both strains to the housing conditions differed, for example, BALB/c mice from standard housing conditions showed more locomotion and drinking, whereas in the NMRI strain this was seen in the animals from the enriched conditions. Genetic differences between strains of mice may

influence the effects of different housing conditions as was shown for inbred mice in an earlier study by Van de Weerd et al (1994). Henderson (1970b, 1976) found that mice from enriched environments performed better on a food seeking task, but the size of the effect differed per strain and cross, suggesting genetic variability. Enrichment apparently interacts with the genotype of the animals used. This should be kept in mind when studying these effects.

A general problem in enrichment studies, is the fact that animals in enriched housing conditions are able to perform a wider range of behaviour than animals in standard environments and these different time budgets are difficult to compare (Novak et al 1995). The LABORAS system only registrates the pre-defined behavioural categories. Other (unknown) behaviours will be put into the undefined category. Behaviours such as for example nest building, which can be performed by the enriched housed mice because they have nesting material will thus be categorised as undefined behaviour. However, when comparing the levels of behaviour in the category ‘undefined’ for the two housing conditions, levels for the enriched housed animals were not higher. But since also other, for the system unknown behaviours are registrated in this category (e.g. digging), overall effects may remain undetected. In future, the system will be improved in order to registrate a more refined ethogram.

In conclusion, effects of environmental enrichment on the circadian rhythms of mice were not detected. However, consistent behavioural differences between the two strains of mice and differences in time budgets of mice housed in two different environments were found. The LABORAS system seems promising for future behaviour research.

Acknowledgements
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GENERAL DISCUSSION

As described in the introduction of this thesis one of the main goals of environmental enrichment is to provide animals with opportunities to better perform their species-specific behaviour, thereby implying that in this way their welfare is enhanced (Beaver 1989; Benn 1995; Chamove 1989a; Hart 1994; Markowitz & Gavazzi 1995; Mench 1994; Poole 1988; Scharmann 1991). But how can the welfare implication of enrichment be established?

The use of preference tests to evaluate the effect of environmental enrichment is based on the assumption that natural selection has shaped the decision-making process of animals in such a way that the resultant behaviour sequences are optimally adapted to the current environmental circumstances (Fraser 1996; McFarland 1977). What the impact of these choices on the welfare status is, cannot be measured directly, therefore we have to use indirect ways and a range of measures to evaluate the welfare status of the animal. By using behavioural and physiological parameters, it is possible to measure the effects of (enriched) environmental conditions. Interpretation of such effects in terms of animal welfare is done against the background of other experimental data and theoretical or philosophical assumptions (Sandøe & Simonsen 1992). The assumption that animals will choose those circumstances in which they feel 'good', is based on the analogy postulate. This postulate assumes that humans and animals which are comparable with regard to many aspects of their physiology and anatomy, are also comparable with regard to the ability of experiencing pain and distress (Stafleu et al 1992). This line of reasoning may also allow to conclude that an animal experiences it as positive to achieve the objective it is willing to work for, and that by attaining this objective its welfare is improved (Broom 1988; Van Rooijen 1983/84). On the other hand, the impossibility to perform certain behaviour may increase the effects of stressful situations and cause suffering (Jensen & Toates 1993). Environments which allow animals to perform more of the natural behavioural repertoire, also allow a larger range of behavioural choices and may thus enhance the well-being of the animals.

Poole (1992) is questioning whether the provision of a single object can be considered as environmental enrichment, since enrichment should allow the performance of a complex behavioural repertoire. This is provoking the question whether the term enrichment is sufficiently defined. Rushen (1995, Applied Ethology Discussion List, Internet) prefers the term environmental *improvement* instead of enrichment and divides the animal's environmental requirements into those that are essential and those that are beneficial. Others prefer environmental *modification* as a more neutral term. According to Duncan (1995, Applied Ethology Discussion List, Internet) environments should allow the performance of essential

species-specific behaviours, which he regards as an essential design feature and not as environmental enrichment. Newberry (1995) has doubts about the use of the term enrichment because it implies improvement, whereas the term is frequently used for the process of environmental change whatsoever. According to her views, the term should be applied only to the (successful) outcome of these environmental changes. It is easy to qualify any improvement to the current barren environments we provide for animals as enrichment, but from a scientific point of view enrichment is about discussing motivation and the effects of frustrating or satisfying these motivations (De Passillé 1995, Applied Ethology Discussion List, Internet). At present, the term enrichment covers a range of modifications of the environment, from satisfying a strong motivation (behavioural need) to making things more interesting for the animals. It seems useless trying to find a term which covers the whole process currently considered as environmental enrichment, since this term is already used widespread in the literature. But it seems making sense to restrict its use to those environmental changes for which animals choose in a preference test and/or of which the positive effects have been shown.

The need of environmental enrichment for laboratory animals is questioned by Beilharz (1994). According to him laboratory animals such as rats and mice do not need an extremely complex environment because they should become adapted to simple laboratory environments in which they are less costly to maintain. Beilharz assumes that the behaviour of these animals quickly adapts genetically to the captive environments. However, as was shown by Van Oortmerssen (1971), laboratory mice do not demonstrate strain-specific, functional adaptations to the artificial laboratory environment. The behavioural differences which he found between inbred strains, have originated in nature as adaptations to different biotopes before the strains became domesticated. Animals with complex, flexible behaviour can adapt to most environments without a change of their genotypes. The entire behavioural repertoire described for wild mice still occurs in laboratory mice, provided that they are given the appropriate environment to live in (Van Oortmerssen 1971).

Adams & Boice (1981) and Boice (1977) showed that the burrowing activities of wild and laboratory rats and mice of inbred strains were similar in every regard, including dimensions and sequence of construction. Burrowing behaviour is comparable to nest making, the movements used in both activities are rather similar (Brain & Rajendram 1986). In the study described in Chapter 3 of this thesis, mice which had never been in contact with nesting materials were allowed to choose between cages with nesting materials or a cage without nesting material similar to the one in which they were housed since weaning. They all preferred a cage with nesting material and within a few hours they constructed a

nest. Other authors have reported the start of nest building activities even within minutes after nesting material was put in the home cages of laboratory mice (Schneider & Chenoweth 1970; Van Oortmerssen 1971; Watson 1993).

Al these reports suggest that laboratory mice and rats, despite inbreeding, still have complex behavioural abilities which are not very different from their wild counterparts. Thus it seems warranted to look more closely to current laboratory housing systems and to seek for adaptations that can meet the natural behavioural requirements in a better way.

In this thesis emphasis is put on the evaluation of simple adaptations of existing housing systems. Preference tests were used as one of the tools to evaluate these enrichments. The preferences of mice for two different types of enrichments were determined: one which could satisfy the need to hide in dark secluded places (different types of nest boxes) and one which could satisfy the need to make sleeping nests (different types of nesting materials). The study in Chapter 5 revealed that the preference of male and female mice for nesting materials was much stronger than for nest boxes. The mice even accepted a (previously avoided) grid floor to have access to the nesting material. The fact that the animals are able to manipulate the materials, thus controlling several aspects of their environment, might be an important motivation for the preference for this type of enrichment.

Enrichment may influence brain anatomy, behaviour and physiological systems of the body (see references in Introduction). Behavioural changes may even be so obvious that in a ‘blind’ experiment, human observers were able to identify the previous housing conditions (either standard or enriched) by only observing the behaviour of rats in a neutral environment (Renner & Hackett Renner 1993). Results of the studies described in this thesis indicate that the degree of complexity of the enrichment plays a role in the level of the behavioural and physiological effects. Cages enriched with a combination of nesting material, a climbing grid and a nest box changed the time budgets of mice, as well as their behaviour in an open field test, hole board test and cage emergence test (Chapters 2, 7 and 8). But when the preferred nesting material was provided as the only cage enrichment, no significant effects on the behaviour of mice in an open field test or aluminium foil test, nor influences on several physiological variables were found (Chapter 6). Several possible causes for enrichment effects have been described (see overview by Van Rijzingen 1995). The combination of physical, auditory, olfactory, tactile, visual and/or social stimuli, rather than a single factor seems to be important in generating an enrichment effect.

For generations, laboratory mice have bred equally well with and without nesting material (Van Oortmerssen 1971). Therefore one might say that nesting material is not essential for survival and reproduction. But it certainly adds

something to their specific needs, because they are strongly motivated to use it. For that reason, nesting material should be considered as enrichment. Nesting material is a general applicable enrichment for all mice, not only for females in breeding facilities. Furthermore, it can be provided to groups of mice but also to individually housed mice, which do not have the companionship of cage mates to huddle with for warmth and shelter. The long-term provision of (preferred) nesting material showed no major effects on the physiology or behaviour of the mice (Chapter 6). In view of the fact that the animals preferred nesting materials in preference tests and were also highly motivated to gain access to nesting materials, there seems to be no good reason to deprive laboratory mice from this form of enrichment.

Besides consequences for the animals, implementation of an enrichment programme may also have consequences for animal husbandry. In certain enriched housing systems the animals are more difficult to monitor, e.g. because they can hide in the shelters or nesting material which are provided. It is also possible that it is more difficult to remove the animals from their cages, because they have more space for fleeing or they have shelters or climbing facilities to take refuge in. It may cause severe stress if the animals have to be chased for this purpose, which might counteract positive effects of enrichment. In the study described in Chapter 6 the effects of enrichment on the behavioural response to handling was studied. It was expected that enriched housed animals were easier to handle because they were more tranquil, but the results were inconclusive.

Handling and management effects may differ between species. Gerbils raised in environments provided with a shelter were more difficult to capture and restrain and were more aggressive towards the experimenter than gerbils from standard laboratory environments (Clark & Galef 1980). On the other hand, studies on rabbits in group housing systems revealed that, although the animals in general flee to a hiding place, this facilitates their capture (Love 1994; Whary et al 1993). Enriched environments may give animals a more secure feeling, because they have more control over their environments, e.g. they can avoid or flee from frightening stimuli.

Enrichment should not only be stimulating for the animals, but it must also be manageable. This means that the enrichment must be easy to implement, to remove, clean, and replace. This is important for the workload of the personnel, and for their motivation and willingness to work with and to improve the enrichment programme (Van de Weerd & Baumans 1995). When including the design, maintenance and evaluation of enrichment programmes into the formal job responsibilities of animal care staff it may generate greater motivation and job satisfaction and a greater bond between caretakers and their animals (Benn 1995;

Bloomsmith et al 1991; Love 1994; Markowitz & Gavazzi 1995; Van de Weerd & Baumans 1995).

Although environmental enrichment might be of benefit for the animals, introducing an enrichment programme may jeopardise the standardisation of experiments. Standardisation of animal experimentation can be described as defining the properties of the animal (or animal population) and its environment, and the subsequent keeping constant or regulating (controlled varying) of these properties (Beynen et al 1993). The consequence is, that the response of the animals to treatment is as standardised as possible and the reproducibility of group mean results from one experiment to another is increased. The comparability of results within and between laboratories is also improved by standardisation (Beynen et al 1993; Clough 1987). The genetic background of experimental animals is in general standardised by selective breeding programmes. Genetic uniformity of the animals, however, does not mean that the animals will be phenotypically identical. Their response to experimental procedures can still vary, because this is only one of the factors influencing measured values. Other sources of variation are the pre- and postnatal environment and so-called intangible variance (Beynen et al 1993; Gärtner 1990; Van Zutphen et al 1993).

Environmental standardisation is not achieved when laboratories all develop their own individual strategies of enrichment. Comparison of results from different laboratories may be difficult if for example, one laboratory provides large enriched cages for groups of animals while another provides only one enrichment object to individually housed animals. Enrichment in itself can easily be standardised. The composition of nesting material for example, the amounts provided, the rate of replacement and hygiene (most materials can be autoclaved or sterilised) can all be well-defined.

Enrichment should not be a process of randomly applying objects which seem attractive for animals, but it should be a well designed and critically evaluated programme with well defined objectives. Care should be taken that enrichment strategies are implemented from the perspective of an animal, considering animal demands, and not only for the benefits of the caretakers (Newberry 1995; Rose 1994; Stauffacher 1994).

The introduction of enrichment may change certain characteristics of animals, and as a consequence, experimental results may not be comparable with previously found results. This, however should not hamper the introduction of enrichment, as it may be questioned whether the maintenance of animals in unresponsive environments makes them adequate models for extrapolating results to humans (Markowitz & Gavazzi 1995). Animals in an enriched environment are more competent to control their environment and therefore learn

to cope with novel and unexpected changes, as they are better adapted to their environment (Chamove 1989a; Rose 1994; Wemelsfelder 1994). Animals from standard housing conditions when brought into experimental situations are expected to react with more fear and stress than ‘enriched’ counterparts, which are more used to stimuli due to enrichment and habituate faster to new circumstances. In this way, more variation in reaction to novelty can be expected from animals from standard housing conditions in comparison with animals from enriched conditions. The results of experiments with enriched housed animals may therefore show less variation. Several authors have stated that animals from enriched housing conditions are physiologically and psychologically more stable and are therefore considered as more refined animal models, which ensure better scientific results (Bayne 1996; Benn 1995; Rose 1994; Spinelli & Markowitz 1985). Eventually the use of ‘enriched’ animals might lead to a reduction in the number of animals necessary for obtaining valid experimental results.

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SUMMARY

Current laboratory housing systems have mainly been developed on the basis of ergonomic and economic factors. These systems provide adequate, basic physiological requirements of animals, but only marginally fulfil other needs, such as the performance of natural behaviour or social interactions. Many signs indicate that the current housing conditions affect the well-being of animals negatively. Environmental enrichment, which provides a more structured environment, may solve some of the problems caused by the restrictive laboratory environments. Enriched environments allow the animals to increase the performance of behaviours which are within the range of the animal's species-specific behavioural patterns. This will improve the biological functioning of animals and as a consequence their well-being may increase.

When introducing enrichment it is important to evaluate the effects of the enrichment programme used. This can be done by observations of the animals in their home cages, by submitting animals to preference tests or behavioural tests or by measuring physiological variables.

In the first study described in this thesis, the effects of relatively simple environmental enrichment provided in standard laboratory cages on the behaviour of mice were investigated. Male mice of two inbred strains (C57BL/6J and BALB/c) were used. After being housed under either enriched or standard conditions the mice were subjected to three behavioural tests: a cage emergence test, a hole board test and an open field test. Effects of enrichment on the behaviour of the mice were found, although the effects differed per strain.

Choice tests have been used to assess laboratory animals' preferences for different environments or for aspects of the environment. The preferences of male and female mice of the two inbred strains for six different types of nesting material have been evaluated. No significant differences in preference were found between the strains nor between the sexes. All mice showed a clear preference for cages with paper towels or tissues. The results also suggest that the nature (paper or wood) of the nesting material is less important than its structure, which determines the nestability of the material.

In a second preference test series, six nest boxes made of different materials were offered as enrichment. A nest box provides an opportunity to withdraw actively from frightening stimuli outside or inside the cage, hide for aggressive conspecifics or for overexposure to light. In general, mice showed a preference for cages with a nest box made of grid metal or of perforated metal. When testing a nest box with one open side versus a nest box with two open sides, most mice preferred the nest box with one open side. In such a nest box the usual orientation of the animals was with their heads directed towards the opening.

In order to gain some insight in the strength of preference for these types of enrichment, the most preferred nesting material and the most preferred nest box (from the previous test series) were tested against each other. All mice showed a strong preference for the nesting material. In a second experiment, a choice was offered between nesting material combined with a grid floor (previously found to be avoided) and a nest box combined with bedding material. Even under these circumstances all mice chose the cage with the nesting material. This indicates

that the presence of nesting material, which can be manipulated, fulfils a need of mice.

In a following study the effect of the long-term provision of enrichment was studied. Groups of male and female mice of the two strains were housed under either standard or enriched conditions (provided with the preferred nesting material) for eleven weeks. During this period several behavioural and physiological parameters were monitored to determine the impact of environmental enrichment. All mice used the nesting material to build nests throughout the study. The main result of this study was that mice from enriched conditions weighed more than mice housed under standard conditions, although the latter consumed more food. The long-term provision of the nesting material showed no major effects on the physiology or behaviour of the mice. These studies led to the conclusion that providing a cage with nesting material can be a relatively simple method to contribute to the well-being of laboratory mice, without jeopardising the outcome of experiments. Thus there seems to be no good reason to deprive laboratory mice from this form of enrichment.

In order to further investigate the effects of enrichment on the behaviour of laboratory mice, cages were enriched by providing a combination of nesting material, a nest box and a climbing grid. The enriched housed animals showed a higher locomotor activity in the first three minutes of an open field test. With longer test durations however, the reverse was found. The animals from the enriched housing conditions seem to habituate faster to the test situation, reflected in a (rapid) decline in exploratory behaviour, whereas control animals continued to explore the new environment longer.

The behavioural patterns of mice housed under either enriched or standard conditions have been registered during 24 h using LABORAS, a newly developed system for the automated registration of behaviour. Environmental enrichment appeared to have strain-specific effects on the time budget of mice, but not on the circadian rhythm of behaviour.

In conclusion, the experiments described in this thesis have indicated that preference tests are adequate for determining the relevance of enrichment. Environmental enrichment may have profound effects on the behaviour of mice in their home cage and in behavioural tests. The degree of complexity of the enrichment however, plays a role in the level of the behavioural and physiological effects. Combining behavioural and physiological parameters provide data that can be used for interpreting the impact of environmental enrichment on the welfare status of an animal.

Animals from enriched housing conditions seem to be physiologically and psychologically more stable and could therefore be considered as more refined animal models. This means that eventually their use might lead to a reduction in the number of animals necessary for obtaining valid experimental results.

CURRICULUM VITAE

Heleen Ariane van de Weerd was born in 1967 on April 23rd in Amersfoort. From 1974 until 1978 she lived in Oranjestad on Aruba, one of the Dutch Antilles. After returning to The Netherlands, she graduated from high school (VWO - Eindhovens Protestants Lyceum) in 1985. In the same year she started to study Biology at the Agricultural University of Wageningen.

As one of her main subjects she choose Ethology, with Prof P Wiepkema as supervisor. The research for this subject was performed during a six months stay at the experimental horse farm (Proefbedrijf voor de Paardenhouderij) in Brunssum. In 1990 she went for a four months practice Ethology to the Animal Research Institute in Werribee, Australia under the guidance of Dr P Hemsworth. Her second main subject was Laboratory Animal Science under supervision of Prof AC Beynen (part-time professor in Laboratory Animal Science in Wageningen) performed at the Laboratory Animals Centre (Centrum Kleine Proefdieren) in Wageningen. As an extra subject the course on Laboratory Animal Science (in accordance with the Dutch Experiments on Animals Act, article 9) was taken. In September 1991 she graduated as an agricultural engineer (MSc) in Biology at the Agricultural University of Wageningen.

In January 1992 she started as a PhD student at the department of Laboratory Animal Science of Utrecht University, where she performed the research described in this thesis.

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