

## 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 (Bloomsith 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 (Bloomsith 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<sup>®</sup> 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 build 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 lose 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.