

Ethoexperimental Studies of Behaviour in Wild and Laboratory Mice

**Risk Assessment, Emotional Reactivity
and Animal Welfare**

Hanna Augustsson

*Department of Large Animal Clinical Sciences
Unit for Comparative Physiology & Medicine
Uppsala*

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Abstract

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The laboratory mouse is the most frequently used laboratory animal in biomedical research today. This thesis deals with behavioural studies of risk assessment in mice as a means of assessing emotional reactivity. The long-term objective of this work is to find relevant behavioural measures in relation to animal welfare. We were interested in increasing our knowledge about the natural baseline of risk assessment behaviours and therefore the idea of comparing behavioural strategies between wild mice and laboratory mice was realised. A novel environment elicits exploratory motivation in the mouse and entails a trade-off conflict between the possibility of locating important resources and the risk of encountering unidentified dangers. Using a battery of tests, Concentric Square Field, Open Field and Elevated Plus Maze, differences in behavioural strategies of exploration and risk assessment were studied. Wild-derived house mice (*Mus musculus musculus*) were contrasted with domesticated mice of the inbred strains BALB/c and C57BL/6. Taken together, differences in behavioural strategies between wild and laboratory mice were mainly quantitative, however specific behavioural profiles in relation to risk assessment and emotional reactivity were recorded. Wild mice differed from both laboratory strains mainly in an unwillingness to enter open areas. The BALB/c mice generally avoided risk areas and showed a high risk assessment while C57BL/6 mice were more explorative, demonstrating a higher rate of risk taking and performing little risk assessment. No major sex differences were found in the behaviours related to risk assessment and emotional reactivity. However, sex differences were more pronounced in Wild and C57BL/6 mice than in BALB/c mice. In the Light/Dark test, strain was a greater factor than home cage environment (enriched/non-enriched) in terms of risk assessment, emotional reactivity and inter individual variance. Risk assessment was also investigated in a novel test of predator exposure, the Rat Exposure Test. The results were in contrast to the previous findings, as C57BL/6 mice showed more risk assessment, avoidance behaviour and active defence than BALB/c mice in response to the rat. This discrepancy suggest a difference between the responsiveness to novelty or novel places compared to the anti-predator response, at least in these two inbred strains. Moreover, it supports the notion that the environmental features of the test arena, familiarity of the environment and type of aversive stimuli may have a large impact on emotional reactivity and that the strain of the mouse is an important factor in how the situation is perceived. It is concluded that risk assessment is a sensitive marker for emotional reactivity in laboratory mice but that a multivariate approach is necessary for a thorough characterisation in terms of animal welfare.

Keywords: animal well-being, environmental enrichment, domestication, defence, ethology, strain differences, gender, anxiety, emotionality.

Authors address: Hanna Augustsson, SLU, Unit for Comparative Physiology and Medicine, Department of Large Animal Clinical Sciences, P.O. Box 7018, SE-750 07 Uppsala.

*You can give a mouse fear but you cannot give it friendship.
If you want to learn its ways, it must not know you're watching.*

Peter Crowcroft, Mice all over, 1966

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Appendix

The thesis is based on the following papers, which will be referred to by their Roman numerals I-IV.

- I. Augustsson, H., van de Weerd, H.A., Kruitwagen, C.L.J.J and Baumans V. 2003. Effect of enrichment on variation and results in the light dark test. *Laboratory Animals* 37(4) 328-340.
- II. Augustsson, H and Meyerson B.J. Exploration and risk assessment: A comparative study in male house mice (*M. m. musculus*) and two laboratory strains. *Physiology & Behavior*. In Press.
- III. Augustsson, H, Dahlborn K and Meyerson B.J. Exploration and risk assessment in female wild house mice (*M. m musculus*) and two laboratory strains. Manuscript.
- IV. Yang, M., Augustsson, H., Markham C.M., Hubbard, D.T., Webster, D., Wall, P.M., Blanchard R.J. and Blanchard, D.C. The rat exposure test: A model of mouse defensive behaviors. *Physiology & Behavior*. In Press.

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Introduction

The laboratory mouse is the most frequently used laboratory animal in biomedical research today. Numerous inbred strains and outbred stocks is commercially available and the use of genetically modified transgenic and knock-out strains are increasing rapidly. Ensuring laboratory animal health and welfare is imperative to the ethical use of animals in laboratory research. The use of healthy animals is also a crucial factor in producing valid research results.

Although there are numerous studies of the behaviour of laboratory mice, there are important areas that have partly been neglected. The laboratory mouse is derived from the common house mouse (*Mus musculus species*) and it is frequently stated in guidelines of housing that laboratory mice should be able to perform species-specific natural behaviour. Nevertheless, conclusive information on how this shall be achieved, which consequences it has on the animal model and in what aspects the laboratory mouse differs from its wild ancestor is very limited. Moreover, knowledge of the natural behaviour of the mouse is also needed for the development of sensitive animal models for neuroscience and behavioural pharmacology and for a valid interpretation of results. These issues form the background of this thesis.

Laboratory Animal Science

Laboratory Animal Science (LAS) has been defined by Scand-LAS as “*the study of the scientific, legally approved and ethically acceptable use of animals in biomedical research, i.e. a multidisciplinary field encompassing genetic, metabolic (nutritional), microbiological, environmental and sociological (ethological) points of view, husbandry, animal experimental technology and the scientific use of animal species as models for other species or man*” (Öbrink & Waller, 1996). Hence, LAS has numerous sub-disciplines, each with its own focus and basic research. It is also an applied subject in which the multi-disciplinary aspects are taken into account and weighed against each other in the search for practical improvements or alternatives to research using animal subjects.

The three Rs (Replacement, Reduction and Refinement) define the central themes of the field of laboratory animal science (Russel & Burch, 1959). Firstly, *Replacement* through the development of alternatives to the use of animals in research. Secondly, *Reduction* of the number of animals used in research through minimisation of confounding variables in the environment, improved experimental design and the use of healthy genetically defined animals. Thirdly, *Refinement* is achieved through improvement of experimental techniques, alleviation of pain and distress associated with scientific procedures and through development of husbandry regimes that allow the animal to engage in species-specific behaviour and that promotes animal welfare.

Historically, LAS has focused mainly on standardisation of animals and the laboratory environment as well as basic research on the biology and physiology of research animals. During the recent decades animal welfare related issues have

received increasing attention. Standardisation aims to reduce inter individual variation between animals and thereby the number of animals needed per experiment. For laboratory animal housing, standardisation commonly means control and regulation of the temperature, ventilation, light schedule and air humidity within narrow limits. Although this environment provides the laboratory animals with everything needed for physical survival, the animals, which are evolutionary adapted to a natural variation in environmental parameters, may have difficulties in acclimatising to the uniformity of the artificial environment of a laboratory animal facility (Meyerson, 1986). Another issue is that although housing mice in groups in standard cages may enable them to interact socially it does not allow them to '*carry out the actions that would normally reduce risks to life*' (Dawkins, 1990) i.e. defence reaction patterns. In nature, there is an option of leaving the territory of the resident animal, in captivity the only options are aggressive conflict or subordination. Hence, the situation may lead to a socially stressful environment (Blanchard *et al.*, 2001b), physical injury and reduced welfare (van Loo, 2001). Taking into account knowledge of the natural habitat and behaviour of the species concerned may help improve the captive environment (Brain, 1992). One way of ameliorating the negative effects of standardised barren environments is to increase the biological relevance in laboratory animal housing through environmental enrichment (Newberry, 1995). A large number of studies aiming to improve housing for mice have recently been reviewed (Olsson & Dahlborn, 2002).

Ethoexperimental approaches to the study of behaviour

In Europe, there has been a tradition of ethological studies in natural contexts, with low environmental control, but with elaborate descriptive analyses of animal behaviour, exemplified with Lorenz and Tinbergen. In the US, the experimental psychology approach dominated, using laboratory tests with a high degree of control and simplified automatic recordings of spatial location and specific responses associated with behaviourism, as used by Skinner was. Later, during the 60s and 70s and thereafter, several researchers independently began combining and discussing these approaches from both ethology and traditional psychology (Grant, 1963; van Oortmeerssen, 1971; Archer, 1973; Barnett & Cowan, 1976; Brain, 1980; Meyerson & Höglund, 1981)). The term 'ethoexperimental' was later coined as to describe this experimental approach (Blanchard & Blanchard, 1986). The approach was discussed in a workshop visited by researchers from various fields such as behavioural neuroscience, behavioural endocrinology, behavioural ecology and ethology (Blanchard *et al.*, 1988).

The aim of the ethoexperimental approach is to study meaningful behaviours using biologically relevant laboratory test environments and including descriptions of animal behaviour as part of the analysis (Brain, 1988). In the interpretation of results both proximate and ultimate theorems are used (Parmigiani *et al.*, 1998). This approach has been proven to result in improved sensitivity and specificity and lead to the development of several novel methods used in both behavioural neuroscience and behavioural psychopharmacology.

Although simplified automated tests are still used to a high extent in both pharmacology and neuroscience, many behaviourally oriented researchers use and promote an ethoexperimental approach (including among others (Lister, 1990; Brain *et al.*, 1991; Wall *et al.*, 1993; Hendrie *et al.*, 1996; Blanchard *et al.*, 1997; Rodgers, 1997; Parmigiani *et al.*, 1998; Weiss *et al.*, 1998; Kavaliers & Choleris, 2001; Roy *et al.*, 2001)). This approach is also increasingly used in applied ethology and the study of animal welfare.

Wild and laboratory mice

The wild house mouse (Mus musculus species)

The common house mouse was first described by Linnaeus in Uppsala in 1758 and named *Mus musculus Linnaeus*. Within the species, several sub-species has later been defined. The four major sub-species groups are the *M. m. domesticus* (South and Western Europe, North and South America), *M. m. musculus* (Scandinavia, eastern Europe, Russia and Northern China), *M. m. castaneus* (South East Asia) and *M. m. mollosinus* (Japan), which are thought to be a hybrid between castaneus and musculus (Boursot *et al.*, 1993).

The house mouse is one of the most wide spread mammals in the world and the species inhabits climate zones ranging from arctic to tropical areas, however they generally live in close association with humans and cultivated areas. It is a prey species preyed upon by both mammals (rats, foxes etc) and birds of prey. They exhibit a diurnal activity rhythm (nocturnal) and an omnivorous diet including seeds, roots, fruit and insects. The social structure is not strict but may vary depending on environmental constraints (Mackintosh, 1981), colony size (Poole & Morgan, 1973) and the degree of male and female aggressiveness (Brain & Parmigiani, 1990; Parmigiani *et al.*, 1998). Male house mice generally disperse from their natal territory to establish their own territory in the nearby surroundings. The territories vary in size from the area just outside the nesting area to home ranges of about 25-30 meter or even greater (Brown, 1953). The resident territory holder defends the home range from conspecific intruders but may allow subordinate males to reside within the territory when unoccupied space is scarce. Female mice often stay within their natal territory, and mate mainly with the dominant male. Females engage in territorial defence especially when pregnant (Mackintosh, 1981). After parturition they protect the nest and pups from infanticidal attacks (Parmigiani *et al.*, 1998). Both male and female wild house mice exhibit nest building behaviour not only as a part of their parental behaviour. The type of nest may vary from bowl shaped to spherical depending on the genetic background of the mice and the habitat in which they reside (Brown, 1953). Some wild mice use pre-existing nesting cavities such as stone crevices or walls while others live in fields or other open areas where they may dig burrows and tunnel systems. House mouse (*M. m. domesticus*) burrows may range from 10 cm to over 8 m in length and include several entrance holes, tunnel segments and larger cavities functioning as nesting burrows and food caches (Schmid-Holmes *et al.*, 2001).

Origin of laboratory mice

Increasing genetic evidence support a phylogenetic divergence in laboratory mice (Atchley & Fitch, 1991; Wade *et al.*, 2002), which indicates that depending on strain, the laboratory mouse have more or less genetic input from the different subspecies of house mice. This and not only later selective breeding may have influenced the phenotype differentially in different strains (van Oortmeerssen, 1971; Sluyter & Oortmeerssen, 2000). Studies of Y-chromosome DNA suggest input from the *musculus* and *mollosinus* subspecies in many laboratory strains (Bishop *et al.*, 1985; Moriwaki, 1994). This is further supported by historical data which show that fancy mice of both Japanese and European origin were used in the founding colonies of laboratory mice (Festing & Lovell, 1981). Laboratory mice may therefore be considered a mosaic of the different subspecies and should hence be referred to only as *Mus musculus* without any subspecies reference (Bonhomme *et al.*, 1994).

A large proportion of the differences in the behaviour of mouse strains found today may be explained by founder effects, genetic drift and human selection. As the behaviour of wild mice may vary between different locations and habitats and genetic background (Capanna *et al.*, 1984; Brain & Parmigiani, 1990) founder effects may have resulted in laboratory strains genetically adapted for different habitats (van Oortmeerssen, 1971; Sluyter & Oortmeerssen, 2000). For instance, based on differences in nest building strategies and digging behaviour between BALB/c and C57BL mice, it was suggested that these strains might be more or less adapted to surface living and hole living respectively. It was concluded that this was the result of the original genetic input from different subspecies of the house mouse (van Oortmeerssen, 1971). It has later been shown that some of the commonly used laboratory mice such as the C57BL mice, BALB/c, and the outbred Swiss mice belong to different genetic groups (Beck *et al.*, 2000).

Behavioural phenotyping studies are being performed in laboratory mice for the purpose of characterising genetically engineered animals (Crawley, 1999; Rogers *et al.*, 1999), and for molecular purposes (Crawley *et al.*, 1997). Characterising and comparing behavioural strategies used by different mouse strains may tell us about their suitability as animal models for specific psychological features or diseases, but may also give us information about their differential “needs” regarding their captive environment. There is little information on wild house mice using behavioural phenotyping but at least one major study has been conducted (Koide *et al.*, 2000). The study was performed on female subjects from different inbred wild derived strains using behavioural tests for locomotory activity, anxiety, passive avoidance and active avoidance. They found a high degree of behavioural diversity between strains derived from both the same and different subspecies. It is evident that more research is needed before the behaviour of wild house mice could be considered characterised.

Domestication effects on behaviour

Domestication can be defined as an evolutionary process where the original natural and sexual selection pressures acting upon the species are modified to favour adaptation to a captive environment and cohabitation with a human

population (Price, 1999). Domestication involves genetic changes and it is evident that domestication may affect taming ability through the reduction of flight responses and decreased aggressiveness towards humans. However, the process of taming should be regarded as a learned adaptation to the captive environment that can be culturally transferred but not as a part of the domestication process. There have been a number of different approaches to experimentally assess the effects of the domestication of laboratory animals. These include direct comparisons between wild and laboratory animals (Smith *et al.*, 1994), random breeding of wild animals for several generations in captivity (Connor, 1975; Künzl *et al.*, 2003), and selective breeding for a certain trait (Janczak *et al.*, 2003).

In a study of wildness and domestication in house mice, laboratory reared wild mice were compared to three laboratory strains (C57BL/6, DBA/2J and A/J) and studied in a battery of behavioural tests (Smith, 1972). The characteristics of wild mice in that study were poor avoidance learning, long emergence latencies, extensive freezing, high wheel running activity, and frequent escape attempts. The behaviour of wild mice bred in a semi-natural environment was compared to wild mice and the same three laboratory strains (C57BL/6, DBA/2J and A/J) bred in a laboratory environment (Connor, 1975). The behavioural tests used were repeated sessions of resident-intruder tests, a handling test, food-related neophobia, and recapture latency. Wild mice differed from the laboratory strains by showing more conspecific aggression in early trials, less investigation of intruders, more vocalisation when handled and a higher avoidance in recapture trials. Wild mice did not differ from laboratory strains in neophobic aversion to novel food or in biting when handled. In no test did the wild mice from the different environments differ from each other. The same tests were performed again on the wild mice after ten generations of differential breeding (random naturalistic, random laboratory and inbred laboratory). Inbreeding significantly reduced two parameters, aggression and recapture latencies, while habitat only affected biting when handled. Interestingly, wild mice kept in a laboratory environment bit more often than wild mice from the naturalistic habitat. In a review article of the domestication effects on behaviour in different species, it was concluded that *“the single most important effect of domestication is reduced responsiveness and sensitivity to environmental change”* (Price, 1999).

Risk assessment

In a novel environment an animal is driven by two opposing motivations. It is motivated to engage in exploratory behaviour to familiarise itself with the environment and to search for potential resources. However, a novel environment may also contain potential hazards or threats to the animal. Firstly, the environment itself may entail threatening features, such as sharp edges, cliffs, or running water. Secondly, animals of prey species typically coexist with predators, so that they frequently encounter signs of potential predator presence such as odours, sounds, and ambiguous visual stimuli, that all require investigation and evaluation. Thirdly, a novel environment may also be the territory of a conspecific and an encounter may result in fighting and serious injury. Exploration of the environment, therefore, is a trade-off between the chance of finding and utilizing

the resources necessary for survival and reproduction and the risk of being killed by a predator or an aggressive conspecific. The trade-off between risk taking and potential gain may vary in different situations and depending on motivational or emotional state (Lima & Dill, 1990).

Depending on the research field investigating the conflict and trade-offs between risk and reward, different terminology is used (Kavaliers & Choleris, 2001). In behavioural ecology the term vigilance is often used to describe readiness of the animal for potential risks, such as predators and aggressive conspecifics, in the environment. Behavioural trade-offs are usually measured in terms of scanning behaviour, altered foraging behaviour (food handling, diet choice), refuge use, patch selection, group size, group structure etcetera (Lima & Dill, 1990). In laboratory studies the term risk assessment is most commonly used. Risk assessment can be described as gaining information about a novel situation and a means to determine if an actual threat is present. Risk assessment appears to represent a balance of two opposing goals; investigating the threat stimulus and simultaneously remaining as protected as possible from it. Examples of risk assessment behaviours in mice are head dips, stretched attend posture (SAP), flat back approach, and scanning. If risks are detected, the individual switches to a more specific defence strategy. If not, the animal gradually returns to normal, non-defensive behaviours (Blanchard & Blanchard, 1989).

Defensive strategies include escape or avoidance, aggressive defence, freezing and immobility, and submission if the threat is a conspecific (Marks & Nesse, 1994). The most successful strategy depends on the situational factors, such as threat proximity (defensive distance), environmental constraints and individual factors (Blanchard *et al.*, 2001a). In mice, reactions to clearly manifested threat stimuli include flight if an escape route is available, hiding if a shelter can be found, defensive burying if substrate is available, and freezing if still undetected or if neither of the above applies (Rodgers, 1997). Additional measures of risk assessment have involved alternating approach to/withdrawal from threat, and stopping to orient toward a chasing predator (Griebel *et al.*, 1995). Extremely proximal threats usually promote explosive defensive threat/attack behaviours followed by fleeing/jump escape (Blanchard *et al.*, 2001a). Such attacks are usually directed towards the face region of the predator (in contrast to aggressive attacks at conspecifics that are usually directed towards the back of the antagonist (Blanchard *et al.*, 1979; van Loo *et al.*, 2001; Brain & Hui, 2003).

In the laboratory, risk assessment behaviours have been observed in mice in a variety of social (Rawleigh *et al.*, 1993) and non-social situations. These include exploration of novel environments (Rodgers & Dalvi, 1997; Rodgers *et al.*, 1999), predator odour (see (Dielenberg & McGregor, 2001) for a review), novel odour (Kemble & Bolwahn, 1997), and non-attacking predators (Blanchard *et al.*, 1995b; Blanchard *et al.*, 1998). Characterisation of risk assessment and defensive behaviour under laboratory conditions show ethological validity compared to in natural and semi-natural environments and have previously been shown to be fruitful in detecting differences between wild and laboratory rats and mice (Blanchard *et al.*, 2001a).

Emotional reactivity

Although we can all recognise an emotion within ourselves, it is often difficult to describe and categorise. This is true also in the scientific literature. There are many definitions and little consensus of what an emotion really is. However, most definitions refer to the individual's subjective experience of its situation (Ramos & Mormede, 1998). Emotions are expressed both behaviourally and physiologically and are thereby one of the possible modifying agents of a behaviour. Scientifically, the second major problem with emotions is that there is no means of measuring subjective experiences per se. The only available option, both in humans and animals, is to assess the emotional expressions (emotional reactivity).

When measuring emotional expressions it is wise to start by focus on behavioural descriptions of reaction patterns in different situations without presuming any functional relevance to avoid bias in interpretation at an early stage. However, there is abundant evidence indicating that animal reaction patterns are individual, situation specific and modified by emotional and motivational state (for a review see (Boissy, 1995)). Thus, there is a need for a functional interpretation of results and identification of the mechanisms of the underlying processes that have lead to the expression of the recorded behaviours. Depending on research field, different theoretic frameworks are used as a means of elucidating the underlying mechanisms of emotional expression. In a review article on emotional behaviour,, the most relevant and common approaches was summarised and categorised (Belzung & Chevalley, 2002). In experimental psychology, emotional expression is regarded either as a perceptual feedback system, a result of cognitive appraisal, an evolutionary adaptive response or a result of the reinforcing properties of the stimulus. In neuroscience, emotional expression is explained in terms of neurotransmitter systems and the activation or inhibition of specific brain areas. In genetic studies, strain differences, QTL analyses, and the effects of gene function are investigated, and in developmental biology epigenetic (pre-, post-natal experiences) are accentuated. It is unlikely that any of these approaches can explain emotional expression by itself, but that the truth is rather a combination of all of the above.

Emotions such as anxiety and fear may have evolved as an adaptation to modify animal behaviour, thereby preventing the animal from being injured in a potentially dangerous situation. The existence of such innate modifiers of behaviour also increase the likelihood of survival, as they result in a more flexible and suitable behavioural response to environmental stimuli. Risk assessment is related to emotional reactivity by being the information gathering procedure on which the appraisal of the situation is based. Risk assessment is also part of the cognitive decision making process of the cost/benefit analysis on determining optimal behavioural strategy (Pinel & Mana, 1989). As risk assessment is not selected for as strongly in a captive environment it is likely that domestication may affect risk assessment behaviours and emotional reactivity.

Animal welfare and well-being

Definition

The terms animal welfare and animal well-being are often used synonymously but may also be used as separate terms with its own specific meaning (Clark *et al.*, 1997). Animal welfare is the more general term comprising aspects such as general health status whereas an animal's well-being is more closely related to the animal's subjective experience of its situation. The major difference between the terms lies within the conflict between present positive experiences and future health. What is perceived as positive in the short term might not be beneficial for the animal in the long term.

Over the years numerous definitions of animal welfare and animal well-being have been put forward. I have chosen to present only two of those, chosen on the basis of their relevance to the aim of this thesis. One definition of animal welfare states that "*welfare is present when an individual can reliably predict or control relevant events by means of species specific signals or means*" (Wiepkema & Koolhaas, 1993). Others have defined animal well-being as the animal's "*internal somatic and mental state that is affected by what it knows (cognition), its feelings (affect) and motivational state and the responses to internal and external stimuli or environments*" (Clark *et al.*, 1997). In these definitions the terms are used synonymously and although the phrasing is different the definitions are both approaching the term by emphasising emotional and cognitive processing of perceived environmental stimuli and the performance of species-specific reaction patterns.

Assessment

The approaches used to assess animal welfare can be divided into three categories based on the underlying ethical and scientific concerns (Duncan & Fraser, 1997).

The function based approach is the traditional approach to animal welfare and include measures of longevity, growth, absence of stereotypies or other abnormal behaviours as well as physiological stress markers such as glucocorticoids. A potential stressor and the response of the animal are relatively simple to measure with the right methodology but interpreting the results can be difficult (Rushen, 1991). Functional parameters are sometimes considered crude on the grounds that they may not manifest until the welfare of the animal has been severely compromised. Functional parameters are also often more indicators of the absence of welfare rather than of welfare.

The natural approach has been adopted by most guidelines of how to house laboratory mice and other species. For instance, the Swedish Animal Welfare Act (SFS 1988:534, SFS 1998:56) states that laboratory animals should be kept "in such a way as to promote their health and permit natural behaviour". To allow for natural or species-specific behaviour may be a sound aim but the phrasing is weak and open for interpretation. Moreover, not all naturally occurring behaviours are beneficial for animal health and well-being. The Rodent Refinement Working Party (Jennings *et al.*, 1998) attempted to define this requirement in more detail by suggesting that cages for mice ideally should allow for "*resting, grooming, exploring, hiding, searching for food, gnawing, social interaction, nesting,*

digging and going into retreats". This recommendation is specific enough to begin implementing practically, however, scientifically there is still a lack of knowledge about what constitute a natural behaviour in the laboratory mouse and which behaviours are necessary for the animal to perform if its welfare or well-being should not be compromised.

The feelings-based approach relates to the animal's subjective appraisal of its situation. This field of research is receiving increasing attention but where in human studies questionnaires or self-appraisal schemes can be used to assess subjective states this option is not available in animals. However, techniques for measuring choice and motivation in animal are available and are increasingly used. Methods include preference tests (Baumans *et al.*, 1987; van de Weerd, 1996), operant conditioning (Dawkins, 1990; Sherwin, 1998; van der Harst, 2003), anticipation of reward (Spruijt *et al.*, 2001; van der Harst, 2003) and the assessment of subjective emotional states (Désiré *et al.*, 2002). Recently evidence of differential cognitive evaluation of ambiguous stimuli depending on previous experience has been published (Harding *et al.*, 2004), indicating reduced expectation of positive events in rats housed in stressful unpredictable conditions.

These approaches are not mutually exclusive and combined approaches to the study of animal welfare are common. The major problem in animal welfare assessment is the lack of agreement on a clear definition of what actually comprises animal welfare. Until then, assessment criteria of animal welfare and well-being will remain indirect. Basic research on different aspects of behaviours which are potentially related to animal welfare needs to be performed before the assessment criteria can be used to assess practical situations in the laboratory.

Background to this thesis

A need for improved criteria of welfare assessment

The assessment of animal welfare is not yet based upon measurements giving an absolute notion of what represents good or bad welfare for the animal in question. The different assessment criteria used are only representations of what we humans believe to be indicators of animal welfare. Therefore, the assessment of welfare in animals requires knowledge, not only of the normal behaviour and physiology of the species but also of its evolutionary background. It is also of importance to avoid anthropomorphic and across species generalisations when trying to interpret the assessed parameters in terms of the mental state of the animal. A scientific approach is crucial to avoid misguided attempts to improve the conditions for captive animals. The factors affecting the welfare of an animal are interrelated and cannot be easily distinguished from each other. To reach these goals we need to start by focusing on specific elements of this multi-variate group of issues rather than trying to grasp the whole problem at once.

In this thesis an ethoexperimental approach is taken to study behaviours that might be used as tools for evaluation of steps taken to improve animal welfare. Animal welfare is closely related to individual animal's emotional appraisal of its situation and improved means of assessing emotional states in animals are needed.

Animal models of anxiety

An affective disorder can be defined as “*a condition induced by the imbalance between environmental demands and the response capability of the individual to the demands that are perceived as threatening or overtaxing*” (Clark et al., 1997). The similarities between this definition and the factors thought to be related to animal well-being are obvious. Hence, there is reason to believe that animal models of anxiety and affective disorders may be useful in the assessment of animal well-being.

A large number of animal models have been developed to facilitate pre-clinical research on the behavioural pharmacology and neuroscience of emotional reactivity and anxiety disorders (Lister, 1990; Green & Hodges, 1991; Rodgers, 1997). Most of them are pharmacological validated using anxiolytic benzodiazepine drugs. However, pharmacological validation does not necessarily mean that the test is a valid test of anxiety. Behavioural validation is also necessary to dissociate between the pharmacological effects on general activity, motor pattern, and emotional reactivity (Lister, 1990). In measuring emotional reactivity, human psychological and psychiatric terminology has been adopted also in animals. However, discussing animal mental states in an anthropocentric terminology originating from human psychology can be misleading. It is also questionable to attribute a specific behaviour to a specific emotion isolated from context (Boissy, 1995). Behaviours induced by the perception and appraisal of potential threats are usually referred to as related to the emotional state of anxiety whereas behavioural responses to direct threats are thought to represent fear or even panic (Boissy, 1995; Lang *et al.*, 2000; Blanchard et al., 2001a).

Animal models are often classified as either unconditioned or conditioned response tests. According to Rodgers (1997), the major difference between these tests is that conditioned tests allow for a high degree of control over behavioural baselines, because the procedure involves training and learning. The unconditioned tests rely on the animal's spontaneous reactions to aversive stimuli. The individual responses of the animal are therefore more variable but on the other hand the model itself is often more natural and ecologically valid. The unconditioned models could be further classified into exploration, social and anti-predator models based on the type of aversive stimulus used (Rodgers, 1997).

The most widely used unconditioned models of anxiety include exploratory behaviour in unfamiliar environments. For some models no aversive stimuli apart from the novelty of the environment (Open field, Free exploration) is used but in other models aversive stimuli such as bright light (Light/Dark test) or heights (Elevated Plus Maze) are used to induce anxiety like behaviour. Although the aversive elements may be considered artificial, the context could be said to model the approach-avoidance conflict between exploration for resources (food and mates) and staying in the safe home environment thereby avoiding exposure to dangers such as predators and competing conspecifics. A more natural approach to the study of emotional reactivity may entail exposing animals to an environment containing predatory stimuli (Blanchard *et al.*, 1993; Kavaliers & Choleris, 2001). This naturalistic approach has been shown to be very efficient in eliciting anxiety-like behaviour patterns, such as risk assessment behaviour in mice. The choice of

predator stimuli differ among studies but generally include non-attacking live predators (Blanchard et al., 1993; Kemble & Bolwahn, 1997; Blanchard et al., 1998), natural predatory odour cues (Dell'Omo & Alleva, 1994; Berton *et al.*, 1998; Belzung *et al.*, 2001; Hayley *et al.*, 2001), synthetic odours imitating predatory odours (Kemble & Bolwahn, 1997) and predatory vocalisations (Hendrie, 1991).

Risk assessment, emotional reactivity and animal welfare

The present thesis addresses studies of exploration and risk assessment behaviours in mice under various experimental conditions. The choice of the experimental conditions originates from the assumption that explorative activity is, at least partly, controlled by an optimisation between the risks/benefits of the performed behaviour. The ability to correctly assess potential risks in a new environment and to adjust the behavioural coping strategy based on that assessment is crucial for survival and evolutionary fitness of animals in the wild (Lima & Dill, 1990). The ability to assess and adjust to external stimuli is important also for animals in captivity, to minimise stress and divergence from homeostatic balance. Hence, we assume that there is a link between the ability of the mouse to assess (risk assessment) and adjust (emotional reactivity) to novel situations and their well-being in a captive laboratory environment. Several different factors may affect risk assessment and emotional reactivity:

Differences in behavioural strategies in wild versus domestic mice

In the laboratory, the ability to assess risks may not have been selected for as strongly as in the wild. Thus, risk assessment behaviour may serve as a marker for the extent to which laboratory mice have deviated from the wild house mice in an important aspect associated to coping in captivity. An analysis of the differences, qualitative or quantitative, between wild and domesticated mice in basic explorative strategies is important in order to identify how domesticated laboratory mice phenotypes differ from the wild animal in crucial respects.

Sex related differences in behaviour

Some studies on laboratory mice indicate that males and females may have differential perception of novel and social events and use different approaches when exposed to the same task depending on context (Bimonte *et al.*, 2000; Palanza *et al.*, 2001). This may influence their requirements in a captive environment differentially. These potential differences between the sexes of laboratory mice are important to consider from an animal welfare point of view.

In neurobehavioural and psychopharmacological studies, male mice are used more often than female mice and sex differences in behaviour are not commonly investigated. In rats, sex differences have been more thoroughly investigated than in mice. In one study, male and female rats were exposed to three different types of tests of anxiety, the EPM, the Vogel conflict test and a social interaction test (Johnston & File, 1991). It was concluded that the behaviour of males and females differed in these tests but no conclusions on the general level of anxiety could be drawn as the results of the three tests pointed in different directions. It was also noted that these tests may not measure the same thing in males and

females as they were validated only for males. This fact has also been pointed out by others (Fernandes *et al.*, 1999). As argued by Palanza (Palanza, 2001), an ethoexperimental approach including females in the analysis, and considering both ultimate and proximate causations of the recorded behaviours may help improve current animal models of anxiety and depression.

Test and methodological situation

The optimisation of the trade-off between risk taking and potential gain may vary in different situations and depend upon motivational state. For instance, the presence of natural predators generally increases the risk assessment activities and reduces exploratory behaviour. However, the potential benefits of exploration may be greater than the risk in situations where only limited resources (food, partners, shelter) are available. By these means the motivational/mental state of the animal may be experimentally modified.

Impact of home cage environment

In neurological studies, 'enriched' cage environments, compared to standard conditions, have been proven to affect brain areas, such as the amygdala and the hippocampus in rodents, with subsequent effects on emotional reactivity, learning and memory (van Praag, *et al.* 2000). A review of over 40 environmental enrichment studies in mice (Olsson & Dahlborn, 2002) concluded that several studies, among others (Chapillon *et al.*, 1999) indicate that increased cage complexity may decrease emotional reactivity.

An increased complexity in the housing environment may therefore be hypothesised to have favourable effects on both animal welfare and experimental outcome. Correctly applied, cage enrichment may improve the animals' ability to cope with other types of interactions such as experimental procedures (Baumans, 1997) and thereby act to reduce variability between individual animals. However, a commonly expressed concern regarding environmental enrichment is that the introduction of enrichment items into the standardised cages of laboratory animals may increase the variability between animals with the consequence that more animals must be used (Eskola *et al.*, 1999; Mering *et al.*, 2001). Others (van de Weerd *et al.*, 2002) report no adverse effect on variation, which indicates that these concerns may be exaggerated and valid only under certain circumstances.

Aims of the thesis

As discussed above, risk assessment and emotional reactivity may be useful markers of animal welfare and well-being. The usefulness and outcome of these markers for animal welfare is related to an extensive complex of experimental factors. The present thesis addresses studies of exploration and risk assessment behaviours in mice under various experimental conditions. The objective was to investigate:

- Differences in behavioural strategies in wild versus domestic mice
- Strain and sex related differences in behaviour related to risk assessment and emotional reactivity
- Various test and methodological situations
- The impact of home cage environment on emotional reactivity and experimental variance (validity)

The specific aims of the included papers were:

Paper I

- To investigate if enrichment induces an effect on experimental results and on inter-individual variation in the behaviour of two different strains of mice (BALB/c and C57BL/6) in the Light/Dark paradigm or on general parameters such as body weight and food/water intake.
- To assess whether enrichment would alter the effect of a pharmacological treatment (diazepam) in the two strains used

Paper II

- To characterise behavioural strategies in novel environments and investigate in what respects male Wild house mice differ from the laboratory mouse (represented by BALB/c and C57BL/6) in risk/benefit assessment and explorative strategies

Paper III

- To characterise behavioural strategies in novel environments and investigate in what respects female Wild house mice differ from the laboratory mouse (represented by BALB/c and C57BL/6) in risk/benefit assessment and explorative strategies

Paper IV

- To validate a novel predatory exposure model aiming to facilitate behavioural analyses of risk assessment behaviours in mice
- To characterise the behaviour of four commonly used mouse strains (BALB/c, C57BL/6, SWISS, CD-1) in this model.

Materials & methods

Animals

The laboratory mice

Two inbred strains were used in all four studies namely the albino strain BALB/c (BALB) and the pigmented strain C57BL/6 (C57BL). These strains were chosen both on the basis of previous research (van de Weerd *et al.*, 1994; van de Weerd *et al.*, 1997a; van de Weerd *et al.*, 1998) on these strains in relation to the environmental enrichment used in Paper I and also based on their previously reported differential emotional reactivity (Griebel *et al.*, 1993; Beuzen & Belzung, 1995; Lepicard *et al.*, 2000; Belzung & Griebel, 2001). In a review of studies of anxiety like behaviour in mice, Belzung & Griebel (2001) concludes that BALB is the only mouse strain that consistently shows higher levels of anxiety compared to other strains. Based on this finding they proposed that BALB mice might be considered a genetic model of “trait anxiety”. The C57BL strain is characterised as a ‘non-emotional’ or low-reactive strain in common behavioural tests of anxiety but this strain has also been proposed as a good model for panic like behaviour (Griebel *et al.*, 1997). Moreover, the C57BL strain is also the most common foster strain in the generation of genetically modified mice. This together with previous experience of the behaviour of these strains made us choose these strains again for comparison with the wild mice in Paper II and III and with the two outbred lines in Paper IV.

In Paper IV, two outbred lines were included namely Swiss-Webster (Swiss) and CD-1 mice. These two lines are related to each other, CD-1 mice are from an outbred stock originally stemming from a colony of Swiss mice. The choice of Swiss-Webster mice was based on its extensive previous use in predator exposure tests (Blanchard *et al.*, 1995b; Griebel *et al.*, 1996b) and its similarities with wild mice (Parmigiani *et al.*, 1989). In retrospect, this strain would have been very interesting to include also in Paper II and III. The CD-1 strain has also previously been used in predatory models of anxiety (Dell’Omo & Alleva, 1994; Blanchard *et al.*, 1998).

The wild mice

The only subspecies of wild house mouse living in Sweden is the *M. m. musculus* type. As the laboratory mouse is still considered a mosaic of different subspecies (Bonhomme *et al.*, 1994), we concluded that, together with the obvious practical aspects, this subspecies would be suitable for our purposes. To establish a founder colony for Paper II and III, approximately 30 wild house mice of both gender were caught using cage traps (250x78x65 mm, Ugglan special, Grahnb, Sweden) at four different locations (Jälla, Ensta, Knivsta, Slavsta) within 50 km from the city of Uppsala, Sweden. All traps were set in the vicinity of farms. To minimise inbreeding, female mice captured at one location were housed with a male captured at another location. Only first or second generation laboratory born mice were used.

In our studies of wild house mice, all subjects were reared and kept in captivity. This enabled us to have a greater control of age and environmental influences between the wild mice and the laboratory mice. One could question whether this affected the wild mice to the extent that they could no longer be regarded as representative of wild house mice. There are previous studies that indicate only minor differences in behaviour among wild mice reared in captivity for a few generations (Connor, 1975). Moreover, a recent study of another species (wild cavies and domestic guinea pigs) also found no behavioural differences between wild-trapped and 30th generation laboratory reared offspring of the wild cavies. Wild cavies of both backgrounds differed from guinea pigs in showing shorter exploration latencies in a free exploration task, less socio-positive and more aggressive behaviour (Künzl et al., 2003). This indicates that the effects of domestication are not achieved only in a few generations and that our mice could be considered representative to wild house mice in this respect.

Behavioural tests

Five different tests of exploration, risk assessment and emotional reactivity were used in this thesis. Three of them were tests widely used in behavioural pharmacology (Light/dark test, Open Field, and Elevated plus maze). The other two were novel tests (Concentric Square Field and Rat Exposure Test).

The Light/Dark test (LD)

The LD-test, (Crawley & Goodwin, 1980), is based on the conflict of residing in a dark (safe) area or exploring of a brightly lit (unsafe) area. It has been used both in its original form but also in modified forms (Costall *et al.*, 1989; Onaivi & Martin, 1989; Hascoët & Bourin, 1998). The methodology used by different laboratories varies in test duration, site of release of the mouse (light or dark compartment), size of compartments, tunnel or no tunnel, illumination level, clean vs soiled apparatus and parameters measured (Hascoët *et al.*, 2001). It was concluded that the method was useful as a test of anxiolytic or anxiogenic drugs but that simultaneous sedative or stimulatory effects may make it difficult to separate effects on emotional reactivity and general activity.

The Open Field test (OF)

The Open Field test is a tool to measure both “emotionality” and the animal’s general level of explorative activity or ambulation. The general procedure is that the animal is placed in an empty arena in which it is allowed to explore for a period of time. Factors such as, the size and shape of the arena, level of illumination, and duration of testing vary considerably between studies and may have effects on the behaviour of the animals (Lister, 1990; Choleris *et al.*, 2001). The animal’s performance is usually measured as peripheral and central activity, immobility and defecation (Lister, 1990) but other behavioural parameters have also been used (Archer, 1973). Mice have a tendency to stay close to vertical structures, such as the walls of the open field arena, and this tendency is commonly referred to as positive thigmotaxis or wall-seeking (Choleris *et al.*, 2001). Moreover, mice avoid open spaces such as the central parts of the field.

Immobility or freezing is a strategy to avoid being detected and defecation is thought to reflect “emotional elimination” (Archer, 1973) i.e. the tendency for animals to defecate or urinate when in a threatening situation as not to impede flight. The Open Field has been criticized for its inability to dissociate between parameters for general activity and exploration (Archer, 1973). A thorough ethological analysis has been published which includes risk assessment behaviours such as SAP and returns, rearing, grooming, and jumping (Choleris et al., 2001). An image of the apparatus used in Papers II and III can be found in Paper II.

The Elevated Plus maze (EPM)

This test is together with the OF one of the most widely used test of exploration and anxiety in pharmacological research. As in the case of the LD-test and the OF, the structure of the arena varies between studies and this may influence the animal’s behaviour (Hogg, 1996; Rodgers & Dalvi, 1997). In the EPM, emotional reactivity is dissociated from locomotion by relating the number of open arm entries to the total number of arm entries (Lister, 1990). The introduction of ethologically relevant measures of exploration and risk assessment (Rodgers & Cole, 1993) such as stretched attend posture, head dips and rearing improved the sensitivity of the method. Moreover, the connection between these measures and physiological stress reactions has also been confirmed (Rodgers et al., 1999). Principal component analyses on behavioural parameters used in the EPM have been reviewed and re-evaluated, resulting in a recommendation for the use of the parameters: open and closed arm entries, % duration in open and closed arms, unprotected head dips and SAPs and rearing (Wall & Messier, 2001).

The Concentric Square Field (CSF)

This test was originally established in order to score the functional effects of experimental brain lesions achieved by trauma (Clausen *et al.*, 2001) or microembolization in the rat (Roos *et al.*, 2003). It has also been used to measure the effects of maternal separation on exploratory behaviour in rats (Roman *et al.*, 2003). The effects of pre-trial stimuli (restrain, food deprivation, social encounters), predator stimulation during the test session and strain differences have also been explored in the rat (Meyerson, In prep). Papers II and III represents the first uses of the Concentric Square Field in mice.

The CSF was established as a multivariate test suitable for measuring risk - benefit assessment in explorative activity. The rationale to use risk/benefit assessment for this purpose was based on the assumption that risk/benefit assessment behaviour should comprise widespread neuronal circuits including perceptible, cognitive and motor abilities. A multivariate test situation was developed in which the animal is allowed to choose between zones that it perceives as more or less aversive. In this test it is registered whether the animal chooses to enter and spend time in an open central arena or a corridor system including a dark enclosed room, a small area requiring some physical effort to enter and a brightly lit elevated bridge construction. An image of the apparatus used in Papers II and III can be found in Paper II.

The Rat Exposure Test (RET)

The test is essentially a simplification of the Visible Burrow System, an ethoexperimental model that has been shown to have the properties to elicit anti-predator defence behaviour and risk assessment in mice after cat exposure (Blanchard et al., 1995b). The negative aspects of this model is that it is both time consuming and labour intensive to perform. This new Rat Exposure Test consists of a small dark chamber attached via a transparent tunnel to a Macrolon Type III cage divided in two parts by a wire mesh. After three sessions of habituation, a rat is introduced to the mesh enclosed part of the cage. Spatial and multiple ethological measures of risk assessment (stretched attend posture (SAP), freezing, avoidance, defensive burying) are registered, to allow for characterisation and differentiation between treatments. The test has also been pharmacologically validated using chlordiazepoxide, an anxiolytic drug that resulted in reduced risk assessment and avoidance but increased freezing (Blanchard *et al.*, 2003b). A schematic side view of the apparatus can be found in Paper IV.

Differences and similarities between tests

The LD-test, the CSF, the OF and the EPM all include exposure to novel environments whereas the RET test is performed in a familiar environment. The aversive elements used in the novel environments are: bright light in the LD-test, bright light and elevation in the CSF, openness in the OF, and elevation in the EPM. These are all constant predictable physical features of the environment. The RET test, on the other hand, uses a living rat as the aversive stimuli, a stimuli that is both natural, unpredictable and a real threat to the exposed animals. All the tests used are to some degree measuring exploration, risk assessment and emotional reactivity.

Table 1 is based on the functional analysis used in Papers II and III but now incorporating all tests used in this thesis. A thorough description of the different functional parameters can be found in Paper II. In summary, the first category ACTIVITY include calculations of ambulation in the tests measured by frequency of entering non-aversive zones or direct measurements of locomotion as in the LD-test. In the second category, EXPLORATION, parameters related to the latency to enter non-aversive zones and behaviours related to directed exploration such as head dips and rearing are included. In the third category, APPROACH/AVOIDANCE, the latency to enter potentially aversive zones, the frequency of entering and the duration of each visit in these zones and zones in which assessment was made from were included. Furthermore, risk assessment behaviours and other defensive reactions (SAP, defensive burying, freezing) and grooming are included. The fourth and last category, OPEN/SHELTER, summarises parameters relating to the latency, frequency and duration of periods spent in open areas such as in the centre of the arenas and the time spent in the sheltered areas. The implications of this summary are further outlined in the results and discussion sections.

	ACTIVITY	EXPLORATION	APPROACH - AVOIDANCE	OPEN - SHELTER
LD	TOTAL DISTANCE VELOCITY DURATION LOCOMOTION IMMOBILITY	CROSSINGS <i>FRQ REAR</i>	LAT ENTRY LIGHT DURATION LIGHT <i>FRQ SAP</i> <i>TUNNEL TIME</i>	
CSF	FRQ TOT CORR	LAT LEAVE LAT DCR LAT HURDLE FRQ REAR	FRQ SAP SLOPE, BRIDGE LAT, FRQ BRIDGE INT LAT BRIDGE - SLOPE FRQ SLOPE DUR/VISIT BRIDGE DUR/VISIT DCR	LAT, FRQ, DUR CENTRAL CIRCLE LAT, FRQ, DUR DCR FRQ, DUR TOT CENTRE
OF	FRQ TOT ENTRIES	LAT GOAL ZONE <i>FRQ REAR</i>	LAT ENTRY FRQ START BOX DUR/VISIT START BOX <i>FRQ SAP</i>	DUR START BOX LAT, FRQ, DUR CENTRE DUR CENTRE/ARENA FRQ, DUR MIDDLE CIRCLE
EPM	FRQ CAE	LAT CA LAT OA FRQ TOT DIP FRQ REAR	% DUR CENTRE FRQ TOT SAP % PSAP FRQ GROOM % PDJIP % OA % CA	
RET		FRQ REAR	TUNNEL TIME TOTAL CONTACT FRQ, DUR SAP STRETCH APPROACH FREEZING DEFENSIVE BURYING DUR SURFACE CHAMBER TIME	CHAMBER TIME

Table 1. Functional table of parameters related to activity, exploration, approach avoidance and open-shelter. Behavioural parameters that are used in the different papers and that are closely related to the functional parameters are printed in **bold**, parameters used but less clearly related to a functional category, in normal text and potentially useful parameters in *italics*.

Behavioural registration methods

All studies were performed using continuous sampling of behaviours during the test period. However, both manual and automated registration methods were used. For the LD test both manual and automated recordings were used simultaneously but in the other tests either manual (CSF, EPM, RET) or automatic (OF) recording was used. All manual recording was done from videotapes.

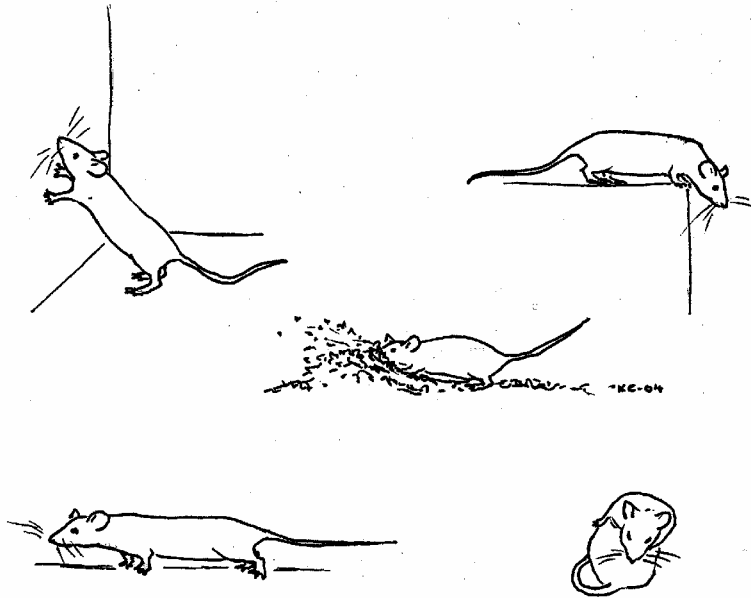


Figure 1. Sketches of some of the behaviours registered in the different papers in the thesis: rearing (upper left), head dip (upper right), defensive burying (middle), stretched attend posture (lower left), and grooming (lower right).

Automated tools

LABORAS™ (Laboratory Animal Behaviour Observation, Registration and Analysis System, Metris, The Netherlands) was used in Paper I. The system is based on measurement of vibrations induced by movement of the animal within its cage. It is capable of measuring position of the animal, speed and travelled distance as well as some behavioural elements (van de Weerd *et al.*, 2001).

Ethovision (Noldus Information Technology bv) was used in Paper II and III to measure spatial location in the OF. The system is based on automatic registration of the animal via Black/White image contrast. Originally, our intention was to use this method for both CSF and OF for spatial location and to get a measure of distance travelled and velocity and a possibility to investigate how these variables were affected by habituation to the test arena. However, unfortunately problems with providing enough contrast for the different coloured mice reduced validity and precision of these variables to a degree that we could not use Ethovision in the CSF at all. For spatial location in the OF however, Ethovision produced reliable measurements compared to manual recordings.

Manual tools

Three different registration tools were used: The Observer (Noldus Information Technology bv) is a widely used, commercially available, windows-based software for behaviour registrations. It was used for manual registration of spatial location and SAP in Paper I. Score is a much simpler but practical software, developed by Pär Nyström, Department of Psychology, Uppsala University. This program was used in Papers II and III. Hindsight is a DOS-based software, developed by Dr Scott Weiss. The software has been used by several behavioural research groups and was also used in Paper IV.

Experimental procedures

Paper I

The behaviour of 216 naive adult male mice of two different strains (BALB/c and C57BL/6) was studied. The animals were housed in groups of four in ‘non-enriched’, ‘enriched’ (nesting material) or ‘super-enriched’ (nest-box, nesting material, wooden gnawing stick and PVC tube) cages. After 5 weeks the animals were assigned to one of three treatments: control (no injection), sham (saline injection i.p) or diazepam (1mg/kg bw i.p) and tested in the Light/Dark test for 5 minutes. In addition to spatial measures, behavioural measures of risk assessment were registered.



Figure 2. Photos of the three housing conditions. From left to right: Non-enriched, Enriched, and Super-enriched.

Paper II

A total of 39 adult male mice (14 Wild (first- or second-generation laboratory born wild-derived house mice), 13 BALB, and 12 C57BL) were tested in three behavioural tests, the Concentric Square Field (CSF), a modified Open Field (OF) and a conventional Elevated Plus Maze (EPM). The rationale of running the animals in all three methods was to achieve an estimate of the general level of activity and explorative motivation of the mice. The animals were tested over three consecutive days in the order CSF, OF, EPM. The test periods were 15 minutes in the CSF, 20 minutes in the OF and 5 minutes in the EPM. In addition to spatial measures, behavioural measures of exploration and risk assessment were

registered. The parameters were categorised according to their relevance to activity, exploration, approach–avoidance, and the use of open areas/shelter.

Paper III

This study was performed on female mice using exactly the same methodology as we previously used for male subjects in Paper II. A total of 14 BALB mice, 16 C57BL mice and 14 first- or second-generation laboratory born wild-derived house mice were included.

Paper IV

Two experiments were presented in this paper. Experiment I was performed to characterise risk assessment behaviours in the RET. A total of 23 BALB mice were used. The procedure consisted of three 10-minute sessions of habituation over three consecutive days. On the fourth day, either a male Long-Evans rat systemically injected with 5.0 mg/kg of d-amphetamine or a plush toy rat was introduced behind the mesh enclosed part of the cage. Spatial and multiple ethological measures of risk assessment (stretched attend posture, freezing, avoidance, and defensive burying) were registered to allow for characterisation and differentiation between treatments. Experiment II compared risk assessment and other defensive behaviours of four strains of mice, two outbred strains (SWISS, CD-1) and two inbred strains (BALB and C57BL), in the RET. The apparatus and procedures were identical to those of Experiment 1, with the exception that only rat exposure trials were run, i.e. no animals were exposed to toy-rat stimuli.

Comments on methodology

A pilot study was conducted for the purpose of guiding the protocols of Paper II and III, in relation to the order of performance to the three tests. This study aimed to assess if and to what extent there was a carry over effect when the animals were subjected to the three methods. A total of 18 adult (5 week old) male BALB mice housed in groups of three in Macrolon III cages, were divided into three different treatment groups of six animals were used. The animals were moved directly from the animal room to the test room.

The tests were performed over three successive days and the groups were defined as follows:

- Group 1: CSF, OF, EPM
- Group 2: EPM, CSF, OF
- Group 3: OF, EPM, CSF

The experimental protocols were identical to the ones described in Papers II and III with the following exceptions. In the CSF, the bridge slopes were covered with a black rubber mat in Pilot I instead of the grey rubber mat used in the studies which followed. The LAT, FRQ, DUR Centre, DCR, Bridge, the Corridor zones and Hurdle were scored manually from videotapes using Etholog v 2.25. In the Open field, the mouse was allowed to explore the start box for 5 minutes. The test

began with the removal of the plastic shield covering the entrance hole to the arena and lasted for 30 minutes. No stimulus was placed in the goal box. The location of the experimental animal was recorded by an infrared-sensitive video camera-computer linkup automatically interpreted by the software TELOF. The open field was divided into six pre-programmed areas. The entering and leaving of an area were scored (variables: latency to first visit, frequency of visits and duration of visits into the area). In the EPM, the mice were allowed to explore the arena for ten minutes. The behaviour of the mice was videotaped and scored manually using Etholog v 2.25. The parameters included were: LAT, FRQ, DUR open arms, closed arms, centre. FRQ SAP and head dip were also recorded.

For CSF, significant differences were found for LAT Bridge ($p < 0.01$) where Group 2 had a longer latency than Group 1 and 3. A difference was also found for DUR Bridge ($p < 0.05$) where Group 2 had a shorter duration than Group 1. No differences were detected between the different groups in OF total activity, that is the scores recorded when the animal moves in the various zones. Nor was there a significant difference as to latency to leave the start box, latency to first visit in the goal box zone or time spent in the peripheral zone. For EPM, no significant differences between groups were found in any of the parameters registered.

Test order had a limited effect on the behaviour of BALB mice in the three tests. Considering the relatively small sample size ($n=6$) the existence of actual differences due to test order cannot be fully excluded. Recognising that the parameters that did differ between test order were recorded in the CSF we concluded that this test was the most sensitive to previous experience and should therefore be performed as the first test in the test battery to reduce the likelihood of any carry over effect.

Statistics

In general, statistical treatment of data was done in the following way. Raw data was tested for normality distribution. Data that did conform to normality was tested using ANOVA followed by post hoc testing. Data that did not conform to normality was tested using non-parametric statistics followed by post hoc comparisons. For details on specific methods see respective paper. In all papers differences were considered significant at $p < 0.05$.

In Paper I, the statistics was supplemented with an analysis of variation using test chosen and performed by a professional statistician. The three different statistical measures of variation used in this study: the mean absolute deviation (MAD), the coefficient of variance (CV) and the Solo Power Analysis sample size (SS). MAD is defined as the mean distance that individual observations have with respects to their group mean. It is independent of overall mean level. CV is defined as standard deviation divided by mean, and is a measure of variation, relative to the overall level of response. SS is a function of CV, namely $SS = \text{constant} * CV^2$. No statistical test was performed between the different measures for variation (MAD, CV, SS).

In Paper II and III, the traditional statistics was supplemented with a multivariate data analysis using a pattern recognition based approach (Soft

Independent Modelling of Class Analogy, PCA SIMCA-P+10.02 software, Umetrics). The PCA creates a condensed summary of the behaviours across the three groups of mice, analysed graphically by means of two plots. A score plot showing a summary of the relationship among the individuals and a loading plot identifying variables important for creating these relationships.

Results

In this section a summary of the results from the four appended papers is presented in terms of a functional analysis based on Table 1. Some previously unpublished data is also presented here.

Functional analysis

Many of the functional parameters summarised in Table 1 were measured in all of the tests used in this thesis. The functional analysis means that the collected behavioural scores in terms of latency, frequency, duration etc have been brought into a functional context. This functional context is an evaluation of the descriptive behavioural parameters. Taken together, they may also tell us something about emotional reactivity.

Activity

Male BALB/c mice showed lower levels of activity than C57BL mice in the LD-test but no differences were found between these mouse strains in the CSF, OF and EPM. Female BALB mice had lower levels of activity than C57BL in the EPM.

Male Wild mice had a lower general activity level than the laboratory strains in CSF, OF and EPM. Female Wild mice had a lower general activity only in the OF. In the EPM, Wild females had the lowest mean number of entries into closed arms, however, this was only significantly lower than C57BL.

Comments

On a functional level, Wild male mice were interpreted as having a low activity level. What is not clear from this conclusion is that although wild mice had a lower ambulation between different non-aversive zone, they were also very reactive as shown in a high vigilance level and quick reactions to disturbances. This could not be measured using our methodology and consequently this is just a personal impression. The activity parameter in the OF is affected not only by activity pattern within the arena, but also by how much time the animal spends in the start box. The arena was entered on a voluntary basis and many Wild mice spent a large part of the test period in the start box. Among female mice, many BALB mice also stayed the whole test period in the start box indicating that the basis of comparison for this parameter may be biased.

Exploration

Only in the EPM did BALB differ from C57BL in exploration parameters. BALB males had a lower head dip frequency in the EPM and a higher rearing frequency. BALB females had both a lower head dip frequency and a lower rearing frequency. No differences were found in explorative activity when comparing male Wild and laboratory strains in the CSF, OF and EPM. Of the exploration parameters, Wild females showed a higher frequency of rearing than the laboratory strains. This higher rearing frequency was significantly different to both strains in the CSF, and Wild also had the highest mean in the EPM although only significantly different to BALB mice.

A parameter for which both male and females Wild mice had a lower duration than the laboratory strains, although not originally included under the exploration heading, was total time spent in the corridors. In Wild males the frequency of entering the corridors was also lower but in females the frequencies were equal between mice. This indicates a shorter time per visit in the corridors before moving to another zone. It is possible that the corridors are used only as transit zones on the way to other zones or that it is a strategy of changing location often as to reduce the risk of being detected.

Approach –avoidance

Stretched approach posture (SAP) was measured in all tests except from the Open Field. It was registered also in the LD test but not presented in the original paper. Statistical analysis revealed that BALB mice showed more risk assessment behaviour (SAP) than the C57BL mice ($p < 0.001$). The occurrence of SAP ranged from 7-10 per treatment in BALB mice and 3-8 per treatment in C57BL mice. No difference in risk assessment behaviour was found between housing systems. Hence, in all tests performed in novel environments (LD, CSF, OF, EPM), BALB mice showed more SAP than the C57BL mice. In the RET, BALB showed intermediate levels of SAP whereas C57BL mice showed more SAP than all the other strains.

The behaviours grooming, freezing and defensive burying were categorised as part of the approach-avoidance category. Male Wild mice had a higher duration of grooming in the EPM than laboratory strains. No difference was found in the CSF or for female Wild mice in any of the tests. In the RET, BALB performed virtually no defensive burying, and very low levels of freezing in the first experiment, although slightly higher in the second. C57BL mice showed intermediate levels of defensive burying and the highest levels of freezing of the four strains.

Differences in approach–avoidance was also measured in risk taking pattern. Both male and female BALB mice avoided risk areas while C57BL/6 mice were more inclined towards risk taking. Many BALB did not enter the CSF bridge and many females did not enter the OF arena at all.

Wild males showed less risk assessment behaviours than both laboratory strains, but were cautious before entering a potentially dangerous zone and explored all zones after they had assessed them as risk free. This was evident mainly in the

CSF where Wild males had a longer interval between entering the slope zone for the first time and before entering the bridge compared to C57BL mice. This pattern was less evident in Wild females.

Comments

The SAP is characterised by an elongated body posture where the animal is performing intention movements in different directions with its front paws while retaining the position of its rear paws. If the animals instead of returning to the original position stretches its body and continues its motion forward, the behaviour is sometimes referred to as stretched approach. Although not specifically described or quantified, my impression is that the behavioural sequence of SAP differs slightly between the strains. BALB mice generally stand still with their hind feet while performing repeated SAPs in different directions whereas the C57BL and Wild mice stretches its body for longer periods and sometimes continues in the same direction, as has been defined as stretched approach. For head dips, strain differences could also be described. BALB mice stretched outside the EPM arms but they did only dipped their head. C57BL mice very clearly dipped both head and shoulders down towards the floor. Wild mice performed both very quick head dips and longer dips.

Open –Shelter

Both male and female BALB mice crossed and stayed in the central circle in the CSF more than C57BL. No significant difference between strains was found in the OF. In the EPM, BALB mice spent little time in open arms. In the RET, BALB mice spent the majority of its time on the surface and by the mesh and less time in the chamber than the other strains. C57BL mice spent less time in contact with the mesh compared to the other strains, and spent more time in the chamber than on the surface. A reluctance to enter open areas was found in male Wild mice for both CSF and OF. The same tendency was found in Wild females compared to the laboratory strains although not as clear. In the EPM, this trend was not found in either male or female Wild mice.

Comments

In Paper II, the percentage time spent in the open arms in the EPM was categorised in the Open-Shelter category. However, it now seems more likely that it is a measure of approach-avoidance (risk taking). This reinterpretation is based both on the lack of similarities between the parameters of open area avoidance in the CSF and OF and on previous studies (Rodgers, 1997). There is a potentially important difference between the CSF and OF compared to the EPM. The CENTRAL CIRCLE of the CSF and the OF are within an enclosed arena, whereas the open arms of the EPM are open to the surrounding environment and consequently also a potential escape route. Hence, the risk/benefit ratio of entering these open zones may differ.

Impact of home cage environment

In Paper I, very limited effects on the mean values of exploration were found in relation to the cage environment. In fact, no clear housing effects were found in any of the behavioural parameters measured. Only a strain x housing interaction was found in total distance travelled. The mean score for BALB mice housed in SE cages was higher than BALB mice in the NE and E cages whereas for C57BL mice the mean score for distance travelled was shorter for mice housed in SE cages compared to other alternatives. The only effect of housing conditions was found on body weight, where NE mice gained less weight than E and SE mice. This effect has been found before and was interpreted as an effect of improved thermoregulation (van de Weerd *et al.*, 1997b).

The cage environment also had little effect on variation between individuals. Only for *velocity*, was a significant housing effect found. SE mice had higher variation than E and NE mice. A treatment x housing effect for duration of *locomotion* was found relating to the fact that for different treatments the variation of housing varied. For none of the treatment groups were these housing effects significantly different from any of the other groups.

Differences in behaviour in wild versus laboratory strains

In Paper I, strain differences were found in both the activity and the emotional reactivity of the two mouse strains. The strain differences in the Light/Dark test were much more prominent than the effects of cage environment. In summary, the C57BL mice scored higher than BALB mice in activity related measurements. They also showed a lower emotional reactivity in the pharmacological control situation. After diazepam treatment, BALB mice decreased their emotional reactivity whereas C57BL mice remained unchanged in their behaviour. Strain differences were also found in risk assessment as measured by SAP (data presented here), which was recorded more frequently in BALB mice than in C57BL mice.

In Paper II, the behavioural parameters were categorised according to their relevance to activity, exploration, approach–avoidance, and use of open areas/shelter. Male Wild mice had lower activity and a higher avoidance of open areas than the laboratory strains. No differences were found in exploratory motivation. The BALB mice avoided risk areas and showed a high risk assessment (SAP) while C57BL mice were more explorative, demonstrating a higher rate of risk taking and little risk assessment. Male Wild mice seemed to have a different behavioural strategy of risk assessment in being more cautious before entering a potentially dangerous zone but explored all zones after assessed as non-risky. A Principal Component Analysis of the animals' behaviour in the CSF arena supported these findings by clearly separating the three strains on basis of their behavioural performances.

In Paper IV, all strains showed risk assessment behaviours towards the rat. C57BL mice spent less time in contact with the mesh compared to the other strains, and spent more time in the chamber than on the surface. They showed more risk assessment (SAP) than all the other strains, intermediate levels of

defensive burying and the highest levels of freezing of the four strains. BALB mice spent the majority of its time on the surface and by the mesh and less time in the chamber than the other strains. They showed intermediate levels of risk assessment (SAP), virtually no defensive burying, and very low levels of freezing in the first experiment, although slightly higher in the second. The two outbred strains, the Swiss-Webster and the CD-1, did not differ from each other on either spatiotemporal or ethological measures. They spent equal time in both chamber and surface and more time in total contact than the C57BL, but were not significantly different from the BALB. They performed less SAP and less freezing than C57BL mice and more defensive burying than both C57BL and BALB mice.

Sex related differences

The same methodology was used in Papers II and III. The only difference between the studies were the sex of the animals. Wild males had a pattern of lower locomotory activity in novel environments than the laboratory strains, which was not the case in females. In males, several differences were found in approach avoidance patterns, which led us to the conclusion that male Wild mice had a different risk assessment strategy to the laboratory mice used. This pattern was not as clear in females. Neither did we find any clear patterns of differential risk taking behaviour in male and female mice. However, what was evident in both studies was that Wild mice of both sexes avoided open areas to a higher degree than the laboratory strains.

The CSF method provides the animal with a greater number of behavioural alternatives than the OF and the EPM. With the objective of characterising and contrasting the behaviour of the Wild house mouse and two laboratory mouse strains we found it relevant to supplement the traditional statistical evaluation and our functional analysis with a multivariate data analysis, PCA. Individuals were used as 'cases' and the behaviour parameters as variables.

Males

The score plot distribution showed a case pattern clearly attributable to the strain affiliation of the mice. The corresponding loading plot showed that the grouping of the Wild mice was mainly due to the parameters: DUR/VISIT DCR, DUR DCR, LAT BRIDGE and LAT CENTRAL CIRCLE and to a lesser degree to FRQ GROOM. For the C57BL mice, the parameters DUR and FRQ BRIDGE and SLOPE coincided with the grouping pattern seen in the score plot. The parameters related to HURDLE cannot be attributed to either Wild or C57BL but are clearly distinguished from BALB. The strongest behaviour variables (in terms of distance from the origin) that influenced the score plot location for the BALB mice were DUR TOT CORR, DCR, and CENTRAL CIRCLE, and FRQ CORR, CENTRE, CENTRAL CIRCLE and DCR. The behaviours SAP and REAR were also found in the right half of the chart. Wild mice differed from both BALB and C57 but BALB and C57BL were also clearly different from each other. Further, the loading plot shows that the behaviours that formed this grouping are in good accordance with the outcome of the traditional statistical evaluation and our functional analysis.

Females

The PCA clearly separated Wild and BALB females along the x-axis but most C57BL mice were found in the central area of the score plot. This indicates that their profile is characterised by average values relative to the individuals that are marked at a longer distance from the plot centre. Thus, Wild females could not be clearly separated from both laboratory strains. The parameters that distinguished Wild females the most in the loading plot were FRQ SLOPE, BRIDGE, HURDLE and REAR, DUR SLOPE and LAT CENTRAL CIRCLE. There was a trend in the functional analysis indicating differences in exploration or approach-avoidance between female Wild and laboratory strains (BALB, C57BL). However, this trend was not fully supported by the statistical evaluation. Except for the FRQ BRIDGE parameter, the PCA supported the trend of active exploration combined with a hesitance to enter open areas in the Wild female mouse. The general pattern was clearer for open/shelter and indicated that female Wild mice have a higher avoidance of open areas than the laboratory strains.

Sex differences

The data from the male and female mice were also taken together and subjected to a new PCA analysis to enable comparisons between the sexes in CSF behaviour strategy. The score plot (Fig 3a) shows that Wild males cluster in the upper left quadrant, and female Wild mainly the lower left quadrant together with the male C57BL mice. Female C57BL are located along the x-axis but not spread much along the y-axis whereas both male and female BALB are distributed further to the right in both upper and lower quadrant.

The loading plot (Fig 3b) clusters the parameters SAP SLOPE, FRQ CENTRE, DUR CENTRE, DUR CORRIDOR, and FRQ DCR furthest to the right side in the same parts as the BALB mice. BALB mice had significantly higher values in all these parameters except for FRQ DCR. The parameter REAR is positioned solitary close to the y-axis in the lower left quadrant. Wild females had a higher frequency of rearing in the CSF and many of them are also positioned in the lower left quadrant in the score plot. Parameters related to the SLOPE, BRIDGE, and HURDLE are also positioned in this quadrant. High values in these parameters pulls Wild females and C57BL mice in this direction. High values in the parameter DUR BRIDGE separates C57BL and Wild mice from BALB mice towards the left along the x-axis. Male Wild mice are pulled to the upper left corner by their high values in DUR/VISIT in the DCR.

Methodological comparisons

Papers I, II and III generated a behavioural profile of the C57BL strain as having low emotional reactivity and the BALB as having high emotional reactivity. These results are all based on its exploratory behaviour in the novel environments. The results of the RET were in contrast to these findings. In a familiar environment, but in the presence of a rat, C57BL mice showed more risk assessment, avoidance behaviour and active defence than BALB mice. This poses the question of whether

predator versus novel environments responses entail different approach-avoidance conflicts. Environmental features of the test arena, the familiarity of the environment and differences in the responses to different stimuli seem to have a great impact on emotional reactivity.

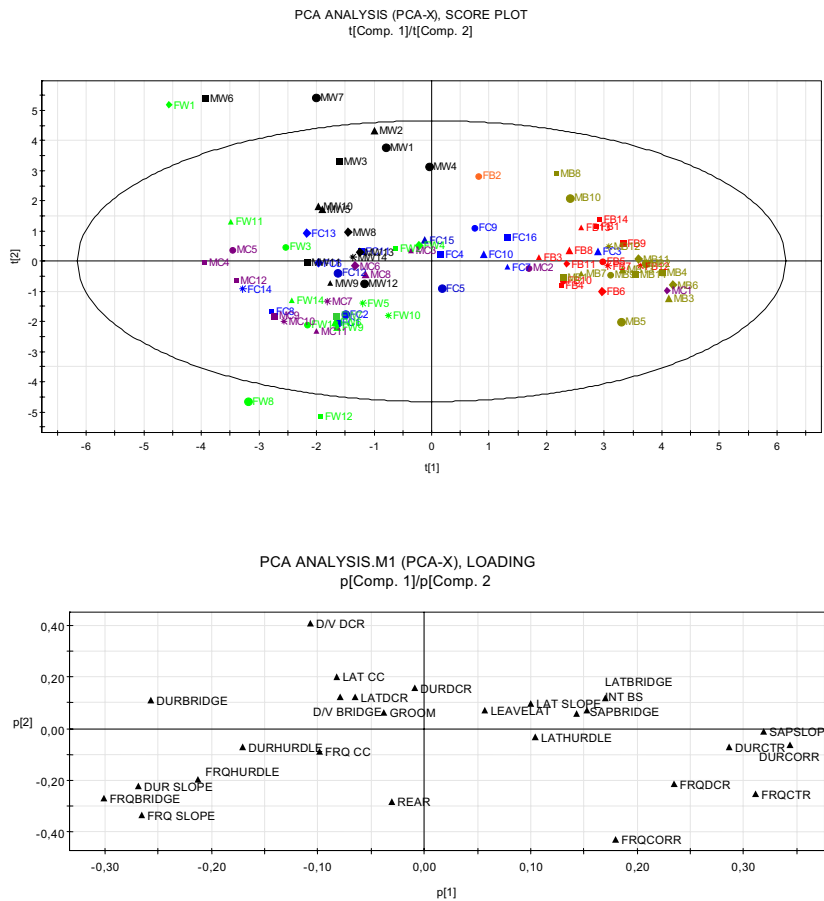


Figure 3. PCA-SIMCA analysis. The score plot is a summary of the relationships between individual animals. The first letter stands for sex (male (M) and female(F)), the second for strain (Wild (W), BALB (B) and C57BL (C)). The numbers indicate individual animals. The loading plot identifies variables important for creating these relationships. The direction of the score plot corresponds to the same direction in the loading plot.

Discussion

This thesis deals with behavioural studies as a means of assessing emotional reactivity. It has focused mainly on explorative behaviour and risk assessment in mice in different types of test situations. The long-term objective of this work is to find relevant measures in relation to animal welfare.

Differences in behavioural strategies in wild and laboratory mice

Genetic uniformity, as found in inbred strains, does not necessarily result in phenotypic uniformity, as discussed above, but may increase or decrease the likelihood for the performance of certain behaviours and decrease variation in behaviour between individuals. We were interested in increasing our knowledge about the natural baseline of risk assessment behaviours and therefore the idea of comparing behavioural strategies between wild mice and laboratory mice was realised.

Wild and laboratory mice

The question in focus was whether there are any evident differences in behaviour strategies between the wild mouse and the domesticated mouse. In other words, how much of their original behavioural strategies remain in the domesticated mouse, in particular behaviours that have an adaptive meaning. A characterization of the similarities and differences in this context seems relevant to enable further steps towards improved animal welfare and it could provide further information on what could be considered natural behavioural strategies. Our studies may also lead to a greater understanding of the domestication effects on the behaviour of laboratory mice used as research models.

In general, differences in behavioural strategies between wild house mice and laboratory strains of mice as measured in Papers II and III seem to be mainly quantitative. No behaviours specific for the Wild mice were registered. Wild mice differed from laboratory mice most clearly in the functional category Open-Shelter. The unwillingness of Wild mice to enter open areas was clear in both male and female Wild mice. Wild mice also showed a higher tendency to seek shelter than laboratory strains. This was evident mainly in males but to some degree also in females. Shelter seeking distinguished Wild males the most in the PCA analysis including both the male and the female mice. The tendency to seek shelter was evident also at times of capture after the end of the test period, when Wild mice of both sexes regularly fled to the DCR or start box. This is not surprising as wild mice, in the wild and in semi-natural enclosures, generally prefer sites with overhead cover and complex ground level structure to open areas (Plesner Jensen *et al.*, 2003). To take cover under structures or in burrows as an avoidance reaction has been found earlier in wild mice after minor human noise disturbance (Plesner Jensen *et al.*, 2003) and in response to predator exposure (Blanchard *et al.*, 1998).

The degree of hiding in laboratory mice also increased following exposure to a predator. In the RET, rat-exposed mice spent about twice as much time in the

home chamber as toy-exposed mice. This result is in agreement with the previous findings that predator exposure induce avoidance in mice (Belzung & Griebel, 2001) and that, when a shelter is provided, hiding is a defensive response (Roy et al., 2001).

Risk assessment and the stretched attend posture

When the possibility to do so is available, risk assessment, freezing, and defensive burying appear to occur from a place of concealment. In the RET, it was notable that rat-exposed mice exhibited 90 % of the total amount of SAP in the tunnel. This may be compared to the finding that SAP is most often seen when the animal is in the central square of the EPM (Rodgers, 1997). In Papers II and III, all mice had a higher percentage protected SAP compared to unprotected SAP. A common feature of the tunnel in the RET and the central square of the EPM is that, in these areas, animals are near or in a protected site but also close to the source of danger. Hence, risk assessment represents a balance of two opposing goals; investigating the threat stimulus and simultaneously remaining as protected as possible from it. This interpretation is very similar to that given for the stretch attend posture itself, that it enables the animal to investigate the threat while being minimally visible to it.

Wild mice were not included in the RET-study. However, the defensive behaviour of wild mice in response to a rat have been compared to Swiss mice in the Mouse Defence Test Battery (Blanchard et al., 1998). The MDTB is a comprehensive test battery within a single arena in which exploration of a novel environment, predator avoidance, flight, active defence and post predator exploration can be measured. A factor analysis of the MDTB has shown that stop orient and reverse during the chase/flight test (where the mouse is chased with a hand held anaesthetised rat) and approach withdraw in the straight alley (non-approaching rat) are considered as risk assessment behaviours (Griebel *et al.*, 1996a). Wild mice in the MDTB, in comparison to Swiss mice, avoided the rat at greater distances, froze more in the straight alley test, and attacked or attempted to escape by jumping when the rat was moved closer to the mouse (Blanchard et al., 1998). In the MDTB, BALB and Swiss mice showed high levels of risk assessment and C57BL low levels of risk assessment in the chase test (Griebel et al., 1996a). However, this does not suggest that the C57BL are less anxious, rather the contrary, as the C57BL mice have markedly higher flight speed than the other strains suggesting that they were already in a panic state. In the straight alley test, BALB, C57BL and Swiss mice all showed equal levels of approach activity to the non-approaching rat.

In comparison with the MDTB, the RET most closely resembles the straight alley test but may be slightly more threatening because it entails a live moving predator. In the RET, all laboratory strains showed oriented risk assessment behaviours towards the rat. This is a further indication that the natural defensive repertoire remains in the genome of laboratory mice. In the MDTB, the effects of domestication on defence were found to be most prominent in avoidance distance, freezing and jump attacks when wild mice were compared to the combined mean scores of six different laboratory strains (Blanchard et al., 2001a).

Grooming

Grooming is a behaviour that may have several functional purposes, including body care, displacement activity or a self-calming procedure (Spruijt *et al.*, 1992). Both male and female Wild mice had higher level of grooming than laboratory mice. Some authors suggest that grooming, at least in the EPM, is related to displacement or possibly approach-avoidance conflict (for a review see (Wall & Messier, 2001). Two ways of distinguishing between grooming as body care and as a conflict behaviour are the length of each grooming bout and the completeness of the behavioural sequence (Spruijt *et al.*, 1992). Conflict grooming mainly occur in short bouts and may therefore only include grooming of the head regions. Body care grooming is a low-priority behaviour, which only occurs when the animal is relaxed and then it is performed completely and at length. Male wild mice had a higher frequency, but also longer grooming bouts in the EPM, which may indicate body care rather than displacement activity or approach avoidance conflict.

Defensive burying

Defensive burying is a behaviour consisting of digging and pushing substrate so as to cover an aversive stimuli, which thereby could be avoided (De Boer & Koolhaas, 2003). In a natural environment, defensive burying might be a means of closing up a tunnel entrance or building a wall to protect against predators or intruders. In the laboratory, defensive burying is shown by both rats and mice in response to aversive stimuli such as an electric shock probe (Sluyter *et al.*, 1999), marbles (Njung'e & Handley, 1991) or live aversive stimuli (Londei *et al.*, 1998). For rat-exposed mice in Paper IV, defensive burying often involved mounding sufficient substrate in the tunnel that it apparently blocked the mouse's view of the rat chamber. The mouse would stretch over the substrate mound, take a look out at the chamber, and then rapidly retreat behind the mound, an action very similar to the "approach-withdraw" actions of mice in the MDTB (Blanchard *et al.*, 2003a). This further support a connection between risk assessment and shelter or concealment.

Both experiments in Paper IV indicated that BALB mice show an extremely low level of defensive burying in both studies. This raises the interesting question of how the genetic makeup of the BALB strain may compare to strains showing more normal levels of defensive burying. Digging behaviour in different strains has been investigated in the context of nest building (van Oortmeerssen, 1971). It was found that BALB mice showed very low frequencies of digging and push-dig compared to C57BL mice. Based on this and differences in nest building strategies between these strains he suggested that these strains might be more adapted to surface living (BALB) and burrow living (C57BL). Later findings support this suggestion. It has been shown that C57BL mice construct more elaborate burrows than BALB mice in a semi natural environment (Adams & Boice, 1981; Dudek *et al.*, 1983). BALB mice also generally build more spherical or dome shaped nests whereas C57BL mice build more bowl shaped nests (Broida & Svare, 1982; van de Weerd *et al.*, 1997b). Taken together, this indicates that there may be a link

between the burrowing behaviour and the burying behaviour of a strain, which is related to their defensive repertoire.

Freezing

Freezing was measured specifically only in the RET. In the RET, freezing occurred mainly in the home chamber or in the tunnel when mice were exposed to the rat. Mice always froze facing the chamber exit, in the direction of the rat stimulus. This is similar to observations made on rats in a Visible Burrow System (Blanchard & Blanchard, 1989) and reinforces a view that animals may be vigilant while maintaining immobility. The time in immobility was measured automatically in the LD-test. It is therefore impossible to deduce whether this was freezing reactions or simply lack of movement due to other factors. BALB mice had higher values than C57BL, but no differentiation between spatial location (light or dark compartment) was made. In the RET, BALB had the lowest values of the four strains and C57BL the highest. In the CSF, OF and EPM, freezing was not recorded and rarely seen.

Sex related differences

In general, female mice are used more frequently than male mice in biomedical research (LASA, 1998). The reason for this is not that female subjects are more interesting as a research subject, but that problems due to aggression in home cages are less in females than in males (van Loo, 2001). In psychobiological research, however, the majority of research is performed using male subjects. Using only males may result in an incomplete understanding of neurobehavioral systems and thus be detrimental for the interpretation and implementation of findings into pharmacological treatments (Blanchard *et al.*, 1995a). Hence, the issue deserves more attention. The characterization of differences in behavioural strategies of exploration and risk assessment between male and female wild and laboratory mice in Paper II and III is a step in that direction.

Although males and females were subjected to an identical experimental protocol we refrained from a direct statistical evaluation of sex differences, which would have required that the experiment had originally been set up for such comparisons. Nevertheless, some interesting similarities and differences are revealed when comparing the results of the two studies. Similarities may be true or a result of the methods used. As mentioned above, quantitative but no obvious qualitative differences were detected that could be attributed to sex in the Wild and the two laboratory strains investigated. The characteristic strain differences seen in male mice also occurred in females, although they were less pronounced. It is reaching too far to discuss them from an evolutionary point of view, but it is tempting to assume that risk assessment and emotional reactivity might have different bases in sex-specific behavioural strategies of survival and reproduction. Evidently more research has to be done on this and with respect to animal welfare.

The PCA analysis for both males and females revealed overlapping profiles in the lower left quadrant for most female Wild and male C57BL mice as well as several female C57BL mice. The greatest differentiation according to sex was

found in Wild mice and male Wild mice also showed the least overlap with other groups. BALB mice were found in a strain related cluster with little differentiation according to sex.

Previous studies of male and female wild mice in traditional anxiety tests are rare, but there are a few interesting findings reported. The major differences found between wild derived *M. m. domesticus* males and females in the elevated plus maze by (Holmes *et al.*, 2000) were a preference for the open arms in male wild mice whereas females spent an equal amount of time in the closed and open arms. In this study, Wild females showed a clear preference for the closed arms. In that study (Holmes *et al.*, 2000), both freezing and jump attempts were repeatedly recorded in wild mice. Neither jump attempts nor any clear cases of freezing were observed in our studies. In the free exploratory paradigm, no sex differences in the time spent in the arena or in risk assessment between wild derived *M. m. domesticus* males and females was found (Parmigiani *et al.*, 1999). The test most similar to the free exploratory test in our study was the open field, which could be voluntarily entered from the start box. Contrary to our findings, the wild mice in (Parmigiani *et al.*, 1999) spent more time in the arena than C57BL mice but similar amount of time to Swiss and DBA/2 mice. They also recorded more risk assessment in C57BL and DBA/2 compared to wild and Swiss mice.

Methodological comparisons

State versus trait anxiety

The grade of familiarity has been suggested to be a key feature differentiating between “trait” anxiety models and “state” anxiety models (Belzung & Berton, 1997). Models using forced confrontation with unfamiliar environments (LD, CSF, EPM) measure state anxiety whereas models where the animal may choose from a familiar and a novel environment constitutes models of “trait” anxiety (free exploration)(Griebel *et al.*, 1993; Belzung & Berton, 1997). In a review of studies of anxiety like behaviour in mice, Belzung & Griebel (2001) concludes that BALB/c mice is the only mouse strain that consistently have shown higher levels of anxiety compared to other strains. Based on this finding they proposed that BALB/c mice might be considered a genetic model of “trait anxiety”. The result of the RET study contradicts this suggestion.

The main difference between Papers I, II, III on one side and Paper IV is of course the presence of the predator. However, another feature of the RET differentiating it from the other tests is that it is performed in an arena the mouse have previous experience of. The mice were not only familiarised with the RET arena, it also contained soiled bedding material from the home cage of the mouse. This could be compared to the free exploration paradigm where the mice are confronted by the choice between a pre-familiarised area and entering a novel area. A test that also has some resemblance to the free exploration paradigm is the modified OF used in Papers II and III. However, unlike in the free exploration paradigm (Belzung & Berton, 1997), and in the OF (Paper II and III), BALB mice in Paper IV showed low levels of risk assessment behaviours. This indicates that

although BALB show neophobic and anxiety-like behaviour in many situations, this strain may not always be a good model of trait anxiety.

Exposure to potential versus real threats

As mentioned, the results of Papers I, II and III were not compatible with the pattern of higher avoidance and risk assessment for C57BL mice compared to BALB mice in response to the rat in Paper IV. BALB mice in the RET study showed low levels of risk assessment behaviours. Nor was it in agreement with the general notion from situations involving novel arenas that C57BL mice have lower levels of anxiety compared to BALB mice (Griebel et al., 1993; Beuzen & Belzung, 1995; Lepicard et al., 2000; Belzung & Griebel, 2001). However, the RET data is in agreement with previous findings that C57BL mice started to avoid an approaching rat stimulus at a significantly longer distance compared to other strains, including BALB and Swiss (Griebel et al., 1997). C57BL mice also displayed much higher flight speeds than the BALB mice. Moreover, there are reports that the plasma corticosterone response to predator exposure is less pronounced in BALB than in C57BL mice (Hayley et al., 2001).

The systematic discrepancy between the results of paper IV and earlier findings suggest a difference between the responsiveness to novelty or novel places compared to the antipredator response, at least in these two inbred strains. Moreover, it supports the notion that optimisation of the trade-off between risk taking and potential gain varies in different types of situations. A novel environment elicits exploratory motivation in the mouse and entails a conflict between potential unidentified dangers and the possibility of locating important resources. The aversive elements of an encounter with a predator seem to be perceived differently to an encounter with a novel environment containing no or only potential signs of threat, thereby producing qualitatively different emotional states.

Although aggression and social interactions have not been the focus of this thesis there are too many potentially interesting connections with our findings to leave this aspect out entirely. It has been suggested that there may be a relationship between levels of aggression and behavioural responses to potentially dangerous situations (Parmigiani et al., 1999). For instance, (Guillot & Chapouthier, 1996) found that mice of more aggressive strains had a higher level of anxiety like behaviours as measured by the LD test. In that study, C57BL mice had the lowest level of attacking males and the highest number of transitions in the LD test. BALB mice were considered intermediately aggressive compared to the other strains and showed the lowest number of transitions. The most aversive potential threat in a new environment may not be a predator but a conspecific territory holder (Hendrie et al., 1996). If so, the intensity of the threat in a novel situation might be defined by the level of resident aggression.

Impact of home cage environment

The term 'environmental enrichment' is used both in neuroscience and laboratory animal science but with some potentially important difference in meaning. In

neuroscience, the enrichment protocol is mainly based on novelty induced stimulation and the objects used as ‘enrichment’ items are changed regularly to measure the effects on neuronal plasticity (for a review see (van Praag *et al.*, 2000)). This is a different approach to what is commonly promoted for enhancing the welfare of laboratory animals. In the latter case a standardised set up of items, validated for having a lasting positive effect on parameters related to laboratory animal welfare, is used. It could be argued that what today is considered standard instead should be termed “impoverished”. The term impoverished (IC) is used in neuroscience studies of enrichment to describe the conditions of individually housed subjects in conventional cages (Mohammed *et al.*, 2002). The term Standard conditions (SC) is used for socially housed subjects in conventional cages. Enriched conditions (EC) are usually larger than usual cages with many different kinds of enrichment item (such as running wheels, tubes, nesting material etc) and contain a larger number of animals. Some of these items are changed daily. The question of what should be the baseline cage environment is yet to be answered.

We investigated whether environmental enrichment induced an effect on experimental results and on inter individual variation in the behaviour of two strains of mice (BALB/c and C57BL/6) in the Light/Dark paradigm. Our data did not demonstrate a reduction in emotional reactivity between housing conditions. No effect on inter-individual variance, due to housing condition, was detected. For example, in a study on the effect of rearing environment on later reactivity (Chapillon *et al.*, 1999) showed that BALB/c mice reared under enriched conditions were less fearful in anxiety tests than mice of the same strain from standard conditions. This was the case for both ‘state anxiety’ using the elevated plus maze and ‘trait anxiety’ using the free exploratory paradigm. For C57BL/6 mice the greatest effect was found on ‘state anxiety’.

There are several potential reasons to why no such differences emerged in our study. Even though we could not show effects on emotional reactivity in the LD test for the mice housed in enriched environments, others have and it is possible that we, using a more sensitive test, also could have detected differences. Another possibility is that the enriched environment might have both positive and negative properties, which will eliminate each other with respect to a measurable expression in behaviour. The housing related response could also have been masked by other factors such as handling or other environmental factors (Cheslera *et al.*, 2002).

In this study, enrichment was kept constant apart from the unavoidable changes due to the animal’s own manipulation of the objects. The neuroscience approach has resulted in increased neurogenesis, dendritic branching and synaptic density with consequent effects on learning, memory, emotional reactivity and habituation to novel environments (Mohammed *et al.*, 2002). However, the effects on the brain of using the welfare approach is less investigated (Würbel, 2001). It has previously been argued that animals, which are evolutionary adapted to a natural variation in environmental parameters, may have difficulties in adjusting to the regularity of standardised cage conditions (Meyerson, 1986). It is still an open question whether enrichment can be kept constant and standardised or whether

novelty is a crucial part of the “enriching” value. It is likely, however, that enrichment that provides the animals with possibilities to act and react in a species specific manner have a lasting enrichment value.

It could also be argued that the period of enrichment was during a less sensitive period in the life of a mouse or that the period was too short to produce any effect. In this study, all mice were reared under non-enriched conditions and assigned as adults to one of the three constant housing conditions five weeks before the experiment. However, other studies have found effects on exploratory behaviour of mice when constant enrichment was introduced at a later age (van de Weerd *et al.*, 1994; Dahlborn *et al.*, 1996). It has also been argued that a mismatch between post natal and adult environment may result in pups that are adapted to one environment but are less prepared for the types of threatening stimuli encountered in the adult environment (Würbel, 2001).

Synthesis, implications for animal welfare and future prospects

The research presented in this thesis form a possible conceptual basis for further research on animal welfare related issues. The results point at some potentially important aspects that should be further investigated.

Multivariate methodology

One factor leading to the lack of differences between non-enriched and enriched conditions in Paper I may have been that the method used, the LD-test, was not ideal for measuring emotional reactivity. Among the drawbacks of this model is that behavioural postures can only be measured in the light compartment. For rats, a novel method (CSF) had been designed to assess elements of exploration and risk assessment. The model had not previously been used in mice but it seemed suitable for the purpose of our study. To achieve an estimate of the general level of activity and explorative motivation in the mice, we supplemented this method with two other more validated methods with a similar set-up in Papers II and III. One was the EPM, a behavioural model where many studies had been performed regarding risk assessment and the inclusion of ethological parameters. The other model used was a combination of the traditional OF and the free exploration paradigm. This more ethoexperimental and multi-test approach lead to a more detailed and diversified behavioural characterisation of the mice than what would have been possible using only one test. In Paper IV, when the aversive stimuli was a rat and not a novel environment, this meant a further insight into emotional reactivity and risk assessment.

It has been repeatedly argued that it is questionable to attribute a specific behaviour to a specific emotion isolated from context (Lister, 1990; Boissy, 1995; Ramos & Mormede, 1998; Calatayud & Belzung, 2001). Hence, the importance of starting with a descriptive study of behaviour, and then in a context interpret behavioural parameters into functional categories cannot be overemphasised. Only after that, it should be attempted to infer something about emotional state. A recent study investigated the generality and consistency in coping strategies to a variety of stressors in Long Evans Hooded rats (Campbell *et al.*, 2003). They

found that an animal's exploratory motivation is general to a variety of novel environments or objects but not necessarily predictive of behavioural strategy when exposed to an actual threat i.e. when defence or escape is a more relevant response than exploration. This is in agreement with our findings that mice react differently to different type of situations and that it may be unwise to refer to a strain as high reactive or low reactive. It also further evidence of the importance of studying animals in environments that allows for several different behavioural choices and using a variety of aversive stimuli as results of only a single test may be misleading.

Strain and sex specific behavioural strategies

It is clear from our studies that there are strain specific strategies in exploratory behaviour, risk assessment and emotional reactivity. Our behavioural characterisations also indicate that there may be sex specific strategies. This may have implications for animal welfare. There are studies describing effects of strain on reactions to different stressors (Anisman *et al.*, 2001), and differences in how different strains perceive and use a specific enrichment item (van de Weerd *et al.*, 1994; Nevison *et al.*, 1999). Other studies indicate that individually housed male mice tend to show higher propensity for exploration and a lower level of anxiety compared with group-housed males. Individually housed female mice had higher risk assessment and less risk taking than group housed mice (Palanza *et al.*, 2001). It is evident that the influences and interactions of strain, sex and housing on emotional reactivity and risk assessment is a complex issue that merits further investigation and that across strain and across sex generalisations should be avoided.

How should differences in risk assessment be interpreted in terms of animal welfare?

The present thesis indicate that risk assessment and emotional reactivity may be useful markers of animal welfare and well-being. However, to infer how risk assessment behaviour would be modified in animals experiencing high or low welfare requires further studies. In a study by (Quartermain *et al.*, 1996), risk assessment after acute stressors was assessed in a set-up similar to the OF used in Papers II and III. Stressed mice had a shorter entry latency and reduced locomotory activity in the OF. Stressed mice also often moved directly into the centre of the arena. It was concluded that acute stress disrupts normal risk assessment behaviour. Although at first counterintuitive, it is interesting that both animals housed in enriched environments and acutely stressed animals may react with shorter entry latencies. However, the motivational and emotional state when exploring an environment that is perceived as safe and leaving an environment that is considered unsafe is likely to differ. The reactions of the acutely stressed animals may be compared to behaviours related to panic which also may be interpreted as inappropriate and erratic. Hence, even though both enrichment and acute stress may be expressed as reduced entry latency, other factors in their behaviour profile are likely to differ. Moreover, chronically stressed animals may react with apathy and reduced reactivity and might therefore be less reactive in a behavioural test than control animals. It is also likely that different types of acute

and chronic stressors (social, psychological, physiological) may result in different behavioural profiles. This further indicate that a multivariate methodology is necessary to dissociate between the many underlying factors that may influence the behavioural expression of the animal.

Conclusions

This thesis has dealt with behavioural studies of risk assessment in mice as a means of assessing emotional reactivity. The long-term objective of this work was to find relevant measures in relation to animal welfare.

Differences in behavioural strategies between wild and laboratory mice seem to be mainly quantitative but strain specific behavioural profiles in relation to risk assessment and emotional reactivity was recorded. Wild mice differed from both laboratory strains mainly in unwillingness to enter open areas.

Strain of the mouse was an important factor in how the situation is perceived. In novel environments, BALB/c mice generally avoided risk areas and showed a high risk assessment while C57BL/6 mice were more explorative, demonstrating a higher rate of risk taking and little risk assessment. The results of the Rat Exposure Test were inconsistent to the findings in Papers I, II and III. C57BL/6 mice showed more risk assessment, avoidance behaviour and active defence than BALB/c mice in response to the rat. Strain was also a greater factor than home cage environment (enriched/non-enriched) on risk assessment, emotional reactivity and inter individual variance in the Light/Dark test.

No major sex differences were found in behaviours related to risk assessment and emotional reactivity. However, sex differences were more pronounced in Wild and C57BL/6 mice than in BALB/c mice.

Risk assessment is a sensitive marker of emotional reactivity in laboratory mice. However, environmental features of the test arena, familiarity of the environment and type of aversive stimuli may have great impact on emotional reactivity. A multivariate approach is therefore necessary for a thorough characterisation in terms of animal welfare.

Populärvetenskaplig sammanfattning

Musen är det mest använda försöksdjuret inom biomedicinsk forskning idag. Ett stort antal inavlade och utavlade stammar finns kommersiellt tillgängliga och framställningen och användningen av genetiskt modifierade stammar ökar kraftigt. Att säkerställa att djur som används inom forskningen mår väl och är friska är viktigt både ur etisk synvinkel och för att forskningsresultaten ska bli så rättvisande som möjligt. Kunskaper om musens beteende är viktiga för att kunna främja dess välfärd och välbefinnande och uppfylla det lagstadgade kravet i djurskyddslagen att tillgodose en burmiljö som ”ger dem möjlighet att bete sig naturligt”.

Beteendestudier på möss utförs främst inom neurovetenskap, psykofarmakologi och toxikologi där mössens beteende i speciellt utvecklade test studeras. Beteendestudier används även inom tillämpad etologi som en parameter för att bedöma djurs välfärd och välbefinnande. Trots att det finns ett otal studier av musens beteende i olika situationer saknas det fortfarande kunskap inom vissa områden. Ett område som är centralt för att kunna tolka laboratoriemusens beteende på ett korrekt sätt är kunskap om den vilda husmusens beteende samt vilka likheter och skillnader som finns mellan dess beteende och den domesticerade laboratoriemusen. Möjligheten att reagera på ett arteget sätt i hotande situationer har stor betydelse för djurets upplevelse av kontroll över sin situation och följaktligen dess välbefinnande.

Eftersom djurets subjektiva upplevelse av sin situation är omöjlig att mäta direkt måste man fokusera på dess uttryck, det vill säga djurets beteende. Det man mäter är kvantitativa (frekvens, duration, latens) och kvalitativa skillnader (olika beteendestrategier) i djurens beteende. Att lära sig att bättre bedöma dessa tillstånd genom att studera utforskande beteenden och musens riskbedömning i olika experimentella situationer har varit målsättningen för denna avhandling.

Fokus inom denna avhandling har varit att studera

- o likheter och skillnader i beteende hos den vilda husmusen (*Mus musculus musculus*) och den domesticerade laboratoriemusen (här representerade av två inavlade stammar BALB/c och C57BL/6)
- o stam och könsskillnader i beteenden relaterade till riskbedömning och emotionell reaktivitet
- o olika metodologiska testsituationer
- o effekten av burmiljöns utformning på experimentell variation och emotionell reaktivitet

Avhandlingens engelska titel speglar innehållet på följande sätt: metodiken som använts i alla studier är en kombination av traditionella test för att mäta emotionalitet, oro/ångest och liknande känslotillstånd hos djur (i de fall de används som modeller för mänskliga emotioner). ”Etho” i ”Ethoexperimental” syftar på att till dessa traditionella metoder har också använts mer grundläggande etologisk metodik och förklaringsmodeller såsom en mer utförlig beskrivning och mätning av djurens beteendemönster. Det innebär också att man använder sig av

mer naturliga inslag i själva testsituationen (exempelvis att använda rovdjursstimuli istället för ett högt ljud för att framkalla en emotionell reaktion hos djuret) och att man använder sig av kunskaper om djurets naturliga beteendemönster för att tolka sina resultat. Undertiteln är vald för att spegla den problematik som avhandlingen syftar att belysa nämligen hur djurets riskbedömning och emotionella reaktivitet kan vara kopplade till dess välbefinnande.

Skillnader mellan vilda husmöss och laboratoriemöss

När en mus utsätts för en hotfull situation uppvisar den en rad olika beteenden som syftar till att inhämta mer information om de riskfaktorer som finns i omgivningen. Exempel på riskbedömningsbeteenden är ”*stretched attend posture*” (se fig 1 i kappan) då musen sträcker ut sig med kroppen längs med marken för att kunna se, lukta/sniffa och höra vad som sker längre fram utan att bli upptäckt. En sådan riskbedömning sker kontinuerligt så att det utforskerande (undersökande) beteendet skall kunna genomföras under så säkra förhållanden som möjligt. Hot utlöser också strategiförändringar i förhållande till andra beteenden. Man kan exempelvis finna en minskning i förekomsten av utforskerande beteenden och en förlängd tid innan de besöker potentiellt farliga områden. Hur väl individen kan anpassa sig till en miljö är beroende av både individens emotionella tillstånd, dess förmåga att bedöma risker och den omgivning som begränsar individens handlingsmöjligheter. Vår teori är att en hög anpassningsförmåga är nödvändig för att kunna hantera en omväxlande laboratoriemiljö på ett för musen bra sätt och att egenskapen därmed är viktig ur välfärdssynpunkt. Frågeställningen berör hur denna förmåga skiljer sig mellan den vilda husmusen och den domesticerade laboratoriemusen.

Två studier, baserade på samma teori och metodologi, utfördes som två separata försök: Arbete II med hanar och Arbete III med honor som försöksobjekt. Ett batteri av tre tester: Concentric Square Field (CSF), Open Field, Elevated Plus Maze användes. I alla de tre olika testerna finns möjlighet att mäta vissa gemensamma faktorer såsom allmän aktivitet, exploration, riskbedömning och tendens att besöka öppna ytor respektive mer ”trygga” boliknande strukturer. Resultaten indelades därför under dessa fyra funktionella rubriker. Ett av testen, CSF, innefattar möjligheter till fler beteendeanternativ än de övriga testen. Därför genomförde vi också för detta test en multivariat analys (PCA-SIMCA) som beskriver både individernas inbördes relationer och vilka beteendeparametrar som skiljer sig mellan de olika grupperingarna.

Genom våra tester fick vi fram en utforskerande profil för de olika stammarna där vi kvantitativt och kvalitativt kunde beskriva skillnader i beteendestrategier mellan de vilda mössen och de båda laboratoriemusstammarna. Vildmushannarna skiljde sig från båda laboratoriemusstammarna i flera olika beteendeparametrar, främst i kvantitativa termer. De hade bland annat en lägre benägenhet att utforska öppna ytor och en lägre lokomotorisk aktivitet än laboratoriemusen i en ny miljö. Detta kan jämföras med BALB/c-mössen som hade en hög frekvens av riskbedömningsbeteenden och ett lågt risktagande. C57BL/6-mössen hade relativt få riskbedömningsbeteenden och ett högt risktagande. Vildmushonorna och honorna från de båda laboratoriemusstammarna skiljde sig inte lika tydligt ifrån

varandra som hanarna gjorde. Däremot fann vi flera likartade mönster i beteendet mellan honor och hanar som var beroende av deras stamtillhörighet. Detta gällde till exempel tendens att besöka öppna ytor och riskbedömningsprofil. Könsskillnaderna var störst hos vildmössen och minst uttalade hos BALB/c.

Effekter av miljöberikning

Miljöberikning är den svenska benämningen på den engelska termen "Environmental Enrichment" vilket syftar till att ge burmiljön en komplexare och mer artanpassad utformning. Detta rekommenderas generellt för att öka djurs välbefinnande. Möss som hålls i berikade miljöer har i tidigare studier visat sig vara mindre lättskrämde och mer benägna att undersöka sin omgivning. Det är därför sannolikt att berikning leder till att djuren inte bara mår bättre i sin hemmiljö utan också har lättare att anpassa sig till skötselrutiner och provtagningar. Som motpol till att berika miljön för laboratoriemöss står strävan att standardisera burmiljön för att minska omgivningsvariablernas påverkan på djuret som modell. Det finns en oro i forskarvärlden för att berikning skulle kunna öka variationen mellan djuren och att ett större antal djur därmed skulle behöva användas. Detta skulle leda till en etisk konflikt där det individuella djurets välbefinnande då måste ställas mot önskemålet att minska det totala antalet djur som används i forskningen.

I Arbetet I, användes också BALB/c och C57BL/6 som varit inhysta i olika hemmiljöer (oberikad bur, berikad med enbart bomaterial samt berikad med bomaterial, bolåda, klättringsnät). Vi undersökte effekten av dessa olika hemmiljöer på mössens beteende i ett traditionellt test för utforskningsbeteende och emotionell reaktivitet (Light/Dark test). Detta test består av en tvådelad box där den ena sidan är i mörker och den andra starkt belyst. Vi studerade också effekter på kroppsvikt. Resultaten visade inga signifikanta effekter av inhysningsmiljö på mössens beteende eller på variationen mellan mössen. Berikade möss hade dock en högre kroppsvikt. BALB-mössen uppvisade en högre frekvens av riskbedömningsbeteenden, lägre aktivitet och tydligare undvikande av den ljusa delen av boxen vilket indikerar att de uppfattade situationen som mer hotfull än C57BL-mössen. Trots att det rörde sig om inavlade stammar var alltså stamtillhörighet en större variationsfaktor än burmiljön. Resultaten för de båda stammarna med avseende på riskbedömning och emotionell reaktivitet var i linje med Arbetet II och III.

Olika typer av hot - olika stressande för olika stammar?

Möss reagerar olika beroende på om det finns ett potentiellt eller reellt hot i omgivningen. Ett evolutionärt reellt hot för en mus är närvaro av ett rovdjur exempelvis i form av en råtta. En nyutvecklad metod för att mäta riskbedömning och försvarsstrategier hos möss utvärderades i Arbetet IV. Testet kallas "The Rat Exposure Test" eftersom metodiken går ut på att utsätta musen för en råtta, som är en av dess naturliga predatorer. Testarenan består av en konventionell musbur som är avdelad i två avdelningar med ett galler. På den ena sidan finns en tunnel som leder till en mindre kammare i vilken musen kan gömma sig. Först validerades testet med antingen en riktig råtta eller en leksaksråtta placerad i rättavdelningen

och sedan utfördes en stamjämförelse (med två inavlade stammar: BALB/c, C57BL/6 och två utavlade: CD-1, SWISS) där mössen enbart exponeras för råttan.

Metoden framkallade tydliga riskbedömningsbeteenden hos alla stammar och mössen undvek att uppehålla sig nära råttan. Vad som däremot framkom var att riskbedömningsprofilen, hos C57BL/6- och BALB/c-mössen, i det här testet skiljde sig diametralt från deras riskbedömningsprofil i våra tidigare arbeten. Här var C57BL/6 den stam som visade mest undvikande och riskbedömningsaktivitet och BALB/c den stam som var mest risktagande och utforskerande. Den största metodologiska skillnaden mellan studierna var att i de första studierna var hotet bara potentiellt medan det här rörde sig om ett reellt predatorhot. I det senare testet hade också mössen fått undersöka miljön innan råttan introducerades. De metodologiska skillnaderna hade en avgörande betydelse för vilka slutsatser som kunde dras från de olika studierna rörande de olika stammarnas emotionella reaktivitet. Detta visar på nödvändigheten av att använda flera olika typer av beteendetester vid karaktäriseringen av en viss stam för att undvika förhastade och eventuellt felaktiga slutsatser. Det visar också att beteenden som relaterar till riskbedömning och emotionell reaktivitet är känsliga parametrar väl värda att studera i detta sammanhang.

Slutsatser med avseende på djurens välbefinnande

Riskbedömning är en känslig markör för emotionell reaktivitet som också kan vara intressant som en markör för välfärd och välbefinnande hos laboratoriedjur. Stam, kön och metodologiska faktorer har stor inverkan på hur en viss situation uppfattas av djuret. En multivariat analys som inkluderar flera olika testsituationer och olika typer av aversiva stimuli är nödvändig för att kunna karaktärisera en stams emotionella reaktivitet.

Det fanns både likheter och skillnader i beteende mellan vildmössen och laboratoriemössen. Likheterna tyder på att mycket av det naturliga reaktionsmönstret finns kvar även hos domesticerade laboratoriemöss som avlats i fångenskap under lång tid. Med avseende på de beteenden som undersökts i denna avhandling finns inte mycket som talar för att de laboratoriemusstammar som undersökts skulle avvika på ett sätt som gör att de har lättare, jämfört med vild husmus, att anpassa sig till den inhysningsmiljö som idag erbjuds.

Vi kunde inte påvisa några skillnader med avseende på interindividuell variation mellan de möss som hölls i oberikade respektive berikade burar. Detta indikerar att en väl validerad berikning inte nödvändigtvis behöver öka variationen mellan djuren. Det finns inte heller något som utesluter att en berikningsåtgärd som visat sig innebära en positiv förändring i djurets välfärd kan standardiseras och i framtiden ingå i vad vi kallar konventionell djurhållning.

Att de två musstammarna som studerades i alla fyra studierna hade helt olika emotionella reaktionsmönster i nya miljöer jämfört med inför predatorhot indikerar att det kan vara vanskligt att generellt karaktärisera en viss stam som emotionellt hög-reaktiv respektive låg-reaktiv (vilket ofta görs). Dessa skillnader antyder också att det är möjligt att olika stammar kan ha olika upplevelse av laboratoriemiljön.

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