Exploring Brain Gene Expression in Animal Models of Behaviour

JULIA LINDBERG
Dissertation presented at Uppsala University to be publicly examined in Zootissalen, Zoologiska institutionen, EBC, Norbyvägen 16, Uppsala, Thursday, September 27, 2007 at 13:00 for the degree of Doctor of Philosophy. The examination will be conducted in English.

Abstract


The genetic basis for behavioural traits is largely unknown. The overall aim of this thesis was to find genes with importance for behavioural traits related to fear and anxiety. Microarray analysis was used to screen expression profiles of brain regions important for emotional behaviour in dogs, wolves, foxes and mice. In a first experiment, dogs and their wild ancestors the wolves were compared. Our results suggested that directed selection for behaviour might have resulted in expression changes in few genes acting on several brain functions, possibly affecting behaviour. However, the observed expression differences were confounded with environmental effects. This was addressed in a second study on domesticated silver foxes. By correlating behaviour and brain gene expression in foxes selected for tameness to non-selected foxes raised in the same environment, we found large behavioural differences but only few genes with differential expression in the brain. Fifteen of the 40 genes showing evidence of expression difference were related to haem or haemoglobins. Further studies showed an additive genetic effect on brain gene expression, similar to the additive genetic inheritance of behaviour, indicating an involvement in domestication. Transcriptional profiling was also used for finding genes involved with the sleep disorder narcolepsy. Narcoleptic Doberman pinschers homozygous for the canarc-1 mutation were compared to their unaffected heterozygots revealing reduced expression of three genes, TAC1, PENK and SOCS2, with relevance to the narcoleptic phenotype. Finally gene expression was investigated in relation to anxiety-related traits in a mouse model. Surprisingly, as in the fox study, genes coding for haemoglobins indicated differential expression in the brain between animals with different anxiety levels. Our combined results suggest that genes like haemoglobins, best known for their function in oxygen transport in blood, may also participate in brain functions related to decreased anxiety in domestic animals.

Keywords: behavioural genetics, gene expression, brain, microarray analysis, domestication, animal model, haem, Canis familiaris, Vulpes vulpes, Mus musculus

Julia Lindberg, Department of Evolution, Genomics and Systematics, Norbyv. 18C, Uppsala University, SE-75236 Uppsala, Sweden

© Julia Lindberg 2007

ISSN 1651-6214
ISBN 978-91-554-6948-1
urn:nbn:se:uu:diva-8177 (http://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-8177)
Publications

This thesis is based on the following papers, which will be referred to in the text by their roman numerals:


V Saetre P*, Lindberg J*, Wirén A, Holm K, Bakken M and Jazin E. Genetic alterations in haemoglobin levels and behaviour in mice selected for high litter size. *Manuscript*

* Equal contribution

Reprints were made with permission from the publishers
## Contents

- **Introduction** ..................................................................................................... 9
- **Rodent and canine models for behavioural research** .................................. 9
- **Behaviour during domestication** ............................................................ 12
- **Genetic basis for evolution of behaviour** ............................................. 13
- **Changes in behaviour due to disease** .................................................... 14
- **Brain and behaviour** ................................................................................. 14
  - **The hypothalamus** ............................................................................. 16
  - **The amygdala** ................................................................................ 17
  - **The frontal lobe** ............................................................................... 17
- **Studying behavioural genetics** ................................................................. 17
- **Measuring gene expression** ..................................................................... 18

### Research aims

- **Specific aims for included papers** .......................................................... 20

### Present investigations

- **Paper I. From wild wolf to domestic dog: gene expression changes in the brain** ................................................................................................................ 21
  - **Methods** .......................................................................................... 21
  - **Results** ........................................................................................ 22
  - **Discussion** .................................................................................. 22
- **Paper II. Selection for tameness has changed brain gene expression in silver foxes** ........................................................................................................... 23
  - **Methods** .......................................................................................... 23
  - **Results** ........................................................................................ 24
  - **Discussion** .................................................................................. 24
- **Paper III. Selection for tameness modulates the expression of heme related genes in Canidae brains** ................................................................. 24
  - **Methods** .......................................................................................... 25
  - **Results** ........................................................................................ 25
  - **Discussion** .................................................................................. 25
- **Paper IV. Expression of TAC1, PENK and SOCS are down regulated in narcoleptic dog brain** ................................................................. 26
  - **Methods** .......................................................................................... 26
  - **Results** ........................................................................................ 26
  - **Discussion** .................................................................................. 27
Paper V. Genetic alterations in haemoglobin levels and behaviour in mice selected for high litter size ........................................................................................................... 27
  Methods ........................................................................................................ 27
  Results ......................................................................................................... 28
  Discussion ................................................................................................. 28
  General discussion ..................................................................................... 29
  Future perspectives ................................................................................... 30
  Concluding remarks .................................................................................. 31

Summary in Swedish ......................................................................................... 32
  Artikel I. Från vild varg till domesticerad hund: ändrat genuttryck i hjärnan .............................................................. 32
  Artikel II. Selektion för tamhet ger ändrat genuttryck i hjärnan hos silverräv .......................................................... 33
  Artikel III. Selektion för tamhet ändrar uttrycket av hemgener i hjärnan hos silverräv .................................................. 34
  Artikel IV. Nedreglerat uttryck av TAC1, PENK och SOCS i hjärnan hos narkoleptiska hundar ........................................ 34
  Artikel V. Genetiska förändringar av haemoglobinnivåer och beteende i möss selekterade för stor kullstorlek .................. 35
  Slutsatser .................................................................................................... 36

Acknowledgements ........................................................................................ 37

References ..................................................................................................... 39
<table>
<thead>
<tr>
<th>Abbreviations</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACTB</td>
<td>Actin beta</td>
</tr>
<tr>
<td>ACTH</td>
<td>Adrenocorticotropic hormone</td>
</tr>
<tr>
<td>ANCOVA</td>
<td>Analysis of co-variance</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>AQP4</td>
<td>Aquaporin 4</td>
</tr>
<tr>
<td>CALCB</td>
<td>Calcitonin-related polypeptide, beta</td>
</tr>
<tr>
<td>cDNA</td>
<td>Complementary DNA</td>
</tr>
<tr>
<td>CO</td>
<td>Carbon monoxide</td>
</tr>
<tr>
<td>CRH</td>
<td>Corticotropin-releasing hormone</td>
</tr>
<tr>
<td>CRYM</td>
<td>Crystallin mu</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>EPM</td>
<td>Elevated plus maze</td>
</tr>
<tr>
<td>eQTL</td>
<td>Expression QTL</td>
</tr>
<tr>
<td>F1</td>
<td>First generation offspring</td>
</tr>
<tr>
<td>F2</td>
<td>Second generation offspring</td>
</tr>
<tr>
<td>GAPDH</td>
<td>glyceraldehyd-3-phosphate dehydrogenase</td>
</tr>
<tr>
<td>HBA</td>
<td>Haemoglobin alpha</td>
</tr>
<tr>
<td>Hbb-b1</td>
<td>Haemoglobin beta b1</td>
</tr>
<tr>
<td>Hbb-b2</td>
<td>Haemoglobin beta b2</td>
</tr>
<tr>
<td>HBE</td>
<td>Haemoglobin epsilon</td>
</tr>
<tr>
<td>HBG1</td>
<td>Haemoglobin gamma A</td>
</tr>
<tr>
<td>HCR</td>
<td>Hypocretin</td>
</tr>
<tr>
<td>Hcrtr-2</td>
<td>Hypocretin receptor 2</td>
</tr>
<tr>
<td>HEBP1</td>
<td>Haem binding protein 1</td>
</tr>
<tr>
<td>HPA-axis</td>
<td>Hypothalamus-Pituitary-Adrenal axis</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic resonance imaging</td>
</tr>
<tr>
<td>mRNA</td>
<td>Messenger ribonucleic acid</td>
</tr>
<tr>
<td>mtDNA</td>
<td>Mitochondrial DNA</td>
</tr>
<tr>
<td>NO</td>
<td>Nitric oxide</td>
</tr>
<tr>
<td>NPY</td>
<td>Neuropeptide Y</td>
</tr>
<tr>
<td>OCD</td>
<td>Obsessive-compulsive disorder</td>
</tr>
<tr>
<td>PCA</td>
<td>Principal component analysis</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
</tr>
<tr>
<td>PENK</td>
<td>Proenkephalin</td>
</tr>
<tr>
<td>PTSD</td>
<td>Post traumatic stress disorder</td>
</tr>
<tr>
<td>Term</td>
<td>Definition</td>
</tr>
<tr>
<td>---------</td>
<td>------------------------------------------------</td>
</tr>
<tr>
<td>qPCR</td>
<td>Quantitative real-time RT-PCR</td>
</tr>
<tr>
<td>QTL</td>
<td>Quantitative trait loci</td>
</tr>
<tr>
<td>Rgs2</td>
<td>Regulator of G-protein signalling 2</td>
</tr>
<tr>
<td>SAP</td>
<td>Stretched-attendant posture</td>
</tr>
<tr>
<td>SOCS2</td>
<td>Suppressor of cytokine signalling 2</td>
</tr>
<tr>
<td>TAC1</td>
<td>Tachykinin precursor 1</td>
</tr>
<tr>
<td>TTR</td>
<td>transthyretin (prealbumin, amyloidosis type I)</td>
</tr>
</tbody>
</table>
Introduction

Genetics and neuroscience research have co-existed for a long time. Despite this, the molecular mechanisms behind what modulates our fundamental behaviours are still unknown. Behaviour like anxiety, fear and aggression, are of a complex nature and thought to be orchestrated by both environment and multiple genes, which each contributes to the phenotype. Behavioural genetics aims to understand the regulation of what is regarded as the normal aspects of behaviour in animals, as well as the manifestations of mental illness in man. With the fast development of molecular tools during the last decade, new possibilities have emerged that facilitate studies of behavioural genetics in a functional genomics context. By studying the spectra of normal behaviours in animal models and states of neurological illness in both animal and man, new understanding of how behaviour is shaped by evolutionary processes, domestication or origin of disease can be gained. Furthermore, behavioural genetics can also generate new knowledge on basal brain function, which in the future may improve current forms of therapy for psychiatric illness. The papers included in this thesis are based on investigations of gene expression studies in brain, as a means to search for molecular pathways involved in behavioural changes observed as a cause of selective breeding.

Rodent and canine models for behavioural research

The use of animal models in research is essential to understand and test processes of gene interactions and gene function within biological systems and whole organisms. The most commonly used model organism for basic mammalian biology, genome evolution and human disease is the house mouse (*Mus musculus*) (Tecott, 2003). With its small size, large litters and short generation time it is easy to keep in laboratory environment. Much due to its usefulness, it has in recent years been subjected to whole genome sequencing (Waterston et al., 2002) and effort has been aimed at characterising transcriptomes (Okazaki et al., 2002) and gene function through targeted mutations
The last common ancestor of mouse and man has been estimated to originate 75 million years ago, and about 99% of the genes in mouse have a human homologue (Austin et al., 2004). More than 300 inbred strains of mice have been created since the beginning of the last century (Paigen, 2003a), each with its own characteristics, providing an important tool for dissecting genetic involvement in behavioural phenotypes and Quantitative Trait Loci (QTL) mapping (Paigen, 2003b). Alongside the establishment of mouse strains, a number of behavioural tests have been developed and standardised to evaluate basic reactions like anxiety, aggression and fear (Lister, 1987; Porsolt et al., 1977; Thurmond, 1975).

Today, the most used animal models for human psychiatric disorders are rodents (Dennis, 2005). However, rodent’s reactions might not always translate well to human behaviour. Though mouse and man share fundamental behaviours, the natural and species-specific behaviour of the animal under study must be taken into account, and may not correlate to the repertoire of human behaviours. This is particularly true for other well-studied animal models like the nematode (Caenorhabditis elegans) or the fruit fly (Drosophila melanogaster) which may limit their use for cognitive behavioural research (though they have proven to be superior in aspects of exploring the bases of signalling pathways and cellular networks (Grishok et al., 2000; Harding et al., 1985)).

The dog (Canis familiaris) might in this sense be a more suitable model for behavioural genetic studies, as they are closer to humans than mice, both with regard to evolutionary distance and in the fact that they share our everyday life. The dog has a short evolutionary history, as descendants from the grey wolf (Canis lupus) (Vila et al., 1997; Savolainen et al., 2002; Leonard et al., 2002). Archaeological findings of dog remains have been dated to be about 15 – 30 000 years old, but mitochondrial data imply that dogs with a more wolf-like appearance probably existed already 100 000 years ago (Savolainen et al., 2002; Vila et al., 1997).

Despite this short history of evolution, dogs show an extraordinary diversity of both morphological and behavioural traits (Parker et al., 2004). This is manifested not only as breeds with great variation in size, shape and specific behaviours (e.g. herding or retrieving), but also in personality traits like emotionality, aggressiveness, sociability and activity level (Scott & Fuller, 1965; Svartberg & Forkman, 2002;
Svartberg, 2005). Many of today’s different dog breeds have undergone severe bottlenecks and some originate from only a handful of individuals (Sutter & Ostrander, 2004). As a result of the strict selection for breed specific traits and use of popular sires (Sundqvist et al., 2006), dog breeds can therefore to some extent be regarded as the canine form of inbred mouse strains (Lindblad-Toh et al., 2005).

In a study by Parker et al, 414 dogs were screened with 96 microsatellite markers distributed genome wide, and individuals could with 99% accuracy be correctly assign to its breed (Parker et al., 2004), indicating that breeds are genetically different. The records kept by breed organizations world wide holds a rich source of information on pedigrees for registered dogs of more than 400 acknowledged breeds, that can be used for large population genetic studies.

Dogs are not only sharing our daily life and environment, they also develop conditions comparable to human psychiatric disorders like post-traumatic stress disorder (PTSD), obsessive-compulsive disorder (OCD), social phobia and Alzheimer’s Disease (Overall, 2000). Both dog and man can also be successfully treated with the same pharmacological compounds to reduce symptoms of distress (Wynchank & Berk, 1998). Some of the conditions in dogs seem to have a breed-specific prevalence, indicating that there are genetic components involved (Moon-Fanelli & Dodman, 1998).

Dogs and humans share a unique social relationship. The social behaviour of dogs is thought to have developed during domestication, since behavioural experiments show they perform tasks based on social skills. Research has shown that they understand our unspoken cues like pointing or gazing exceedingly better than wolves, but do equally well (or worse) in other tasks (Hare et al., 2002; Miklosi et al., 2003). Unlike most other animals used as models in behavioural research, dogs have a diverse repertoire of communicating their feelings of well-being or distress, using vocalizations, facial expressions, tail-wagging and body posture.

Another canine relative, the fox (Vulpes vulpes) (Figure 1), has also been subjected to selective breeding; initially for fur production but later also specifically for behaviour with the aim to mimic the domestication process (Trut et al., 2004). A fur colour variant of red foxes, known as the silver fox, has since the beginning of the 20th century been used for fur production. These silver foxes was the base for a
breeding program to study the domestication initiated in the 1960’s by Russian scientist Dimitri Belyaev (Trut, 1999). Farmed foxes showing weak aggressive- and fear responses towards humans were repeatedly selected to parent the next generation of animals (Trut et al., 2004). As a result of this strict selection exclusively for behavioural traits, foxes started to show a dog-like contact seeking behaviour towards humans after six generations of breeding. In concert, both physiological and morphological changes appeared (Trut et al., 1974; Plyusnina, 1991; Popova et al., 1991). After 40 generations of selection, these tame foxes perform as well as dogs in tests measuring the animal’s ability to interpret human social cues (Hare et al., 2005). This selection program still continues at the Institute of Cytology and Genetics at the Russian Academy of Sciences (Novosibirsk, Russia) and holds a unique resource for exploring the behavioural and genetic aspects of domestication.

![Figure 1. Phylogenetic tree (based on sequencing of mitochondrial DNA control region) to illustrate relationship of four members of the Canidae family used in papers I, II and III.](image)

**Behaviour during domestication**

Animal domestication has been defined as the process by which a species adapts to man and to a life in a captive environment (Price, 1984). The adaptation can be seen as a combination of heritable genetic changes occurring over generations and individual experiences during the life and development of each animal (Price, 1984; Price, 1999). An
initial threshold in the domestication process is the animal’s ability to survive and reproduce in captivity. The natural selection posed by the new environment will favour individuals that adapt to the situation while behaviours important for survival in nature may loose much of its significance. Consequently, the behavioural characteristics associated with the domesticated phenotype are commonly seen as a general reduction in responsiveness to environmental and physiological stimuli (Price, 1984).

Domestication is however not solely beneficial for the animal. Beside gaining shelter and being provided food, living together with humans has also brought companion animals increased susceptibility to many diseases which also affect the human population, like obesity (Bergman et al., 2007) and cancers (Kumaraguruparan et al., 2006).

**Genetic basis for evolution of behaviour**

The genetic basis for the rapid behavioural evolution observed in domestic species is currently unknown. One theory, which accounts for the limited amount of mutations in functional genes occurring during rapid differentiation, suggests that changes in regulatory regions could be a major source of variation (Britten, 1971). It has been hypothesized that modification of regulators of gene expression may be more important for biological differences between species than structural changes in proteins (King & Wilson, 1975). A growing number of studies are indicating that natural genetic variation can cause significant differences in gene expression (Oleksiak et al., 2002; Brem et al., 2002; Cowles et al., 2002).

Brain gene expression is suggested as an important factor for manifestations of behavioural traits. Studies have reported that a shift in transcript levels of e.g. hormonal receptors and neuropeptides can be manifested as changes in complex social behaviours such as grooming and partner preference in prairie voles (Hammock et al., 2005) or reproductive behaviour in whiptail lizards (Woolley et al., 2004). It has also been proposed that some of the behavioural differences seen between humans and our closest primate relatives could be due to acceleration in transcriptome evolution in the human brain (Enard et al., 2002; Gu & Gu, 2003). The Russian domestication experiment with its directed selection for tameness in silver foxes may be thought of as a rapid evolution of specific behavioural traits, potentially influenced by gene regulation mechanisms.
Changes in behaviour due to disease

Both rodent and canine animal models are important as disease models, since identification of susceptibility genes for diseases in human populations can be a difficult challenge (Kas et al., 2007). Interactions between genes and environmental and developmental factors can often be difficult to control when performing studies in human subjects. This can be especially limiting for studies of complex disorders like psychiatric disease, where the genetic heterogeneity in sample populations and subjectivity in disorder evaluation adds to the complexity (Kendler, 2006).

One example where behavioural changes in both mice and dogs have successfully assisted in exposing the underlying mechanism of a complex disease, is the finding of hypocretin deficiency as the cause of narcolepsy (Lin et al., 1999; Chemelli et al., 1999). This sleep disorder is mostly sporadic in humans with a relatively low concordance (20-32%) between monozygotic twins (Mignot, 1998). By the discovery of heritable symptoms of narcolepsy in a group of Doberman dogs, it was possible to find a causative mutation by breeding strategies and positional cloning. Knock-out studies in mice further verified this finding (Chemelli et al., 1999). The discovery of a hypocretin receptor (Hrctr-2) dysfunction in these animals put focus on the signalling system that later also was revealed being disturbed in the human form of the disease (Nishino et al., 2000).

Brain and behaviour

All behaviour is manifested in the brain through a complex network of neurons organized in regions with different cytoarchitecture (Kandel & Squire, 2000), where each region and cell type has its own function and genetic expression (Lein et al., 2007). Small shifts in expression levels in brain of key molecules like hormones and receptors, has shown possible to dramatically change complex behaviour (Hammock et al., 2005). Since the individual differences in brain and behaviour are produced both by genetic and environmental effects, and often acts through the modulation of mRNA transcription, expression studies can be one means of searching for genes important for behaviour.

The main focus of this thesis is the change in behaviour that occurs as a result of domestication. The behavioural phenotype of domestic
animals has shown to be accompanied with changes in neurochemistry and modified reactivity of neural systems related to stress and emotionality (Price, 1999). This may be explained by the initial stage of domestication, when capturing and restraining by humans puts severe stress on the individual animal. The individuals with best adaptive ability will thus be favoured, and genetic substrates for tame behaviour will be inherited in the next generation (Price, 1999).

The response to stress is thought to be an important aspect of domestic behaviour (Lindqvist et al., 2007) and we have decided to study expression in hypothalamus and related regions, as it is the crucial site for integration of the stress response (Finn et al., 2003). For the majority of papers in this thesis, we have also explored gene expression in two additional brain regions important for behavioural response, that both are inter-connected with the hypothalamus; the amygdala and the frontal lobe (Figure 2).

Figure 2. Ventral side of fox brain. Sampled regions marked: the hypothalamus (H), amygdala (A) and frontal lobe (FL).
The hypothalamus

The hypothalamus is the coordinator of autonomic and neuroendocrine responses. The hypothalamic-pituitary-adrenal (HPA) axis (Figure 3) is a neuroendocrine system facilitating an adaptive physiological response to situations experienced as stressful to the individual (Minton, 1994). The stress response of the autonomic nervous system has the ability to prepare the organism for a “fight or flight” reaction to avoid what is perceived as threatening, and this response can subsequently be restored by the homeostatic functions of the HPA-axis. The physiological reaction to stress is correlated to an increase of circulating glucocorticoids like cortisol and corticosterone that is excreted from the adrenal cortex. A rise in cortisol excretion is indicative of fear and therefore often used for assessing stressful reactions in animals (Hydbring-Sandberg et al., 2004). Difference in plasma cortisol levels and pituitary hormones has also been reported between silver foxes selected for tameness and non-selected foxes in association to human handling, suggesting that the responsiveness of the HPA-axis has been affected by selection for tameness (Harri, 2003; Rekila et al., 1999; Trut et al., 1974; Gulevich et al., 2004; Moe & Bakken, 1997).

Figure 3. The HPA-axis response. Hypothalamic corticotropin releasing hormone (CRH) acts on the pituitary to produce adrenocorticotropic hormone (ACTH) that is released and transported by the blood. The adrenal cortex responds to ACTH by releasing glucocorticoids (cortisol) that acts as a negative feedback on the brain stress response.
The amygdala

The amygdala is a collection of several nuclei within the temporal lobes of the cerebral hemispheres (Figure 2). This part of the limbic system is important for regulation of emotions like anxiety, and in attaching emotional context to learning (Rosen, 2004). The amygdala is also involved in the long-term memory storage of experiences associated with emotional events (Kilpatrick & Cahill, 2003; Wilensky et al., 2006). The neural connections between amygdala, hypothalamus and basal forebrain systems that are involved in motivational control and basal motivational states, may thus have important influences on functions of emotions (LeDoux, 1993; Hariri et al., 2003).

The frontal lobe

The frontal cortex (Figure 2) is a brain region important for regulating executive functioning in relation to primary sensory inputs, and in the planning of appropriate cognitive response (Fuster, 2002). With its extensive innervations to other brain regions (e.g. hypothalamus and amygdala), the frontal lobe has been suggested to be involved in several cognitive and behavioural responses like features of human psychiatric diseases, e.g. Schizophrenia (Yamada et al., 2007), aggression (Davidson et al., 2000), and coordinating the suppression of emotional memories (Depue et al., 2007). It is also thought to be involved in social interactions of both pigs and apes (Poletto et al., 2006; Schenker et al., 2005) and the understanding of other individuals actions (Nelissen et al., 2005).

Studying behavioural genetics

Studying phenotypic natural variation is one means to elucidate what genes or chromosomal regions are important for a behavioural trait. By breeding and crossing individuals that display heritable extremes of a behavioural phenotype (e.g. high or low level of anxiety), on can identify regions of the genome which is associated to the variation of the trait without any a priori knowledge of which genes are involved (Willis-Owen & Flint, 2006). Beside studies of natural variation
among individuals, it has also shown useful to induce random mutations in germ line cells, using mutagenic chemicals (Vitaterna et al., 1994) or transposable elements (Ding et al., 2005) in order to screen for phenotypes of interest. The advantage of the QTL method is that it directly addresses the genetic loci underpinning the behavioural trait. However, this might be a daunting task when traits of a complex nature often are under the influence of many genes of small effects. The method calls for large sample sizes, and mapping candidate genes and finding sequence variants has been slow (Flint et al., 2005).

For fine-mapping regions of interest, the most promising strategy until now has been the use of outbred strains of mice with known genetic background. Together with quantitative complementation, a method that uses comparisons of mice with mutations and wild type alleles of a candidate gene, this strategy revealed the Rgs2 gene to be involved in anxious behaviour in mice (Yalcin et al., 2004).

For the papers included in this thesis, we have chosen another approach, which instead makes use of global transcriptional profiling in brains of animals subjected to directed selection for behavioural traits. Expression profiling has the prospect of directly finding the molecules involved in the behaviour, and can be used as an initial step in the search for genes with putative functions for a trait.

Measuring gene expression

With recent year’s development of new powerful methods of screening tens of thousands of genes simultaneously, using e.g. microarray analysis, new possibilities for behavioural genetic studies has emerged (Luo & Geschwind, 2001). The use of expression studies in post mortem brain tissue, for making inferences of the living brain, has shown possible as transcript levels of most genes are preserved in relation to reference genes (Castensson et al., 2000; Preece et al., 2003). However, due to environmental effects influencing mRNA levels, it is crucial to consider the post-mortem time, handling and storage of the samples, as degradation of mRNA might be rapid and thereby obscure actual differences in transcript levels (Catts et al., 2005). Also, age, sex or nutritional status of the individual can affect transcriptional levels.
For all studies included in this thesis, microarray analysis is used as an initial tool to search for genes with differential expression in pools of individuals. This method allows for ranking the genes with respect to their likelihood of being differentially expressed between defined groups of animals. Verification of results has subsequently been confirmed by quantitative real-time RT-PCR (qPCR).
Research aims

The general aim of this thesis was to use gene expression studies of post mortem brain tissue as a means to search for candidate genes involved in behavioural traits.

Specific aims for included papers

Paper I Exploring global levels of mRNA in three regions of the brains of dog and its wild relatives, to search for a signature of domestication in expression profiles.

Paper II Analysing behaviour and brain gene expression in a model for domestication, comparing silver foxes selected for tameness with control foxes that were kept under identical conditions.

Paper III Study the inheritance of haem-related genes in three groups of silver foxes; selected for tame behaviour (S), non-selected for tame behaviour (NS), and F1 offspring generated from crossing S and NS foxes.

Paper IV Using brain gene expression in a genetic model of canine narcolepsy, to identify molecular alterations downstream of the canarc-1 mutation.

Paper V Investigating behaviour and brain transcriptional profiles in a mouse model of production traits, showing difference in emotional reactivity.
Present investigations

Paper I. From wild wolf to domestic dog: gene expression changes in the brain

In this study we explored gene expression in the brains of three closely related species within the Canidae family, the domestic dog (*Canis familiaris*), the wolf (*Canis lupus*) and the coyote (*Canis latrans*). We hypothesised that the large fundamental behavioural differences that exist between dogs and wolves could be manifested as differences in brain gene expression, as these two species are nearly identical on DNA level.

Methods

Post mortem brain samples of amygdala, hypothalamus and frontal lobe from ten dogs, five wolves and ten coyotes were pooled with respect of species and brain region. First, region-specific expression was assessed by hybridising each of the nine sample pools together with a common reference sample, to human cDNA probes (N=7762) printed onto a microarrays. Major patterns of expression of 156 genes from this experiment were explored using a principal component analysis (PCA). In a second step, we used the same sample pools for a pairwise comparison between species. Genes were ranked according to their evidence of being differentially expressed, using a penalized F-statistic test, in both microarray experiments. Replica plates holding cDNA of each individual sample and tissue were prepared and used for verifying expression with qPCR, using species-specific primers. The qPCR results were analysed with a mixed ANCOVA model. To adjust for variability of mRNA quantity in the sample, reference genes with supposedly stable expression, such as actin beta (ACTB) or glyceraldehyd-3-phosphate dehydrogenase (GAPDH) were used as covariates in the model.
Results
The microarray analysis revealed 156 genes with evidence of region-specific expression. A principal component analysis of this data showed that gene expression was substantially larger between brain regions than between species as 83% of the total variation was explained by region differences and only 12% by species differences. It was also revealed that hypothalamus showed a clearly different expression from that of the frontal lobe and the amygdala, explaining 68% of the total variation. By designing a second microarray experiment we improved the statistical power to explore differences between species. With this strategy we found 114 genes with evidence of species-specific expression. The expression profile of hypothalamus in dogs had a clear divergence from the other two species, with eight genes being species-specifically expressed compared to one in coyotes and none in wolves. Four genes that were chosen for qPCR analysis, CRYM, NPY, CALCB and AQP4, verified the results of the microarrays.

Discussion
Using cross-species hybridisation when performing microarray analysis has shown to be useful when the genome sequence of the species under study is unknown. For this study we estimated the identity between ~20 000 human and the canine mRNAs to an average of 88% for coding sequences. At the time of the study, no canine arrays were available. Even today human cDNA microarrays holds a superior number of annotated genes in comparison to available canine arrays (Holzwarth et al., 2005). Cross-species hybridization may bias the detection to abundant transcripts with high sequence similarity to human genes. However, we regarded this approach to be sensitive enough for an initial screening. We also used a strategy of pooling material before microarray hybridisation. This can be a concern, as a single sample sometimes explains a disproportionate part of the total variance. However, these limitations are addressed by confirmation experiments with a qPCR assay, where a subset of genes are verified in each individual brain tissue sample, using Canidae-specific primers.

With this study we suggested that strong selection for behavioural traits could have resulted in expression changes in few genes acting on several brain functions, with a possible effect on behaviour. The find-
ing of two neuropeptides with a dog-specific expression in hypothalamus could thereby indicate a domestication related response for these genes. However, with the use of samples originating from both wild and domestic animals, we cannot exclude confounding effects of environmental differences that might have caused the observed expression divergence in dogs.

Paper II. Selection for tameness has changed brain gene expression in silver foxes

The aim of this study was to investigate global gene expression in brains of two groups of domesticated silver foxes (Vulpes vulpes) in relation to their difference in behavioural response towards humans. We compared foxes selected for tame behaviour for more than 40 generations to a group of non-selected foxes, all living together under identical conditions.

Methods

First, an initial behavioural test was performed, scoring animals of the two groups of silver foxes and their F1 offspring for reactivity to human contact. Behavioural parameters were scored and difference between groups were analysed with an ANOVA model. To contrast behaviour with brain gene expression we examined the transcriptional profiles of three groups of foxes selected, non-selected and wild using microarray analysis. For this comparison, tissue samples of nine individuals were pooled with respect to region (amygdala, hypothalamus and frontal lobe), and hybridized to human cDNA arrays, holding 29 750 probes. Evidence of differential expression between groups of foxes for one or more brain regions, were estimated using a similar model as in paper I. Three genes (HBG1, HEBP1 and TTR) were chosen for verification in three individual tissue samples from 50 foxes, with qPCR analysis as previously described in paper I.
Results

Foxes of the selected and non-selected groups showed large differences in behaviour, with intermediate response in the F1 offspring. This showed that an additive genetic component was underlying these behavioural traits. The microarray analysis revealed 2469 clones with evidence of differential expression between wild foxes and the two groups of domesticated foxes, while only 40 clones differed between selected and non-selected foxes. The expression difference across brain regions was also found to be similar. Surprisingly, six of the 40 clones identified as differentially expressed, were related to haem.

Discussion

In this paper we suggest that the striking difference in behaviour between selected and non-selected foxes is associated with limited differences in the brain transcriptome. Furthermore, the comparison between wild and farmed foxes demonstrates that environmental effects have a large impact on brain gene expression that must not be overlooked when making inference of global brain expression. Surprisingly, we did not find expression differences in the genes previously reported to be altered in animals of this fox model (Gulevich et al., 2004; Popova et al., 1991). The reason for this might be that the effect of these transcripts was diluted in the relatively large tissue sample used for hybridisation to the arrays, or that purely post-transcriptional regulation acted on these systems. An alternative explanation could be that the tame foxes in these earlier studies were contrasted to animals purely selected for aggression. This could explain the observed differences in glucocorticoids and serotonin as more reflecting expression of aggression or fear, than tameness. Haem-related genes with an expression that was genetically correlated to the behavioural response was further analysed in paper III.

Paper III. Selection for tameness modulates the expression of heme related genes in Canidae brains

In this paper we verify that several haem-related transcripts (previously identified in paper II), are differentially expressed in the brains
of silver foxes (*Vulpes vulpes*). Gene expression levels for heme binding protein 1 (HEBP1), hemoglobins alpha (HBA), beta (HBB) and epsilon (HBE), were analyzed using qPCR in both wild red foxes and farmed silver foxes (selected, non-selected and F1 crosses). We also performed a mitochondrial DNA (mtDNA) analysis to estimate the sequence divergence as a measure of genetic background between the two groups of silver fox.

**Methods**

To estimate genetic variability in the groups of foxes, sequencing of the left domain of mtDNA control region were performed. A neighbour-joining tree was constructed with sequences of wild and farmed foxes. To verify expression difference found in haem related genes in paper II, qPCR analysis was performed (as previously explained in paper I and II).

**Results**

The expression of haem-related genes could be well described by an additive polygenic effect. The silver foxes selected for tame behaviour showed a reduced expression of all four genes compared to non-selected foxes, with the F1 offspring having intermediate mRNA levels. Furthermore, mtDNA analysis revealed that the genetic background of selected and non-selected silver foxes was very similar. The difference found in expression levels was therefore probably not due to large sequence divergence between the groups, but the result of selective breeding for tameness.

**Discussion**

The results from this study indicated that the expression of haem-related genes have been modulated by selection for tameness. The finding of a possible involvement of haem genes in behaviour is unexpected, however not unfeasible. Molecules like haemoglobin may potentially affect the behaviour of canids by directly or indirectly interacting with CO and NO signalling, or after enzymatic cleavage, with brain opioid receptors. The results reported in this paper will need to be verified by functional studies that might reveal if this group of genes adds to behaviours related to the domestic phenotype.
Paper IV. Expression of TAC1, PENK and SOCS are down regulated in narcoleptic dog brain

In this paper we use the analysis of brain transcripts to screen a canine model for narcolepsy, a neurological disorder of known pathogenesis. Narcolepsy causes dramatic behavioural alterations in both humans and dogs, with excessive sleepiness and cataplexy triggered by emotional stimuli. Deficiencies in the hypocretin system are well established as the origin of the condition; both from studies in humans who lack the hypocretin ligand (HCRT) and in dogs with a mutation in hypocretin receptor 2 (HCRTR2). However, little is known of downstream alterations due to the impaired signalling, and expression studies in brain may thus add information of such molecular modifications.

Methods

By using microarray technology we have screened the expression of 29760 probes in the brains of six Doberman pinschers with a heritable form of narcolepsy (homozygous for the canarc-1 [HCRTR-2-2] mutation), and their six unaffected heterozygous siblings. Due to the limited number mRNA that could be extracted from the animals, a pooling strategy was used, with sample pools of post mortem samples from the regions of amygdala, hypothalamus and pons. Expression differences between the two groups of siblings were analysed with an ANOVA model with the factor ‘status’ (narcoleptic or unaffected) and the interaction ‘status*region’. Genes were ranked based on penalized F-statistics for their evidence of being differentially expressed between narcoleptics and controls. Candidate genes were subsequently chosen for qPCR verification using the same methodology described in paper I and II.

Results

We identified two neuropeptide precursor molecules, Tachykinin precursor 1 (TAC1) and Proenkephalin (PENK), that together with Suppressor of cytokine signalling 2 (SOCS2), showed reduced expression in narcoleptic brains. The difference was particularly pronounced in the amygdala, where mRNA levels of PENK were 6.2 fold lower in narcoleptic dogs than in heterozygous siblings, and TAC1 and SOCS2 showed 4.4 fold and 2.8 fold decreased in expression, respectively.
The results obtained from microarray experiments were confirmed by qPCR.

Discussion
In this paper we report difference in expression profiles in the brains of narcoleptic dogs. Although a relatively low resolution of the microarray analysis, due to pooling and technical variation, we were able to verify the results of the array experiment for three of the genes, TAC1, PENK and SOCS2. The neuropeptides TAC1 and PENK have in previous studies shown to respond with increase expression after use of amphetamine-like stimulants to increase wakefulness. It is therefore possible to hypothesize that expression differences discovered in this study might be connected with the excessive daytime sleepiness not only in dogs, but also in other species, possibly including humans.

Paper V. Genetic alterations in haemoglobin levels and behaviour in mice selected for high litter size

In this study we investigated behaviour with an additive effect correlated to brain gene expression that have resulted from selection for reproduction, in two strains of mice. Mice with a high number of offspring (H-line mice) were compared to randomly bred controls (C-line mice). Beside changes in fecundity, mice of the H-line have shown to display an altered behavioural response in novel situations, compared to control mice.

Methods
By creating crosses of the two lines (F1 and F2 animals and reciprocal backcrosses to parental lines), we screened for behaviours and mRNA levels with evidence of an additive mode of inheritance. Behaviour was assessed using the elevated plus maze (EPM), and behavioural response associated with anxiety and exploratory drive was scored during a five minute period. Correlations of ten behavioural variables were explored with PCA. Microarray analysis was subsequently per-
formed, using brain tissue samples from the limbic regions that were pair-wise hybridised to arrays with 15,000 mouse probes. Genes of interest were chosen for qPCR verification among the 49 clones with evidence of an additive expression profile. Finally, sequencing of cDNA and in situ hybridisations of tissue sections were used.

Results
The principal component analysis of behaviour demonstrated strong correlation of behavioural variables. The first two components explained 61% of the total variation, and included variables related to open arm activity or exploration. The microarray analysis revealed an overrepresentation of clones coding for iron-binding genes, with a genetic additive difference in expression level. We chose to further analyse transcripts coding for haemoglobin alpha (Hba), beta 1 (Hbb-b1) and beta 2 (Hbb-b2) using qPCR. From this analysis, opposite expression patterns were found for Hbb-b1 and Hbb-b2. Since qPCR results indicated that the parental lines might carry different Hbb haplotypes (Erhart et al., 1985), sequencing of individual cDNAs was performed. This confirmed that the H-line belonged to the HbbD haplotype, while C-line mice were of the HbbS haplotype. In situ hybridizations of riboprobes for Hbb and Hba revealed a scattered distribution of expression throughout the brain, corresponding to that reported in the Allen Brain Atlas (Lein et al., 2007).

Discussion
With this study, we have once again found a genetic correlation of behaviour and expression of haemoglobin in brain (as reported in paper II and III). In the light of recent reports on Hbb haplotype and glutathione metabolism (Hempe et al., 2007), as well as the ability to change behaviour with altered expression of glutathione related genes in mice (Hovatta et al., 2005), we hypothesise that haemoglobins might be one key to the observed change in behaviour in mice, as well as in other mammals.
General discussion

The work presented in this thesis show that strong, directed selection for behaviour do indeed affect gene expression in brain, with possibility of being substrate for evolution of behaviour. Although microarray analysis is a more rapid method for finding genes than classic QTL analysis, it will also generate large amount of data, suggesting numerous genes with putative functions for the phenotype. In our studies of animals subjected to short-term selection, we unexpectedly did not find known modulators of anxious behaviour, like neuropeptides and hormones. Instead, the surprising, and independent findings of altered levels of haemoglobin in relation to anxious/exploratory behaviour in foxes and mice indicated that this group of genes might be of importance for some aspects of these traits.

As haemoglobin genes are mostly recognised for their involvement in oxygen transport, the results are thought-provoking. Interestingly, a QTL with a behavioural effect has been found on mouse chromosome 7 approximately 2 cM from the position of the Hbb (Henderson et al., 2004). This QTL was however suggested to relate to the nearby albino locus (tyr) and be due to impaired vision in white mice, that might affect the outcome in the behaviour test.

Little is known of haem-related gene function in the brain. Evidence from other studies indicates that components of haem metabolism can intervene with the HPA-axis, and through this mechanism influence behaviour (Mancuso, 2004). Haemoglobins are also known to encode proteins that can give rise to biologically active molecules when degraded, so called ‘haemorphins’ (Nyberg et al., 1997). These endogenous peptides have opioid-like effects and have shown evidence to influence behavioural traits like spatial learning and passive avoidance in rats (Albiston et al., 2004; Lee et al., 2004).

Haemoglobin genes are known to be prone to mutations and a large number of alleles are described in human populations (Weatherall & Clegg, 2002). The variability found in human haemoglobins is thought to have a recent origin, being an adaptation to infections of the malaria parasite some 10,000 years ago (Tishkoff et al., 2001). If the adaptability of these genes also could include short-term directed selection of other traits, like behaviour in animals, remains to be verified.
Future perspectives

Performing gene expression studies in the context of behavioural genetics is quite often limited by the access of samples. As sampling in most cases means collection of post mortem samples, tissue from dog and human subjects are precious. Future method development of less invasive techniques would therefore be of highest interest.

Recently, studies on human neuropsychiatric diseases have indicated that the expression profile of the brain seems to be reflected also in leukocytes (Vawter et al., 2004; Middleton et al., 2005; Borovecki et al., 2005) which would open the possibility to obtain more human samples and reduce the number of animals being euthanized. The method of using blood samples would also have the benefit of repeated sampling of each individual over time. Intriguing new studies of monitoring transcription in live animals have also recently been reported, were conjugates of paramagnetic probes for magnetic resonance imaging (MRI) of specific mRNA produced in brain, are used (Liu et al., 2007). This technique will however not be of practical use until several methodological flaws concerning delivery and clearance of the conjugate, have been solved.

An alternative method for expression analysis called eQTL has recently gained interest (Brem et al., 2002; Schadt et al., 2003; Chesler et al., 2005). By regarding mRNA levels as quantitative traits it complement traditional QTL analysis with the possibility to correlate behaviour and expression profiles of multiple genes (Gibson & Weir, 2005). The benefit of combining tissue-specific expression and QTL analysis may help discovering neurobiological pathways and gene regulatory networks and also suggest genetic linkage to a chromosomal region.

Behavioural genetics studies will always need confirmation of candidate gene function in living subjects. The future prospects for the genes found and presented in this thesis would consequently involve studies in live animals (rodent or canids) in order to verify that they indeed have an impact on behaviour. In three of the studies (paper II, III and V) and two animal models, we found genes relating to haemoglobin. A tissue-specific conditional knock-out of these genes in restricted brain regions of interest would be a possible approach. Specifically silencing or over-expressing of genes in order to test for be-
haviour and down-stream effects of expression would be another potential method.

The work recently reported by Hovatta et al, suggested that enzymes with implications for glutathione levels and oxidative stress are involved in anxious behaviour in mice (Hovatta et al., 2005). As haemoglobin can modulate the metabolism of glutathione (Hempe et al., 2007), it may be worth comparing enzyme levels in the mouse strains used in paper V.

Concluding remarks

The main finding of this thesis is that selection for behaviour modifies gene expression in brain, which could have implications for the rapid evolution of behaviour seen under domestication. The possibility of haemoglobins as genes with putative functions for modulating anxious behaviour is novel in this aspect. The significance of this finding can first be evaluated after functional studies are performed. Finding genes that affect behavioural traits in animal models could in a long-term perspective have putative significance for some aspects of human psychiatric disorders, leading to new pharmacological therapies. These findings could also have direct applications for breeding programs and treatment of behavioural disorders in the animal itself, and thus can they also be beneficial for animal welfare.
Trots att genetik och neurobiologisk forskning har samexisterat under lång tid, har de molekylära mekanismerna bakom vad som formar våra mest grundläggande beteenden ännu inte kartlagts. Komplexa beteenden som ångest, rädsla och aggression styrs sannolikt av många gener, som alla bidrar och tillsammans modulerar beteendet. Med de senaste årens snabba utveckling av molekylära metoder har nya möjligheter öppnats för funktionell beteendegenetisk forskning. Studier av normala beteendevariationer i djurmodeller och neurologiska sjukdomstillstånd hos både djur och människa, kan ge ökad förståelse för hur beteenden modifieras under evolutionära processer, domesticering eller vid uppkomsten av sjukdom. Det kan även leda till ny kunskap om hjärnans basala funktion, och i en möjlig förlängning också till förbättrade terapiformer för psykiskt lidande.

I artiklarna som denna avhandling baseras på, används genuttryck i hjärnan som metod för att utforska beteende i olika djurmodeller, med särskild vikt på beteendeförändringar orsakad av riktad selektion.

Artikel I. Från vild varg till domesticerad hund: ändrat genuttryck i hjärnan

Målet med denna studie var att undersöka genuttrycksskillnader i hjärnan hos tre närbesläktade arter: hund (Canis familiaris), varg (Canis lupus) och prärievarg (Canis latrans). Beteendesskillnader mellan hundar och vargar har uppstått under en relativt sett mycket kort period av domesticering, då skillnader i DNA sekvens inte hunnit förändras nämnvärt. Vi undersökte därför om ändrade nivåer av genaktivitet i hjärnan istället kunde förklara den ändring i beteende som uppkommit av domesticering.

Artikel II. Selektion för tamhet ger ändrat genuttryck i hjärnan hos silverräv

I denna studie undersöcktes genuttryck i hjärnan hos grupper av domesticerade silverrävar (Vulpes vulpes) som uppvisar beteendeskillnader i kontakt med människor. Dessa grupperna bestod av rävar som avlats för tamhet i mer än 40 generationer och rävar som inte selekterats för beteende, samt en grupp bestående av deras första generation (F1) avkommor. Då rävarna levde tillsammans under identiska förhållanden, antogs att miljöns påverkan på genetiket var litet. Med ett beteende test påvisades stora beteendeskillnader mellan tama och icke-selekterade rävar, och intermediära responser hos korsningar. För att kontrastera genuttryck i hjärnan hos dessa rävar till beteendet, undersöktes expressionsskillnader mellan tama och icke-selekterade rävar, och intermediära responser hos korsningar. För att kontrastera genuttryck i hjärnan hos dessa rävar till beteendet, undersöktes expressionsskillnader med hjälp av microarrayanalys, där även vilda rödrävar jämfördes. Hjärnprov från nio individer ur varje grupp poolades med avseende på region (amygdala, hypotalaums och frontal lob), och hybridiserades parvis till humana cDNA arrayer.

Totalt 2469 cDNA kloner uppvisade uttrycksskillnader mellan vilda och domesticerade rävar, medan endast 40 kloner skiljde mellan rävar selekterade för tamhet och de icke-selekterade rävarna. Sex av de 40
kloner som uppvisade skillnader mellan de två grupperna av domesti-
cerade rävar visade sig vara gener som kodar för varianter av hemo-
globin eller andra hemoproteiner. Med denna studie visade vi att den
stora skillnad i beteende som observerats mellan rävar selekterade för
tamhet och icke-selekterade rävar, kunde associeras med en uttrycks-
skillnad endast i ett fåtal gener. Samtidigt påvisade vi med jämförel-
sen av domesticerade och vilda rävar, att miljöpåverkan har mycket
stor inverkan på genuttrycket i hjärnan.

Artikel III. Selektion för tamhet ändrar uttrycket av
hemgener i hjärnan hos silverräv.

I denna studie verifierades genuttrycket av gener identifierade i Arti-
kel II, i individuella hjärnprover från silverräv. Genuttrycksnivåer för
hembindande protein 1 (HEBP1), hemoglobin alpha (HBA), beta
(HBB) och epsilon (HBE) analyserades med qPCR i både silverrävar
selekterade för tamhet, icke-selekterade rävar och deras F1 korsningar,
samt vilda rödrävar. Med hjälp av mitokondrie DNA analys uppskat-
tades även de olika rävarsgruppernas genetiska sekvenslikhet.
Silverrävar som selekterats för tamhet uppvisade ett reducerat uttryck
av alla fyra hemgener jämfört med icke-selekterade rävar, medan de-
ras F1 avkommor hade intermediära mRNA nivåer. Analysen av mi-
tokondrie DNA indikerade att den genetiska bakgrunden var begrän-
sad bland de selekterade och icke-selekterade rävar och därmed inte
var en trolig förklaring till de observerade skillnaderna i genuttryck.
Resultatet från denna studie visade att uttrycket av hemrelaterade ge-
er i hjärnan har ändrats genom den riktade selektionen för tamhet.

Artikel IV. Nedreglerat uttryck av TAC1, PENK och
SOCS i hjärnan hos narkoleptiska hundar

I denna studie användes microarrayteknik för screening av genuttryck
i hjärnan hos hundar avlade att fungera som en genetisk modell för
narkolepsi. Bakgrunden till sjukdomen är väletablerad och beror på
störningar i signalering av en neuropeptid (hypocretin), medan de mo-
lekylära förändringarna nedströms om detta system fortfarande är
okänd. Genom att jämföra hundar homozygota för canarc-1 mutatio-
nen som orsakar sjukdomen i denna modell, med deras friska hetero-
zygota syskon identifierades tre gener, TAC1, PENK och SOCS2. 
Alla dessa uppvisade ett nedreglerat uttryck i hjärnan hos narkoleptis-
ka hundar.
Resultaten kunde konfirmeras med qPCR och visade att effekten var 
särskilt uttalad i amygdala. De två neuropeptiderna TAC1 och PENK 
har i tidigare studier visat sig öka i uttryck i samband med amfetamin-
stimulering för ökad vakenhet i hundar, vilket skulle kunna indikera 
e ett samband till den narkoleptiska fenotypen, möjligeven i männi-
ska.

Artikel V. Genetiska förändringar av 
haemoglobinnivåer och beteende i möss selekterade för 
stor kullstorlek
I denna artikeln utforskas beteende och genuttryck i hjärnan hos indi-
vider från två muslinjer (H och C), där den ena (H-linjen) selekterats 
för stor kullstorlek, och i tidigare studier uppvisat avvikande beteende. 
Genom att inkludera avkommor genererade från F1 korsningar och 
tillbakakorsningar till parentallinjerna, screenades beteende och 
mRNA nivåer i hjärna utifrån en additativ modell för att söka efter 
gener med genetiskt nedärvat uttryck. Beteende som indikerar ökad 
nivå av rädsla hittades främst hos djuren i H-linjen. 
Med hjälp av en efterföljande microarray analys hittades en stark 
överrepresentation av hemoglobingener, liknande dem vi funnit i en 
tidigare studie av tama silverrävar (Artikel II och III). Med qPCR ana-
lys upptäcktes motsatta skillnader i uttryck av två hemoglobin beta 
kedjor (Hbb-b1 och Hbb-b2) mellan H och C-linjer. Detta indikerade 
att parentallinjerna hade någon av de olika haplotyper som beskrivits 
för hemoglobin beta i möss. Sekvensering av cDNA konfirmerade att 
alla möss från H-linjen hade haplotypen för HbbD, och möss från C- 
linjen haplotypen för HbbS. Då HbbD haplotypen i tidigare studier 
visat koppling till glutationmetabolism, och uttryck av glutionrelate-
rade gener även visat sig kunna ändra beteende relatater till rädsla i en 
anann musmodell, föreslår vi möjligheten att hemoglobin kan vara 
e en grupp av gener som på kort tid kan ändra beteende i möss, såväl 
som i andra däggdjur.
Slutsatser

Arbetet som ligger till grund för denna avhandling har påvisat att riktad selektion för beteende ändrar genuttrycket i hjärnan. Detta skulle kunna vara en underliggande orsak till den snabba evolutionen av beteende som observerats under domesticering. Vidare föreslår vi också att hemoglobiner skulle kunna vara en grupp av gener som är involverade i modulering av beteenden relaterade till rädsla. Signifikansen av detta fynd kan dock först utvärderas efter uppföljande funktionella studier. Fortsatta expressionsstudier i djurmodeller kan på sikt vara en viktig metod för att för att hitta gener med betydelse för psykiska sjukdomar i mänskliga, men skulle även kunna användas i arbeten inriktade mot förbättrad djurhälsa.
Acknowledgements

First and foremost I would like to thank my two supervisors, Elena Jazin and Peter Saetre. You have really complemented each other in the best of ways, and I have learned so much from working with you. With huge amounts of enthusiasm and excellent guidance, you have made my time as a PhD-student equally challenging, rewarding and fun!

Thanks also to Professor Hans Ellegren for accepting me as a graduate student at the department of Evolutionary Biology, and to Professor Rolf Ohlsson and Professor Reinald Fundele at the Department of Development and Genetics, for ‘housing’ me during my last year at EBC.

Many thanks to all former and present members in the research group of Behavioural Genetics: Anja Castensson for our boxing sessions, and long walks in Hågadalen (whenever I could convince you to do a bit more relaxed kind of exercise). Lina Emilsson, for your extremely good sense of humour that made our office by far the loudest in the corridor! Karro Åberg, for your focused attitude and for sharing my interest in horses. Eva Lindholm, for all our nice lunch discussions on personal and scientific matters. Björn Reinius, for bringing new skills to the group (who would have thought that there ever would be such a thing as “the skull-sawing-device”?), for serving me coffee and listening to my complaints ;-) . Lin Jiang, for sharing your delicious Chinese food and good spirit.

All former students that have been involved in various aspects of the projects included in the thesis, especially Karolina Wallenborg, Anna Wirén, Kalle Holm, Izabella Baranowska and Anita Petterson.

Thanks also to all staff and students at the Department of Evolutionary Biology, for a very nice atmosphere at the lab. Special thanks to Carles Vilà for his involvement in our studies on canids, and to Susanne
Björnerfeldt for those famous expeditions to Norway, but mostly for being so supportive and always cheering me up in times of doubt ;-)  

All people working at the Department of Development and Genetics, especially my present and former room mates, Yang Yu and Tian Geng, for nice company, discussions and enormous amounts of cookies.

Thanks also to the staff that make everyday-life at the departments run smoothly: Helena Malmikumpu, Gunilla Kärf, Carolina Wallström-Pan and Rose-Marie Löfberg.

This work would not have been possible without the collaboration of a number of people that participated in financing, finding or collecting samples. I am especially grateful for the efforts of Kerstin Olsson, Åke Hedhammar and Kenth Svartberg at SLU, Erik Ågren at SVA, Jennifer Leonard at EBC, Emmanuel Mignot and Seiji Nishino at Stanford University (USA), Morten Bakken and Birgitte Seehus at the University of Life Science (Norway), Guido Pollevick at the Masonic Medical Research Laboratory (USA), and Ádám Miklósi and Barbara Klauzs at Eötvös Loránd University (Hungary). Thanks also to all dog owners that agreed to donate samples for this research.

Finally, thanks to all friends off-work for keeping me in touch with the “outer world”, especially Ulrika Danielsson, Johanna Minten, Mette Risberg-Lööw, and the Biotech-girls (or should I say biotech-moms?). Last but definitely not least, thanks to my constantly growing family for all your care and support!
References


42


A doctoral dissertation from the Faculty of Science and Technology, Uppsala University, is usually a summary of a number of papers. A few copies of the complete dissertation are kept at major Swedish research libraries, while the summary alone is distributed internationally through the series Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Science and Technology. (Prior to January, 2005, the series was published under the title “Comprehensive Summaries of Uppsala Dissertations from the Faculty of Science and Technology”.)