A study of depression in Huntington’s disease

Terence Yeow-Chwen Pang

(81405)

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Howard Florey Institute

Department of Anatomy & Cell Biology
Faculty of Medicine, Dentistry & Health Sciences
University of Melbourne

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Abstract

Huntington’s disease (HD) is an inherited neurodegenerative disorder that is caused by a mutation of a single gene, huntingtin. The disease is more commonly known for the characteristic choreiform movements that develop in the later, more advanced stages of the disease. However, cognitive deficits and psychiatric symptoms are frequently observed prior to the onset of the motor symptoms. Little is known about the pathological bases for the neuropsychiatric features which include increased irritability and heightened aggression. Depression affects 30-50% of HD patients and is the most commonly diagnosed psychiatric symptom. This is proportionally higher than in the general population and it is possible that inherent pathological changes in the HD brain render a HD-gene positive individual more susceptible to depression.

Using a variety of behavioural tests, the R6/1 transgenic mouse model of HD was found to display altered responses reflective of depression-related behaviour, indicating that the HD mutation confers a genetic susceptibility for developing depression. The behavioural alterations were more robust in female HD mice reflecting a possible sex-dependent manifestation of the depression symptoms in the human HD population that has yet to be investigated. The onset and rate of progression of HD is strongly influenced by the environment and the development of depression is similarly impacted upon by environmental factors (e.g. stress, negative life events). The experimental paradigms of environmental enrichment and wheel-running slow the development of motor and cognitive symptoms in R6/1 HD mice and the present study reports that both paradigms also correct the depression-related behavioural phenotype. This study also found that HD mice had muted responses to two common classes of antidepressant drugs, highlighting the need for a detailed examination of the efficacy of drug treatments in HD patients.

Depression susceptibility is linked to genetic variance in the human population and studies of gene candidates in mutant mice report the detection of behavioural phenotypes similar to the present study. The depression-related behavioural phenotype of the R6/1 HD model was found to be associated with early down-regulations in mRNA levels of the
serotonin (5-HT) 1A and 5-HT 1B receptors in the cortex and the hippocampus. Additionally, female HD mice had reduced cortical 5-HT transporter gene expression. Collectively, these findings indicate that a disruption of serotonergic signaling in the HD brain contributes to the development of depression in HD. Brain-derived neurotrophic factor (BDNF) gene expression is down-regulated in the HD brain, however the expression pattern of exon-specific splice variants was previously unknown. This study reports that BDNF mRNA levels are reduced in the hippocampus by an early age but also reports that individual exon-specific transcripts are differentially down-regulated in males and females, although the functional relevance of this remains to be investigated.

Overall, this study has demonstrated that the R6/1 transgenic mouse model of HD is ideal for further investigating the occurrence of depression in pre-motor symptomatic HD. It has also identified alterations in gene expression of key components of neuronal signaling which might be linked to the molecular basis of depression.
Declaration

This is to certify that

i. the thesis comprises only my original work towards the PhD except where indicated in the Preface,

ii. due acknowledgement has been made in the text to all other material used,

iii. the thesis is less than 100,000 words in length, exclusive of tables, maps, bibliographies and appendices.

Terence Pang
September 2008
Preface

I would like to acknowledge the following people for their contributions to this work. Ms. Michelle S. Zajac provided the sequences for the 5-HT 2A/C real-time primers and also offered invaluable insight into the performance of high quality real-time PCR. She was also involved in stimulating discussions of the interpretations of the real-time qPCR results.

The histone modification work was in collaboration with the Epigenetics lab at the Murdoch Children’s Hospital, jointly headed by Drs. Jeff Craig and Richard Saffery. Initial optimization of the ChIP protocol was performed by Mr. Paul Canham. Ms. Blaise Weinrich and Mr. Boris Novakovich assisted in performing the ChIP procedure. The BDNF DNA methylation study was performed in collaboration with Dr. Nick Wong, also of the Epigenetics lab at the MCRI.
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Chapter 1 Background

In his seminal paper published in 1872, George Huntington described a condition that afflicted a select group of his patients, causing them to exhibit dance-like writing motions (Huntington, 1872). He also noted that ‘it never skips a generation to again manifest itself in another; once having yielded its claims, it never regains them.’. More than a century on, despite the tremendous effort placed in understanding the ailment known as Huntington’s disease (HD), there are no effective means of treatment and a cure remains elusive.

1.1 Huntington’s disease

HD is an inherited neurodegenerative disorder which affects approximately one in 10,000 individuals, mostly of European origin. There are ~30,000 sufferers in the United States of America with an additional 75,000 gene carriers. Here in Australia, 7-10 individuals per 100,000 people are affected by HD. It is caused by a mutation in the huntingtin gene and is transmitted in an autosomal dominant manner so offspring have a 50% chance of inheriting the disease-causing allele should one of their parents be HD-positive. The disease typically manifests during the third and fourth decades of life and is characterised by a triad of psychiatric, cognitive and motor symptoms. There is no cure and death occurs 10-20 years after clinical diagnosis.

1.2 The HD gene - huntingtin

HD is the result of a mutation of the huntingtin (htt) gene which is located within the 4p16.3 locus on the short arm of chromosome 4 (Huntington's Disease Collaborative Research Group, 1993). Following the systematic haplotype analysis of 75 disease families, the novel gene was identified and termed IT15. On HD chromosomes, it was found to contain an unstable polymorphic trinucleotide repeat. The polymorphic stretch of CAG repeats reside in the 5’ coding region of the gene and translates into an expanded
polyglutamine (polyQ) tract in the 348kD htt protein (Persichetti et al., 1995) that is widely expressed but unrelated to any known gene.

1.3. Huntingtin gene expression

The htt gene is widely expressed in human tissue with the highest level of expression in the brain (Huntington's Disease Collaborative Research Group, 1993; Li et al., 1993; Strong et al., 1993). In situ hybridization has been used to demonstrate that htt RNA in the human brain is mainly detected in neuronal cells although there is some level of glial expression (Li et al., 1993; Strong et al., 1993). There are regional differences in the density of htt RNA labeling with moderate expression in the striatum but particularly high levels in the dentate gyrus neurons of the hippocampus as well as the granule and Purkinje cells of the cerebellum (Strong et al., 1993). The distribution of RNA does not correspond with the pattern of neuropathology of HD (discussed in Section 1.7.2) which indicates that the gene expression pattern of the expanded trinucleotide sequence does not directly explain the regional selectivity of neuropathology. Instead, the selective pattern of neuropathology appears to reflect somatic mosaicism with different levels of CAG expansion in different tissue. Initial data from four pairs of monozygotic twins reported no somatic instability present in blood, lymphocyte and brain DNA but gametic mosaicism in sperm samples (MacDonald et al., 1993). However, subsequent analyses of the different tissue regions in the brain found evidence of somatic mosaicism in the basal ganglia and the cerebral cortex (the regions which develop the most neuropathology) while the cerebellar cortex had the lowest degree (Telenius et al., 1994). Early on in the disease process, somatic instability is more evident in the striatum than in the cerebral cortex and reflects the susceptibility of particular neuronal populations in the striatum as the nitric oxide synthase-positive interneurons which are relatively spared in HD have smaller mutation length gains (Shelbourne et al., 2007). The extent of somatic expansion has also been demonstrated to be age-dependent in a chimeric mouse model of HD in which it was detected in 40-week old mutant mice in the absence of neuronal death but became more evidently more pronounced as the mice aged (Ishiguro et al., 2001).
1.4. Regulation of huntingtin gene expression

The precise nature by which \textit{htt} gene expression is regulated is still unclear. The complexity of gene regulation is demonstrated by the numerous transcription factor binding sites within the gene sequence (see Figure 1-1). The \textit{htt} promoter region lacks a TATA and a CAAT box, is GC rich and contains several consensus sequences for Sp1, activating proteins (AP)-2 and AP-4 transcription factor binding sites (Holzmann et al., 2001). There are also multiple putative p53-responsive elements in the \textit{htt} gene and activation of p53 (also a tumor suppressor gene) has been shown to selectively up-regulate \textit{htt} expression in the cortex and striatum (Feng et al., 2006). Coincidentally, both brain regions are particularly vulnerable in HD pathology suggesting the possibility that abnormal \textit{htt}-p53 interaction is involved in the selective pathology of HD.

1.5. Huntingtin protein localization

The \textit{htt} protein is expressed by human lymphoblastoid, neuroblastoma and fibroblast cell lines, as well as neural and non-neural rodent tissue (Trottier et al., 1995). In the brain, the cortex and cerebellum are the regions with the highest levels of \textit{htt} protein with moderate expression in the striatum and midbrain. The protein is also detected in peripheral tissue such as the liver, lung, intestine, spleen and kidney but is most highly expressed in the testes and heart. There is strong punctate staining of \textit{htt} throughout the human brain, and these are mainly localized to nerve fibres, varicosities and neuronal perikarya. Immunostaining reveals that the protein is present in the substantia nigra pars reticulate, layers IV and VI of the cortex, as well as the Purkinje cell layer of the cerebellum. Interestingly, \textit{htt} expression is sparse in the striatum where there is a heterogeneous distribution of the protein which is confined to neurons and neuropil within the matrix component (Ferrante et al., 1997). There has been a report of different subcellular localization of the \textit{htt} protein in human, mouse and rat brains; however the functional relevance of this is not understood (Wood et al., 1996).
Figure 1-1 Transcription factor binding sites within the huntingtin (htt) gene.
The *htt* gene is located on chromosome 4 within the p16.3 locus and is 169, 280 base pairs in length. There are multiple transcription factor-binding sites located along the *htt* gene sequence. Transcription factors are listed on the left column and their corresponding binding location along the *htt* gene is indicated by the bars on the right. Information adapted from the UCSC Genome Brower.
1.6 Function of the huntingtin protein

The entire functional capacity of the huntingtin protein has also yet to be fully appreciated. htt is an essential protein involved in early embryonic development (Duyao et al., 1995; Nasir et al., 1995; Zeitlin et al., 1995). Homozygocity for the murine htt homologue (deletion of both alleles) results in embryonic fatality but mice with one functional copy of Htt via heterozygous inactivation develop normally. The homozygous embryos terminate before E8.5 with signs of abnormal gastrulation prior to organogenesis and somite formation. The loss of htt expression and resulting embryonic lethality has been proposed to be the result of an impairment of the nutritive functions of the visceral endoderm due to the localization of htt to the membranes of vesicles associated with microtubules. This lethal phenotype can be rescued by the introduction of extra-embryonic wild-type embryonic stem cells which express wild-type htt to support the development and survival of neurons in the mutant embryo (Dragatsis et al., 1998; Reiner et al., 2003). Beyond embryonic development, htt has broad roles in the regulation of gene expression and cell survival.

1.6.1. Htt regulates brain-derived neurotrophic factor expression

The ability of htt to regulate gene expression was first uncovered by studies examining the effect of mutant htt protein expression on neurotrophin production. HD pathology involves the selective degeneration of striatum neurons which require brain-derived neurotrophic factor (BDNF) for survival and differentiation (Mizuno et al., 1994; Ventimiglia et al., 1995; Ivkovic and Ehrlich, 1999). BDNF is not produced locally and is transported to the striatum from the cortex by anterograde transport via cortico-striatal afferents (Altar et al., 1997; Conner et al., 1997). In neuronal cell lines and in the brains of transgenic HD mice and HD patients, there is a dramatic reduction of BDNF mRNA and protein levels (Zuccato et al., 2001; Spires et al., 2004a; Pang et al., 2006). Wild-type htt protein has been shown to up-regulate BDNF transcription in a promoter-specific (promoter II) manner while expression of the mutant htt protein results in repression of transcriptional activity at all BDNF promoter sites. The transcriptional regulation of
BDNF by htt is selective as expression of other neurotrophins such as nerve growth factor (NGF) and neurotrophin-3 (NT-3) is unaltered. The negative effects of mutant htt on BDNF transcription are also cell-type specific and is restricted to neuronal cells since mutant htt protein does not affect BDNF production in non-neuronal cells (fibroblasts). It has since been confirmed that the dysregulation of BDNF gene expression is due to a loss of wild-type protein function because the down-regulation of BDNF expression can be reversed by re-expressing wild-type protein in the presence of mutant htt (Reilly, 2001). The specific loss of BDNF protein in the striatum is likely to be the main pathological cause of the motor symptoms related to HD since transgenic mice selectively lacking cortically-produced BDNF (Baquet et al., 2004) also develop a motor phenotype that is similar to the transgenic mouse models of HD (discussed in Section 1.8).

1.6.2. Htt regulates gene transcription by interacting with transcription factors

As mentioned above, expression of BDNF through promoter II is mediated by htt and this transcriptional process involves the interaction of htt with the RE-1 silencing transcription factor/neuron-restrictive silencer factor (REST/NRSF) (Zuccato et al., 2003). REST/NRSF is a transcription factor which targets the repressor element-1/neuron-restrictive silencer element (RE1/NRSE) sequence within a given gene promoter region to inhibit the repression of gene expression. There is an RE1/NRSE element located within the promoter II region of BDNF (Nakayama et al., 1994; Tabuchi et al., 2002) and the inhibition of BDNF gene transcription involves the recruitment of a repression complex comprising of the co-repressor mSin3A (Grimes et al., 2000; Roopra et al., 2000) and various histone deacetylases (Hassig et al., 1997; Heinzel et al., 1997; Laherty et al., 1997; Nagy et al., 1997). Genome-wide screening has identified multiple genes that are under the regulatory control of REST/NRSF (Bannerman et al., 2004) such as proenkephalin, m4 muscarinic acetylcholine receptor (Wood et al., 1996), corticotrophin releasing hormone (Seth and Majzoub, 2001), components of vesicular trafficking and exocytosis (D’Alessandro et al., 2008), tryptophan hydroxylase-2 (the rate limiting enzyme in serotonin biosynthesis) (Patel et al., 2007) and the serotonin (5-HT) 1A
In addition to REST/NRSF, htt also regulates the expression of other genes through its interaction with the transcriptional activator Sp1, the co-activator TAFII130 (TAF10) and other components of the core transcription apparatus (Dunah et al., 2002; Li et al., 2002; Zhai et al., 2005). In the case of Sp1, htt facilitates the binding of Sp1 to the GC-rich elements in gene promoter regions (e.g. nerve growth factor receptor) and this event is an initial trigger the activation of gene transcription (Briggs et al., 1986).

**1.6.3. Htt is involved in vesicular trafficking**

Recent evidence has uncovered a role of htt in vesicular trafficking. Htt facilitates the dynein/dynactin-mediated transport of vesicles along microtubules and is involved in the maintenance of cytoskeletal integrity (Diprospero et al., 2004; Gauthier et al., 2004; Cavistone et al., 2007; Johnson et al., 2007a; Smith et al., 2007). It has been shown to enhance post-Golgi trafficking of various proteins to the plasma membrane through an association with acidic phospholipids (Kegel et al., 2005; Strehlow et al., 2007). The htt protein has been demonstrated to specifically enhance the transport of BDNF-containing vesicles along microtubules via its interaction with huntingtin-associated protein-1 (HAP-1) and the p150(Glued) subunit of dynactin which is an essential component of molecular motors (Gauthier et al., 2004). The dynamic protein-protein interaction acts as a molecular switch for antero- as well as retrograde transport in neurons (Colin et al., 2008) and contributes to the anterograde transport of BDNF from the cortex to the striatum.

**1.6.4. Htt is involved in anti-apoptotic pathways**

Htt promotes cell survival by modulating the signals that trigger programmed cell death (apoptosis). Apoptosis is triggered by caspase (cysteine-proteases) activity and htt has been demonstrated to interact with caspase-3 (Zhang et al., 2006). Initiation of caspase-3 activity through tumor-necrosis factor receptor (TNFR) signaling ultimately results in cell
death and htt interacts with activated caspase-3 to inhibit this down-stream pro-apoptotic signal. The presence of wild-type htt protein is essential for regulating caspase-3 mediated signaling as htt depletion leads to overt cell death or increased vulnerability to cell death.

In addition to cell survival, htt is also implicated in cell differentiation and neurogenesis (Bai et al., 2003; Su et al., 2004; Ballas et al., 2005). It has also been shown to be an iron-response protein which regulates iron homeostasis during development (Hilditch-Maguire et al., 2000; Lumsden et al., 2007). These findings are consistent with the occurrence of neuronal dysfunction and death in the presence of mutant htt protein that is associated with perturbation of the cation balance within perinuclear membrane organelles such as the mitochondria (Hilditch-Maguire et al., 2000; Benchoua et al., 2006; Solans et al., 2006; Simmons et al., 2007).

### 1.7. HD pathology

#### 1.7.1. HD as a result of CAG repeat expansion in htt gene

The number of CAG repeats in the human htt gene is polymorphic and normally ranges between 6 - 35 CAG repeats. HD typically results from having 40 or more repeats and there is incomplete penetrance in the range of 36-39 repeats. Most cases of HD are adult-onset and patients do not develop clear clinical symptoms until their third or fourth decades of life. There is an inverse correlation between the number of CAG repeats and the age of onset of HD, i.e. the greater the CAG repeat expansion, the earlier the age of disease onset (Andrew et al., 1993; Duyao et al., 1993; Snell et al., 1993; Trottier et al., 1994). Expansions of more than 60 repeats are associated with an early onset and faster-progressing form of juvenile HD. The disease usually progresses over a period of 10-20 years following clinical diagnosis and is ultimately fatal. The exact CAG repeat length is itself subject to some level of intergenerational variation due to gametic instability (MacDonald et al., 1993; Wheeler et al., 1999) although recent evidence indicates the
existence of mosaicism due to somatic CAG instability (Gonitel et al., 2008); however the exact mechanisms underlying these events remain unknown.

Incomplete penetrance i.e. not every individual with 36-39 CAG repeats in their \( htt \) gene will develop HD, suggests that the development of this monogenic disease is subject to the influence of non-genetic factors. In a landmark study of Venezuelan kindreds with HD, it was observed that only 40% of variability in the age of disease onset was directly attributable to an individual’s CAG repeat length (Wexler et al., 2004). That implied that non-genetic factors or alternatively ‘environment factors’ such as the level of physical exercise and educational background exerted a significantly greater effect on disease development. In agreement and further demonstrating that non-genetic factors modulate the development of HD, there has been a study of a pair of monozygotic twins suffering from HD with the same number of CAG repeats and each twin manifested different rates of disease progression while developing different disease symptoms (Georgiou et al., 1999). The significant influence of the environment has been demonstrated in rodent models of HD (discussed later) whereby the experimental paradigm of environmental enrichment and increased physical activity are both sufficient in delaying the onset and progression of disease symptoms (Carter et al., 2000; van Dellen et al., 2000; Pang et al., 2006). These results highlight the need to better understanding how mechanisms involved in the environmental modulation of HD pathology as this knowledge might open up potential avenues for the development of therapeutic interventions.

1.7.2. HD neuropathology

Despite the wide expression of the \( htt \) gene and protein throughout the mammalian body and its non-selective cellular distribution, the well-described pathology of HD is largely restricted to the brain. Broadly, post mortem HD brains have reduced weights, display obvious signs of degeneration of the cerebral cortex (including selective thinning of cortical regions) and basal ganglia, enlargement of the ventricular space as well as a loss of white matter (de la Monte et al., 1988). Atrophy of grey matter is progressive and occurs in the cerebral cortex, striatum (comprising the caudate and putamen), insula, and cerebellum. Late-stage neuronal loss is evident especially within the caudate nucleus
(Vonsattel et al., 1985) and is attributed to the initiation of programmed cell death (apoptosis) (Petersen et al., 1999) which has also been observed in some mouse models of HD (Reddy et al., 1999). The extent of neurodegeneration is correlated with disease severity, in particular the selective atrophy of the frontal lobes, caudate and thalamus (Ruocco et al., 2008).

The region typically associated with HD neurodegeneration is the striatum, where there is a selective loss of preproenkephalic (PPE) medium-sized spiny neurons corresponding with a reduction in the level of PPE mRNA (Albin et al., 1991). This decline in PPE-positive neurons has been directly correlated to disease severity. However, in the early stages of the disease, a decrease in PPE mRNA levels can already be detected in the absence of neuronal death (Richfield et al., 1995b) suggesting that morphological changes such as dysmorphic dendritic arborisation contribute to neuronal dysfunction and the early symptoms of HD (Ferrante et al., 1991). The PPE-positive striatal neurons are more vulnerable and are affected to a greater extent than a separate population of preprotachykinin (PPT) expressing striatal neurons despite an overall reduction in PPT mRNA levels (Richfield et al., 1995a). Other histological hallmarks of HD include a decrease in enkephalin immunoreactivity in the external globus pallidus (Albin et al., 1991) as well as a preferential loss of striato-external pallidal projection neurons during the pre- and early symptomatic stages while the internal projections remain relatively spared (Albin et al., 1992).

Another prominent hallmark of HD pathology is the gradual appearance of neuronal protein aggregates or inclusion bodies which are localized throughout the nucleus, cell body including the cytoplasm, dendrites, axons and synaptic terminals (Petrasch-Parwez et al., 2007). These aggregates contain N-terminal fragments of the mutant htt protein and were first observed in pre-motor symptomatic transgenic HD mice (Davies et al., 1997) before being subsequently confirmed to be present in the cortex and striatum of post mortem HD brains (DiFiglia et al., 1997; Becher et al., 1998). The formation of inclusion bodies is also an observed event in cultured cells that express the mutant htt protein
Surprisingly, aggregates occur in only about 1-4% of striatal neurons despite it being the region most affected in HD. Instead, aggregates are more commonly observed in cortical neurons. A proportion of aggregates are immuno-positive for ubiquitin, indicating that they are marked for proteolysis by the ubiquitin proteasome system (Gutekunst et al., 1999). These aggregates are formed only by the cleavage of the mutant htt protein with the expanded polyglutamine tract by endogenous caspases which results in protein fragments that aggregate (Kim et al., 1999). The extent and rate of aggregation is directly correlated with polyglutamine length and is believed to be due to misfolding of the protein fragments (Cooper et al., 1998). Cells become more vulnerable to toxicity following the accumulation of protein aggregates which implies that the presence of protein aggregates compromises the health of neurons and this is confirmed by the demonstration that delaying the appearance of neuronal inclusions leads to increased cell survival, delayed disease progression and increased survival rates in transgenic HD mice (Ona et al., 1999). However, there is also extensive evidence that aggregates per se are not neurotoxic and under some circumstances could even be neuroprotective (Arrasate et al., 2004).

1.7.3. Presymptomatic changes to the HD brain and its associated disease symptoms

While gross structural changes to the brain occur by the more advanced stages of HD, gradual neuropathology is detectable during the early stages prior to the appearance of overt symptoms. Aided by advancements in brain imaging techniques, changes in white and grey matter were uncovered in brains of preclinical HD patients. A combination of magnetic resonance imaging (MRI), voxel based morphometry (VBM) and diffusion tensor imaging (DTI) has revealed that there is a reduction in white matter volume which precedes grey matter atrophy during the presymptomatic stages of HD and that the severity of pathology is correlated with the number of CAG repeats as well as the estimated time to disease onset (Ciarmiello et al., 2006; Jech et al., 2007). The temporal reduction of white matter is not limited to a particular brain region and is evident in the frontal cortex, striatum, internal capsule and the corpus callosum (Ciarmiello et al., 2006;
Recently, the loss of cortical white matter in presymptomatic HD patients has been implicated as the main factor contributing to the impaired performance of HD patients on tasks dependant on prefrontal cortex function such as verbal working memory (Wolf et al., 2007; Wolf et al., 2008a; Wolf et al., 2008b).

MR imaging has also been repeatedly used to show that the basal ganglia undergoes a progressive volumetric reduction during the presymptomatic stages of HD, increasing with proximity to estimated disease onset (Aylward et al., 1994; Aylward et al., 1996; Aylward et al., 1997). There are dramatic reductions of caudate and putamen grey matter volume (Aylward et al., 1994; Harris et al., 1996; Aylward et al., 1997; Kipps et al., 2005) which reflect the topographical pattern of atrophy corresponding to the dorso-ventral gradient of neuronal loss (Kassubek et al., 2004). Significant volumetric changes can be detected up to ten years prior to the onset of clinical symptoms (Harris et al., 1999) and persist throughout the symptomatic stages of the disease (Aylward et al., 2000). Changes in the caudate have also been detected with positron emission tomography (PET) (Grafton et al., 1992) and are concurrent with decreased glucose metabolism (Grafton et al., 1992; Kuwert et al., 1993) and reduced cerebral blood flow measured by single photon emission computed tomography (SPECT) (Harris et al., 1996). The grey matter changes extend to other regions of the HD brain and are also prominent in the thalamus, midbrain, insula and globus pallidus (Aylward et al., 1994; Thieben et al., 2002; Kipps et al., 2005). While the functional relevance of these changes has yet to be fully appreciated, studies utilizing functional MRI (fMRI) further implicate them in the gradual decline in cognitive capabilities of presymptomatic HD subjects (Kim et al., 2004; Paulsen et al., 2004; Reading et al., 2004).

The hippocampal formation is one region of the HD brain that receives less attention perhaps due to it being less severely affected compared to the basal ganglia or cortex. It was not until recently that the overall volume of the hippocampus was examined and found to be smaller in HD brains (Geuze et al., 2005). Previously, there had only been a single study describing a reduction in neuronal density specific to the CA1 subregion in
late-stage symptomatic HD patients (Spargo et al., 1993). The hippocampus is essential for memory (reviewed by Bird and Burgess, 2008; and Colgin et al., 2008) and reduced hippocampal volume is associated with depression pathophysiology (Campbell and Macqueen, 2004). This indicates that changes in the hippocampus might be relevant to some of the cognitive and psychiatric features of HD. The development of working models of HD has facilitated the further study of HD-related hippocampal pathology and these models are discussed in the following section 1.8.
1.8. Mouse models of HD

Knowledge that the trinucleotide repeat expansion in the 5’ end of the *htt* gene caused HD (Huntington’s disease Collaborative Research Group, 1993) was subsequently followed by the development of several working models of HD that provided researchers with tools with which to better understand the molecular mechanisms involved in HD pathology. Recent advances have been made in developing larger mammalian models of HD such as a rat (von Horsten et al., 2003), non-human primate (rhesus macaque) (Wang et al., 2008; Yang et al., 2008) and ovine (sheep) (Snell RG, *personal communications*) models. However, mouse models remain the most widely used models because these reliably recapitulate the progressive, selective nature of HD neuropathology while developing similar cognitive and motor symptoms as the human condition within a practical time period (see Figure 1-2) and are easily bred which facilitates larger-scale investigations.

1.8.1. Transgenic R6 mice (‘fragment’ models)

The most popular transgenic mouse models of HD are the R6 lines. These transgenic mice ubiquitously express the 5’ end fragment of the human HD gene that contains between 115 (R6/1 line) and 150 (R6/2 line) CAG repeats (Mangiarini et al., 1996). However, it is also important to note that there is gametic and somatic instability with the repeats tending to be more instable upon paternal transmission (Mangiarini et al., 1997). That has been confirmed by recent unpublished reports of the R6/2 colonies which express more (in excess of 300 repeats) or less (~74 CAG repeats) than the original 150 CAG repeats (Morton AJ, *personal communications*). The R6 transgenic lines develop a progressive neurological phenotype including choreiform-like movement, involuntary stereotypic movements and tremor. The R6/2 line has accelerated onset and progression of disease symptoms. Motor deficits are detected at 5-6 weeks of age such as development of an involuntary stereotypic motion of the hind limbs when suspended by their tails (this behavioural phenotype is commonly termed rear-paw or hind-limb
The progressive development of the human condition can be categorized into four different stages reflecting the presence and severity of various symptoms. Often, cognitive deficit and psychiatric changes are detected in Stage I/II prior to the onset of motor deficits by stage III. The R6 lines recapitulate the appearance of cognitive deficits prior to motor impairment. As reflected in the figure, the R6/2 line is an early onset, faster progressing model compared to the R6/1 line. While motor deficits can be detected in the hdh150 knock-in line between 70-100 weeks of age, this line has not yet been evaluated for cognitive changes. The YAC128 line develops a subtle motor learning deficit at 8 weeks of age (a more extensive evaluation of cognitive ability is required for this line) and motor impairment is detected at 24 weeks of age. Note that depression-related behaviours have not been described in any of the mouse models to date.

Figure 1-2 Recapitulation of the human HD condition by various transgenic mouse models.
clasping) before the overt symptoms are more prominent by 8 weeks and death occurs by 14 weeks of age (Mangiarini et al., 1996). Due to the early and aggressive nature of disease symptoms, the R6/2 line is probably more reflective of juvenile-onset HD. In comparison, R6/1 HD mice is much slower progressing and the rear-paw clasping phenotype is not observed until the mice are ~16 weeks old (Mangiarini et al., 1996; Pang et al., 2006). R6/1 HD mice also have an extended life expectancy of up to 40 weeks of age (Mangiarini et al., 1996; Spires et al., 2004b; van Dellen et al., 2008). It is probably due to accelerated development of disease symptoms in the R6/2 line that has made it a more attractive model for experimentation compared to the R6/1 line. A search of National Center for Biotechnology Information (NCBI) database (www.pubmed.gov) reveals that 246 articles have been published citing the R6/2 mouse model compared to only 61 citing the R6/1 line. While the R6/2 model has been ideal for investigating the late and terminal stages of the disease as well as testing the effects of various compounds on late-stage pathology, the juvenile onset of aggressive neuropathological changes hinders any attempt to investigate the pre-symptomatic and early stages of disease development. For such studies, the R6/1 model is far superior and appropriate with its delayed onset and slower progression of disease symptoms in addition to offering investigators a larger window of time to examine the early stages of this disease.

Despite the huge difference between their rates of disease progression, both R6 lines are highly similar in other aspects of the disease such as displaying significant weight loss with age and disease progression while maintaining normal feeding habits and caloric intake. It has been shown that the loss of body weight is due to increased metabolism together with abnormalities of the major organ systems involved in metabolic regulation such as the hypothalamus, stomach and adipose tissue (Fain et al., 2001; van der Burg et al., 2008). Those changes might reflect the underlying reasons for the weight loss of HD patients which has been mainly associated with loss of muscle mass (Goodman et al., 2008). The hypothalamus is also involved in the regulation of water consumption and R6/2 mice were reported to have increased drinking activity correlating with decreased vasopressin immunoreactive neurons in the paraventricular nucleus of the hypothalamus (Wood et al., 2008). However, a contradictory increase in the level of serum vasopressin
was found in HD subjects by the same study despite signs of greater xerostomia (sensation of dry mouth). It is still not clear how the neural circuitry of thirst is altered in the HD brain and further investigations will be required. Interestingly, in the absence of any evidence for the R6/1 line (Josefsen et al., 2008) or HD patients (Wood et al., 2008), the R6/2 line has been shown to develop diabetes mellitus. R6/2 HD mice have higher blood glucose levels by 12-weeks of age coupled with dramatic reductions of glucagon and insulin (Hurlbert et al., 1999). This disturbance of glucose homeostasis has been attributed to a reduction of islet beta-cell mass compounded by disrupted exocytosis of insulin containing vesicles (Bjorkqvist et al., 2005). However, HD patients have normal levels of insulin expression and histologically normal pancreatic islets (Bacos et al., 2008) and do not have protein aggregates forming in the pancreatic beta cells unlike the R6/2 mice (Hunt and Morton, 2005). Therefore, it appears that the occurrence of diabetes is isolated to the R6/2 mouse line and is distinct from HD pathology. Further alterations in the hypothalamic-pituitary-adrenal axis have been described in the R6/2 line (Bjorkqvist et al., 2006) that mirror previously reported endocrine changes in HD patients (Heuser et al., 1991; Leblhuber et al., 1995; Markianos et al., 2007), but those findings have yet to be replicated.

The neuropathology observed in the brains of both R6/1 and R6/2 transgenic mice is consistent with human HD. HD brains weigh less and exhibit a loss of cerebral and striatal volume in the absence of neuronal death (van Dellen et al., 2000; Hockly et al., 2002; Stack et al., 2005; van Dellen et al., 2008). There is also shrinkage of specific brain regions such as the anterior cingulate cortex and the hippocampus, the latter accompanied by a progressive disruption of adult neurogenesis in the dentate gyrus (Lazic et al., 2004; Grote et al., 2005). It was in these transgenic mouse models that the existence of neuronal intranuclear inclusions was first detected (Davies et al., 1997; Kosinski et al., 1999; Meade et al., 2002) before subsequent confirmation in the human brain (DiFiglia et al., 1997). The R6 lines progressively develop cognitive and motor impairments similar to the symptoms of the human condition. R6/2 HD mice are impaired on cognitive tasks that are dependent on frontal cortex and hippocampal function before 8 weeks of age (Lione et al., 1999) which reflects the preclinical cognitive changes in human HD. Similarly, 12-
week old R6/1 HD mice develop an inability to perform hippocampal-dependant cognitive tasks before displaying signs of motor symptoms by 16 weeks of age (Nithianantharajah et al., 2008). In the absence of cell death until the advanced-stages of the disease, it is quite evident that most HD symptoms are a result of neuronal dysfunction which is represented by broad disruptions of the dopaminergic (Cummings et al., 2006; Kung et al., 2007), glutamatergic (Usdin et al., 1999) and cholinergic signaling systems (Picconi et al., 2006) during the early stages of HD progression in HD transgenic mice. Together with evidence of dendritic spine pathology in the HD cerebral cortex, striatum and hippocampus (Spires et al., 2004b; Lazic et al., 2006), all these alterations translate into altered neocortical function and impaired neuronal plasticity which are well-documented occurrences in both R6 lines and directly related to the development of motor and cognitive impairments as well as the behavioural abnormalities (Murphy et al., 2000; Centonze et al., 2001; Cybulksa-Klosowicz et al., 2004; Mazarakis et al., 2005; Milerwood et al., 2006; Picconi et al., 2006; Cummings et al., 2007; Lazic et al., 2007; Walker et al., 2008).

The selective nature of HD pathology and impairment of neuronal function has been attributed to altered gene expression and protein trafficking in the HD brain e.g. BDNF (Canals et al., 2004). Changes in gene expression have been consistently demonstrated in the cerebral cortex, striatum (Luthi-Carter et al., 2000; Luthi-Carter et al., 2002b; Hebb et al., 2004), hippocampus (Luthi-Carter et al., 2003; Obrietan and Hoyt, 2004) as well as in other brain regions including the thalamus (Kusakabe et al., 2001) and cerebellum (Deckel et al., 2002; Luthi-Carter et al., 2002b) of both R6/1 and R6/2 mouse lines. It is important to note that these alterations are not uniform across the brain and also share the regional selectivity of neuropathology. Many of the changes in gene expression are detected in the brains of presymptomatic R6 mice which indicate that transcriptional dysregulation is a key process contributing to HD pathology. Several genes identified as having reduced expression are crucial components of cellular signaling cascades such as BDNF (Zuccato et al., 2001), the various subunits of the NMDA receptor (Luthi-Carter et al., 2003), dopamine D1 and D2 receptors, the adenosine A2A receptor (Luthi-Carter et al., 2002b) and protein kinase C-β II (Harris et al., 2001). The alteration of gene expression is similarly reflected at the protein level although there are certain exceptions
which could be informative of the mechanisms underlying HD pathology. For example, R6/1 mice have reduced BDNF mRNA levels in the anterior cortex despite elevated protein levels (Pang et al., 2006). As mentioned above, the htt protein is involved in vesicular transport so one interpretation of the abnormal accumulation of BDNF in the anterior cortex is that there is a disruption of protein transport in addition to transcriptional deficiency. Disrupted gene expression ultimately results in a disturbance of the downstream effects of receptor-mediated signaling, such as the induction of immediate early gene expression (Spektor et al., 2002). There have been reports that the restoration of gene expression is beneficial and ameliorates HD disease symptoms (Gharami et al., 2008) so specific targeting of those particular genes would be an appealing strategy for the development of HD therapeutics.

In summary, the R6 mouse lines recapitulate key facets of HD neuropathology and symptomatology, and as such are important media for the development and assessment of potential therapeutic treatments. They are currently the most popular and widely used working model of HD and are a vital tool in the ongoing investigation of the molecular changes that contribute to the development of this disease.

### 1.8.2. Knock-in HD models

The *mus musculus* genome expresses a homologue of the human *HTT* gene on chromosome 5 (Barnes et al., 1994). The murine gene (*Htt*) is highly conserved and shares a high level of identity (85-91%) with the human DNA coding sequence and protein. There are only 7 CAG repeats in *Htt* compared to 13-35 in normal human *htt* and the insertion of a much longer repeat expansion of 70-150 CAG repeats (several lines expressing different lengths have been produced) into the *Htt* gene elicits a HD-like behavioural phenotype in these ‘knock-in’ mice (Wheeler et al., 1999; Lin et al., 2001; Menalled et al., 2003). These mutant hdh knock-in mice have altered energy metabolism profiles (Gines et al., 2003), deficits in implicit and motor learning (Trueman et al., 2007, 2008), and exhibit an age-dependent, late-onset motor phenotype with significant gait
abnormalities (Lin et al., 2001; Menalled et al., 2002; Wheeler et al., 2002; Menalled et al., 2003; Heng et al., 2007). Consistent with human HD neuropathology, the mice develop neuroanatomic abnormalities such as reactive gliosis and increased glial fibrillary acidic protein (GFAP) immunoreactivity in the striatum (Lin et al., 2001) and the hallmark formation of neuronal intranuclear inclusions predominantly in the striatum which progresses from diffuse nuclear localization of htt protein observed within striatal neurons, formation of htt immunoreactive NIIs before a significant loss of striatal neuron numbers (Menalled et al., 2002; Menalled et al., 2003; Tallaksen-Greene et al., 2005). An abundance of NIIs are also found throughout the cortex e.g. layers III and IV of the somatosensory cortex and in layer II of the piriform cortex. The knock-in lines also have a loss of striatal dopamine D1 and D2 receptor binding sites which precedes the appearance of gross striatal pathology (Lin et al., 2001; Heng et al., 2007) and striatal neuronal dysfunction (Miller et al., 2008), further recapitulating other features of the human condition. Similar to the R6 models, altered gene and protein expression is also detected in mutant Hdh knock-in mice (Fossale et al., 2002; Strand et al., 2005; Gines et al., 2006; Kuhn et al., 2007; Woodman et al., 2007) but due to slower rates of disease onset and progression of these knock-in models, the faster progressing R6 models remain the working models of choice for longitudinal studies and preclinical trials.

1.8.3. Yeast artificial chromosome models

Yeast artificial chromosome (YAC) libraries were useful screening platforms during the process of localizing the huntingtin gene prior to its identification (Bates et al., 1992; Youngman et al., 1992). Several lines of transgenic mice have been developed that express different lengths of polyglutamine expansion by means of YAC (Hodgson et al., 1999; Van Raamsdonk et al., 2007) and have been demonstrated to be accurate models of human HD as they show a similar progressive development of cognitive, motor deficits and neuropathology with the human condition. These mice have cognitive impairments which precede motor deficits which progressively worsen with age (Van Raamsdonk et al., 2005) and show remarkable similarities in gene expression changes in the striatum compared to the other mouse models of HD (Kuhn et al., 2007). Mutant YACHD mice
display the typical signs of HD-related pathology with degeneration of striatal medium-sized spiny neurons, translocation of N-terminal htt protein fragments to the nucleus and formation of nuclear inclusions. In the striatum, electrophysiological studies have demonstrated that there is early disruption of glutamatergic signaling (Milnerwood and Raymond, 2007) in YACHD mice. The augmentation of post-synaptic currents mediated by the NMDA receptor is believed to be involved in the selective degeneration of the medium-sized spiny neurons. NMDA receptor binding is also increased in YACHD mice (Benn et al., 2007) and there are higher levels of NR1 and NR2B subunits localized to the synaptic membrane (Fan et al., 2007). YACHD MSSNs display heightened sensitivity of the NR2B-subtype NMDA receptor (Zeron et al., 2002) which results in greater intracellular calcium levels during synaptic activity (Tang et al., 2005). The presence of greater levels of calcium then contribute to increased susceptibility to NMDA-mediated excitotoxicity through the activation of a mitochondrial-associated apoptotic pathway (Zeron et al., 2004; Fernandes et al., 2007). Recently, another transgenic mouse model based on a bacterial artificial chromosome (BAC) with 97 CAG repeats in the htt sequence has been developed which again develops the predicted progressive and selective neuropathology together with motor deficits despite the absence of mutant protein aggregation (Gray et al., 2008). More work is required to further characterise this new and alternative model of human HD.

1.9. Altered molecular biology in HD

It is still unclear how a single gene mutation can lead to such specific neurodegeneration. In fact, the precise pathological mechanism initiated by expression of the mutant htt protein remains largely unknown. Initially, it was thought that the expanded tract interfered with normal protein function; however this ‘loss of function’ hypothesis was found to be highly unlikely since individuals with only one copy of the HD gene do not develop the HD phenotype while individuals homozygous for the HD expansion do not display increased severity of the disease (Gusella and MacDonald, 1994). There is now evidence that the polyQ expansion constitutes a toxic ‘gain-of-function’ as further discussed below.
1.9.1. Altered gene expression and transcription factor-interaction

The use of DNA microarray technology has enabled high throughput screening of gene expression in the HD brain. Studies to date have consistently reported a multitude of gene expression changes detectable in the cortex, striatum and cerebellum of brains from HD mouse models and HD patients (Luthi-Carter et al., 2000; Luthi-Carter et al., 2002b; Crocker et al., 2006; Desplats et al., 2006; Hodges et al., 2006; Kuhn et al., 2007). The dysregulation of gene expression has been demonstrated to be solely dependent on the expression of the mutant htt protein in an inducible striatal cell model of HD (Sipione et al., 2002). That same study also found that transcriptional changes were an early pathogenic event prior to aggregate formation and cell death, and the expression of genes involved in cell signaling, transcription and vesicle trafficking were the first to be altered. Recently, chromosomal profiling has revealed that transcriptional dysregulation occurs in a coordinated fashion across large genomic regions which partially accounts for how large clusters of genes are affected in HD and further suggests that the mutant htt protein has an effect on chromatin structure (Anderson et al., 2008). Interestingly, the extent of transcriptional dysregulation is inversely proportional to the length of the repeat mutation (unlike its relationship with predicted age of disease onset) with fewer changes in gene expression associated with a longer length of polyglutamine repeat expansion (Chan et al., 2002). While the changes in gene expression identified thus far have largely been limited to the brain, attempts at identifying peripheral blood-borne biomarkers of HD have also found evidence of mRNA changes but these are only detectable during the late, symptomatic stages of the disease (Borovecki et al., 2005; Runne et al., 2007).

As mentioned previously, the wild-type htt protein facilitates BDNF gene transcription through promoter II by inhibiting the repressive RE1/NRSE element within the promoter region. In contrast, mutant htt protein represses BDNF gene expression at all promoters examined to date (Zuccato et al., 2001; Pang et al., 2006). This has been found to be due to an abnormal interaction of the mutant htt protein with REST/NRSF resulting in reduced repressional activity of REST/NRSF (Zuccato et al., 2007). It was confirmed when the expression of a dominant negative-construct of REST/NRSF with an attenuated
mutant htt-REST/NRSF interaction (but still maintaining a DNA binding domain) was found to be sufficient to restore BDNF mRNA levels back to wild-type levels in cell culture (Zuccato et al., 2007). Given that the loss BDNF expression is implicated in the development of the devastating motor symptoms of HD, identifying a means to restore the normal regulatory capacity of REST/NRSF in the presence of mutant htt protein could be a potential treatment option of huge impact.

Altered gene expression in HD is also the direct consequence of abnormal interactions of the mutant htt protein with various transcription factors. The cleaved, soluble N-terminal fragments of the mutant htt protein have been found to bind the transcription activator Sp1 which results in decreased binding of Sp1 to the promoters of susceptible genes in HD (Dunah et al., 2002; Chen-Plotkin et al., 2006; Cornett et al., 2006). Sp1 is an oxidative stress-induced transcription factor that protects cortical neurons against oxidative stress and DNA damage (Ryu et al., 2003). Therefore it is plausible that this disruption of Sp1-mediated gene expression plays a major role in inducing the neuronal dysfunction that leads to cortical degeneration. Interestingly, cells expressing the mutant htt appear to up-regulate Sp1 expression as a means of compensation and the suppression of this Sp1 over-activity has been shown to be beneficial (Qiu et al., 2006). The reasons for this contradictory effect of Sp1 expression is not known at present.

The insoluble fragments of the mutant htt protein aggregate and form neuronal intranuclear inclusions which are able to sequester a variety of transcription factors including TATA-binding protein (TBP) (van Roon-Mom et al., 2002), Sp1, CREB-binding protein (CBP) and p53 (Steffan et al., 2000; Nucifora et al., 2001; Dunah et al., 2002). The resulting disruption of transcriptional activity and repression of gene expression (e.g. reduced proenkephalin mRNA levels) culminates in neuronal toxicity in a process that also involves histone hypo-acetylation which is an epigenetic modification associated with inactivation of transcription activity (Jiang et al., 2006; Ryan et al., 2006). Similar to Sp1, p53 expression has been found to be increased in the brains of HD patients, N171-82Q transgenic mice and immortalized striatal neurons originating from
mhtt knock-in mouse embryos (Trettel et al., 2000; Bae et al., 2005). However, unlike Sp1 which is anti-apoptotic (Ryu et al., 2003), the elevation of p53 expression is likely to be involved in late-stage cell loss since the over-expression of p53 elicits neuronal death in neuronal cultures (Jordan et al., 1997). p53 functions as a tumor suppressor gene and initiates apoptosis (Clarke et al., 1993), however, in a paradoxical manner, the alteration of p53-mediated cellular signaling in HD (Song et al., 2002) has been proposed to contribute to the extremely low incidence of cancer in HD patients (Sorensen et al., 1999).

1.9.2. Altered receptor-mediated gene transcription in HD

The mechanisms through which the mutant htt protein exerts an effect on gene expression are gradually coming to light. One possibility which is consistent with the occurrence of neuronal dysfunctional as the main pathogenic event in HD is that the presence of mutant htt disrupts normal neurotransmission and this in turn alters the down-stream cellular response which would usually involve activate gene transcription. Dopaminergic neurotransmission is well-documented to be altered in the HD brain (Bibb et al., 2000), probably as a result of altered dopamine D1 and D2 receptor mRNA levels in the striatum (Augood et al., 1997; Cha et al., 1998). As such, reduced expression levels of immediate early genes are detected in the HD mouse, compared to the wild-type, striatum following antagonistic-stimulation of D2-receptor mediated signaling cascades while D1-receptor agonism induces wild-type-equivalent levels of IEG mRNA despite reduced D1-receptor expression (Spektor et al., 2002).

1.9.3. Altered neurotransmission in HD

Dopaminergic signaling is altered in the striatum and cortex of the HD brain and disruption of dopaminergic neurotransmission is believed to be strongly involved in the development of the motor symptoms of HD (Hickey et al., 2002; Gantois et al., 2007). The levels of dopamine are reduced in the striatum of late-stage R6/2 transgenic mice and late-stage HD brains (Reynolds et al., 1999), corresponding with a decrease in the expression and enzymatic activity of the rate limiting enzyme for dopamine biosynthesis,
tyrosine hydroxylase (Yohrling et al., 2002). Striatal levels of dopamine are unexpectedly elevated during the earlier stages of the disease and have been suggested to act synergistically with glutamate via the D1 receptor to induce the elevated calcium signals required to initiate apoptosis (Tang et al., 2007). It is possible that in an attempt to counter-balance the abnormal elevation of dopaminergic signaling, there is down-regulation of striatal dopamine receptors and reduced release of dopamine-containing vesicles from synaptic terminals (Johnson et al., 2006; Heng et al., 2007). This dampening of dopaminergic signaling is then detected as reduced dopamine-dependent striatal LTP in the R6/2 mouse model (Kung et al., 2007). While the role of dopamine in cortical degeneration is not as prominent as its role in striatal loss, abnormal dopamine receptor signaling has been implicated in altered cortical plasticity in HD mice (Cummings et al., 2006; Cummings et al., 2007) although more work will be required to conclusively demonstrate this.

The selective vulnerability of striatal gamma-aminobutyric acid (GABA)ergic medium-sized spiny projection neurons has been attributed to an eventual increase in the frequency of spontaneous inhibitory postsynaptic GABAergic currents (sIPSC) (Cepeda et al., 2004) while spontaneous excitatory postsynaptic currents (sEPSC) remain unchanged (Centonze et al., 2005). In the absence of a change in GABA receptor binding (Benn et al., 2007), these HD-related alterations of GABAergic current suggests that the conductance of GABA is increased in the medium-sized spiny neurons and this has recently been demonstrated to be so (Ade et al., 2008). The source of this disruption of GABAergic signaling is still unknown although, interestingly, bath application of exogenous BDNF on striatal slices is sufficient to significantly reduce the frequency of the sIPSC (Cepeda et al., 2004).

Little else is known about serotonergic signaling in the HD brain and its association with disease symptoms and pathology. There have been several studies of blood concentration levels of serotonin (5-HT, 5-hydroxytryptophan) which offer conflicting results, partly due to the small numbers of HD patients examined. Several of these studies report higher
5-HT levels in the blood of HD patients (Belendiuk et al., 1980; Ehsanullah and Turner, 1981) while others (including the most recent publication) have found no change (Tukiainen et al., 1981; Christofides et al., 2006). However, there is some evidence to suggest that tryptophan (the essential amino acid from which 5-HT is synthesized) metabolism is impaired in HD patients. Following tryptophan depletion, HD patients respond normally with reduced levels of blood 5-HIAA whilst maintaining 5-HT levels (Christofides et al., 2006). However, upon tryptophan loading, HD patients do not respond appropriately with the increase in 5-HT and 5-HIAA levels indicating that the production of 5-HT is affected in HD. It is important to note that these observations involve peripheral measures and it is still uncertain whether the peripheral activity of tryptophan hydroxylase is altered in HD.

In the R6/2 brain, there is a decrease in the bioactivity of tryptophan hydroxylase and, as expected, there are reduced levels of 5-HT and 5-HIAA (Reynolds et al., 1999; Yohrling et al., 2002). While a reduction in the bioavailability of this key neurotransmitter is sufficient to impair signaling, there is also a concurrent decrease in the density and binding efficiencies of various 5-HT receptors including the 5-HT 1A and 1B receptors as observed in R6/2 mice and post-mortem HD brains (Waebler and Palacios, 1989; Gonzalez-Heydrich and Peroutka, 1991; Steward et al., 1993; Castro et al., 1998; Yohrling et al., 2002), further potentiating the disruption of serotonergic signaling in the HD brain.

Coincidentally, the dysregulation of the serotonergic signaling system is implicated in the pathophysiology of depression which is a common feature of presymptomatic HD (discussed later). Antidepressant drugs (e.g. the selective serotonin reuptake inhibitor sertraline) are routinely used to treat the psychiatric symptoms of HD (Ranen et al., 1996; Como et al., 1997) and the benefits of this class of drugs have more recently been demonstrated in the N171-82Q and R6/1 mouse models which respond with delayed disease onset and progression (Duan et al., 2004; Grote et al., 2005). Future investigations are needed to specifically examine the relevance of disrupted serotonergic
signaling to the development of depression in HD and address the effectiveness of antidepressant drugs in treating HD-related depression.

1.9.4. Altered electrophysiological responses in HD

The accurate spatiotemporal recapitulation of the molecular and neurological changes in the HD brain across models of HD has facilitated the better understanding of disease pathology, especially given the limited scope for such investigations in live human subjects. Altered electrophysiological responses and profiles reflect HD-related changes in neuronal biology and can be detected following exposure to various stimuli. The altered stimulus-induced neuronal responses are not limited to particular sub-regions of the brain and can be detected across the broad cortical regions, striatum and hippocampus. For example, measurements of the electrophysiological responses in the somatosensory cortex initiated by passive sensory stimulation of the median and tibial nerves consistently demonstrate the occurrence of reduced cortical signaling of early-stage HD patients (Noth et al., 1984; Abbruzzese et al., 1990; Kuwert et al., 1993; Beniczky et al., 2002; Lefaucheur et al., 2002). There is progressive deterioration of the response amplitude (Lefaucheur et al., 2006), the extent to which correlates with the length of the repeat expansion (Beniczky et al., 2002) and is associated with abnormal activation of subcortical regions such as the basal ganglia (Boecker et al., 1999). In yet another remarkable demonstration of the precision of the disease models, somatosensory cortical plasticity deficits have also been reported in the R6/1 mouse model of HD (Cybulska-Klosowicz et al., 2004; Mazarakis et al., 2005).

Based on studies of the mouse models, the HD hippocampus has also been shown to develop gradual deficits in long-term potentiation (LTP) and long-term depression (LTD) (Murphy et al., 2000; Milnerwood et al., 2006; Lynch et al., 2007) with the reduction in LTP being attributed to a disruption in the transmission of excitatory synaptic signaling, possibly due to altered trafficking of NMDA receptor subunits (Usdin et al., 1999; Fan et al., 2007) similar to the changes detected in MSSNs. The onset of these
electrophysiological changes has been shown to coincide with the progressive impairment of spatial cognitive ability and corresponds with the well-established principle that LTP and LTD are essential components of hippocampal-dependent learning and memory (Murphy et al., 2000) but various other molecular changes at hippocampal synapses could also be contributing to the observed abnormalities of synaptic plasticity (Cummings et al., 2006).

1.9.5. Altered BDNF signaling in HD

The loss of BDNF in the HD brain is central to the development of disease symptoms and pathology. As mentioned previously, the wild-type htt protein has a regulatory role in BDNF gene transcription (see 1.6.1) and mediates the dynamics of microtubule vesicular trafficking of BDNF protein (see 1.6.3). Presence of the mutant htt protein with its expanded polyglutamine tract results in reduced BDNF mRNA levels in the cortex by repressing gene expression across all promoter regions examined to date (Zuccato et al., 2001; Zuccato et al., 2005) and impairs anterograde cortico-striatal transport of BDNF protein (Gauthier et al., 2004). This decline in supply of cortically-derived BDNF protein to the striatum constitutes a loss of trophic support to the striatal neurons which is specifically implicated in the striatal pathology that leads to the development of HD motor symptoms (Baquet et al., 2004; Canals et al., 2004). Further proof that changing levels of cortically-derived BDNF in the striatum are associated with the motor symptoms was demonstrated when a study ameliorated the motor symptoms in a mouse model of HD by selective restoration of BDNF gene expression in the cortex alone (Gharami et al., 2008). While the loss of BDNF accounts for the striatal pathology, the peculiar vulnerability of striatal neurons in HD has yet to be fully explained. Current understanding of this is limited to knowledge that it is independent of striatal BDNF protein levels and solely due to expression of the mutant htt protein by striatal neurons since the gene expression changes associated with HD are not abolished upon normalization of striatal BDNF protein levels (Brown et al., 2008).
The central reduction of BDNF levels is accompanied by peripheral changes detected in the blood. HD patients have been shown to have lower serum BDNF levels than healthy non-HD controls which is a common pathological observation in depression (Ciammola et al., 2007). Low serum BDNF levels in HD patients are further associated with longer CAG repeat lengths and longer duration of illness. In mouse and rat models of HD, progressive changes in BDNF mRNA levels are observed to reflect disease progression (Conforti et al., 2008); however, further investigation will be required to understand the functional consequence of these changes.

The loss of BDNF expression due to the transcriptional changes and resulting down-regulation of BDNF mRNA levels is further compounded by the impact of the mutant protein on vesicular transport of BDNF along microtubules from the cortex to the striatum as transport dynamics are altered (Gauthier et al., 2004; Colin et al., 2008). In addition, the dynamics of vesicular-release and post-Golgi trafficking of BDNF are also compromised (Gauthier et al., 2004; del Toro et al., 2006) and it is hypothesized that these events are collectively represented by an abnormal accumulation of BDNF protein in the cortex during the early stages of HD (Pang et al., 2006).

Neuronal synaptic plasticity mediates essential motor and cognitive function and is altered in HD (reviewed by Di Filippo et al., 2007). Electrophysiological recordings from R6/2 HD mouse hippocampal slices demonstrate progressive disturbances of CA1 long-term potentiation (LTP) which are detected in the dentate gyrus at a later age (Murphy et al., 2000). These changes in hippocampal synaptic plasticity are associated with impaired learning and memory reflected by poor performance of these mice in the Morris Water Maze. The age-dependant perturbation of hippocampal synaptic plasticity is also evident in the R6/1 mouse model (Milnerwood et al., 2006). The mechanisms involved in the deterioration of hippocampal LTP induction in HD slices has until recently been unknown. It has now been demonstrated that altering the level of BDNF protein in the hippocampus is beneficial and reverses the reduction in LTP facilitation as well as the loss of activity-dependant actin polymerization in dendritic spines associated with signal
consolidation (Lynch et al., 2007). Taken together, altered hippocampal BDNF levels appear to be associated with the presymptomatic development of cognitive symptoms. Interestingly, while environmental enrichment and wheel-running have been shown to delay the onset of hippocampal-dependent cognitive deficits in HD mutant mice (Pang et al., 2006; Nithianantharajah et al., 2008), the effects appear to be independent of an increase in hippocampal BDNF protein levels (Spires et al., 2004a; Pang et al., 2006) which indicates that the environmental effects are BDNF-independent. Further work will be required to identify the alternative means by which the environment modulates cognitive performance.

It is clear that alterations in BDNF expression are linked to the development of cognitive and motor symptoms of HD (see Figure 1-3). Furthermore, increased BDNF expression is associated with the slowing of disease progression in mouse models following caloric-restriction (Duan et al., 2003) and environmental enrichment (Spires et al., 2004a). Therefore, rescuing or delaying BDNF depletion in the HD brain may be a useful therapeutic avenue to explore. Coincidentally, BDNF is also implicated in the pathology and treatment of depression, which is a common psychiatric ailment of HD patients, so further investigation is required to better understand the relationship of BDNF with the development of depression in HD.
Figure 1-3 An overview of the relationship between disrupted BDNF gene transcription and the symptoms of HD.

BDNF gene expression is disrupted in the HD brain, resulting in reduced BDNF protein levels in the cortex and hippocampus. Reduced cortical BDNF levels coupled with altered vesicular trafficking leads to a loss of neurotrophic support to the striatal neurons. As a result, there is striatal neuronal dysfunction and this is associated with the development of the motor symptoms. The reduction of hippocampal BDNF levels also contributes to neuronal dysfunction associated with the cognitive deficits. Another consequence is the impairment of hippocampal cell proliferation and neurogenesis. Interestingly, running (an activity which boosts BDNF levels) does not exert the same down-stream effects of up-regulating hippocampal BDNF gene expression and neurogenesis in the HD brain (based on the R6/2 mouse model) as has been demonstrated in wild-type brains.
Towards a better understanding of depression in HD

While having long been regarded as a disorder of the motor system, the current clinical diagnosis of HD also involves the recognition of additional cognitive deficits and psychiatric alterations. In some regards, HD is a neuropsychiatric disorder of great diversity that develops into a motor condition. The broad spectrum of cognitive changes that precede motor symptoms include attention deficits, impaired spatial working memory, loss of executive function and dementia (Lawrence et al., 1996; Lawrence et al., 1998; Lawrence et al., 2000; Watkins et al., 2000; Lemiere et al., 2004; Finke et al., 2006; Marshall et al., 2007; Solomon et al., 2007). The decline in cognitive ability has been linked to a disruption of cellular signaling reflected by abnormal EEGs elicited during memory activation (van der Hiele et al., 2007) together with gross shrinkage of the frontal cortex and hippocampus which are involved in memory processing. Interestingly, HD patients have an impairment in the recognition of negative emotions, in particular of disgust (Sprengelmeyer et al., 1996; Gray et al., 1997; Hennenlotter et al., 2004; Johnson et al., 2007b), but the neural basis for this deficit is not known.

Besides the cognitive changes, the wide range of psychiatric manifestations prior to clinical diagnosis contributes to the overall complexity associated with the initial stages of disease commencement. The frequency of depressive symptoms is significantly greater in HD gene carriers, often co-developing with other psychiatric symptoms such as anxiety and obsessive-compulsiveness (Duff et al., 2007; Julien et al., 2007; Marshall et al., 2007). The clinical prevalence for depression in the HD population is estimated at 40-50% (Slaughter et al., 2001; Paulsen et al., 2005) and increases with closer proximity to clinical onset (Julien et al., 2007). The presence of depressive symptoms has been associated with greater impairment of working memory in presymptomatic HD patients (Nehl et al., 2001) and associated with a more rapid decline in functional ability (Marder et al., 2000). Subtle subclinical psychiatric symptoms can be detected in pre-diagnosed HD patients more than 10 years before estimated HD diagnosis. Therefore, the manifestation of this psychiatric feature could potentially be used as a biomarker to differentiate between non-carriers and pre-clinical gene carriers who are either symptom
free or have minor non-specific motor abnormalities. However, an analysis of longitudinal data will be required to determine the reliability and accuracy of this measure.

It has become increasingly obvious that there is a need for greater awareness of these non-physical symptoms as there have been several reported cases of misdiagnosis when individuals were wrongly diagnosed as suffering from depression or schizophrenia before eventually being found to be HD-gene positive after behavioural and neurological deterioration (Hall et al., 1989; Tost et al., 2004). The incidence of such events should decrease following the increased availability of genetic testing. However, there is still the potential for HD-associated psychiatric symptoms to be overlooked in the instance of adopted individuals when biological family history is unknown. Predictive genetic testing has been argued to contribute to the increased incidence of depression in HD which is viewed as an understandable psychological response to the knowledge of carrying a fatal gene mutation. This may be true to some extent and there is evidence to suggest that predictive testing is not psychologically benign (Codori et al., 2004). However, in the absence of accurate records of the prevalence and frequency of depression in HD prior to the option of predictive testing, it is almost impossible to conclude whether depression is more common in individuals who are unaware of being at risk for HD (e.g. adopted individuals whose biological parents are positive for HD).

The hippocampus is a common region of interest implicated in both depression pathology and cognitive ability, in particular for functional memory. Upon examination, there is a striking similarity between the brains of depressed and early-stage HD subjects. Imaging studies have found that HD and depressed subjects have reduced hippocampus volumes (Geuze et al., 2005). The former is further substantiated by similar reports of reduced hippocampal volumes in late-stage R6/1 HD mice (Grote et al., 2005). The changes in the mouse HD brain have been proposed to be a direct result of impaired dentate gyrus cell proliferation and neurogenesis which have been demonstrated in various HD models (Lazic et al., 2004; Gil et al., 2005; Grote et al., 2005; Phillips et al., 2005; Lazic et al.,
Interestingly, studies of cell proliferation in post mortem HD brains have found increased neurogenesis and gliogenesis in the subependymal layer adjacent to the caudate nucleus, suggesting that this process could be in response to the late-stage neurodegeneration occurring in the HD brain (Curtis et al., 2003).

The impairments of hippocampal cell proliferation and neurogenesis in the R6/1 mouse model of HD have been demonstrated to be rescued by treatment with the selective serotonin reuptake inhibitor fluoxetine and housing in environmentally enriched conditions (Grote et al., 2005; Lazic et al., 2006). The behavioural effects of antidepressant administration are also associated with increased hippocampal neurogenesis (Malberg et al., 2000; Duman et al., 2001; Malberg and Duman, 2003; Santarelli et al., 2003; Malberg, 2004) which raises the question whether environmentally-mediated increases in hippocampal neurogenesis (Kempermann et al., 1997) would exert an ‘anti-depressive’ effect on HD mice as has been demonstrated in other rodent models of depression (Brenes Saenz et al., 2006; Koh et al., 2007; Brenes et al., 2008). Additionally, an increase in hippocampal neurogenesis is associated with the ‘anti-depressive’ effects of running in rodents (Bjornebekk et al., 2005), which raises the possibility that a deficit in hippocampal neurogenesis contributes to the development of depression in HD and that wheel-running could also impart a behavioural effect on HD mice via an up-regulation of hippocampal neurogenesis.

The expression of forebrain BDNF has also been demonstrated to be essential for normal hippocampal function (Monteggia et al., 2004) and it is therefore presumed that the disruption of BDNF production in both conditions impact on hippocampal cell function and health and contributes to the volumetric reductions observed. An up-regulation of BDNF in the hippocampus by antidepressant treatment and environmental enrichment (Nibuya et al., 1995; Young et al., 1999) facilitates the increase in neurogenesis (Sairanen et al., 2005; Scharfman et al., 2005). In contrast, wheel-running which also up-regulates hippocampal BDNF expression (Russo-Neustadt et al., 1999; Johnson et al., 2003) and neurogenesis (van Praag et al., 1999a) in wild-type rodents does not appear to have the
same effect in R6/2 HD mice (Kohl et al., 2007). It is unclear why these treatment paradigms would differ in their down-stream cellular outcomes in the HD brain. One possibility that has been raised is that the presence of the mutant huntingtin protein impairs the neurogenic process as well as the production and secretion of BDNF into the local hippocampal environment. The exact mechanisms have yet to be uncovered and further work will be required to demonstrate this.

Altered 5-HT 1A receptor binding implicated in depression (reviewed by Drevets et al., 2007) and differences are also detected between females and males in receptor binding in the hippocampal and cortical regions (Jovanovic et al., 2008). Perturbed 5-HT 1A receptor binding has been reported in the hippocampus and cortex of R6/2 HD mice (Yohrling et al., 2002) which is consistent with a loss of forebrain BDNF expression (Hensler et al., 2007). It is interesting to note that two independent lines of forebrain-specific BDNF knockout mice show clear gender differences with the females displaying greater depressive-like behaviours than males (Monteggia et al., 2007). Those differences could be attributed to sex hormone-specific effects since it has been shown that estrogen has the ability to regulate BDNF expression in the cortex and hippocampus (Bora et al., 2005). No sex differences in behavioural phenotype or molecular changes have been reported in the HD literature as yet but gender will be an important consideration for future analyses of HD-related depression.
1.11. Overall summary

Huntington’s disease is a complex brain disorder with multiple gene expression and cellular changes (see Figure 1-4) contributing to the range of symptoms that develop. Of the spectrum of symptoms, the psychiatric changes are least understood, in particular depression which is the most commonly diagnosed neuropsychiatric feature of HD patients. Further investigation of the molecular changes underlying the development of depression during the presymptomatic stages of HD is warranted.

This thesis explores the use of the R6/1 transgenic HD mice as a model of presymptomatic depression in HD. A variety of behavioural tests will be used to explore depression-related behaviour in rodents in combination with the administration of different antidepressant drugs. The effects of environmental modulation enforced by enrichment or wheel-running on depression-related behaviour will also be examined. Finally, the expression of BDNF and various 5-HT receptors in the HD brains will be quantified to investigate whether changes in specific receptors or transcripts are associated with a behavioural phenotype.

This thesis will show that prior to the development of motor symptoms, R6/1 HD mice develop a phenotype reflective of depression-related behaviour. The behavioural phenotype is more distinct in female HD mice than in the males, suggesting a sexually dichotomous development of mood disorders in HD. The specificity of the HD-related behavioural phenotype will be demonstrated by the normalization of only the female HD behaviour by environmental enrichment and wheel-running while having no effect on male HD or wild-type mice. Close examination of the hippocampus will reveal that a dramatic deficit in cell proliferation is present in the HD brain at a time when hippocampal volume remains unchanged suggesting that changes in hippocampal volume might be due to a reduction in neuron replacement. Also, previous demonstrations of improved memory of HD mice by wheel-running will be shown to be independent of increases in hippocampal cell proliferation. Quantification of mRNA levels of exon-
Figure 1-4 Altered cellular biology in the presence of mutant htt protein.

(A) In the cytoplasm, normal wild-type htt protein is involved in vesicular trafficking of BDNF along microtubules and regulates caspase-3 mediated cell death. In the nucleus, htt protein regulates gene transcription by its interactions with transcription factors e.g. REST/NRSF and Sp1. (B) N-terminal fragments of mutant htt protein aggregate in the cytoplasm and nucleus. The nuclear aggregates sequester various transcription factors while soluble fragments bind to Sp1. Collectively, together with abnormal interactions of mutant htt protein with REST/NRSF, normal gene transcription is disrupted. The expanded polyglutamine tract in the mutant protein also alters its interaction with caspase-3.
specific transcripts of BDNF and the various 5-HT receptors in both the cortex and hippocampus reveals variable changes in gene expression that are specific for brain region, genotype, sex and housing environment. Collectively, the findings of this thesis will emphasize that early gene expression changes in the brain contribute to the development of depression in HD, which can be modeled in R6/1 transgenic mice.
Chapter 2 Materials and Methods

2.1. Mice

All mice used in experiments described in this thesis were bred from the R6/1 colony maintained at the Howard Florey Institute (University of Melbourne) established from founder mice courtesy of Dr. Gillian Bates (King College, London, UK) (Mangiarini et al., 1996). Male R6/1 mice were mated with female wild-type mice (Animal Resource Centre, Perth, Australia) on mixed CBA-C57BL/6 background. Genomic DNA extracted from toe clippings at 3 weeks of age was used to determine the genotype of the offspring (Fig. 2-1). The length of the CAG repeat expansion (126 – 134 CAG repeats) was determined by the sequencing service provided by Dept of Pathology, University of Melbourne. Pups were weaned at 4 weeks of age and randomly allocated into groups of four mice per standard laboratory mouse box with two HD and two wild-type mice per cage (Fig. 2-2A). Mice undergoing the environmental enrichment housing paradigm were housed in larger cages that were normally used to house rats (15 x 28 x 38 cm) from 8 weeks of age (Fig. 2-2B). These cages were ‘enriched’ with a variety of objects of varying material, shape and size. The configuration of the ‘home cage enrichment’ was changed weekly. In addition, mice undergoing environmental enrichment were placed for one hour into large 120-litre boxes thrice weekly which exposed them to additional objects and provided them with extra opportunities for cognitive, sensory and motor stimulation (Fig. 2-2C). Mice allocated to the wheel-running paradigm were also housed in larger cages identical to the enrichment paradigm but only containing two running wheels from 8 weeks of age (Fig. 2-2D). All mice were housed at the Integrative Neuroscience Facility (INF) in the HFI where all behavioural experimentation were conducted. The housing rooms were on 12-hour light-dark cycles from 0700H to 1900H. Access to food and water was ad libitum for all housing conditions. All behavioural experiments and measures described in this thesis were performed on 12-week old mice unless otherwise stated. All experiments described in this thesis were approved and performed in accordance with the guidelines of the HFI Animal Ethics Committee.
Figure 2-1 Representative gel of the PCR products obtained for the purpose of genotyping.

Genomic DNA was extracted from toe clippings using the REDExtract-N-Amp™ Tissue PCR Kit (Product # XNATR, Sigma-Aldrich) according to the manufacturer’s instructions. Each PCR reaction consisted of 2.0 µl of extracted DNA added to 18.0 µl reaction mastermix containing 1.0 µl of each of the following primers (primer sequences courtesy of G. Bates, King’s College) at a working concentration of 0.1 µg/µl. Forward: CGC AGG CTA GGG CTG TCA ATC AAT CAT GCT, Reverse: TCA TCA GCT TTT CCA GGG TCG CCA T. Cycling conditions were: 10mins @ 94°C, 34 x (30secs @ 94°C, 30secs @ 60°C, 30secs @ 72°C), 7mins @ 72°C. PCR products were run out on a 1% agarose gel @ 150V for 20mins.
Figure 2-2 Representative examples of the various housing conditions employed in this thesis.

Standard housing (A), home cage housing for mice undergoing environmental enrichment (B), thrice-weekly extra-housing enrichment (C) and home case housing for mice in wheel-running study (D). Photos for (B) and (C) courtesy of M.Zajac & E. Burrows.
2.2. **Tests for motor symptoms and co-ordination**

An accelerating rotarod (Model 7650, Ugo Basile, Comerio, Italy) was used to assess the motor co-ordination of mice (Fig. 2-3A). Testing was conducted over a period of six consecutive days and involved mice being placed onto the rotating beam which accelerated from 4 to 40 rpm over a span of 300 secs. The time a mouse spent balanced on the rotating beam before falling off was recorded. Mice that clung onto the rotating beam for 3 complete turns were deemed to have fallen off and the time at that point was recorded. The apparatus was wiped down with detergent and 70% ethanol after every trial to remove all traces of urine and fecal odours. The R6 transgenic mouse line develops a rear-paw clasping phenotype reflective of the overt motor symptoms of HD (Carter et al., 1999) (Fig. 2-3B, C). Previously, I had shown that our colony of R6/1 HD mice start to display this motor symptom between 14-16 weeks of age (Pang et al., 2006). To confirm that all the mice used in experiments described in this thesis were pre-motor symptomatic, we checked for the presence of rear-paw clasping in all mice. To do this, mice were suspended by their tails and their hind limbs gently tapped against the edge of the bench 2-3 times and observed. None of the mice used (up to and including 12 weeks of age) showed signs of this motor phenotype.
Figure 2-3 Behavioural assessment of R6/1 HD mice for motor symptoms.

The Ugo Basile accelerating rotor which accelerates from 4 to 40rpm over a period of 300 seconds was used to assess motor co-ordination (A). Wild-type mice splay their hindlimbs when picked up by their tails (B) while motor symptomatic R6/1 HD mice develop a rear-paw clasping phenotype (C) during the later stages of disease progression.
2.3. Measurement of baseline serum corticosterone levels

To measure baseline corticosterone levels, behaviourally naïve HD and wild-type mice were killed by cervical dislocation between 0800 – 1000H for blood collection via cardiac puncture. Blood samples were allowed to clot at room temperature for 30mins before serum was separated by centrifuging the samples for 15mins at 1000g. Serum samples were divided into 100µl aliquots and stored at -20ºC until further use. The concentration of serum corticosterone was determined by immunoassay according to the manufacturer’s instructions (DE3600, R&D Systems, MN, USA).

2.4. Forced-swim test (FST)

The forced-swim test is a popular method of eliciting behavioural despair in rodents and was originally developed as a primary screen for antidepressant drugs with good predictive validity (Porsolt et al., 1977). All mice were brought into the testing room at least 1hr prior to commencement of testing to allow them to acclimatize to the surroundings. Briefly, mice were immersed into 2.0 litre Pyrex beakers of 12cm diameter and 18cm height filled to the 1800ml mark (14.5cm) with 23-25 ºC water (Fig. 2-4A). Each test session lasted for 300secs and was filmed from the side of the beakers and recorded onto DVD for review by an experienced experimenter blinded to the genotype of the mouse. Immobility was defined as the absence of movement apart from those contributing to balance and floatation. The total immobility time over the final 240secs was recorded.
2.5. Tail-suspension test (TST)

The tail suspension test is another behavioural test widely used to assess depressive-like phenotypes in mouse models of depression (discussed by Cryan et al., 2005a). It is based upon mice developing an immobile posture following initial attempts or ‘struggles’ to escape. Similar to the FST, the adoption of an immobile posture during the TST is regarded to represent a failure to persist in escape-orientated behaviour and represents the psychological concept of ‘entrapment’ that is described in clinical depression. The TST has the added benefit of avoiding any confounds induced by hypothermia in the FST. All mice were brought into the testing room at least 1 hour prior to commencement of testing to allow them to acclimatize to the surroundings. Mice had their tails taped with sticky tape and suspended from force-sensing hooks on the TST apparatus (Bioseb, Chaville, France) (Fig. 2-4B). Each test session lasted for 300secs and data collection spanned the final 240secs. It has been recorded that mice on the C57Bl/6 background have a tendency to climb their tails so any mice that did so were removed from the subsequent analysis.

2.6. Novelty-suppressed Feeding test (NSFT)

The novelty-suppressed feeding test was conducted in an arena (1m x 1m x 1m) made from opaque Perspex. 2cm of clean bedding was laid onto the base and a piece of filter paper with a food pallet was placed into the centre of the arena. Mice were food-deprived for 24 hours prior to testing. All mice were brought into the testing room at least 1 hour prior to commencement of testing to allow them to acclimatize to the surroundings. Mice were individually placed into a random corner of the arena (Fig. 2-4C) and timed for their latency to approach the centre of the arena (Fig. 2-4D) and to start feeding on the food pallet. ‘Feeding’ activity was deemed as when the mouse was chewing on the food pallet while gripping onto food pallet with its front paws.
Figure 2-4 Combination of tests used to determine behavioural phenotype of R6/1 HD mice.

The forced-swim test (A) and tail-suspension test (B) are both commonly used to examine depression-related behaviours. The novelty-suppressed feeding test (C) is an anxiety based test which requires a food-deprived subject to enter the centre of an open arena (D) to feed on a food pellet.
2.7. Morris water maze (MWM)

The Morris water maze is a test of spatial working memory requiring a mouse to acquire the position of a partially submerged platform using spatial cues in order to escape from water (Morris, 1984). The maze was a circular open top tank of 88cm diameter and filled with water made opaque by the addition of milk (Fig. 2-5A). The arena was divided into four separate quadrants with unique spatial cues that were attached to the inner walls of the maze (Fig. 2-5B). A collapsible platform was located in the center of each quadrant and submerged 1cm below the water level. All mice were brought into the testing room at least 1 hour prior to commencement of testing to allow them to acclimatize to the surroundings. MWM testing was conducted over a period of five days with the very first exposure of the mice to the arena being a pre-trial session involving no visual cues. During pre-trial training, mice were placed into the water and allowed to swim for up to 15secs before being guided onto the platform. The sole purpose of the pre-trial session was to make the mouse aware of the existence of a platform which it had to find in order to escape the water. Mice were allowed to sit on the platform for 30secs before removal from the arena. The location of the platform for this pre-trial session was unimportant as there were no spatial cues for the mice to take reference from. For the training trials, the platform location was fixed for an individual mouse. Mice were placed into random corners of the maze and allowed a maximum of 60secs to locate the platform failing which they would be guided to the submerged platform and allowed to rest for 15secs. Four training trials were conducted per day over four consecutive days. On the fifth day, a single probe trial was conducted during which the platform removed. Movement patterns of the mice during the probe trial were recorded by a video camera mounted directly above the MWM and tracked using the EthoVision® 3.0 software (Noldus, SDR Clinical Technology, NSW, Australia).
Figure 2-5 Cognitive ability determined by performance on the Morris water maze.

Spatial working memory was assessed with an 88cm Morris water maze (A) which had four collapsible platforms that were partially submerged when the tank was filled with water made opaque by mixing with milk. The spatial cues used in this study and the representative quadrants are represented in (B).
2.8. Antidepressant administration

The FST and TST are routinely used as behavioural screens for novel antidepressant compounds. The acute administration of an effective antidepressant compound prior to either behavioural test reduces immobility times of wild-type mice. The behavioural effects of the selective serotonin-reuptake inhibitor (SSRI) sertraline hydrochloride (Pfizer, CT, USA), and the tricyclic antidepressant desipramine (Sigma-Aldrich, USA) which targets both the serotonergic as well as the noradrenergic systems, were examined in male and female wild-type and HD mice. Sertraline was dissolved in distilled water with 1:100 Tween-20 and desipramine was dissolved in distilled water. Acute administration was given 30mins and 60mins prior to testing on the TST and FST respectively. Both drugs were administered intraperitoneally at concentrations of 20mg/kg and controls were injected with equivalent volumes of vehicle.

In addition, a novel antidepressant compound was provided courtesy of Dr. Jia Zhou (Acenta Discovery, Inc., AZ, USA) for testing. JZ-IV-7 is a SERT/NET inhibitor and was tested on a cohort of female HD and wild-type mice. Mice were given an intraperitoneal injection of drug at 40mg/kg 30mins and the behavioural effects of this drug were assessed using the TST.
2.9. Estimation of dentate gyrus volume and BrdU-positive cell counts

For the identification and quantification of proliferating cells in the dentate gyrus, 5-Bromo-2’-deoxyuridine (BrdU) (Sigma-Aldrich, NSW, Australia) was employed as a proliferative marker. BrdU was dissolved in 0.9% saline to a concentration of 10mg/ml and administered (50mg/kg) over 12 consecutive days starting four weeks prior to collection of brains. Mice were deeply anesthetized for transcardial perfusion with 0.1M PBS followed by 4% paraformaldehyde. Brains were removed and stored in 4% paraformaldehyde overnight and then transferred to 30% sucrose until they sank. Brains were sectioned in the coronal plane on a cryostat at 20µm intervals from -1.34mm Bregma in a 1:10 series, thaw mounted onto slides and stored at -80°C until further use. Sections were quenched in 1M PBS for 10mins, treated with 2M HCl at 37°C for 30mins, rinsed in PBS for 5mins, blocked with Cas-block (Invitrogen, USA) for 2hr at room temperature then incubated with sheep anti-BrdU antibody (1:500, Exalpa Biologicals Inc, MA, USA) in 50% Cas-block overnight at room temperature. Slides were rinsed in PBS, and then incubated with a biotinylated rabbit anti-sheep antibody (1:500, Vector Labs, CA, USA) in 50% Cas-block for 2-3hr at room temperature. Sections were rinsed in PBS and visualization of BrdU-positive cells was by reaction with Vectorstain Elite ABC Reagent (Vector Labs, CA, USA) followed by DAB peroxidase solution (DakoCytomation, Code K3466, CA, USA). Finally, sections were counterstained with cresyl violet and mounted in DPX.

For each brain, 6 sections, each at 200µm intervals (1:10 series), commencing at the most rostral section, were selected. The dentate gyrus was imaged and manually highlighted according to a mouse brain atlas (Paxinos & Franklin, 2nd edition, 2001) at 10x magnification and the area was automatically calculated by the Zeiss Axiovision 4.5 software (Carl Zeiss Microimaging Inc, USA). Volumetric estimations were determined by summing the areas of the dentate gyrus and adjusting for sampling frequency and section thickness. Counts of BrdU-positive cells were performed at 40x magnification from the same sections used for volume estimation. The absolute number of cells
immunoreactive for BrdU were counted on each section and totaled for all sections. All volumetric and cell count analyses were performed blind to genotype and experimental group.

2.10. Tissue collection for RNA analysis

All gene expression analyses described in this thesis were performed on RNA extracted from brains of 12-week old mice. Mice were killed by cervical dislocation and brains were immediately removed. The hippocampus and cortex from both hemispheres were dissected out on ice then snap frozen in liquid nitrogen for storage at -80°C. Frozen brain tissue was disrupted with a sonicator (Heat Systems, XL-Series Microson™, Ultrasonic Cell Disruptor). RNA from the hippocampus and the cortex was extracted using the RNA RNeasy Mini Kit (Cat. No. 74104, Qiagen, Australia) and the RNeasy Midi Kit (Cat. No. 75154, Qiagen, Australia) respectively, according to the manufacturer’s instructions. The optional on-column DNase digest step was also performed with the recommended reagents (Qiagen RNase Free DNase Set, Cat. No. 79254, Qiagen, Australia). All glassware used during the extraction process was treated for RNases by baking overnight at 180°C. DEPC is a strong inhibitor of RNases and 0.01% DEPC water was made up, allowed to stand overnight before being autoclaved to remove traces of DEPC. DEPC water was used to make up 70% ethanol for the purpose of wiping down the work bench to ensure a clean working environment at all times. In addition, the bench area was wiped down with RNaseZap® (Ambion/Applied Biosystems, Cat. #AM9780). Pipettes used exclusively for RNA work and the sonicator were treated overnight with UV light. The sonicator probe was rinsed in 0.01% DEPC water in-between the sonication of individual samples. The final RNA elutes were analysed using an Agilent 2100 Bioanalyser for RNA integrity and concentration (service provided by the Australian Genome Research Facility, VIC, Australia). All RNA samples were stored at -80°C until further use.
2.11. cDNA preparation

Before commencement of real-time analyses, 1000ng of RNA was reverse transcribed using the Taqman EZ RT-PCR Core reagents kit (Cat. No. N808-0234, Applied Biosystems, Australia). Negative control reactions comprised of reactions with no RNA (-RNA) and no reverse transcriptase enzyme (-RT) were included in every round of reverse transcription. The reverse transcription conditions were as follows: 10min @ 25°C, 30min @ 48°C, and 5min @ 95°C. cDNA products were stored at -20°C until further use.

2.12. Design of real-time PCR primers

Real-time PCR primers for exon-specific BDNF transcripts had previously been published (Tsankova et al., 2006). All primer sequences (Table 2-1) were checked for specificity using the BLAT sequence alignment function available at UCSC Genome Browser (http://genome.ucsc.edu). The primer sequences for BDNF5 were found to be incorrect and these were redesigned. BDNF primers were designed such that the forward primer was located within the specific exon in question while the reverse primer rested within the coding exon (Figure 2-6). Gene nucleotide sequences for mus musculus 5-HT receptors 1A (Htr1a, NM008308), 1B (Htr1b, NM010482), 2C (htr2c, NM008312) and 5-HT transporter (SerT, AF013604) were obtained from the National Center for Biotechnology Information (NCBI) database (http://www.ncbi.nlm.nih.gov). Primers were designed using Primer3 Ver 0.4.0 (http://frodo.wi.mit.edu/) or Primer Express Ver 3.0 (ABI Biosystems).
<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward sequence</th>
<th>Reverse sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total BDNF (Coding exon)</td>
<td>GCGCCCATGAAAGAAGTAAA</td>
<td>TCGTCAGACCTCTCGAACCT</td>
</tr>
<tr>
<td>BDNF1 (exon I)</td>
<td>CCTGCATCTGTTGGGAGAC</td>
<td>GCGTTGTCCGTGGAGCTTTTA</td>
</tr>
<tr>
<td>BDNF2 (exon II)</td>
<td>CTAGCCACCGGGGTGGGTGTAAT</td>
<td>AGGATGGTCATCAGCTTTTCTC</td>
</tr>
<tr>
<td>BDNF3 (exon III)</td>
<td>CTTCTTGGAGCCCCAGTTCC</td>
<td>CCGTGACGTATTTACTTTTTC</td>
</tr>
<tr>
<td>BDNF4 (exon IV)</td>
<td>CAGAGCAGCTGCCTTGATGTT</td>
<td>GCCTTGTCGTCGGACGTGTTA</td>
</tr>
<tr>
<td>BDNF5 (exon V)</td>
<td>CTGGGAGGCTTGGATGAGAC</td>
<td>GCCTTCATGCAACCGGAAGTA</td>
</tr>
<tr>
<td>Cyclophilin (Cyc)</td>
<td>CCCACCGTGTTCTTCGACA</td>
<td>CCAGTGCTCAGAGCCTCGAAA</td>
</tr>
<tr>
<td>5-HT 1A receptor (htr1a)</td>
<td>CCCAACGAGTGACCCCAT</td>
<td>CGGCCAAAGTGGAGTAGAT</td>
</tr>
<tr>
<td>5-HT 1B receptor (Htr1b)</td>
<td>CACCAACCTCCTCCACCACT</td>
<td>CCAGAGGCAGATCAGGTAAG</td>
</tr>
<tr>
<td>5-HT 2A receptor (htr2a)</td>
<td>CTGCTGGGTTTCTTTGTGAT</td>
<td>TCTGGAGTTGAAGCAGGCTAT</td>
</tr>
<tr>
<td>5-HT 2C receptor (htr2c)</td>
<td>TGGGCATGGTTGGGCAATA</td>
<td>CGTCCCTCAGTCCCACACCA</td>
</tr>
<tr>
<td>5-HT transporter (SerT)</td>
<td>CTTCACTCCCCGGATGGTT</td>
<td>GTGGAATCTCATCAAAAAACTGCAA</td>
</tr>
</tbody>
</table>

Table 2-1 List of primer pairs used for real-time PCR reactions.

Primer sequences for BDNF1, BDNF2, BDNF3 and BDNF4 were obtained from previous published protocols (Tsankova et al., 2006). Primer sequences for total BDNF, cyclophilin and htr2c were courtesy of M. Zajac. Primer sequences for BDNF5 were jointly designed by T.Pang and M.Zajac.
Figure 2-6 Comparison of the mouse BDNF gene sequence against the human sequence.

The nucleotide sequence of the mouse BDNF gene is closely homologous to the human gene. Non-coding exon sequences examined to date are similar as indicated by the percentages in this figure. The red arrows indicate the real-time PCR product based on primer pairs that were designed to span intron-exon boundaries.
2.13. Optimization of real-time PCR primers

All primer pairs were optimized for working volumes and tested for efficiencies prior to real-time runs. To determine the optimal working volumes of forward and reverse primers, a set of primer dilutions was conducted in which different combinations of each primer ranging between 0.5 – 3.0µl were used. The combination that yielded the lowest Ct-value was used as the optimal working volumes. Following that, primer pairs were assessed for their amplification efficiencies. A series of real-time reactions were performed on serial dilutions of cDNA (1, ½, 1/5, 1/10, 1/20, 1/50 and 1/100). The log-value of the cDNA concentration was plotted against the Ct value and the gradient of the line of best fit was determined. Primer pairs with considered to amplify efficiently if the gradient of the line of best fit ($R^2$-value) ranged between 0.95 (95%) – 1.05 (105%). Data from the primer efficiency studies are presented in Figures 2-7 – 10. Primers for the BDNF transcripts, cyclophilin, htr2a and htr2c were optimized for real-time PCR by M.Zajac.
Figure 2-7 Determining the amplification efficiency of the htr1A primer pair.

The working volumes were 0.5µl forward with 0.5µl reverse primer. Real-time PCR product was detected at later cycles with decreasing amount of cDNA template (A). The reactions yielded one consistent product with no indication of genomic contamination (B). The efficiency of the htr1A primer pair was determined to be 96.81% (C).
Figure 2-8 Determining the amplification efficiency of the htr1B primer pair.
The working volumes were 1.0µl forward with 1.0µl reverse primer. Real-time PCR product was detected at later cycles with decreasing amount of cDNA template (A). The reactions yielded one consistent product with no indication of genomic contamination (B). The efficiency of the htr1B primer pair was determined to be 98.45% (C).
Figure 2-9 Determining the amplification efficiency of the SerT primer pair.
The working volumes were 1.0µl forward with 1.0µl reverse primer. Real-time PCR product was
detected at later cycles with decreasing amount of cDNA template (A). The reactions yielded one
consistent product with no indication of genomic contamination (B). The efficiency of the SerT
primer pair was determined to be 97.44% (C).
Figure 2-10 Determining the amplification efficiency of the cyclophilin primer pair.
The working volumes were 1.0µl forward with 1.0µl reverse primer. Real-time PCR product was detected at later cycles with decreasing amount of cDNA template (A). The efficiency of the cyclophilin primer pair was determined to be 95.9% (B).
2.14. SYBR-green real-time PCR

Real-time reactions were performed to a total volume of 20.0µl per reaction consisting of 10.0µl of SYBR green, the appropriate volumes of forward and reverse primer as determined during the optimization process, 5.0µl of cDNA then made up to the final volume with dH₂O. A mastermix for SYBR green, primers and dH₂O was prepared prior to ensure consistency of reactions. cDNA was pipetted into each well before addition of 15.0µl of mastermix. All samples were run in triplicate. Real-time PCR for the BDNF expression study was performed using the ABI Prism 7700 Detection System (Applied Biosystems, Foster City, CA, USA) (Fig. 11A, B) and the dissociation curve conditions were as follows: Stage 1: 15secs @ 95°C, stage 2: 20secs @ 60°C, stage 3: 15secs @ 95°C with the ramp time for stage 3 set to 20mins (Fig. 11C). The real-time reactions for the serotonin receptors and the serotonin transporter were performed on the ABI 7500 Realtime PCR System (Applied Biosystems, Foster City, CA, USA) (Fig. 2-12A). The real-time cycling conditions were: 1x cycle for 2mins @ 50°C, 1x cycle for 10mins @ 95°C, 40x cycles for 15secs @ 95°C, followed by 1min @ 60°C. Upon completion of real-time runs, dissociation curve analyses were performed to check for consistent melting temperatures of the final PCR product (Fig. 2-12B) and for the presence of any evidence of contamination or primer dimers (Fig. 2-12C). The dissociation curve conditions for the ABI7500 were as follows: 15secs @ 95°C, 60secs @ 60°C, 15secs @ 95°C and then 15secs @ 60°C.
Figure 2-11 Representative figures of real-time PCR results obtained from ABI Prism 7700 Detection System.

Relative abundance of real-time PCR product indicated by grey-scale shading (A). Amplification plot demonstrating amplification of two distinct products with baseline set manually to 15 cycles (B). Representative dissociation curve demonstrating a single product obtained for htr2A primer pair (C). NB: This particular primer set was re-optimised for experiments conducted on the ABI 7500 Realtime PCR System.
Results of real-time PCR reactions for htr2a demonstrate the amplification of two distinct products, the second being cyclophilin (A). Dissociation analysis from the htr2c gene expression study indicating the presence of a single product amplified from the htr2a primer pair (B). An example of multiple products being amplified due to the use of poorly designed primer pairs (C).
2.15. Bisulfite sequencing and analysis of methylation status of BDNF CpG Islands

The UCSC Genome Browser (http://genome.ucsc.edu) was used to locate CpG islands along the *mus musculus* BDNF mRNA sequence (X55573). CpG islands are regions where CpG dinucleotides (a cytosine (C) base followed immediately by a guanine (G) base) are present at significantly higher levels than is typical for the genome as a whole. These are common near transcription start sites and may be associated with promoter regions. Four islands were found along the BDNF gene sequence; two islands were positioned more proximal in the region of BDNF exons I, II and III, another was located around the region of exons IV and V while a fourth and most distal island was located 5’ to the coding exon (Figure 2-13). Further analyses were focused on island 3 which spans 741bp and contains 60 CpG dinucleotides, and island 4 which spans 246bp and has a CpG count of 17.

DNA was extracted from hippocampal homogenates originally intended for RNA extractions following a protocol advised by Dr. Michael Emmerling (Qiagen Pty Ltd, VIC, Australia). Briefly, 100% ethanol was added to the lysate and centrifuged for 5-10mins. The supernatant was pipetted off and the pellet resuspended in PBS. This was then taken through to Step 4 of the QiaAmp DNA mini kit protocol (Qiagen, VIC, Australia). The final DNA elutes were stored at -20ºC until further use.
Figure 2-13 Location of non-coding exons and CpG islands along the BDNF gene sequence (X55573).

The location of known non-coding exons are represented as two distinct clusters (A). There are four CpG islands located within the gene sequence (B). The two highlighted CpG islands (isld3 and isld4) were investigated. Island 3 was the largest within the BDNF gene sequence and contained 60 CpGs and was associated with exons IV, V and VI. Island 4 contained 17 CpGs and was associated with the BDNF coding exon.
Up to 150ng of genomic DNA was subjected to bisulfite conversion using the MethylEasy™ Xceed Rapid DNA Bisulfite Modification Kit (ME002, Human Genetic Signatures, NSW, Australia) according to manufacturer’s instructions. Approximately 20ng of converted DNA was used for PCR amplification of isld3 and isld4. Promega PCR Mastermix (Cat. # A3500, Promega, NSA, Australia) was used for amplification. Two rounds of PCR amplification was performed for each promoter using the following hemi-nested primer sets:

<table>
<thead>
<tr>
<th>Primer Name:</th>
<th>Primer Sequence 5’ – 3’:</th>
<th>T_m(°C):</th>
</tr>
</thead>
<tbody>
<tr>
<td>bi_BDNFisld3_1F</td>
<td>ggagattgtagaggttagtgatatag</td>
<td>54.7</td>
</tr>
<tr>
<td>bi_BDNFisld3_2R</td>
<td>aacaataaaacaacttataataacccaaa</td>
<td>53.8</td>
</tr>
<tr>
<td>bi_BDNFisld3_1R</td>
<td>aaataaacttaataatactccctaaaaa</td>
<td>53.5</td>
</tr>
<tr>
<td>bi_BDNFisld4_1F</td>
<td>aaattataagtagatgggttatatttttt</td>
<td>55.3</td>
</tr>
<tr>
<td>bi_BDNFisld4_2F</td>
<td>gtgagaagagtgatgaggttttttt</td>
<td>54.7</td>
</tr>
<tr>
<td>bi_BDNFisld4_1R</td>
<td>cttttataataataataataataataataa</td>
<td>54.4</td>
</tr>
</tbody>
</table>

The first round of PCR was performed using 1F and 1R primers within each hemi-nested set. In the second round, 1F/2R and 2F/1R primers were used for island 3 and island 4 respectively. The PCR conditions for each round of amplification are as follows: Initial denaturation 94°C for 2mins followed by 5 cycles of denaturation at 94°C for 10secs, annealing at 53°C for 10secs and extension at 72°C for 120secs; followed by 25 cycles of denaturation at 94°C for 10secs, annealing at 53°C for 10secs and extension at 72°C for 90secs. Amplicons were then cloned into pGEM®-T-Easy Vector (Cat. # A1360, Promega, NSA, Australia) for automated DNA sequencing. Positive clones were then selected and sequenced at the Australian Genome Research Facility (Queensland, Australia) using established protocols.
2.16. Chromatin immunoprecipitation (ChIP)

The enrichment of modified histones at BDNF promoters was measured using the EZ-Chip™ from Millipore kit (Cat. # 17-371, CHEMICON/Upstate/Linco, Billerica, MA, USA) according to the manufacturer’s instructions. Antibodies to the active histone marks, H3K9ac and H3K4me3, were also purchased from Millipore. ChIPs were quality controlled by the use of control genes which were either expressed in the hippocampus (synaptophysin), expressed ubiquitously (β-tubulin) or not expressed in the hippocampus (ε-globin). All ChIPs which returned fold difference ratios for any of these genes that lay outside 95% confidence intervals were discarded.

2.17. Statistics

All statistical analyses were performed using Sigmastat 2.03.0 (SPSS Inc) software package. Rotarod performance was analyzed using two-way repeated measures ANOVA. Male and female data sets for body weight and corticosterone levels were analysed separately for genotype differences by one-way ANOVA. Results of the FST and TST were initially analyzed with a two-way ANOVA with gender and genotype as the variable factors. Subsequent male and female data sets were then analyzed separately with one-way ANOVA since sex differences were observed. Male and female performance on the FST and TST following acute drug administration were analysed with two-way ANOVA for the effects of genotype and drug. The FST results of male and female data sets for the environmental enrichment and wheel-running experiments were also analysed with two-way ANOVA for the effects of genotype and environment. The various measurement parameters of the MWM were analysed with two-way ANOVA for effects of genotype and wheel-running. Dentate gyrus volume and BrdU-positive cell counts were analysed with two-way ANOVA. Two-way ANOVA was used to analyse real-time PCR results (first converted into fold change relative to wild-type standard-housed levels using the \(2^{(-\Delta\Delta CT)}\) method) and ChIP enhancement ratios (also normalized to wild-type standard-housed levels). All ANOVA analyses were followed with post hoc Bonferroni’s. The level of statistical significance for all analyses was set at \(\alpha = 0.05\).
Chapter 3 Investigating HD-associated depressive-like behaviours using the R6/1 transgenic mouse model.

3.1 Introduction

Higher rates of mood disorders are often reported in sufferers of degenerative brain diseases (Leroi et al., 2002). A subset of those neurological diseases including Huntington’s, Parkinson’s and Wilson’s diseases specifically involve degeneration of the basal ganglia and are characterised by the manifestation of motor, cognitive and psychiatric symptoms (Rosenblatt and Leroi, 2000). Often, neuropsychiatric changes can be detected before the appearance of overt motor or cognitive deficits but the exact neuropathological origins of these psychiatric changes remain unclear.

Almost all sufferers of Huntington’s disease (HD) show some neuropsychiatric symptoms with the most prominent being depression (reviewed by Anderson and Marshall, 2005). It has been estimated that 30-50% of all HD patients are diagnosed with depression at some point during their condition and that rate of incidence is about twice as high as that of the general population (Slaughter et al., 2001; Paulsen et al., 2005). There is also a marked increase in suicidal ideation and suicide attempts. The occurrence of depression is often observed concurrent with increased anxiety, irritability and apathy, while there is a lesser degree of association with obsessive compulsive symptoms and psychosis (van Duijn et al., 2007).

Generally, carriers of the HD gene mutation display a significantly higher rate of depression than non-carriers (Julien et al., 2007). Furthermore, that increases with proximity to clinical onset which suggests that depression develops during early-HD and worsens with disease progression. However, unlike age of clinical onset which is correlated to the length of the CAG repeat expansion (Duyao et al., 1993; Sieradzan et al., 1997), CAG repeat length does not appear to be a factor in the likelihood of developing
depression (Berrios et al., 2001). Interestingly, the length of the CAG repeat expansion is instead highly correlative with increased irritability and cognitive deficits.

Recent studies examining asymptomatic HD patients have confirmed that there is an increased frequency of depressive symptoms together with higher levels of anxiety during the early, pre-clinical stages of the disease (Duff et al., 2007; Marshall et al., 2007). In fact, subtle psychiatric symptoms were be detected despite patients being more than a decade from the estimated age of clinical onset. Therefore, this was a strong suggestion that the earliest changes to the HD brain that were insufficient to cause motor impairment were contributing the development of depression. The etiology of depression by itself is very complex and many factors implicated in the manifestation of depression include structural changes to the brain as well as gene-environment interactions. Some of these factors have also been implicated in HD pathology and will be addressed in later chapters.

Most importantly, there is the yet-to-be resolved issue of whether the higher incidence of depression in HD is an inherent part of symptomatology or due to the natural concerns of being at risk of the disease and the psychological stress following a positive result on the predictive gene test. The availability of predictive genetic testing allows some of the concerns of being at risk for HD to be addressed but a positive outcome would understandably be associated with an increase in the psychological burden. Studies reporting on the levels of depression and psychological distress pre- and post-testing indicate that predictive gene testing is in itself not a psychologically benign event with elevated rates of major depressive episodes reported by both carriers (~15%) and non-carriers of the HD mutation (Decruyenaere et al., 1999; Codori et al., 2004) compared to the general population. On the other hand, predictive testing does have its benefits as the overall levels of psychological distress of participants decrease over a five year period (Almqvist et al., 2003). However, while depression scores decreased for gene-negative individuals post-testing, those who were found to be gene-positive reported an increase in depression symptoms (Larsson et al., 2006) thereby further strengthening the claim that
the mere knowledge of being gene-positive for HD is a trigger for the development of depression instead of being an innate part of disease symptoms. At the present moment, it is seemingly impossible to resolve this important issue in any patient-based study given the overwhelming psychological confounds.

Despite the numerous observational and anecdotal accounts agreeing that standard approaches are effective in treating the depressive symptoms of HD patients, there has yet to be a large scale, longitudinal study to detail the true effectiveness of those treatments in HD patients such as the efficacy of various classes of antidepressant drugs. Monoamine oxidase inhibitors, selective serotonin reuptake inhibitors (SSRIs), in particular sertraline, and even electroconvulsive therapy have been reported to be effective in alleviating the depressive symptoms of HD patients (Ford, 1986; Lewis et al., 1996; and discussed by Slaughter et al., 2001). Incidentally, sertraline has also been used to treat other psychiatric aspects of HD such as elevated aggression and obsessive compulsive tendencies (Ranen et al., 1996; De Marchi et al., 2001).

Given the significant impact of depression on the lives of HD patients and a lack of substantial information regarding drug treatments from patient-based studies, further investigation of HD-related depression is warranted. The availability and use of rodent models of HD which hold construct and predictive validity minimizes, and possibly even avoids, all impact of psychosocial factors that are inherent to any patient-based study. Hence, examining the behavioural profiles of these animal models and their responses to various pharmaceuticals would be highly informative.

Some physiological aspects of depression have been described in working models of HD. A common finding in depressed individuals is an abnormal and persistent elevation of cortisol levels which is associated with heightened feelings of stress and anxiety. A study of the R6/2 transgenic HD mouse model found a progressive dysfunction of the hypothalamic-pituitary-adrenal axis which regulates stress response and the HD mice
were found to have elevated levels of serum and urine corticosterone (Bjorkqvist et al., 2006). That finding was followed up in a group of HD patients at different stages of disease progression and while urine cortisol levels were indistinguishable from age-matched controls during the pre- to early symptomatic stages, HD patients in the moderate to advanced stages of the disease have significantly greater levels of cortisol. As such, subtle physiological changes in the HD condition might be contributing to the development of depression. However, at present, no study has yet explored the presence of any depression-related behavioural phenotypes in any of the HD models.

Several studies conducted on mouse models of HD have demonstrated the effectiveness of the SSRIs paroxetine, fluoxetine and sertraline in altering the progression of motor and cognitive deficits but unfortunately none addressed the specific issue of treating HD-related depression. The chronic administration of the antidepressants at concentrations equivalent to patient prescriptions successfully attenuated motor dysfunction and delayed a loss in body weight (Duan et al., 2004; Duan et al., 2008; Peng et al., 2008) while improving cognitive ability (Grote et al., 2005) of transgenic HD mice. However, despite those positive results, the evidence from patient-based studies is less supportive of the effectiveness of antidepressants in treating HD symptoms. The use of imipramine (a tricyclic antidepressant) was reported as having no effect on the rate of disease progression within a group of 42 HD patients who were followed over 3 years (Myers et al., 1991) and the use of fluoxetine over 4 months did not improve the functional capacity, neurological score or cognitive ability of 17 non-depressed HD patients (Como et al., 1997). Given the different observations between HD models and patient responses, a closer assessment of the different classes of antidepressants in treating the depressive symptoms of HD patients is warranted.
The aims of this chapter were:

1. To assess pre-motor symptomatic R6/1 transgenic HD mice for depression-related behaviours using a range of behavioural tests.
2. To examine whether there are sex differences in the presentation of depression-related behaviours in HD.
3. To evaluate the effectiveness of different classes of antidepressant drug in eliciting behavioural changes in HD mice.
3.2 Materials and methods

All experiments described in this chapter were performed on 12-week old male and female R6/1 transgenic HD mice and their wild-type littermates. Body weights were recorded and an immunoassay (R&D Systems, USA) was used to determine basal levels of serum corticosterone in blood collected via cardiac puncture from a separate cohort of mice that were naïve to behavioural testing. Motor co-ordination was assessed using an accelerating rotarod (Ugo Basile, Italy) which accelerates from 4 to 40rpm over 300 seconds. Independent cohorts of mice were tested on the forced-swim (FST), tail-suspension (TST) and novelty-suppressed feeding tests (NSFT) to determine whether HD mice displayed altered depression-related behaviours. The FST and TST were performed on one of the cohorts with the TST conducted 3 days prior to the FST while the NSFT was conducted on the second cohort of mice. A separate cohort of mice received an acute i.p. administration of the serotonin selective reuptake inhibitor sertraline (20mg/kg; Pfizer, USA) and the tricyclic antidepressant desipramine (20mg/kg; Sigma-Aldrich, USA) which were administered 30 mins and 60 mins prior to testing on the TST and FST respectively. The novel compound JZ-IV-7 (Acenta Discovery Inc., USA) which selectively inhibits the serotonin and norepinephrine transporters was tested for its ability as a potential antidepressant compound and was administered by acute i.p. administration (40mg/kg) 30 mins before testing on the TST. Two-way ANOVA was used to analyse body weight, serum corticosterone levels and results from the baseline behavioural tests (FST, TST and NSFT) with sex and genotype as the two variant factors. The 6-day rotarod results were analysed using a two-way repeated measures ANOVA. Results of male and female responses to acute antidepressant administration on the TST and FST were analysed separately by two-way ANOVA with genotype and drug as the two variant factors. Significant results were analysed with post-hoc Bonferroni’s t-test with the level of significance set at $\alpha = 0.05$. 
3.3 **Results**

3.3.1 **Motor ability of 12-week old HD and wild-type mice**

R6/1 HD mice develop a rear-paw clasping phenotype from 16 weeks of age which wild-type mice never display (Mangiarini et al., 1996; Pang et al., 2006). All mice were checked for this overt motor symptom and as expected, none of the 12-week old HD or wild-type mice used in this study displayed this clasping symptom. Balancing on the accelerating rotarod is a test of motor co-ordination and R6/1 HD mice develop a deficit in this test by 15 weeks of age (Pang et al., 2006). The performance of HD and wild-type mice on six consecutive days of rotarod testing was recorded and no overall genotype difference ($F_{(1,125)} = 2.753, p = 0.114$) was detected indicating that HD mice did not perform any worse than wild-type mice (Fig. 3-1). Hence, it can be concluded that 12-week old R6/1 HD mice do not show signs of motor dysfunction.

There was, however, a significant difference in performance depending on the day of testing ($F_{(5, 125)} = 9.160, p < 0.001$). Post-hoc analyses revealed that while mice did not show a significant improvement in rotarod performance on their second exposure ($p = 0.303$), they were performing significantly better as a group by the third day ($p = 0.010$) and this improvement persisted on the fourth ($p < 0.001$), fifth ($p < 0.001$) and sixth days ($p < 0.001$) which demonstrates motor learning in both wild-type and HD mice.
Figure 3-1 Motor learning abilities of HD and wild-type mice.

12-week old mice were tested on an accelerating rotarod for six consecutive days and the latency to falling off the rotating beam was recorded. Differences in body weight might alter performance on the rotarod but no sex-specific differences were observed (data no shown) so male and female data was pooled for subsequent analysis. There were no differences between the mean latency to falling off the rotarod of HD (n = 10) and wild-type mice (n = 11) across all six days of testing. All mice performed significantly better on the rotarod from Day 3 (Post-hoc Bonferroni’s t-test).
3.3.2 Body weight and level of serum corticosterone

Body weights were recorded since weight loss is a common feature of both depression and HD (Aziz et al., 2007; Mitchell et al., 2008). Two-way ANOVA revealed no differences due to genotype \( (F_{(1,51)} = 2.709, \ p = 0.106) \) but a significant effect of sex \( (F_{(1,51)} = 74.117, \ p < 0.001) \) (Fig. 3-2A). The average weight of male wild-type mice was significantly greater \( (p < 0.001) \) than female wild-type mice as expected. There was also a significant difference \( (p < 0.001) \) between the average weight of male HD mice and female HD mice.

Motor symptomatic R6/2 HD mice have been reported to have elevated corticosterone levels (Bjorkqvist et al., 2006) so serum corticosterone levels of pre-motor symptomatic R6/1 HD mice was quantified. There were no overall differences due to genotype \( (F_{(1,42)} = 0.0508, \ p = 0.823) \) or between the sexes \( (F_{(1,42)} = 0.0439, \ p = 0.835) \) (Fig. 3-2B).
Figure 3-2 Average body weights and serum corticosterone levels of 12-week old HD and wild-type mice.

(A) There was no difference in the average body weight of HD and wild-type mice for either sex. Male HD (n = 19, 26.9g ± 0.5) and wild-type mice (n = 16, 28.8g ± 0.8) weighed significantly greater than their respective female comparisons (HD: n = 11, 21.3g ± 0.4; WT: n = 6; 20.6g ± 1.2). (B) There were no observable differences in the level of serum corticosterone across all groups (Female HD: n = 16; 27.3ng/ml ± 7.7; Female wild-type: n = 10; 31.0ng/ml ± 9.8; Male HD: n = 7; 27.0ng/ml ± 8.7; Male wild-type: n = 10; 30.1ng/ml ± 8.4). Asterisks indicate post-hoc Bonferroni’s t-test: ***: p < 0.001.
3.3.3 Performance of 12-week old mice on the forced-swim test (FST)

Analyses of the behavioural response of mice on the FST revealed that total immobility time displayed was significantly dependent on both genotype ($F_{(1,52)} = 8.182, p = 0.006$) and sex ($F_{(1,52)} = 4.941, p = 0.031$) (Fig. 3-3A) and there was no significant genotype-sex interaction ($F_{(1,52)} = 1.585, p = 0.214$). Female HD mice spent more time immobile than female WT mice ($p = 0.016$) but there was no difference between the mean immobility times of male HD and wild-type mice ($p = 0.158$). Male WT mice recorded significantly greater immobility time than female WT mice ($p = 0.025$) but there was no sex difference within the HD group ($p = 0.468$).

3.3.4 Performance of 12-week old mice on the tail suspension test (TST)

On the TST, both genotype ($F_{(1,51)} = 18.419, p < 0.001$) and sex ($F_{(1,51)} = 17.479, p < 0.001$) were significant factors influencing total immobility time recorded (Fig. 3-3B) while there was no significant genotype-sex interaction ($F_{(1,51)} = 2.586, p = 0.114$). The average immobility time of female HD mice on the TST was significantly less than the average time of female wild-type mice ($p < 0.001$). Male HD mice also recorded significantly lower immobility times than male WT mice ($p = 0.020$). The immobility times of female and male WT mice were also significantly different ($p < 0.001$) while there was a strong trend towards a sex difference within the HD group ($p = 0.051$).
**Figure 3-3 Results of forced-swim and tail-suspension tests conducted at 12 weeks.**

(A) Female wild-type mice ($n = 6$, 32.3secs ± 15.2) recorded less immobility time on the FST compared to male wild-type mice ($n = 22$, 87.7secs ± 12.5) and female HD mice ($n = 11$, 97.8secs ± 12.8). There was no significant difference between the times recorded by male HD ($n = 14$, 113.1secs ± 13.8) and wild-type mice. (B) In contrast to the FST, female wild-type mice ($n = 6$, 154.1secs ± 19.1) recorded significantly greater immobility time on the TST than female HD mice ($n = 11$, 85.2secs ± 6.0). There was a difference between the immobility times of female and male wild-type mice ($n = 19$, 103.5secs ± 13.2). Male HD mice ($n = 16$, 78.3secs ± 17.6) displayed significantly less TST immobility than male wild-type mice. Asterisks indicate post-hoc Bonferroni’s t-test: *: $p < 0.05$, ***: $p < 0.001$. 
3.3.5 Novelty-suppressed feeding test (NSFT)

The NSFT assesses stress-induced anxiety by measuring the latency of a mouse to feed in a novel open-field environment. In order to determine whether the anxiety levels of HD and wild-type mice were similar, the time taken for mice to enter the centre of the test arena where the food pellet was located was compared (Fig. 3-4A). The time taken by mice to enter the centre of the test arena did not differ between the sexes ($F_{(1,48)} = 2.132$, $p = 0.151$) or genotypes ($F_{(1,48)} = 1.635$, $p = 0.208$). There was no significant sex-genotype interaction ($F_{(1,48)} = 0.000808$, $p = 0.977$).

There were no differences between the sexes ($F_{(1,49)} = 0.509$, $p = 0.479$) or genotypes ($F_{(1,49)} = 1.260$, $p = 0.267$) in the time taken to start feeding on the food pellet that was placed in the centre of the test arena (Fig. 3-4B). There was no significant sex-genotype interaction ($F_{(1,49)} = 0.518$, $p = 0.475$).

The amount of food consumed by the mice post-NSFT is an indicator of the extent of hunger induced as a result of the food deprivation. There were no differences between the sexes ($F_{(1,49)} = 0.0442$, $p = 0.834$) or genotypes ($F_{(1,49)} = 1.015$, $p = 0.319$) in the time taken to start feeding on the food pellet that was placed in the centre of the test arena (Fig. 3-4C). There was also no significant sex-genotype interaction ($F_{(1,49)} = 0.0316$, $p = 0.860$).

The NSFT was later repeated with a modified food deprivation protocol and results are presented in Appendix I.
A  Latency to enter center of test arena

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B  Latency to feed

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C  Post-test food consumption

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Figure 3-4 Performance of HD and wild-type mice on the novelty-suppressed feeding test.

(A) There was no difference in the average length of time taken by female HD (n = 11, 87.5secs ± 12.7) and wild-type (n = 10, 107.5secs ± 21.3) mice to enter the center of the test arena. The mean times of male HD (n = 17, 63.6secs ± 11.7) and wild-type (n = 11, 84.5secs ± 18.3) mice were also similar. (B) The average time taken by female HD mice (n = 11, 257.3secs ± 40.4) to commence feeding in the test arena was not statistically different from female wild-type mice (n = 10, 208.0secs ± 23.8). The times recorded by male HD (n = 17, 219.0secs ± 19.2) and wild-type mice (n = 12, 208.2secs ± 19.1) were also similar. (C) The average amount of food consumed in a 5-minute period post-NSFT by female HD mice (n = 11, 0.154g ± 0.022) was similar to that of female wild-type mice (n = 10, 0.120g ± 0.030). Similarly, there was no difference in the amount of food consumed between male HD (n = 17, 0.143g ± 0.016) and wild-type mice (n = 12, 0.119g ± 0.043) in this control measure.
3.3.6 Effect of acute antidepressants on FST and TST behaviour

The FST and TST have been used to routinely test the efficacy of antidepressant compounds which reduce immobility time upon acute administration of antidepressants (Cryan et al., 2005a; Cryan et al., 2005b). Here, the abilities of two drugs from different classes of antidepressants - the SSRI sertraline and the tricyclic antidepressant desipramine – were tested for their ability to decrease immobility times of HD and wild-type mice on the FST and TST.

3.3.6.1 Effect of acute sertraline or desipramine on female FST performance

Overall, both genotype ($F(1,48) = 19.945, p < 0.001$) and sertraline treatment ($F(1,48) = 9.500, p = 0.004$) were significant sources of variation impacting on female FST performance (Fig. 3-5A). There was no significant genotype-drug treatment interaction ($F(1,48) = 0.00932, p = 0.924$). The FST performances recorded by saline-treated mice reflected the results of the baseline FST experiment (see Section 3.3.3) as HD mice ($n = 12, 98.9secs \pm 11.8$) recorded significantly greater immobility time than wild-type mice ($n = 12, 48.1secs \pm 7.6; p = 0.002$). Acute sertraline treatment significantly reduced the average immobility time of female wild-type mice ($p = 0.046$). Sertraline also significantly reduced the immobility times female HD mice ($p = 0.025$).

Comparison of the desipramine and saline-treated groups revealed no overall differences in FST performance due to genotype ($F(1,43) = 1.814, p =0.186$) or desipramine treatment ($F(1,43) = 0.0000125, p = 0.997$). There was a significant genotype-drug treatment interaction $F(1,43) = 5.034, p = 0.030$). Acute administration of desipramine did not reduce FST immobility times of HD ($p = 0.121$) or wild-type mice ($p =0.120$) compared to the respective saline-treated groups. There was no difference between the mean immobility times of desipramine-treated HD and wild-type groups ($p = 0.547$).
3.3.6.2 Effect of acute sertraline or desipramine on male FST performance

Genotype (F(1,54) = 13.010, p < 0.001) and drug-treatment (F(1,54) = 6.837, p = 0.012) were significant sources of variance influencing FST performance of male mice (Fig. 3-5B). There was also a significant genotype-drug treatment interaction (F(1,54) = 4.942, p = 0.031). In agreement with the results of baseline behavioural phenotyping (Section 3.3.3), the average immobility times of saline-treated HD mice (n = 13, 89.7secs ± 11.4) did not differ from saline-treated wild-type mice (n = 14, 77.0secs ± 11.0; p = 0.279). Sertraline treatment significantly reduced FST immobility times of male wild-type mice (p = 0.001) but did not effect the behavioural responses of male HD mice (p = 0.785). Therefore, there was a significant difference between the average FST immobility times of sertraline-treated HD and wild-type mice (p < 0.001).

Comparison of the saline and desipramine-treated male groups revealed an overall effect of genotype (F(1,45) = 29.528, p < 0.001) but not drug-treatment (F(1,45) = 0.869, p = 0.357) on male FST performance. In addition, there was a significant genotype-drug treatment interaction (F(1,45) = 16.524, p < 0.001). Desipramine treatment significantly reduced the mean FST immobility time of wild-type mice (p = 0.025). In contrast, desipramine treatment led to a significant increase in immobility times of HD mice (p = 0.002) and there was a significant difference in the average immobility times of desipramine-treated HD and wild-type mice (p < 0.001).
Figure 3-5 Acute effects of sertraline and desipramine on FST performance.

(A) Sertraline reduced FST immobility time of female HD (n = 14, 63.5secs ± 14.8) and wild-type mice (n = 11, 14.82secs ± 4.5), but desipramine did not have any observable effects of either group (HD: n = 10, 67.1secs ± 19.4; WT: n = 10, 79.8secs ± 17.6). (B) Mean FST immobility times of male wild-type mice was reduced by sertraline (n = 13, 23.2secs ± 4.7) and desipramine (n = 9, 40.9secs ± 10.7). Sertraline had no effect on male HD mice (n = 14, 85.1secs ± 13.9) and desipramine surprisingly increased the FST immobility time of this group (n = 7, 144.1 ± 9.5). Asterisks indicate post-hoc Bonferroni’s t-test: *: p < 0.05, **: p < 0.01, ***: p < 0.001.
3.3.7 Acute effects of sertraline and desipramine on TST performance

3.3.7.1 Effects of acute sertraline and desipramine treatments on female TST performance

Two-way ANOVA revealed that female TST performance was dependent on genotype ($F_{(1,50)} = 4.461$, $p = 0.040$) and drug-treatment ($F_{(2,50)} = 5.627$, $p = 0.007$) and there was no significant genotype-drug treatment interaction ($F_{(2,50)} = 2.276$, $p = 0.114$) (Fig. 3-6A). Saline-treated mice recorded greater immobility times than sertraline-treated mice ($p = 0.006$) but there were no differences between the saline- and desipramine-treated groups ($p = 0.568$) as well as between the desipramine- and sertraline-treated groups ($p = 0.107$).

Saline-treated HD mice ($n = 5, 72.36\text{secs} \pm 11.3$) recorded significantly less immobility time on the TST compared to saline-treated wild-type mice ($n = 9, 114.17\text{secs} \pm 13.4$; $p = 0.020$). Acute sertraline treatment reduced the average immobility time of wild-type mice ($p = 0.006$) but significantly altered the behavioural responses of female HD mice ($p = 0.412$). There was no difference between the immobility times of sertraline-treated wild-type and HD mice ($p = 0.177$).

Female wild-type mice treated with desipramine recorded significantly lower immobility times compared to the saline-treated group ($p = 0.029$), but the drug did not have a significant effect on female HD mice. There was no difference between the immobility times of desipramine-treated HD and wild-type mice ($p = 0.709$).
### 3.3.7.2 Effects of acute sertraline and desipramine treatments on male TST performance

Genotype ($F_{(1,49)} = 5.288, p = 0.026$) and drug-treatment ($F_{(2,49)} = 5.242, p = 0.009$) were significant factors affecting TST performance of male mice (Fig. 3-6B). There was also a significant genotype-drug treatment interaction ($F_{(2,49)} = 7.084, p = 0.002$). There were overall effects of sertraline ($p = 0.015$) and desipramine ($p = 0.019$) but no difference between the effects of sertraline and desipramine ($p = 1.000$).

As expected, saline-treated male wild-type mice ($n = 7, 135.2\text{ secs} \pm 17.2$) recorded significantly greater immobility times on the TST than saline-treated HD mice ($n = 5, 46.8\text{ secs} \pm 9.6; p < 0.001$). Sertraline significantly reduced immobility time of wild-type mice ($p < 0.001$) but had no effect on HD mice ($p = 0.579$). There was no difference between the average immobility times of sertraline-treated HD and wild-type mice ($p = 0.299$).

Acute desipramine treatment significantly reduced the average immobility time of male wild-type mice ($p < 0.001$). However, male HD mice treated with desipramine ($p = 0.975$) showed no significant change in TST immobility times. There was no difference between the average immobility times of desipramine-treated HD and wild-type mice ($p = 0.629$).
Figure 3-6 Acute effects of sertraline and desipramine on TST performance.

(A) Sertraline reduced TST immobility of female wild-type mice (n = 9, 65.8secs ± 11.2) but not HD mice (n = 9, 44.33secs ± 6.0). Desipramine reduced the mean immobility time of female wild-type mice (n = 10, 75.9secs ± 15.4) on the TST but did not alter female HD (n = 9, 81.6secs ± 9.6) performance. (B) Sertraline (n = 9, 38.7secs ± 7.8) and desipramine (n = 11, 54.3secs ± 13.1) reduced TST immobility time of male wild-type mice. There were no observable effects of either drug on male HD mice (Sertraline: n = 9, 57.4secs ± 12.3; Desipramine: n = 9, 46.1secs ± 14.1). Asterisks indicate post-hoc Bonferroni’s t-test: *: p < 0.05, ***: p < 0.001.
3.3.8 Acute effects of the novel antidepressant compound JZ-IV-7

JZ-IV-7 (Acenta Discovery Inc., USA) is a novel antidepressant compound which selectively blocks both serotonin and norepinephrine transporters and effectively reduces the immobility time of wild-type mice on the TST (J. Zhou & Acenta Discovery Inc., personal communications). We tested the ability of this experimental drug to exert an antidepressant effect on the HD mice.

In the female group, two-way ANOVA revealed significant effects of genotype ($F_{(1,24)} = 19.034, p < 0.001$) and drug-treatment ($F_{(1,24)} = 14.561, p = 0.001$) (Fig. 3-7A). There was no significant genotype-treatment interaction ($F_{(1,24)} = 0.0330, p = 0.858$). As expected, the average TST immobility time of vehicle-treated HD mice ($n = 5, 111.36\text{secs} \pm 13.3$) was significantly lower than that of vehicle-treated wild-type mice ($n = 5, 165.08\text{secs} \pm 10.4; p = 0.008$). Administration of JZ-IV-7 significantly reduced the immobility times of wild-type mice ($p = 0.009$) as well as of the HD mice ($p = 0.019$). However, a significant difference between immobility times of JZ-IV-7 treated HD and wild-type mice persisted ($p = 0.003$).

The drug was also tested on a group of male mice; however it should be noted that several wild-type mice had to be excluded from the results analysis (refer to Chapter 2: Materials and Methods for exclusion criteria). Overall, there were significant effects of genotype ($F_{(1,20)} = 14.863, p = 0.001$) and drug-treatment ($F_{(1,20)} = 4.591, p = 0.047$) (Fig. 7B). There was no significant genotype-drug treatment interaction ($F_{(1,20)} = 4.123, p = 0.058$). There was no difference in the mean TST immobility times of vehicle-treated male HD ($n = 6, 96.1\text{secs} \pm 12.7$) and wild-type mice ($n = 2, 78.2\text{secs} \pm 16.7; p = 0.411$). Treatment with JZ-IV-7 significantly reduced TST immobility times of wild-type mice ($p = 0.024$) but had no effect on male HD mice ($p = 0.939$). As such, there was a significant difference in the average immobility times of male HD and wild-type mice in the drug-treated group ($p < 0.001$).
Figure 3-7 Effects of JZ-IV-7 on TST performance.

(A) JZ-IV-7 reduced immobility times of female HD (n = 7, 68.4secs ± 14.2) and wild-type mice (n = 8, 117.83secs ± 7.7). (B) JZ-IV-7 significantly reduced male wild-type (n = 5, 20.4secs ± 7.7) immobility time on the TST but had no effect on male HD mice (n = 8, 101.9secs ± 10.8). Asterisks indicate post-hoc Bonferroni’s t-test: *: p < 0.05, ***: p < 0.001.
3.3.9 Performance of 8-week old mice on the forced-swim test

To determine whether the HD behavioural phenotype progressively developed with age or was innate to this line of mice, the FST was performed on a separate and behaviourally naïve cohort when the mice were 8 weeks of age.

Two-way ANOVA revealed no significant effect of sex ($F_{(1,25)} = 3.633, p = 0.070$) but a significant effect of genotype ($F_{(1,25)} = 22.716, p < 0.001$) on FST immobility times (Fig. 3-8A). There was no significant genotype-sex interaction ($F_{(1,25)} = 0.829, p = 0.372$). The average FST immobility time of female HD mice was significantly greater than the immobility times of female wild-type mice ($p < 0.001$). The immobility times of male HD mice were also greater than those of male wild-type mice ($p = 0.014$).

3.3.10 Performance of 8-week old mice on the tail-suspension test

There was no effect of genotype ($F_{(1,28)} = 3.836, p = 0.061$) or sex ($F_{(1,28)} = 1.577, p = 0.221$) on TST performance of 8 week old mice (Fig. 3-8B) and there was no significant genotype-sex interaction ($F_{(1,28)} = 1.036, p = 0.319$). There were no differences detected within both female (HD: $n = 10, 103.4$secs ± 10.8; WT: $n = 4, 118.7$secs ± 23.2) and male groups (HD: $n = 11, 107.3$secs ± 11.3; WT: $n = 4, 155.4$secs ± 23.6).
Figure 3-8 Forced-swim and tail-suspension tests at 8 weeks.

(A) 8-week old female (n = 8, 135.3secs ± 8.6) and male HD mice (n = 9, 147.8secs ± 5.1) recorded greater immobility times on the FST compared to wild-type littermates (Female: n = 4, 63.8secs ± 19.8; Male: n = 4, 99.3secs ± 23.2). (B) There were no differences between the groups on the TST. Asterisks indicate post-hoc Bonferroni’s t-test: *: p < 0.05, ***: p < 0.001.
3.4 Discussion

The findings described in this chapter offer new insight into HD-related depression by demonstrating that expression of the HD-causing gene mutation results in the display of altered behaviour in the R6/1 HD mouse model that is indicative of depressive-like symptoms. Just as significant is the detection of those abnormal behavioural responses prior to any observation of motor symptoms which strengthens the argument against psychosocial effects as the main trigger for depressive mood during the early stages of HD. Instead, it supports the notion that HD is a neurodegenerative disorder that presents as a genetic predisposition for depression during the preclinical stages in addition to other possible neuropsychiatric developments.

This study has utilized a transgenic mouse model of HD thereby avoiding many confounding factors that could possibly compromise patient-based studies. Multiple factors must be considered for a well-controlled patient-based study such as male/female subject ratios, medical history (previous and currently prescribed medication), ethnicity, diet and disease state (advancement of disease). Often, the recruitment process is difficult and it is not uncommon that critical patient information such as medication history might be unavailable. A lack of age-matched control subjects also renders such studies statistically under-powered and as a consequence, the results need to be interpreted with caution. The use of transgenic mouse models of HD avoids the psycho-social caveats of patient-based studies since the mice are not consciously aware of the consequences of expressing the HD mutation. Furthermore, standardization of genetic background, housing conditions, social interaction, physical activity and diet can be feasibly achieved.

However, the use of rodent models to study human depression remains controversial. It is argued that depression involves the altered state of emotion which has yet to be convincingly replicated or proven to exist in rodents. Furthermore, the validity of using various behavioural tests to model specific aspects and symptoms of depression which
are observed in depressed patients continue to be fiercely debated. The most frequently employed behavioural tests are the forced-swim, tail-suspension and novelty-suppressed feeding tests which have been shown to be sensitive to antidepressant administration. It should be noted that these three tests address different aspects of depressive behaviour. The FST and TST elicit a behavioural response that is taken to be reflective of the feeling of helplessness and are sensitive to acute antidepressant administration. The NSFT requires mice to overcome a highly anxiogenic environment to quench the feeling of hunger and this is sensitive to chronic and not acute antidepressant treatment. These tests are routinely used in studies investigating the role of various genes believed to be involved in depression and mood disorders, and have gradually been accepted to possess face and predictive validity. In fact, the TST is the choice of pharmaceutical companies as a high throughput, predictive tool to screen for potential novel antidepressant compounds.

The age of the mice used in this study was strictly controlled and it was decided that 12 weeks would be an age reflective of preclinical HD because mice of this early age are not motor symptomatic while cognitive changes were just detectable (Mazarakis et al., 2005; Nithianantharajah et al., 2008). Further confirmation that the mice were at a very preliminary stage of disease progression came from the finding that basal serum corticosterone levels of HD mice at this age were not different from wild-type levels, agreeing with a previous report that corticosterone levels are not elevated until the mid-advanced stages of disease progression in the R6/2 mouse line (Bjorkqvist et al., 2006). The decision to further assess the R6/1 mice at 8-weeks of age was based on suggestions that depression may be detected in HD patients more than a decade before clinical onset. The preliminary screen found the absence of any differences in TST performance at 8 weeks suggesting that the development of the depressive-like behaviours in the R6/1 mouse line is progressive in nature. In comparison, an altered FST performance consistent with the 12 week data was detected in the female HD mice which indicates that the FST might be a more sensitive tool for the detection of depressive-like behaviours in this mouse line. It was surprising to find that 8-week old male HD mice tended to have increased immobility on the FST but this study has since been repeated
independently in a larger cohort of mice and only a female-specific HD phenotype was
detected (Renoir & Hannan, personal communications).

Female HD mice had a more robust ‘depression’ phenotype since behavioural alterations
were observed on all three behavioural tests while HD males only displayed altered
behaviour on the TST. On the other hand, this observation could be reflecting a greater
tendency of females with HD to develop depressive-related behaviours than males with
HD. While the exact reasons for this sexual dichotomy have yet to be fully understood, it
would agree with depression data based on the general human population where
depression is twice more likely to develop in females than in males. One possible
explanation for the behavioural difference is that females respond differently in situations
of stress and this was observed within the wild-type group on both the FST and TST as
there were significant differences in the immobility times recorded by the female wild-
type mice from the male wild-types.

The significance of the observed sex differences in wild-type behaviour on these
‘depression’ tests cannot be overstated. While it is widely recognized that females are
more susceptible to developing depressive symptoms, the reasons for this sex difference
are not well understood. It is believed that sex hormones are involved, in particular the
female sex hormone estrogen which has been shown to exert antidepressant-like effects
on the FST (Estrada-Camarena et al., 2003, 2004; Frye et al., 2008; Walf and Frye, 2008).
It is important that the underlying differences between the male and female systems are
clearly understood as it would potentially have a great impact on therapeutic approaches
and treatment strategies. It is worth noting that pharmaceutical companies persist in using
only male mice during their trials of novel antidepressant compounds. The existence of a
high degree of variance in behavioural results obtained from populations of female
rodents is often cited as the main reason for male-only studies. However, given that the
female population has a greater tendency to develop depression and that their behaviour
is distinctly different from the males, future drug studies should include female rodents to
unmask any differences in drug response between males and females (further discussed below).

Female HD mice behaved differently from female wild-types on the FST and TST showing that expression of the HD mutation altered the behavioural responses to stressful situations. The NSFT was repeated (Appendix I) when no differences were detected on the first test raising the possibility that the initial food deprivation protocol was unsuccessful. By modifying the food deprivation period, a consistent loss of 15% body weight by all mice was achieved and there was also an overall reduction in the latency to feed in the NSFT. However, there was no change to post-NSFT feeding indicating that overall appetite and motivation to perform the test was not altered. This subsequent NSFT revealed yet another female HD-specific response whereby female HD mice were taking significantly longer to initiate feeding in the NSFT arena while the male HD response was not different from male wild-type mice. Together, these altered behavioural responses were not due to changes in the anxiety levels of the R6/1 HD mice since it had been previously established that this line of mice do not have an anxiety phenotype (Nithianantharajah et al., 2008). There are many facets of depressive moods including altered stress response and elevated anxiety. Based on the present observations, it appears that stress-induced depression is more relevant than anxiety-induced mood alterations in HD. Studies following this up in cohorts of HD patients by analyzing the presentation of depression will be required to confirm this hypothesis.

The one possible caveat to the NSFT result is that it involves exposing the test subject to a bout of stress as it requires a period of food deprivation before the actual test. Food deprivation has been shown to result in increased serum corticosterone levels in mice (Komori et al., 1996; Mantella et al., 2005) and as previously mentioned, an altered stress response could potentially account for the different performance on the NSFT. To overcome this potential confound, an alternative test to employed would be the saccharin/sucrose preference test which is an indicator of anhedonia, a core symptom of depression describing the inability to gain pleasure from normally pleasurable
experiences. In this test, an unstressed test subject is provided the free-choice of consuming sweetened or unsweetened drinking water with a display of a clear preference for the sweetened water being the expected response. An assessment of the R6/1 behavioural response on this test has subsequently been performed and consistent with all the findings described thus far, a female HD-specific anhedonic tendency is observed at 8-weeks of age (Renoir & Hannan, personal communications), providing further evidence of the sex-specific depressive-like behavioural phenotype in this line of mice.

It was interesting to observe that while female HD behaviour was distinctly different from wild-types on all three behavioural tests, male HD mice only displayed altered TST behaviours. It is not known how such a paradoxical situation may exist since both tests are essentially based upon eliciting similar behavioural responses under the same stressful situations but it is possible that subtle differences might exist between these two tests which cannot be explained at present time. Furthermore, the overall results of the TST were unexpected since reduced TST immobility is often interpreted as a display of ‘anti-depressive’ behaviour. However, this merely highlights the danger of over-interpreting results or drawing conclusions regarding behavioural phenotypes based on a single test. It also emphasizes the need for multiple test paradigms during the process of behavioural phenotyping, reaffirming the strategy for this study which incorporated three separate tests.

A direct comparison of TST and FST behaviours revealed seemingly contradictory behaviour by the female HD mice. However, this is not a novel behavioural profile as similar behavioral patterns have been reported in mouse models used to investigate the molecular pathology of mood disorders. Both serotonin 1A and 1B receptor knockout lines display conflicting TST/FST behavioral profiles as well as sex-differences that are similar to our observations (Mayorga et al., 2001; Jones and Lucki, 2005). An extrapolation of our observations implicates a possible involvement of one or both receptor subtypes in the HD depressive-like phenotype. More specifically, it suggests that either a functional disruption of the serotonin receptors or a loss of serotonin receptor-
mediated signaling has a role in eliciting depression behaviours in HD. There is good evidence of impaired serotonin receptor-1A and -1B function in post mortem HD brains (Castro et al., 1998) and in the R6/2 mouse model of HD (Yohrling et al., 2002) and a global reduction in serotonin levels (Reynolds et al., 1999). Future work will be required to determine whether receptor dysfunction, reduced bioavailability of the serotonin neurotransmitter or impaired downstream signaling from the serotonin receptors contribute directly to the development of psychiatric symptoms in early stage HD. Those findings would be crucial for understanding the molecular changes associated with HD pathogenesis as well as having important implications in the process of determining appropriate therapeutic approaches to treating the affective symptoms in HD since SSRIs are one of the most commonly prescribed classes of antidepressants. Analysis of the serotonergic signaling system in R6/1 HD mice is described in Chapter 6.

It was surprising to observe that desipramine did not reduce immobility time of female wild-type mice on the FST and TST, suggesting that a suboptimal concentration of desipramine may have been used. However, there was a desipramine-induced behavioural response elicited in the male wild-type mice at the same dosage, confirming that the drug doses administered in this study were sufficient. In addition, this concentration (20mg/kg) has been shown to have an antidepressive effect in female mice based on previous publications (Bernardi et al., 1989; Monteggia et al., 2007). There is still the possibility that the dosage used here (20mg/kg) was not high enough since one study found that a slightly reduced dose of 16mg/kg was not sufficient to reduce TST immobility time and that a dose of 32mg/kg was more effective (Crozatier et al., 2007). There is also the question of why sertraline was effective in female wild-types in the absence of a desipramine effect. As a tricyclic antidepressant, desipramine inhibits the noradrenaline and serotonin reuptake transporters and possesses an affinity for the muscarinic and histamine H1 receptors while sertraline acts by specifically blocking the serotonin transporter. It is possible that interactions of the other receptors in the female system are different from that in males, and this complete lack of a desipramine effect in the female group highlights the possible differences in response of the male and female systems to this tricyclic antidepressant. Desipramine is unlikely to be the only antidepressant to have
such different effects on males and females; hence this again emphasizes the need for drug trials to be conducted on both sexes instead of just males as the proficiency of novel antidepressant compounds may be different in females compared to males.

Female HD mice showed a drug response profile similar to wild-types as sertraline reduced FST immobility time but not desipramine. However, the effect of sertraline was limited to the FST since immobility time on the TST was not significantly reduced. This observation of a positive behavioural change on one test but not another observation is difficult to interpret while the subtle differences between the FST and TST remain uncertain. However, it does offer hope that the use of sertraline might have some beneficial effects in treating female HD patients with depression. Our observation of the male HD response to antidepressant treatment was less positive as neither sertraline nor desipramine had the desired behavioural effects on the FST and TST. Surprisingly, there was also a negative effect of desipramine which resulted in increased immobility time of the treated HD males. Taken together, the absence of any positive drug effects signals a breakdown of the molecular signaling mechanisms required for effective drug activity in the male HD system. This is most likely reason for this is the lack of bioavailability of the neurotransmitters that both antidepressants were intended to boost as serotonin and noradrenaline levels are progressively reduced in the HD brain (Reynolds et al., 1999). However, patient studies have revealed that noradrenaline levels are increased in the advanced stages of HD while remaining unchanged in cerebrospinal fluid implying that the integrity and function of the central noradrenergic pathways remain intact throughout the disease. Further investigation will be necessary to elucidate the exact relationship between the decline in these signaling systems and disease progression as this would facilitate a more effective treatment of depression symptoms with the different classes of antidepressants.

Antidepressants have been shown to have beneficial effects on the survival, as well as cognitive and motor aspects, of HD mouse models (Duan et al., 2004; Grote et al., 2005; Peng et al., 2007). However, it has been demonstrated here that they fail to produce a
robust behavioural response in HD mice. To understand why these differential effects exist, it will be essential to understand the actions of antidepressants on the HD brain. Antidepressants (in particular SSRIs) induce molecular and cellular changes with the most prominent being an increase in the expression of the neurotrophic factor, brain-derived neurotrophic factor (BDNF) in the hippocampus (Nibuya et al., 1995). Within the dentate gyrus sub-region of the hippocampus, antidepressants trigger an elevation of cell proliferation and neurogenesis (Malberg et al., 2000). All those processes have been shown to be disrupted in the HD brain, albeit in more advanced stages of the disease than what was investigated in this study (Zuccato et al., 2001; Lazic et al., 2004; Grote et al., 2005; Pang et al., 2006). Future work will be required to find out whether BDNF expression, hippocampal cell proliferation and neurogenesis are indeed altered in 12-week old R6/1 HD mice, as well as whether these are different in males and females which could account for the differences in displayed behaviours and drug responses. Further to that, the partial effect of sertraline within the female HD group could be due to the interaction of sex hormones at the level of the serotonin receptors as the serotonergic system has been shown to cross-link with BDNF-dependant signaling pathways via the BDNF receptor, TrkB. Further work will be required to elucidate whether TrkB-mediated signaling is altered in the HD condition which would validate its involvement in disease pathology.
3.5. Conclusion

This study has shown that prior to developing motor symptoms and changes in the HPA axis, R6/1 HD mice develop a genotype-related depressive-like phenotype. This reflects the elevated occurrence of depression in the human patient population and establishes the validity of the R6/1 HD mouse model as a working model of HD-related depression. The observed behavioural differences between the sexes as well in the different responses to antidepressants by the HD mice might also exist within the HD patient population and future patient-based studies will be required to confirm these findings. This initial behavioural investigation will provide the basis for further investigations of the molecular changes occurring in early HD that contribute towards the development of HD-related depression.
Chapter 4 Modification of the behavioural phenotype and cognitive deficits of R6/1 HD mice by environmental stimuli.

4.1 Introduction

Huntington’s disease (HD) is caused by an expansion of the CAG repeat sequence in the *huntingtin* gene located on chromosome 4p16.3 (Huntington’s disease Collaborative Research Group, 1993). The length of the trinucleotide repeats is correlated with age of onset (Andrew et al., 1993; Duyao et al., 1993). However, while the length of the CAG repeat expansion is the most important factor in predicting age of onset, there is still a great degree of variability (50-60%) in terms of age of onset that is attributed to non-genetic factors (Andrew et al., 1993; Wexler et al., 2004). Also, the disease has been documented to present in monozygotic twins differently (Georgiou et al., 1999). Studies of mouse models of HD have demonstrated that the environment exerts a major impact on the disease by delaying the onset and progression of motor symptoms as well as rescuing the early cognitive deficits (van Dellen et al., 2000; Hockly et al., 2002; Pang et al., 2006; Nithianantharajah et al., 2008). Given that neuropsychiatric symptoms are a major component of HD, it would be important to determine the nature and extent of the environmental influences, especially for depression.

The previous chapter reported that prior to becoming motor symptomatic, female HD mice displayed depression-related behaviours thereby strongly suggesting that carrying the Huntington’s disease-causing gene mutation constituted a genetic predisposition for developing depression-associated behaviours that appears to be more prevalent in females. Attempts to normalize the behavioural phenotype of the HD mice by acute administration of antidepressant drugs were not entirely successful, possibly reflecting a breakdown of the cellular or molecular events that are required for the drug-mediated changes in behaviour. In this chapter, the modulation of the development of HD-related depression by the environment was explored.
Many studies have attempted to explain the predisposition for depression as a function of genetic variance with several gene polymorphisms having been identified as genetic susceptibility factors (Dorado et al., 2007; Jokela et al., 2007; Orstavik et al., 2007; Enoch et al., 2008). Also implicated as a major component of depression etiology is a wide variety of non-specific environmental factors including negative life experiences (traumatic life events or death), social life-style and diet (Rice et al., 2003; Takkinen et al., 2004; Boomsma et al., 2005; Liang and Eley, 2005; Hettema et al., 2006; Lau and Eley, 2006; Al Mamun et al., 2007; Gregory et al., 2007; Nes et al., 2007). Monozygotic twin studies provide further evidence that the occurrence of depression is strongly influenced by the environment as there is incomplete concordance in the manifestation of depression (Takkinen et al., 2004; Nes et al., 2007). While depression is modulated by environmental factors, the exact reasons for gender bias in depression with females twice more likely to develop depression than males have yet to be fully understood. This dichotomy is independent of environmental influences (Bierut et al., 1999) and it has been suggested that the environmental factors which shape the development of depressive symptoms for both males and females are similar (Agrawal et al., 2004).

While antidepressants remain the most common form of treatment for depression, the involvement in a regimented exercise schedule as a means of alleviating depressive mood has been gaining popularity in recent times (reviewed by Barbour et al., 2007). Exercise, especially running, have been shown to be as efficacious as antidepressants in reducing depressive symptoms (Nabkasorn et al., 2006; Blumenthal et al., 2007). These findings have also been extended into the laboratory setting and the ‘anti-depressive’ effects of environmental enrichment and exercise have been replicated in various rodent models (Bjornebekk et al., 2005; Brenes Saenz et al., 2006; Bhansali et al., 2007; Brene et al., 2007; Koh et al., 2007; Brenes et al., 2008).

An estimated 30-50% of HD patients present with signs of depression at some point during the course of disease (Slaughter et al., 2001; Paulsen et al., 2005) which is a higher rate of incidence than in the general population. The development of depression is
clearly not purely dependent on genetic factors since not every HD patient is affected. It is very difficult to estimate the extent of the environmental influence on the manifestation of depressive symptoms in HD patients and this issue would ideally be addressed with studies of twin pairs wherein the rates of depression can be compared between monozygotic and non-identical twins. The most feasible alternative would therefore be to conduct investigations of gene-environment interactions surrounding depression in HD using the various models of HD. Having established that a depression-related behavioural phenotype with sexual dichotomy can be detected in the R6/1 line (Chapter 3), it is now possible to conduct a study that precisely controls the genetic and environmental conditions which would be impossible for any patient-based study.

Current knowledge of depression pathology leans heavily towards the involvement of the hippocampal formation in this mood disorder. Of particular interest is the repeated findings of reductions in hippocampal volumes associated with depression in both males and females (Frodl et al., 2002; Colla et al., 2007; Janssen et al., 2007; Maller et al., 2007; MacMaster et al., 2008). Differential exposure to environmental influences has a clear impact on depression neuropathology as seen from a study of monozygotic twins discordant for depression in which it was found that the depressed twin was more likely to have a smaller hippocampal volume than the non-depressed twin (de Geus et al., 2007). The exact pathological changes involved in the reduction of hippocampal volumes have yet to be fully understood (discussed by Czeh and Lucassen, 2007) but have been strongly associated with a disruption of the neurogenic process. The rate of proliferation and neurogenesis within the dentate gyrus of the hippocampus is the key event behind the ‘hippocampal neurogenesis’ hypothesis for depression which proposed that the affective changes in depression are directly related to a decrease in hippocampal cell proliferation and neurogenesis. However, this has been challenged by a report that neural stem cell proliferation is not altered in brain tissue from depressed patients (Reif et al., 2006). The evidence supporting the involvement of hippocampal neurogenesis in depression stems from studies investigating the working mechanisms of antidepressants in combination with rodent models of depression. The chronic stress model of depression is a commonly utilized rodent model of depression based on the induction of depressive mood following
repeated exposure to stressful experiences (similar to environmentally-driven depression in humans) and this experimental paradigm results in a pathological reduction in hippocampal cell proliferation and neurogenesis (Czech et al., 2001; Westenbroek et al., 2004; Rosenbrock et al., 2005; Jayatissa et al., 2006). The positive effects of antidepressant treatment are concurrent with an increase in hippocampal neurogenesis (Malberg et al., 2000; Santarelli et al., 2003) and perhaps not coincidentally, the ‘anti-depressive’ effects of running also require the active process of hippocampal cell proliferation (Bjornebekk et al., 2005).

The neuropathology of depression is strikingly similar to that which occurs in HD, especially when examining the evidence centered on the hippocampus. Patient imaging studies have shown that hippocampal volume is decreased by the advanced stages of the condition (Geuze et al., 2005). This has been replicated in the R6/1 transgenic mouse model (Grote et al., 2005) which has further been used to attribute the volumetric change to the disruption of hippocampal cell proliferation (Lazic et al., 2004) and neurogenesis (Grote et al., 2005). These neurogenic changes have also been demonstrated to occur in the R6/2 transgenic model (Gil et al., 2005). Cell death does not appear to be the primary cause of this volumetric shift as it is not prominent until the terminal stages of HD when apoptosis is evident due to DNA damage (Smith et al., 2006; Anne et al., 2007). It is interesting to note that post-mortem studies on tissue from advanced stage HD brains have reported an up-regulation of cell proliferation and neurogenesis in the subependymal layer of the lateral ventricle (Curtis et al., 2003; Curtis et al., 2005) although the exact implications of those findings have yet to be understood. Further studies will be required to characterise the state of cell proliferation in relation to hippocampal volume at the earlier stages of HD.

The occurrence of depression in HD has also been correlated with a decline in cognitive ability (Nehl et al., 2001). HD patients have deficits in visual-spatial memory related to changes in corticostrial circuitry (Mohr et al., 1991; Lawrence et al., 1996; Lawrence et al., 2000). However, cognitive processing also involves the hippocampal circuitry. It had
previously been established that R6/1 HD mice have memory deficits on hippocampus-dependant cognitive tasks such as spontaneous alternation in the T-maze and recognition of a novel arm in the Y-maze by the time they are motor symptomatic (Pang et al., 2006). However, subtle cognitive deficits can be detected during the preclinical stages of HD (Lawrence et al., 1998). In support of that, R6/2 HD mice display a learning impairment in the Morris water maze (another test of spatial learning and memory) (discussed by D’Hooge and De Deyn, 2001) prior to the development of a motor phenotype (Lione et al., 1999; Murphy et al., 2000) and this deficit is associated with changes in hippocampal CA1 synaptic plasticity (Murphy et al., 2000). There is a specific decline in the ability to process hippocampal-dependant cognitive tasks as further demonstrated by the ability of R6/1 HD mice to perform the hippocampal-independent version of the object recognition test, but not the hippocampal-dependent version (Nithianantharajah et al., 2008). These deficits of hippocampal-dependent cognition are delayed by environmental enrichment (Nithianantharajah et al., 2008) and wheel-running (Pang et al., 2006) which once again highlights the beneficial effects of positive environmental stimulation previously demonstrated in many other mouse models (Arendash et al., 2004; Dahlqvist et al., 2004; Gobbo and O’Mara, 2004; Jankowsky et al., 2005; Martinez-Cue et al., 2005; Costa et al., 2007; Huang et al., 2007).

Wheel-running improves cognitive ability and Morris water maze performance of wild-type rodents (Fordyce and Wehner, 1993; van Praag et al., 1999b) although there appears to be an upper limit to the extent that physical exercise can induce potential cognitive benefits (Rhodes et al., 2003). The improvement of cognitive performance following bouts of running is associated with increased hippocampal cell proliferation and neurogenesis (van Praag et al., 1999b; van Praag et al., 1999a; Van der Borght et al., 2007) and a disruption of those processes leads to cognitive impairment (Madsen et al., 2003; Raber et al., 2004; Rola et al., 2004; Snyder et al., 2005; Winocur et al., 2006). Interestingly, it has been shown that hippocampal cell proliferation and neurogenesis is disrupted in the brains of older HD mice (Lazic et al., 2004; Gil et al., 2005; Grote et al., 2005; Phillips et al., 2005). It is not known whether and how these cellular processes are
disrupted during the early stages of the disease which might relate to the onset of the cognitive deficits in HD.

Given the evidence that environmental factors exert a strong influence on the development of depression and cognitive ability, the effects of environmental enrichment and wheel-running on the depressive-like phenotype of R6/1 HD mice were assessed. Quantification of hippocampal volume and cell proliferation at that same age was performed in order to determine whether either parameter was associated with the behavioural phenotype. Finally, the spatial learning and memory capabilities of HD mice were further investigated.

The aims for this study were:

1. To investigate the effects of environmental enrichment and wheel-running on the depressive-like phenotype displayed by 12-week old R6/1 HD mice.
2. To analyze cell proliferation and volume of the dentate gyrus in 12-week old HD mice housed under standard and wheel-running housing conditions.
3. To evaluate spatial location memory in HD mice using the Morris Water Maze and establish the effects of wheel-running on cognitive ability.
4.2 Materials and Methods

All experiments described in this chapter were performed on male and female R6/1 transgenic HD mice and their wild-type littermates bred from the colony at the Howard Florey Institute. Litters were weaned at 3 weeks of age into boxes of four mice per box. 8-week old mice were randomly allocated to wheel-running, environmentally enriched or standard-housed conditions. Wheel-running mice were provided free access to two running wheels per box. Environmentally enriched mice were provided extra materials and bedding in their home cages in addition to various items and toys that were changed once per week. In addition, three times per week, enriched mice were transferred into an external enrichment chamber for one hour which offered extra opportunities for exploration and interaction. The forced-swim (FST) was conducted on 12-week old mice from the wheel-running and environmentally enriched cohorts with separate groups of standard-housed controls. The Morris Water Maze (MWM) was conducted on 16-week old mice (Males: 3-4 per group; Females: 6-8 per group) housed under standard or wheel-running conditions. Mice were trained over four days to locate a partially submerged platform guided by four intra-maze spatial cues. A single probe trial was conducted on the fifth day during which the platform was removed and mice allowed to swim in the maze for 60 seconds. For the cell proliferation study, mice were given i.p. injections of BrdU (50mg/kg) for 14 consecutive days starting at 8 weeks. At 12 weeks, the mice were perfused with 4% paraformaldehyde and brains were collected and processed for histochemical and volumetric analysis. Visualization of BrdU-positive cells was achieved by immunoreaction with an anti-BrdU antibody followed by reaction with DAB (Vector Laboratories). All DAB-stained cells within the dentate hilus were counted. The dentate gyrus was manually delineated at 20x magnification on a Zeiss microscope using the Zeiss Axioscope software and adjusted for section thickness and serial sectioning to obtain an estimate of dentate gyrus volume. Male and female performance on the FST and MWM probe trial was analysed with two-way ANOVA with genotype and housing environment as the two variant factors. Performance during the MWM training period was analysed with two-way repeated measures ANOVA. Significant results were analysed with post-hoc Bonferroni’s t-test with the level of significance set at $\alpha = 0.05$. 
4.3 Results

4.3.1. Enrichment reduces FST immobility time of female HD mice

In Chapter 3, female R6/1 mice (HD) were documented to adopt abnormally extended periods of immobility during the FST. It has previously been shown that the experimental paradigms of environmental enrichment and wheel-running reduce immobility times of wild-type rodents on the FST (Magalhaes et al., 2004; Koh et al., 2007; Llorens-Martin et al., 2007). Therefore, male and female HD mice were tested on the FST after four weeks of being housed under environmentally enriching conditions.

Overall, there was an effect of genotype ($F_{(1,22)} = 16.317, p < 0.001$) but not the environment ($F_{(1,22)} = 1.715, p = 0.206$) on FST performance of female mice (Fig. 4-1A). However, there was a significant genotype-environment interaction ($F_{(1,22)} = 4.993, p = 0.038$). In agreement with the findings described in Chapter 3, the average immobility time of standard-housed female HD mice ($n = 6$, 167.8secs ± 12.9) on the FST was significantly greater than standard-housed female wild-types ($n = 5$, 56.6secs ± 26.1; $p < 0.001$). Female HD mice that had been housed under environmentally enriched conditions recorded significantly reduced FST immobility times ($n = 7$, 105.0secs ± 15.8; $p = 0.015$). Surprisingly, environmental enrichment did not significantly alter the average immobility time of female wild-type mice ($n = 5$, 73.0secs ± 15.0; $p = 0.545$). There was no difference between average immobility times of enriched HD and wild-type females ($p = 0.210$).
Both genotype ($F_{(1,32)} = 3.137, p = 0.087$) and housing environment ($F_{(1,32)} = 3.609, p = 0.067$) were not significant factors effecting the FST performance of male mice (Fig. 4-1B). Unlike in the female group, there was no significant genotype-environment interaction ($F_{(1,32)} = 3.187, p = 0.085$). Replicating findings described in Chapter 3, standard-housed male HD ($n = 10, 166.5\text{secs} \pm 18.8$) and wild-type mice ($n = 9, 164.8\text{secs} \pm 17.7$) recorded similar average FST immobility times. Environmental enrichment did not alter the FST performances of male HD ($n = 9, 164.8\text{secs} \pm 14.2; p = 0.944$) or wild-type mice ($n = 7, 111.3\text{secs} \pm 17.8; p = 0.410$).
Figure 4-1 Effects of environmental enrichment on forced-swim test performance. (A) Female HD mice housed under environmentally enriched conditions recorded reduced immobility times. (B) There was no effect of environmental enrichment on FST performance of male mice. Asterisks indicate post-hoc Bonferroni’s t-test: *: p <0.05, ***: p < 0.001.
4.3.2 Running reduces FST immobility time of female HD mice

Apart from environmental enrichment, increased levels of physical exercise by means of wheel-running has also been suggested as an ‘anti-depressive’ activity in wild-type rodents (Russo-Neustadt et al., 2001; Bjornebekk et al., 2005). The anti-depressive effects of running were tested here by providing HD mice with free-access to running wheels in their home cages for four weeks before being tested on the FST.

Genotype ($F_{(1,18)} = 19.383, p < 0.001$) and environment ($F_{(1,18)} = 7.105, p = 0.018$) were both significant factors affecting the overall FST results (Fig. 4-2A). There was no significant genotype-environment interaction ($F_{(1,18)} = 1.070, p = 0.317$). As expected, the average FST immobility time of standard-housed female HD mice ($n = 4; 164.0\text{secs} \pm 18.5$) was significantly greater than the average immobility time of the female wild-types ($n = 5; 82.0\text{secs} \pm 8.8; p = 0.002$). Similar to the effect of environmental enrichment, female HD runners ($n = 5; 108.2\text{secs} \pm 16.5$) recorded significantly lower immobility times compared to the standard-housed group ($p = 0.023$). The FST performance of female wild-type runners ($n = 5; 57.4\text{secs} \pm 15.8$) was not significantly altered ($p = 0.253$). There was a significant difference in average immobility times of female HD and wild-type runners ($p = 0.027$).

Within the male group, there was no overall effect of genotype ($F_{(1,22)} = 1.120, p = 0.303$) or environment ($F_{(1,22)} = 0.0897, p = 0.768$) on FST performance (Fig. 4-2B). However, there was a significant genotype-environment interaction ($F_{(1,22)} = 6.042, p = 0.024$). Surprisingly, there was a significant difference between standard-housed male HD ($n = 6, 133.7\text{secs} \pm 12.4$) and wild-type mice ($n = 4, 91.8\text{secs} \pm 12.2; p = 0.025$). Wheel-running did not reduce the average immobility times of male HD ($n = 6, 100.8\text{secs} \pm 12.3; p = 0.060$) and wild-type mice ($n = 6, 117.5\text{secs} \pm 10.4; p = 0.152$) compared to their respective standard-housed controls. There was no significant difference between the average immobility times recorded by HD and wild-type runners ($p = 0.323$).
Figure 4-2 Effects of wheel-running on forced-swim test performance.
(A) The average FST immobility time of female HD mice was reduced following four weeks of wheel-running. (B) There were no overall genotype or environment effects on male FST performance. Asterisks indicate post-hoc Bonferroni’s t-test: *: p < 0.05.
4.3.3 HD-related depressive phenotype independent of hippocampal integrity

The volume of the hippocampal formation is reduced in HD (Geuze et al., 2005) and a
disruption of the proliferative (Lazic et al., 2004) and neurogenic (Grote et al., 2005)
processes is believed to contribute to that change. Volumetric changes have also been
detected in brains of depressed patients (Sheline, 1996; Sheline et al., 1996; Frodl et al.,
2002; MacQueen et al., 2003; Sheline et al., 2003; Campbell et al., 2004; Videbech and
Ravnkilde, 2004; Geuze et al.) although the exact causes remain unresolved. Active
hippocampal neurogenesis is required for the behavioural effects of antidepressant drugs
(Sahay and Hen, 2008; Surget et al., 2008) hence the association of depressive behaviour
with disrupted hippocampal neurogenesis.

The observed ‘anti-depressive’ effect of environmental enrichment on female HD mice
coupled with an up-regulation of hippocampal neurogenesis by environmental
enrichment (Lazic et al., 2006) led to the examination of the effects of wheel-running on
hippocampal volume and cell proliferation in the HD brain, an activity which has already
been shown to increase hippocampal cell proliferation in wild-type rodents (Bjornebekk
et al., 2005).

Since female and male HD mice were behaviourally distinct on the FST, an initial
analysis was performed to determine if there were any corresponding sex differences in
hippocampal cell proliferation in standard-housed mice. Overall, there was a strong effect
of genotype ($F_{(1,10)} = 558.161, p < 0.001$) but no significant effect of sex ($F_{(1,10)} =
0.000861, p = 0.977$) (Fig. 4-3A). There was no genotype-sex interaction ($F_{(1,10)} = 0.538,$
$p = 0.487$). Male HD mice had significantly less number of BrdU-labeled cells in the
dentate gyrus compared to male wild-type mice ($p < 0.001$). Female HD mice also had
significantly less cells that were positively labeled with Brd-U compared to female wild-
type mice ($p < 0.001$). Therefore, results from the male and female groups were pooled
for further analysis.
There were highly significant effects of genotype ($F_{(1,22)} = 56.293$, $p < 0.001$) and environment ($F_{(1,22)} = 24.729$, $p < 0.001$) on cell proliferation numbers (Fig. 4-3B). There was also a significant genotype-environment interaction ($F_{(1,22)} = 20.800$, $p < 0.001$). Unexpectedly, post-hoc analysis revealed only a strong trend towards a difference between the number of Brd-U+ cells counted in standard-housed HD and wild-type mice ($p = 0.056$). There was a significant increase in the number of Brd-U+ cells detected in wheel-running wild-type mice ($p < 0.001$) compared to the numbers in standard-housed wild-type. In contrast, this selective effect of wheel-running was limited to the wild-type hippocampus as running had no visible effect on the level of proliferation in HD mice ($p = 0.779$). Hence, there was a significant difference in the numbers of Brd-U+ cells counted in HD and wild-type mice of the wheel-running group ($p < 0.001$).

Overall, the volume of the dentate gyrus was significantly influenced by genotype ($F_{(1,22)} = 29.954$, $p < 0.001$) and environment ($F_{(1,22)} = 32.472$, $p < 0.001$) (Fig. 3C). There was also a significant genotype-environment interaction ($F_{(1,22)} = 17.709$, $p < 0.001$). The average volume of the dentate gyrus of standard-housed HD mice did not differ from that of standard-housed wild-type mice ($p = 0.393$). Instead, there was a significant difference in dentate gyrus volumes within the wheel-running group ($p < 0.001$) since wheel-running led to a significant increase in the volume of the dentate gyrus in wild-type runners ($p < 0.001$) while in contrast, the dentate gyrus volume of HD runners was not changed ($p = 0.316$).
A. Cell proliferation in the dentate gyrus

- Male: HD > WT
- Female: HD > WT

B. Effect of wheel-running on cell proliferation

- Standard housed: HD < WT
- Wheel-running: HD < WT

C. Est. volume of the dentate gyrus

- Standard housed: HD = WT
- Wheel-running: HD > WT
Figure 4-3 Effect of wheel-running on cell proliferation and dentate gyrus volume.

(A) Differences in cell proliferation male (HD: n = 3, 17.7 ± 1.9; WT: n = 3, 82.7 ± 1.8) and female mice (HD: n = 2, 15.5 ± 11.0; WT: n = 3, 84.7 ± 2.9). (B) A comparison of cell proliferation in the dentate gyrus of standard-housed (n = 6, 83.7 cells ± 1.6) and wheel-running wild-type mice (n = 6, 300.3 cells ± 43.0). Wheel-running HD mice (n = 6, 26.2 cells ± 4.1) did not have greater number of newborn cells compared to the control group (n = 5, 16.8 cells ± 2.1). (C) Wheel-running increased dentate gyrus volume of wild-type (SH: n = 6, 3.21 mm³ ± 0.07; WR: n = 6, 4.45 mm³ ± 0.21), but not HD mice (SH: n = 5, 3.06 mm³ ± 0.10; WR: n = 6, 3.24 mm³ ± 0.05). Asterisks indicate post-hoc Bonferroni’s t-test: ***: p < 0.001.
4.3.4 No improvement of Morris water maze performance by wheel-running

HD-related depression is associated with greater impairment of working memory (Nehl et al., 2001). HD mice had previously been shown to develop memory deficits on the T- and Y-mazes (Pang et al., 2006). Here, working memory was assessed on a separate cognitive task - the Morris water maze (MWM). The beneficial effect of running on cognitive ability was also explored as short periods of running have been shown to improve MWM performance of wild-type mice in association with increased hippocampal cell proliferation and neurogenesis (van Praag et al., 1999b; van Praag et al., 1999a).

4.3.4.1 Swimming speeds

The onset of motor symptoms was likely to impact on the ability of HD mice to perform the water maze task. Mice were checked for rear-paw clasping (the stereotypic motor phenotype of this mouse line) at 16 weeks of age and 45.5% of standard-housed HD mice and 20% of HD runners were found to display this physical symptom. However, this did not impede their ability to swim as there was no overall difference in swimming speeds between the genotypes ($F_{(1,38)} = 0.137$, $p = 0.714$) (Fig. 4-4A). There was also no significant effect of the environment ($F_{(1,38)} = 3.341$, $p = 0.076$) and no genotype-environment interaction ($F_{(1,38)} = 0.178$, $p = 0.676$).
Figure 4-4 Swim speeds and learning phase performance of mice on Morris water maze.

(A) There were no significant differences between the average swimming speeds of standard-housed HD (n = 11, 22.0cm/sec ± 4.0) and wild-type mice (n = 9, 21.8cm/sec ± 1.0), as well as between wheel-running HD (n = 9, 16.4cm/sec ± 1.2) and wild-type mice (n = 10, 18.4cm/sec ± 1.3). (B) Performance of standard-housed and wheel-running groups of mice during the MWM training period.
4.3.4.2. Performance during training days

Having determined that HD mice had the ability to swim normally, further analyses revealed that HD mice had a learning and memory deficit which was not rescued by wheel-running.

Firstly, the average times taken by mice to locate the hidden platform during the four training sessions on each of the four days of training was examined as an indicator of learning. Only results of the 2\textsuperscript{nd} to 4\textsuperscript{th} days of training were analysed as the results from the 1\textsuperscript{st} day were lost due to unforeseen technical difficulties. Analysis of the performance of standard-housed mice with 2-way repeated measures ANOVA revealed overall differences due to genotype ($F_{(1,59)} = 30.292, p < 0.001$) and the days of training ($F_{(2, 59)} = 4.267, p = 0.022$) (Fig. 4-4B). There was no significant genotype-training day interaction ($F_{(2, 59)} = 0.277, p = 0.760$). Standard-housed HD mice averaged significantly greater latencies than standard-housed wild-types to find the platform on each of the training days (Day 2: $p < 0.001$, Day 3: $p < 0.001$, Day 4: $p < 0.001$). As a whole, the average time of all standard-housed mice to locate the platform decreased from Day 2 to Day 4 ($p = 0.021$). It was therefore surprising to find that the individual group performances of wild-type ($p = 0.258$) and HD mice ($p = 0.079$) did not significantly improve from Day 2 to Day 4 of the training period.

There was an overall genotype difference ($F_{(1,59)} = 12.710, p = 0.002$) influencing the performance of the wheel-running mice but there was no overall difference between the days of training ($F_{(2,59)} = 0.171, p = 0.843$). There was no genotype-training day interaction ($F_{(2,59)} = 1.676, p = 0.201$). The average time taken by wheel-running HD mice to find the platform during the training phase was significantly greater than that of wheel-running wild-type mice (Day 2: $p = 0.015$, Day 3: $p = 0.011$, Day 4: $p = 0.003$).
4.3.4.3. Platform quadrant location during probe trial

During the probe trial, the submerged platform was removed and mice were allowed to swim in the MWM for a total of 60 seconds. There was an overall significant effect of genotype ($F_{(1,38)} = 24.494, \ p < 0.001$) but not of the environment ($F_{(1,38)} = 0.887, \ p = 0.353$) on the time it took mice to swim into the platform quadrant (Fig. 4-5A). There was no significant genotype-environment interaction ($F_{(1,38)} = 0.550, \ p = 0.463$). Standard-housed HD mice (6.3secs ± 1.5) took a significantly longer amount of time to locate the platform quadrant than standard-housed wild-types (1.2secs ± 0.4; $p = 0.005$). Wheel-running HD mice (8.3secs ± 1.8) also took a significantly longer amount of time to swim into the platform-quadrant than wild-type runners (1.4secs ± 0.4; $p < 0.001$).

4.3.4.4. Time spent and distance swum in platform quadrant during probe trial

Overall, HD mice spent less time swimming in the platform quadrant ($F_{(1,38)} = 6.877, \ p = 0.013$) while there was no effect of wheel-running ($F_{(1,38)} = 0.946, \ p = 0.337$) and no significant genotype-running interaction ($F_{(1,38)} = 0.138, \ p = 0.712$) (Fig. 4-5B). Standard-housed HD mice (13.2secs ± 1.4) spent significantly less time in the platform-quadrant than the wild-types (18.3secs ± 2.1; $p = 0.039$). There was no significant difference between the average times spent by HD (12.2secs ± 1.8) and wild-type runners (16.0secs ± 1.5; $p = 0.124$) in the platform quadrant.

HD mice also recorded significantly shorter swimming path lengths ($F_{(1,38)} = 11.135, \ p = 0.002$) than wild-type mice (Fig. 4-6A). Running did not alter overall path lengths ($F_{(1,38)} = 1.616, \ p = 0.212$) and there was no significant genotype-running interaction ($F_{(1,38)} = 0.451, \ p = 0.506$). Standard-housed HD mice (200.7cm ± 36.0) swam a shorter distance in the platform-quadrant than standard-housed wild-type mice (323.3cm ± 26.0; $p = 0.007$). There was no difference between HD (182.4cm ± 26.0; $p = 0.671$) and wild-type runners (263.9cm ± 29.2; $p = 0.071$).
Figure 4-5 Latency to platform quadrant and time spent swimming in platform quadrant.

(A) HD mice took significantly longer to swim into the platform quadrant. (B) Standard-housed HD mice spent less time swimming in the platform quadrant. Asterisks indicate post-hoc Bonferroni’s t-test: *: p < 0.05, ***: p < 0.001.
4.3.4.5. Time taken to locate platform area during probe trial

In addition to taking longer to locate the correct platform-quadrant, HD mice also took a longer time overall to locate the exact platform-area (F(1,38) = 13.252, p < 0.001) (Fig. 4-6B). Running had no overall effect (F(1,38) = 0.722, p = 0.401) and there was no significant genotype-environment effect (F(1,38) = 0.189, p = 0.666). Standard-housed HD mice (19.3secs ± 4.9) averaged significantly longer times to locate the platform area than standard-housed wild-type mice (4.8secs ± 1.5; p = 0.028). There was also a significant difference between the average latency-to-platform area times of HD (25.2secs ± 7.4) and WT runners (6.7secs ± 1.6; p = 0.007).

4.3.4.6. Number of platform area crossings during probe trial

Overall, HD mice averaged significantly fewer crosses of the platform-area than WT mice (F(1,38) = 36.317, p < 0.001) (Fig. 4-7A). Running did not impact on the frequency of platform crossings (F(1,38) = 0.0636, p = 0.802) and there was no significant genotype-environment interaction (F(1,38) = 0.0310, p =0.861). Both standard-housed (6.4 ± 1.0; p < 0.001) and wheel-running wild-type groups (6.5 ± 1.0; p < 0.001) crossed the platform-area significantly more frequently than their respective HD comparisons (Standard-housed HD: 1.9 ± 0.3; Wheel-running HD: 2.2 ± 0.5).

4.3.4.7. Duration swimming over platform area during probe trial

Overall, HD mice spent significantly less time swimming at the exact location of the platform area than wild-type mice (F(1,38) = 26.222, p < 0.001) (Fig. 4-7B). Running did not have an effect on this parameter (F(1,38) = 1.335, p = 0.256) and there was no significant genotype-running interaction (F(1,38) = 0.182, p =0.673). Both standard-housed (3.3 ± 0.7; p = 0.002) and wheel-running wild-type groups (4.1 ± 0.6; p < 0.001) spent significantly greater amounts of time within the platform-area than their respective HD comparisons (Standard-housed HD: 1.0 ± 0.2; Wheel-running HD: 1.4 ± 0.4).
Figure 4-6 Distance swum in platform quadrant and latency to platform area.

(A) The total distance swum in the platform quadrant by standard-housed HD mice was less than wild-type mice. (B) HD mice took significantly longer to swim into the platform area. Asterisks indicate post-hoc Bonferroni’s t-test: *: p < 0.05.
Figure 4-7 Number of platform crossings and duration over platform area.

(A) The total number of crossings of the platform area by standard-housed HD mice was less than wild-type mice. (B) HD mice spent significantly less time swimming within the platform area. Asterisks indicate post-hoc Bonferroni’s t-test: *: p < 0.05, ***: p < 0.001.
4.4 Discussion

The results described in this chapter show that the behavioural phenotype of female HD mice on the forced-swim test can be normalized by environmental enrichment and wheel-running. HD mice had reduced dentate gyrus volume and hippocampal cell proliferation but while environmental enrichment rescues both these deficits (Lazic et al., 2006), neither benefited from wheel-running in spite of a strong effect on the wild-type hippocampus. Finally, while environmental enrichment and wheel-running has been shown to improve cognitive performance on various hippocampal-dependent cognitive tasks (Pang et al., 2006; Nithianantharajah et al., 2008), HD mice had a severe impairment on the Morris water maze which was not rescued by wheel-running.

Both environmental enrichment and wheel-running exerted a sex-specific 'anti-depressive' behavioural effect on female, but not male, HD mice on the forced-swim test. This is the first evidence that the environment modulates the display of depressive-like behaviour in HD and further supports the notion that environmental factors strongly influence the development of depression. The results also agree with other studies that wheel-running and other forms of physical exercise are anti-depressive activities that benefit various rodent models of depression (Russo-Neustadt et al., 1999; Solberg et al., 1999; Bjornebekk et al., 2005; Koh et al., 2007; Brenes et al., 2008) and improves mood in humans (Brown et al., 1992; Morris and Salmon, 1994; Blumenthal et al., 1999; Babyak et al., 2000; Bartholomew et al., 2005; Ernst et al., 2006; Nabkasorn et al., 2006; Blumenthal et al., 2007; Brene et al., 2007). Here, it is clear that physical exercise is beneficial for treating HD-related depression and these findings could potentially be transferred into the clinical environment to aid the development of future therapeutic programs that treat the neuropsychiatric symptoms.

It was perhaps not surprising that the FST performances of wild-types and male HD mice were unaltered by environmental enrichment and wheel-running since they were
considered not to have a “depressive” phenotype. While no study to date has specifically addressed the effect of antidepressants on non-depressed individuals, anecdotal evidence suggests that antidepressants have no significant effect on non-depressed individuals because there is no “depression” to be treated as such. In other words, behavioural changes indicative of effective treatment are observed only if the subject being treated is truly expressing depression. In this case, since the wild-type response is regarded as baseline and male HD mice do not differ, then it can be concluded that there was no depressive phenotype that stood to be corrected by environmental modulation.

A further extrapolation of this specific environment-mediated behavioural modification is that the environmental effects are translated into specific molecular changes to the female HD brain that have a part in the development of depressive behaviour. For example, it has been demonstrated that running triggers changes in mRNA levels of neuropeptide Y and the opiates, dynorphin and enkephalin, in a strain of rat commonly used as a model of depression (Flinders Sensitive Line) that is not detected in the ‘nondepressed’ Flinders Resistent Line (FRL) strain (Bjornebekk et al., 2006). The exact molecular mechanisms behind the modification of behaviour by antidepressant drugs continue to be debated but there have been suggestions that antidepressants may not work by direct modulation of mood and anxiety, but rather by changing emotional processing to facilitate positive perspectives (Harmer, 2008; Harmer et al., 2008). It is difficult to decipher whether rodents are capable of such high-order cognitive processing and future investigation is warranted.

Assuming that the female-HD phenotype is wholly attributable to the effect of the HD, yet another interpretation of the FST results would be that the behaviour of male HD mice is resistant to environmental modification which would be similar to the lack of response by male HD mice to antidepressant drugs as described in the Chapter 3. This would not be the first report of different responses to environmental modifiers by male and female mice as there have been several studies that have reported different effects of enrichment on the exploratory behaviours, anxiety levels and cognitive performances of
male and female rodents (Daniel et al., 1999; Bardo et al., 2001; Elliott and Grunberg, 2005). Similarly, wheel-running alone has also been reported to have different effects on the anxiety levels and cognitive performance of male and female transgenic mice modeling Alzheimer’s disease (Pietropaolo et al., 2008). Future studies will be required to understand the effects that positive environmental stimuli have on particular molecular pathways which ultimately translate into that ‘anti-depressant’ behavioural change. Also, it would be important to identify any differences between the male and female HD brains might contribute to the different environmental responses.

Depression is associated with reduced hippocampal volume and chronic antidepressant treatment increases hippocampal neurogenesis. Consequently, a deficit in hippocampal neurogenesis is implicated as a cause of depression pathophysiology. Chronic stress models of depression have been demonstrated to produced behavioural changes together with reduced levels of neurogenesis, both of which are reversed by antidepressant treatments. (Pham et al., 2003; Lee et al., 2006). Young R6/1 HD mice have normal levels of cell proliferation which is disrupted by the later stages of the disease (Lazic et al., 2004; Grote et al., 2005; Lazic et al., 2006). This pathophysiological change has previously been linked to the cognitive changes in HD. This study now shows that in addition to cognitive decline, the deficit in hippocampal neurogenesis might also have a role in the development of the depressive phenotype as well as reduction of hippocampal volume which has been revealed from clinical imaging studies (Geuze et al., 2005). Evidence for the latter stems from the finding that a severe deficit in cell proliferation in the HD hippocampus occurs despite no detectable reductions in dentate gyrus volume, clearly indicating that the gradual shrinkage of the hippocampus is secondary to a prolonged disruption of the neuronal replacement process.

Given that an increase in hippocampal neurogenesis is associated with the ‘anti-depressive’ effects of antidepressant drugs, it was no surprise that environmental enrichment corrected the HD behavioural phenotype since enrichment had previously been shown to rescue the deficit in neurogenesis in late-stage HD mice (Lazic et al.,
In contrast, wheel-running exerted an ‘anti-depressive’ behavioural effect despite an inability to up-regulate neurogenesis in HD mice. The absence of a running effect on the R6/1 model agrees with a recent study that reported a complete lack of an effect of running on the R6/2 HD model (Kohl et al., 2007). Taken together, there is clear indication that the anti-depressive effects of running occur independently of an up-regulation of hippocampal neurogenesis. The results in this thesis would then be the first report of an ‘anti-depressive’ effect in the absence of a cellular change and would point to the existence of specific running-activated signaling pathways which directly affect behaviour but are non-neurogenic. Future studies would be required to identify such specific signaling pathways and one possible avenue to explore would be a direct comparison of the gene expression profiles of environmentally enriched and running HD mice. Such work would further enable the delineation of the precise molecular signals that trigger hippocampal neurogenesis and those required for a behavioural change. Findings stemming from this work could potentially lead to the development of therapeutics focused around these genes that would benefit both HD and depression sufferers.

It was interesting to find that male HD mice were as deficient in hippocampal neurogenesis as female HD mice. This finding eliminates the possibility that the female HD behavioural phenotype is directly due to a decline in hippocampal neurogenesis which is the basis for the ‘neurogenesis hypothesis of depression’. Furthermore, the absence of a running-induced up-regulation of hippocampal neurogenesis in female HD mice indicates that rescue of the depressive-like phenotype is independent of running-induced hippocampal neurogenesis. However, it is unclear at present whether this constitutes a debunking of the neurogenesis hypothesis of depression. One possibility is that despite their similarities, it is probable that the underlying pathology of HD-related depression and clinical depression are different. As such, the proliferation deficit may contribute to the development of the phenotype but it is by no means likely to be the primary mediatory factor. The absence of a male phenotype also suggests that there is some level of dissociation between the sex-specific behavioural phenotype and hippocampal neurogenesis. It is highly possible that sex hormones are involved in this
behavioural difference and future studies will be required to further investigate the sex-difference in the R6/1 HD model (discussed by McEwen, 2002; Galea et al., 2006; Hajszan et al., 2007). However, all signs point to HD-related depression being atypical due to the lack of concordance at the level of cellular pathology, behavioural phenotype and response to antidepressant treatment (as reported in Chapter 3). This has strong implications for the clinical setting as alternative forms of treatment might need to be considered when treating HD-related depression.

HD mice are impaired on hippocampal-dependent cognitive tasks and environmental enrichment (Nithianantharajah et al., 2008) as well as wheel-running (Pang et al., 2006) have been shown to correct at least some of those cognitive deficits. The Morris water maze is a test of long-term spatial learning and memory and while R6/2 mice have been shown to be impaired on this task (Lione et al., 1999; Murphy et al., 2000), no work had yet been done on the R6/1 line. Here, HD mice were severely impaired in their ability to navigate the water maze cues to learn and remember the location of the partially submerged platform on which they could escape the water. The findings are in agreement with the specific impairment of spatial location memory displayed by HD patients (Lawrence et al., 2000). It is certain that the findings are a true reflection of the cognitive state of HD mice and not due to motor impairment since the swimming speeds were all similar. The greater tendency for female HD mice to adopt an immobile posture in water would have been another confound for the MWM but no mice were observed to float during this test (personal observations).

It was interesting to observe that wheel-running did not improve the MWM performance of HD mice because environmental enrichment had previously been found to be capable of significantly reducing the time it takes for HD mice to locate the platform quadrant in the MWM (Zajac & Hannan, personal communications). This is yet another parameter that is differentially affected by environmental modification, similar to rescue levels of hippocampal neurogenesis in HD mice by environmental enrichment but not wheel-running. Improved performance on the water maze has been shown to be directly
associated with increased hippocampal neurogenesis (Czurko et al., 1997). Given that running but not enrichment-induced hippocampal neurogenesis is disrupted in HD mice, it is therefore not surprising that enrichment but not running is capable of imparting a beneficial effect on the MWM performance of HD mice.

Surprisingly, there was also no improvement of wild-type MWM performance following wheel-running despite the expected increase in hippocampal neurogenesis which has been directly correlated with improved spatial working memory (van Praag et al., 1999b). One possible explanation for the lack of a running effect is that the wild-type mice had been running for an 8 week period. The amount and duration of running has been shown to impact on MWM performance as mice bred for high-levels of running do not show improved MWM performance despite the same elevated levels of neurogenesis (Rhodes et al., 2003). It has been suggested that the birth of new hippocampal neurons in a period of up to 28 days prior to learning a memory provides the basis or a ‘space’ for new memories to be encoded and the neurons which are involved in encoding a memory will survive while non-encoding neurons will die (thereby maintaining hippocampal volume within a preset physiological limit) (Snyder et al., 2005; Sisti et al., 2007). A level of cell turnover beyond a maximal threshold during periods of chronic running would lead to a disruption of the memory consolidation process normally involving a stable pool of newborn neurons.
4.5 Conclusion

This study has shown that the depression-like phenotype developed by female HD mice during the early stages of the disease can be reversed by increasing the level of environmental stimulation and physical activity. A reduction in hippocampal cell proliferation was identified as the possible cause of hippocampal shrinkage during the later stages of the disease. However, this reduction was not causatively linked to the depression phenotype because it was found in both male and female HD mice. Environmental enrichment triggers an increase in neurogenesis in the HD hippocampus via a mechanism that is independent of the signaling pathways stimulated by physical activity alone, reflecting differential hippocampal processing of signals that originate from the broader cortical regions.
Chapter 5 Investigation of the factors contributing to altered BDNF gene expression in the HD hippocampus.

5.1 Introduction

The previous two chapters have described the detection of depression-related behaviours specific to female R6/1 transgenic HD mice and how those behaviours are modified and corrected back to wild-type levels by increased cognitive stimulation (environmental enrichment) and physical activity (wheel-running). This chapter will seek to address the molecular changes that might contribute towards the female-specific HD behavioural phenotype.

Microarray profiling has revealed that the expression of a wide array of genes is altered in post mortem HD brains and mouse models of HD (Luthi-Carter et al., 2000; Luthi-Carter et al., 2002b; Luthi-Carter et al., 2002a; Hodges et al., 2006; Kuhn et al., 2007; Anderson et al., 2008). Most studies to date have been focused on gene expression changes in the striatal, cortical and cerebellar regions during the later stages of HD (Iannicola et al., 2000; Luthi-Carter et al., 2003; Obrietan and Hoyt, 2004) and have been associated with the cognitive and motor symptoms. Little is known about gene expression changes in other distinct brain regions during the initial stages of HD that might be related to the development of neuropsychiatric symptoms, such as the hippocampus.

Brain-derived neurotrophic factor (BDNF) is an essential neurotrophin that is strongly implicated in the development of HD pathology. The huntingtin protein is required for BDNF gene transcription (Zuccato et al., 2001) and anterograde trafficking of the protein from the cortex to the striatum (Gauthier et al., 2004; Colin et al., 2008). These critical processes are disrupted in the HD brain and as a result, there is a marked reduction of BDNF levels in the striatum. This loss of tropic support to striatal neurons eventually leads to abnormal dopaminergic signaling and contributes to the development of the severe motor symptoms of HD (Canals et al., 2004; Pineda et al., 2005). Studies have
demonstrated the neuroprotective and restorative effects of exogenously applied BDNF in various HD models (Volpe et al., 1998; Lynch et al., 2007). Most recently, over-expression of BDNF in the forebrain of transgenic HD mice was shown to result in increased BDNF levels in the striatum along with an amelioration of motor dysfunction and gross loss of brain weight (Gharami et al., 2008). As such, there is optimism that therapeutic interventions based on the use of BDNF would be successful for the treatment of HD patients.

BDNF is implicated in the pathophysiology of major depression. BDNF expression is reduced in postmortem brain samples (Knable et al., 2004) while serum levels of BDNF are also reduced in depressed patients and can be treated with antidepressants (Hellweg et al., 2008; Huang et al., 2008; Matrisciano et al., 2008; Sen et al., 2008). BDNF is central to the neurotrophic hypothesis of depression which suggests that altered expression of this molecule is involved in depression pathology and is key to the treatment of the condition (reviewed by Duman and Monteggia, 2006). A loss of BDNF expression in the forebrain of mice resulted in a female-specific development of depression-related behaviours and non-responsiveness to antidepressant administration (Monteggia et al., 2007). Interestingly, and as described in Chapter 3, R6/1 HD mice are also found to exhibit a similar behavioural phenotype. It is therefore possible that a loss of BDNF expression in the HD brain is directly linked to the development of the neuropsychiatric aspects of this disease, in particular depression.

The exact mechanisms that regulate the expression of BDNF in the brain remain unclear at present. The BDNF gene is complex as there are multiple 5’ non-coding exons (Timmusk et al., 1993; Liu et al., 2006; Aid et al., 2007; Pruunsild et al., 2007). Alternative splicing occurs and a set of unique BDNF mRNA transcripts is formed following the recombination of a single non-coding exon with the main coding exon. However, all transcripts are subsequently translated into the same protein. The purpose of these multiple exons has been suggested to serve as a means of translating the spatial and temporal information contained within a particular neuronal signal (Tongiorgi et al., 1997;
Tongiorgi et al., 2004; Pattabiraman et al., 2005; Chiaruttini et al., 2008). It is well-established that BDNF gene expression is reduced in the HD brain (Zuccato et al., 2001; Pang et al., 2006). Little is known about how the expression of specific BDNF splice variants is altered. One study of the R6/2 transgenic HD model revealed that a progressive loss of BDNF exons II, III (IV in the present study) and IV (VI in the present study) in the cortex corresponded to increasing disease severity (Zuccato et al., 2005).

Various studies have reported that stress reduces BDNF expression in the brain (Nibuya et al., 1995; Smith et al., 1995a, b; Ueyama et al., 1997). Recently, it has also emerged that stressors linked to the development of depression-related behaviours in rodent models induce differential regulation of BDNF exon-specific transcripts (Nair et al., 2006; Tsankova et al., 2006; Fuchikami et al., 2008). Furthermore, treatments which alleviate those depressive behaviours such as antidepressant drugs (Khundakar and Zetterstrom, 2006; Tsankova et al., 2006; Yasuda et al., 2007) and physical activity such as running (Russo-Neustadt et al., 2000; Garcia et al., 2003) lead to an up-regulation of BDNF transcription in an exon-specific manner. As such, it is possible that the ‘antidepressant’-like effects of environmental enrichment and wheel-running on female HD mice observed in Chapter 2 could be due to an up-regulation of particular BDNF exon-specific transcripts and this warrants further investigation.

While the specific mechanisms by which BDNF gene expression is disrupted in HD remain unclear, a general picture of the broader causes of disrupted gene transcription in HD has begun to emerge. Aggregates formed from N-terminal fragments of the mutant huntingtin protein sequester transcription factors thereby reducing their bioavailability (Chen-Plotkin et al., 2006). Mutant htt protein also interferes with the activity of the enzyme histone acetyltransferase which acetylates the core histones rendering DNA more accessible to transcription factors (Steffan et al., 2001). This latter event results in the hypoacetylation of histones associated with many of the genes down-regulated in the HD (Igarashi et al., 2003; Sadri-Vakili and Cha, 2006). These observations are supported by the success of various histone deacetylase (HDAC) inhibitors in treating the pathological
symptoms of various HD models (Ferrante et al., 2003; Hockly et al., 2003; Steffan and Thompson, 2003; and reviewed by Butler and Bates, 2006).

A dysregulation of the epigenetic environment impacts on gene expression and is implicated in the pathology of depression (reviewed by Tsankova et al., 2007). The epigenetic regulation of BDNF transcription involves the modulation of DNA methylation. An increase in BDNF synthesis correlates with a decrease in methylation levels of CpG dinucleotides within the regulatory region of the BDNF gene sequence together with a dissociation of the methyl-CpG-binding-protein (MeCP2) repression complex from the gene promoter region (Martinowich et al., 2003). While the exact contribution of a dysregulation of BDNF gene methylation to depression pathology is not firmly established, several studies have provided evidence for its involvement. A microarray profiling study focused on CpG islands throughout the human genome and identified an increase in DNA methylation at distinct loci associated with major psychosis including bipolar disorder. A mouse model of depression evoked by perinatal exposure to methylmercury also found that depression-related behaviours together with a decrease in hippocampus BDNF mRNA levels corresponded to hypermethylation of the BDNF promoter regions (Onishchenko et al., 2008). Recently, MeCP2 levels have been found to be increased in the brains of R6/2 HD mice (G. Sadri-Vakili, personal communications). Hence, an extrapolation of those findings raises the possibility that disruption of BDNF gene transcription in the HD brain could be due to hypermethylation of the BDNF promoter regions.

In addition to DNA methylation, other forms of epigenetic regulation involve chromatin modification, specifically the modification of core histone proteins around which DNA is wound. In a chronic social defeat stress model of depression, the onset of social defeat was found to be coupled with a specific down-regulation of BDNF gene expression in the hippocampus and an increase in dimethylation of the lysine 27 residue of histone H3 (a modification associated with repression of transcriptional activity) (Tsankova et al., 2006). Furthermore, the same study revealed that increased histone acetylation regulated
the effects of chronic antidepressant treatment in reversing the depressive behaviour. Therefore, it is possible that the reversal of depression-related behaviours of female HD mice might involve epigenetic modifications of histones associated with the BDNF gene.

The experiments described in this chapter examined the changes in BDNF gene expression at the age when depression-related behaviours were detected in female, but not male, HD mice. A study of the epigenetic status around the BDNF gene sequence was undertaken focusing on the level of DNA methylation and modification of core histone proteins. We hypothesized that the development of the depression phenotype of HD mice correlated with a down-regulation of particular exon-specific transcripts of BDNF in the hippocampus in a female-specific manner and that the rescue of this behavioural phenotype by environmental enrichment was directly related to an up-regulation of the same transcripts. Furthermore, we also hypothesized that an alteration of the level of DNA methylation and/or differing levels of histone modification along the BDNF gene contribute to the repression and down-regulation of BDNF expression in the HD brain.

The aims of this chapter were:

1. Characterise the expression patterns of the coding exon and the exon-specific transcripts of BDNF in the hippocampus of wild-type and HD mice.
2. To investigate the effects of environmental enrichment on the mRNA levels of BDNF in the hippocampus of wild-type and HD mice.
3. To investigate the level of DNA methylation at specific regions along the BDNF gene sequence in the hippocampus of wild-type and HD mice.
4. To investigate the modification of core histones associated with specific BDNF non-coding exons in the HD mouse model.
5.2 Materials and Methods

8-week old wild-type and R6/1 HD mice were housed under standard, wheel-running or environmentally enriched housing conditions for four weeks until mice were 12-weeks old. Mice were killed by cervical dislocation and brains removed for fresh dissection. All dissected brain tissue was frozen immediately in liquid nitrogen and stored at -80ºC until further use. RNA was extracted from the hippocampus using the Qiagen RNeasy Mini kit (Qiagen, NSW, Australia) and final RNA elutes were analysed with a bioanalyser (Australia Genome Research Facility, Victoria, Australia) to ascertain the quality of the extracted RNA. cDNA was reversed transcribed with Taqman EZ RT-PCR Core reagents (Perkin-Elmer Applied Biosystems, VIC, Australia) for real-time analysis of BDNF expression which was performed using primers designed specifically for the coding exon and exon-specific transcripts. Cyclophilin was selected as the endogenous control gene and all primers pairs were optimized prior to real-time runs. SYBR green real-time reactions were carried out using the ABI Prism 7700 Detection System (Applied Biosystems, Foster City, CA, USA). Melt curve analyses were performed to check for consistent final products and the absence of contamination or primer dimers. Genomic DNA from hippocampal tissue of standard-housed mice were subjected to bisulfite conversion using MethylEasy™ Bisulfite Modification Kit (Genetic Signatures, Australia) according to manufacturer’s instructions followed by PCR amplification of CpG islands 3 and 4 with Promega PCR Mastermix. Amplicons were then cloned into pGEMT-Easy Vector (Promega) for automated DNA sequencing and positive clones were selected for sequencing (Australian Genome Research Facility, Queensland, Australia). Histone modification at the BDNF promoters was measured using an Upstate chromatin immunoprecipitation (ChIP) kit (Millipore, Billerica, MA) according to manufacturer’s instructions. Antibodies to H3K9Ac and H3K4me were also purchased from Millipore. ChIPs were quality controlled by the use of control genes which were either expressed in the hippocampus (synaptophysin), expressed ubiquitously (β-tubulin) or not expressed in the hippocampus (ε-globin). All ChIPs for which fold difference ratios for any of these genes lay outside 95% confidence intervals were discarded.
5.3 Results

5.3.1. Expression of BDNF coding exon in the hippocampus of wild-type and HD mice

There was no overall difference in BDNF coding exon expression (i.e. total BDNF mRNA levels) between the sexes ($F_{(1, 17)} = 3.226; p = 0.094$) but there was a significant genotype effect ($F_{(1, 17)} = 36.671; p < 0.001$) (Fig. 5-1A). There was no sex-genotype interaction ($F_{(1, 17)} = 3.093; p = 0.100$). Expression of BDNF coding exon in male HD mice ($n = 4; 60\% \pm 6.5; p = 0.012$) was 40% less than in male wild-types ($n = 4; 100\% \pm 10.2$). Female wild-type expression ($n = 5; 133\% \pm 12.5$) was slightly greater than in male wild-types while female HD levels ($n = 5; 61\% \pm 5.4$) were similar to male HD levels and significantly less than female wild-type expression ($p < 0.001$).

The effect of environmental enrichment on hippocampal expression of BDNF coding exon in both sexes was assessed separately with expression normalized to the respective standard-housed wild-type group. Within the male group, there were significant overall effects of genotype ($F_{(1, 16)} = 44.326; p < 0.001$) and environment ($F_{(1, 16)} = 5.121; p = 0.041$) but no genotype-environment interaction ($F_{(1, 16)} = 1.897; p = 0.192$) (Fig. 5-1B). As reported above, standard-housed male HD mice had a 40% reduction in coding exon levels compared to standard-housed male wild-type levels ($p = 0.003$). Enriched male wild-types ($n = 4; 127\% \pm 9.7$) had significantly greater levels of coding exon expression than standard-housed male wild-types ($p = 0.026$) and this was also significantly greater than expression levels of enriched male HD mice ($n = 5; 67\% \pm 3.7; p < 0.001$). There was no difference between the levels of coding exon of standard-housed and enriched male HD mice.
A  Sex differences in BDNF coding exon expression

B  BDNF coding exon expression in male hippocampus

C  BDNF coding exon expression in female hippocampus
Figure 5-1 Expression of BDNF coding exon in the hippocampus.

(A) A comparison of total BDNF mRNA levels in male and female mice of both genotypes as reflected by levels of BDNF coding exon. Results were normalized to male wild-type expression. Both male and female HD mice had reduced levels of BDNF coding exon in the hippocampus compared to wild-type mice. (B) Environmental enrichment increased the levels of BDNF coding exon in wild-type hippocampus but did not alter expression in the HD hippocampus. (C) Unlike in the male group, environmental enrichment did not significantly alter the expression of BDNF coding exon in the female wild-type hippocampus. There was also no significant effect of enrichment on BDNF coding exon mRNA levels in the female HD hippocampus. Asterisks indicate post-hoc Bonferroni’s t-test: *: p < 0.05, ***: p < 0.001.
There was an overall genotype effect on BDNF coding exon expression in the female group \( (F_{(1, 19)} = 49.816; p < 0.001) \) but no environment effect \( (F_{(1, 19)} = 0.000261; p = 0.987) \) (Fig. 5-1C). There was no genotype-environment interaction \( (F_{(1, 19)} = 3.450; p = 0.082) \). Expression of coding exon in standard-housed female HD mice \( (n = 5; 46\% \pm 4.1; p < 0.001) \) was significantly reduced compared to standard-housed female wild-type levels \( (n = 5) \). Enriched female HD mice \( (n = 5; 57\% \pm 6.0) \) also had significantly reduced levels of coding exon compared to enriched female wild-type \( (n = 5; 89\% \pm 3.7) \) levels \( (p = 0.002) \).

5.3.2. Expression of exon-specific BDNF transcripts in the hippocampus

Having found an overall change in BDNF expression in the hippocampus of HD mice, the next investigation was focused on quantifying the relative expression of individual BDNF exon-specific transcript variants as well as investigating whether environmental enrichment has differential effects on their expression.

5.3.2.1. Expression of BDNF exon I in hippocampus of wild-type and HD mice

There was no sex difference \( (F_{(1, 18)} = 0.848; p = 0.372) \) in exon I levels but there was a significant effect of genotype \( (F_{(1, 18)} = 13.263; p = 0.002) \). There was no significant sex-genotype interaction \( (F_{(1, 18)} = 1.255; p = 0.280) \) (Fig. 5-2A). Male HD mice \( (n = 4; 46\% \pm 14.0; p = 0.104) \) had a 54% reduction in exon I expression compared to male wild-type mice \( (n = 5) \). Expression of exon I in female HD mice was similar to male HD mice \( (n = 5; 46\% \pm 9.6) \) and this was significantly reduced compared to female wild-type levels \( (n = 5; 159\% \pm 34.1; p = 0.003) \).

In the analysis of BDNF exon I expression in male mice, there was a significant effect of genotype \( (F_{(1, 18)} = 23.400; p < 0.001) \) but not environment \( (F_{(1, 18)} = 1.926; p = 0.185) \) (Fig. 5-2B). There was also no genotype-environment interaction \( (F_{(1, 18)} = 2.433; p = 0.140) \). The 54% reduction in exon I expression in standard-housed male HD mice
compared to standard-housed wild-type levels was statistically significant (p = 0.040).
Enriched male HD mice (n = 5; 43% ± 6.2) had 57% less exon I expression compared to
standard-housed wild-type levels and this was significantly less than expression levels of
enriched male wild-types (n = 5; 148% ± 20.0; p < 0.001).

Similar to the male findings, there was a significant genotype effect (F(1, 19) = 15.182; p =
0.001) on BDNF exon I expression in female mice but not an environment effect (F(1, 19) =
0.00986; p = 0.922) (Fig. 5-2C). There was no genotype-environment interaction (F(1, 19) =
0.500; p = 0.490). Compared to standard-housed female wild-type levels (n = 5; 100% ±
23.8), there was a significant reduction in BDNF exon I levels in standard-housed
female HD mice (n = 5; 29% ± 6.7; p = 0.005). Enriched female HD mice (n = 5; 41% ±
12.3) had significantly less exon I expression than enriched female wild-type mice (n = 5;
90% ± 20.3; p < 0.001).
Sex differences in BDNF exon I expression

A

BDNF exon I expression in male hippocampus

B

BDNF exon I expression in female hippocampus

C
Figure 5-2 Expression of BDNF exon I transcript in the hippocampus.

(A) There were no significant sex differences in BDNF exon I expression detected within the wild-type and HD groups. (B) The level of BDNF exon I transcript was significantly reduced in male HD mice. Enriched mice did not have significantly altered levels of BDNF exon I transcripts despite enriched wild-type mice having 48% greater expression. (C) BDNF exon I transcript levels were also reduced in female HD mice. There was also no significant effect of environmental enrichment on wild-type and HD mice. Asterisks indicate post-hoc Bonferroni’s t-test: *: p < 0.05, **: p < 0.001.
5.3.2.2. Expression of BDNF exon II in hippocampus of wild-type and HD mice

There were no differences in BDNF exon II expression due to sex ($F_{(1, 18)} = 0.218; p = 0.647$) or genotype ($F_{(1, 18)} = 3.446; p = 0.083$) (Fig. 5-3A). No sex-genotype interaction was detected ($F_{(1, 18)} = 1.630; p = 0.221$). Expression of BDNF exon II in male HD mice was relatively similar ($n = 4; 88\% \pm 20.3$) to male wild-type levels ($n = 5$). Female wild-type mice ($n = 5; 146\% \pm 31.7$) had higher exon II expression than male wild-types while female HD mice had less ($n = 5; 75\% \pm 13.0$).

There was no genotype effect ($F_{(1, 18)} = 2.727; p = 0.119$) and no environmental influence ($F_{(1, 18)} = 1.082; p = 0.315$) on BDNF exon II expression in male mice (Fig. 5-3B). There was also no genotype-environment interaction ($F_{(1, 18)} = 0.803; p = 0.384$). Enriched male wild-type mice had higher exon II expression ($n = 5; 131\% \pm 15.5$) while enrichment did not alter the levels in male HD mice ($n = 5; 90\% \pm 13.5$).

Unlike the male group, an overall genotype effect ($F_{(1, 19)} = 4.584; p = 0.048$) was found in the female group (Fig. 5-3C). However, there was no environmental effect ($F_{(1, 19)} = 0.0629; p = 0.805$) and no genotype-environment interaction ($F_{(1, 19)} = 1.737; p = 0.206$). Here, a significant reduction in BDNF exon II expression was detected in the standard-housed female HD group ($n = 5; 52\% \pm 9.8; p = 0.026$) compared to standard-housed female wild-type expression levels ($n = 5; 100\% \pm 24.1$). BDNF exon II expression was relatively similar in enriched female wild-types ($n = 5; 85\% \pm 15.3$) while expression in enriched female HD mice was 22% greater than their standard-housed counterparts ($n = 5; 74\% \pm 7.3$).
A  Sex differences in BDNF exon II expression

B  BDNF exon II expression in male hippocampus

C  BDNF exon II expression in female hippocampus
Figure 5-3 Expression of BDNF exon II transcript in the hippocampus.

(A) There were no significant sex-differences in expression within the wild-type and HD groups. (B) Environmental enrichment did not significantly alter expression of BDNF exon II transcripts in male mice. (C) Levels of BDNF exon II transcript was reduced in standard-housed female HD mice and there was no effect of environmental enrichment. Asterisk indicates post-hoc Bonferroni’s t-test: *: p < 0.05.
5.3.2.3. Expression of BDNF exon III in hippocampus of wild-type and HD mice

There were significant effects of sex ($F_{(1, 18)} = 6.458; p = 0.023$) and genotype ($F_{(1, 18)} = 12.783; p = 0.003$) on BDNF exon III expression (Fig. 5-4A). In addition, there was a significant sex-genotype interaction ($F_{(1, 18)} = 4.680; p = 0.047$). Exon III levels in male HD mice ($n = 4; 79\% \pm 18.1$) were similar to male wild-types ($n = 5; 100\% \pm 10.8; p =0.347$). Female wild-type mice ($n = 5; 176\% \pm 21.6$) had significantly greater levels of BDNF exon III transcript than male wild-types ($p = 0.004$). Female HD mice ($n = 5; 86\% \pm 7.8$) did not differ in exon III expression from male HD levels ($p = 0.799$) while their expression level was significantly less compared to the female wild-types ($p < 0.001$).

In the analysis of the effects of environmental enrichment on BDNF exon III expression in male mice, there was a trend towards an overall genotype effect ($F_{(1, 18)} = 3.951; p = 0.065$) (Fig. 5-4B). However, there was no environmental effect ($F_{(1, 18)} = 1.059; p = 0.320$) and no genotype-environment interaction ($F_{(1, 18)} = 0.241; p = 0.630$). Enriched male wild-types had 22\% greater exon III expression than standard-housed wild-types ($n = 5; 122\% \pm 19.2$) while enriched male HD mice had a similar expression level with their standard-housed counterparts ($n = 5; 86\% \pm 8.3$).

There was also an overall genotype effect ($F_{(1, 19)} = 13.367; p = 0.002$) on BDNF exon III expression in the female group (Fig. 5-4C). There was no environment effect ($F_{(1, 19)} = 1.727; p = 0.207$) but there was a significant genotype-environment interaction ($F_{(1, 19)} = 6.909; p = 0.018$). Standard-housed female HD mice ($n = 5; 49\% \pm 4.6$) had a large reduction in exon III levels compared to standard-housed female wild-types ($n = 5; 100\% \pm 12.7; p < 0.001$). Expression in enriched female HD mice ($n = 5; 60\% \pm 6.6$) was not significantly different from the standard-housed group despite a significant reduction of BDNF exon III levels in enriched female wild-type mice ($n = 5; 68\% \pm 7.1; p = 0.013$).
Sex differences in BDNF exon III expression

A

Sex differences in BDNF exon III expression

% Male WT levels

Male
Female

WT
HD

B

BDNF exon III expression in male hippocampus

% SH WT mRNA levels

Standard Housing
Environmental Enrichment

B

BDNF exon III expression in female hippocampus

% SH WT mRNA levels

Standard Housing
Environmental Enrichment

C

BDNF exon III expression in female hippocampus

% SH WT mRNA levels

Standard Housing
Environmental Enrichment

* * *
Figure 5-4 Expression of BDNF exon III transcript in the hippocampus.

(A) Female wild-type mice had significantly greater levels of BDNF exon III transcript in the hippocampus compared to male wild-types. (B) Male HD and wild-type mice had similar levels of BDNF exon III transcript which were not significantly altered by environmental enrichment. (C) Standard-housed female HD mice had significantly reduced expression of BDNF exon III transcript and while environmental enrichment reduced expression in female wild-types, there was no effect of enrichment on female HD mice. Asterisks indicate post-hoc Bonferroni’s t-test: *: p < 0.05, ***: p < 0.001.
5.3.2.4. Expression of BDNF exon IV in hippocampus of wild-type and HD mice

Overall, there was no significant sex difference in BDNF exon IV expression ($F_{(1, 18)} = 1.575; p = 0.229$) (Fig. 5-5A). However, there was a significant genotype effect ($F_{(1, 18)} = 5.469; p = 0.034$). There was no sex-genotype interaction ($F_{(1, 18)} = 2.707; p = 0.121$). Male HD mice ($n = 4; 91\% \pm 13.2$) had similar levels of BDNF exon IV expression compared to male wild-type mice ($n = 5; 100\% \pm 8.0$). Female wild-type mice ($n = 5; 138\% \pm 15.6$) had higher expression of exon III than male wild-types. The expression of exon IV in female HD mice ($n = 5; 87\% \pm 11.8; p = 0.011$) was significantly less than female wild-type levels.

Both genotype ($F_{(1, 18)} = 6.442; p = 0.023$) and environment ($F_{(1, 18)} = 10.932; p = 0.005$) were significant overall factors influencing BDNF exon IV expression in male mice (Fig. 5-5B). There was no genotype-environment interaction ($F_{(1, 18)} = 3.036; p = 0.102$). There was no difference in exon IV levels between standard-housed wild-type ($n = 5; 100\% \pm 8.0$) and HD mice ($n = 4; 91\% \pm 13.2$). Environmental enrichment significantly boosted exon IV expression in male wild-type mice ($n = 5; 154\% \pm 15.7; p = 0.002$) but did not significantly alter the expression in HD mice ($n = 5; 108\% \pm 2.2; p = 0.299$). There was a significant difference between exon IV expression of HD and wild-type mice in the enriched group ($p = 0.007$).

Unlike the male group, overall BDNF exon IV expression in female mice was not affected by genotype ($F_{(1, 19)} = 2.928; p = 0.106$) or environment ($F_{(1, 19)} = 0.644; p = 0.434$) (Fig. 5-5C). However, there was a significant genotype-environment interaction ($F_{(1, 19)} = 5.015; p = 0.040$). Exon IV expression was reduced in standard-housed HD mice ($n = 4; 100\% \pm 11.7$) compared to standard-housed wild-type levels ($n = 5; 64\% \pm 8.9; p = 0.013$). However, there was no difference between HD and wild-type levels ($p = 0.714$) in the enriched group due to a significant increase in expression levels of enriched HD mice ($n = 5; 92\% \pm 8.8; p = 0.047$) compared to standard-housed HD mice.
concurrent with a slight decrease in expression levels in enriched wild-type mice (n = 5; 87% ± 8.3; p = 0.325).

5.3.2.5. Expression of BDNF exon V in hippocampus of wild-type and HD mice

There was a trend towards an overall effect of sex (F(1, 18) = 4.425; p = 0.053) on BDNF exon V expression levels in addition to a strong genotype effect (F(1, 18) = 62.066; p < 0.001). There was no sex-genotype interaction (F(1, 18) = 2.686; p = 0.122) (Fig. 5-6A).

Exon V expression in male HD mice (n = 4; 50% ± 7.7) was only 50% of male wild-type levels (n = 5; 100% ± 6.4; p < 0.001). Female wild-type mice (n = 5; 131% ± 11.0) also had significantly greater exon V expression compared to female HD mice (n = 5; 54% ± 5.9; p < 0.001).

There were significant overall effects of genotype (F(1, 18) = 58.953; p < 0.001) and the environment (F(1, 18) = 7.564; p = 0.015) on BDNF exon V expression in male mice (Fig. 5-6B). There was also a trend towards a genotype-environment interaction (F(1, 18) = 3.436; p = 0.084). There were significant differences in the expression of BDNF exon V within both standard-housed (p = 0.001) and enriched (p < 0.001) groups. Enriched male wild-type mice (n = 5; 139% ± 13.1) had significantly greater exon V expression compared to standard-housed wild-types (p = 0.004). However, exon V expression in enriched male HD mice (n = 5; 58% ± 4.4) did not differ from standard-housed HD levels (p = 0.547).

The overall expression of BDNF exon V in female mice was also significantly affected by genotype (F(1, 19) = 79.070; p < 0.001) (Fig. 5-6C). However, unlike the male group, there was no significant effect of the environment (F(1, 19) = 0.0754; p = 0.787) and there was no genotype-environment interaction (F(1, 19) = 1.886; p = 0.189). Exon V expression was significantly reduced in standard-housed (p < 0.001) and environmentally enriched HD mice (p < 0.001) compared to their respective wild-type controls.
Sex differences in BDNF exon IV expression

A

BDNF exon IV expression in male hippocampus

B

BDNF exon IV expression in female hippocampus

C
Figure 5-5 Expression of BDNF exon IV transcript in the hippocampus.

(A) No sex differences in BDNF exon IV transcript levels were detected within the standard-housed groups of mice. (B) Environmental enrichment significantly increased exon IV transcripts in male wild-type, but not HD, hippocampus. (C) Enrichment did not significantly alter wild-type expression but significantly increased exon IV transcript levels in the female HD hippocampus. Asterisks indicate post-hoc Bonferroni’s t-test: *: p < 0.05.
A  Sex differences in BDNF exon V expression

B  BDNF exon V expression in male hippocampus

C  BDNF exon V expression in female hippocampus
Figure 5-6 Expression of BDNF exon V transcript in the hippocampus.

(A) Male and female HD mice had reduced expression of BDNF exon V transcripts. There were no sex differences observed within both wild-type and HD groups. (B) Environmental enrichment significantly increased exon V transcripts in male wild-type, but not HD, hippocampus. (C) Enrichment did not significantly alter wild-type and HD expression. Asterisks indicate post-hoc Bonferroni’s t-test: *: p < 0.05, **: p < 0.001.
5.3.3. Epigenetic changes in HD do not correlate with BDNF expression changes

5.3.3.1. DNA methylation along the BDNF gene sequence

Changes in gene expression can be brought about by changes in the epigenetic marks of DNA methylation and/or covalent histone modification. The level of DNA methylation in the BDNF gene has previously been correlated with the genotype of an SNP associated with major psychosis (Mill et al., 2008). However, little else is known about the epigenetic status of the BDNF gene in HD.

Given that gene repression is commonly mediated by DNA methylation, we investigated the possibility that there is increased methylation along the BDNF gene sequence. Using the UCSC Genome Browser (http://genome.ucsc.edu), the sequence for the *mus musculus* BDNF gene (NM_012513) was retrieved. The various exons were localized into two clusters (Cluster 1: exons I, II and III; Cluster 2: exons IV, V, VI) that were upstream of the main coding exon (Fig. 5-7A). There were four CpG rich regions (CpG islands) located near the exon clusters and the coding exon (Fig. 5-7B). Two CpG islands (island3 and island4) were selected for closer analysis using bisulfite-based DNA sequencing (Fig. 5-7C, 5-7D). Island3 is the largest of the CpG islands with 60 C-G dinucleotide pairs and is associated with the promoter region closest to exons III, IV and V. Island4 contains 17 C-G dinucleotide pairs and is associated with the coding exon.

In agreement with a previous study which showed almost complete unmethylation around the BDNF exon IV promoter (Martinowich et al., 2003), C-G dinucleotides within island3 were largely unmethylated in hippocampal genomic DNA from both wild-type and HD male mice (Fig. 5-7E). This was also similar to the methylation pattern observed for wild-type and HD female mice. In contrast to those findings, island4 was almost completely methylated in both wild-type and HD male mice, as well as in wild-type and
Figure 5-7 DNA methylation around CpG islands located within the BDNF gene.

(A) Mouse BDNF Locus. (B) Locations of CpG islands with respect to BDNF exons. (C) Location of bisulphite PCR assays used to investigate BDNF CpG island3 and CpG island4. (D) Location of CpG dinucleotides within each assay. (E) Representative DNA methylation depiction of CpG islands across the BDNF locus obtained from clones derived from genomic DNA from the hippocampus of HD and wild-type mice. Each row represents an allele sequenced. CpG dinucleotides are depicted as circles and are filled (black) to represent methylated CpG dinucleotides. Open (white) circles represent unmethylated CpG dinucleotides.
HD female mice. Hence, the reduction of BDNF gene expression in HD is not a result of increased transcriptional depression due to increased DNA methylation.

5.3.3.2. Histone modification of histone associated with the BDNF gene

The regulation of BDNF exon IV expression was further investigated as this is the only exon which is up-regulated following environmental enrichment and wheel-running (Zajac et al., personal communications) in female HD mice, both of which also corrected the depressive-like phenotype in HD mice as described in Chapter 3. Epigenetic regulation around the BDNF exon IV promoter region has been suggested to be associated with the induction of depressive-like behaviour in mice (Tsankova et al., 2006). Here, core histone modification around BDNF exon IV in the HD brain was assessed using chromatin immunoprecipitation (ChIP).

Three specific histone modifications indicative of active transcriptional activity were examined – acetylation of the lysine 9 residue of histone H3 (H3K9ac) (Koch et al., 2007), methylation of lysine 4 of histone H3 (H3K4me) (Koch et al., 2007) and methylation of lysine 27 of histone H3 (H3K27me) (Barski et al., 2007; Benevolenskaya, 2007). The level of trimethylation of the lysine 9 residue of histone H3 (H3K9me3) which is indicative of transcriptional repression (Barski et al., 2007) was also examined.

5.3.3.2.1. ChIP analysis of control genes for H3K9ac

Firstly, the level of key histone modifications of constitutively active and inactive genes was examined as a control for the ChIP process (Fig. 5-8A). The synaptic vesicle glycoprotein synaptophysin (SYP) was selected as one active control since it is active in the hippocampus (Berton et al., 2006) and its expression is not altered in the HD brain (Nithianantharajah et al., 2008). A second active control was the cytoskeletal molecule β-tubulin and the inactive control of choice was ε-haemoglobin (HEB1) which is expressed during embryonic development.
ChIP enhancement ratios for SYP-associated H3K9ac did not differ due to genotype ($F_{(1,12)} = 0.159; \ p = 0.700$) but there was an overall difference between the sexes ($F_{(1,12)} = 7.560; \ p = 0.022$). Male HD mice ($n = 5; \ 11.10 \pm 2.48$) had an enhancement of H3K9ac over female HD mice ($n = 3; \ 3.08 \pm 1.35; \ p = 0.044$) but there was no significant difference between male ($n = 3; \ 11.74 \pm 3.20$) and female ($n = 2; \ 4.63 \pm 1.39$) wild-type mice ($p = 0.133$).

The level of β-tubulin-associated H3K9ac was similar for male and female wild-type mice (Male WT: $n = 4; \ 33.50 \pm 7.08$; Female WT: $n = 3; \ 34.16 \pm 6.29$). There was no overall sex difference ($F_{(1,14)} = 0.513; \ p = 0.489$) but there was a trend towards a genotype difference ($F_{(1,14)} = 4.737; \ p = 0.052$) as HD mice had lower H3K9ac enhancement ratios than wild-type mice (Male HD: $n = 5; \ 25.99 \pm 4.06$; Female HD: $n = 3; \ 17.33 \pm 3.29$).

Very low enhancement ratios (less than 1.0 for all groups) were obtained for HEB1 and this was as expected for an inactive gene.

**5.3.3.2.2. ChIP analysis of BDNF exon IV for H3K9ac**

The enhancement ratios for BDNF exon IV-associated H3K9ac (Fig. 5-8B) were similar to those of SYP. There were no overall genotype ($F_{(1,14)} = 0.931; \ p = 0.355$) or sex differences ($F_{(1,14)} = 1.046; \ p = 0.328$) despite an approximately 2-fold enhancement of H3K9ac in male HD mice ($n = 5; \ 10.26 \pm 3.92$) compared to male WT mice ($n = 4; \ 4.33 \pm 0.80$). Exon IV-associated H3K9ac of female HD ($n = 3; \ 4.16 \pm 0.32$) and WT ($n = 3; \ 4.65 \pm 0.44$) mice was similar.
Figure 5-8 ChIP results of H3K9ac for control genes and BDNF exon IV from hippocampal genomic DNA.

(A) Levels of H3K9ac associated with control genes synaptophysin, β-tubulin and ε-haemoglobin (HBE1). (B) No significant differences in levels of H3K9ac at BDNF exon IV. Asterisks indicate post-hoc Bonferroni’s t-test: *: p < 0.05.
5.3.3.2.3. ChIP analysis of control genes and BDNF exon IV for H3K4me

The enhancement patterns of H3K4me for the three control genes were similar to that of H3K9ac with both SYP and β-tubulin showing moderate levels enhancement while HBE1 levels were suppressed (Fig. 5-9A). There were no overall genotype \( (F(1,15) = 0.397; p = 0.540) \) or sex \( (F(1,15) = 0.0480; p = 0.830) \) differences in H3K4me levels at SYP but there was a significant genotype-sex interaction \( (F(1,15) = 8.357; p = 0.014) \). SYP-associated H3K4me was enhanced in male HD mice (\( n = 5; 13.55 ± 2.26 \)) compared to female HD mice (\( n = 4; 7.41 ± 2.15; p = 0.036 \)). However, male WT mice (\( n = 4; 6.59 ± 0.64 \)) had lower H3K4me enhancement levels than female WT mice (\( n = 3; 11.87 ± 1.76; p = 0.101 \)).

There was an overall genotype effect \( (F(1,13) = 5.096; p = 0.048) \) on H3K4me of β-tubulin and a trend towards a sex difference \( (F(1,13) = 3.561; p = 0.089) \) but there was no genotype-sex interaction \( (F(1,13) = 1.615; p = 0.233) \). Post-hoc analysis revealed a difference in the enhancement ratios of female WT (\( n = 2; 81.80 ± 29.63 \)) and HD mice (\( n = 4; 33.93 ± 8.69; p = 0.044 \)) but no difference between male WT (\( n = 3; 38.95 ± 20.36 \)) and HD mice (\( n = 5; 25.57 ± 5.42; p = 0.462 \)).

In agreement with results of the H3K9ac ChIP, enhancement ratios for H3K4me at HBE1 were very low (less than 2.0 for all groups). Interestingly, at this low level of activity, an overall sex difference was detected \( (F(1,12) = 9.074; p = 0.015) \). However, the functional relevance of this is more than likely to be insignificant due to the extremely low level of HBE1 expression in the brain.

There were no overall differences in the level of H3K4me at BDNF exon IV due to genotype \( (F(1,15) = 3.145; p = 0.102) \) or sex \( (F(1,15) = 0.926; p = 0.355) \) (Fig. 5-9B). There was also no genotype-sex interaction \( (F(1,15) = 1.292; p = 0.280) \). The mean level of H3K4me at BDNF exon IV in the hippocampus of male HD mice (\( n = 5; 8.30 ± 0.82 \))
was approximately twice that of male wild-type mice (n = 4; 4.14 ± 1.54) while the levels in female wild-types (n = 3; 4.73 ± 0.48) and HD mice (n = 4; 5.19 ± 1.72) were similar.

5.3.3.2.4. ChIP analyses for BDNF exons I, II, III and V

The analyses of histone modification around the BDNF gene was expanded to examine BDNF exons I, II, III and V from hippocampal genomic DNA. The results are summarized below in Tables 5-1 (H3K9ac) and 5-2 (H3K4me). Overall, no significant differences between the genotypes and the sexes were found between the enhancement ratios of H3K9ac and H3K4me associated with BDNF exons I, III and V. Interestingly, differences in histone modification levels between the genotypes and sexes at BDNF exon II were detected which were specific for the modification event. There was an overall effect of genotype with HD mice having lower enhancement ratios for BDNF exon II H3K9ac. However, post-hoc statistical analysis did not reveal any significant differences between the two genotypes. In contrast, there was an overall sex difference in BDNF exon II H3K4me levels with males having higher enhancement ratios. Post-hoc analysis revealed no significant difference within the wild-type group, but male HD mice had a significantly elevated ChIP enhancement ratio compared to female HD mice.
Figure 5-9 ChIP results of H3K4me for control genes and BDNF exon IV from hippocampal genomic DNA.

(A) Levels of H3K9ac associated with control genes synaptophysin, β-tubulin and ε-haemoglobin (HEB1). (B) No significant differences in levels of H3K4me at BDNF exon IV. Asterisks indicate post-hoc Bonferroni’s t-test: *: p < 0.05.
<table>
<thead>
<tr>
<th>Target</th>
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<th>Genotype-Sex Interaction</th>
<th>Group</th>
<th>n</th>
<th>ChIP enhancement ratios</th>
<th>Post-hoc p-values</th>
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<td>BDNF exon I</td>
<td>$F_{(1,14)} = 0.174$</td>
<td>$F_{(1,14)} = 1.298$</td>
<td>Male WT 4</td>
<td>5.24 ± 1.41</td>
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<tr>
<td></td>
<td>$p = 0.685$</td>
<td>$p = 0.279$</td>
<td>Male HD 5</td>
<td>5.54 ± 1.16</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Female WT 3</td>
<td>4.55 ± 1.38</td>
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<td></td>
<td></td>
<td></td>
<td>Female HD 3</td>
<td>3.13 ± 1.32</td>
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<tr>
<td>BDNF exon II</td>
<td>$F_{(1,13)} = 5.573$</td>
<td>$F_{(1,13)} = 1.318$</td>
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<td>8.43 ± 1.50</td>
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<td></td>
<td>$p = 0.040$</td>
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<td>Male HD 5</td>
<td>5.15 ± 0.46</td>
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<td></td>
<td></td>
<td></td>
<td>Female WT 3</td>
<td>7.18 ± 3.45</td>
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<td></td>
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<td></td>
<td>Female HD 3</td>
<td>2.55 ± 0.88</td>
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<tr>
<td>BDNF exon III</td>
<td>$F_{(1,12)} = 0.203$</td>
<td>$F_{(1,12)} = 0.0866$</td>
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<td>7.58 ± 2.93</td>
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<tr>
<td></td>
<td>$p = 0.663$</td>
<td>$p = 0.775$</td>
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<td>Female WT 3</td>
<td>5.24 ± 1.58</td>
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<td></td>
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<td>Female HD 2</td>
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<td>BDNF exon V</td>
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<td>$p = 0.373$</td>
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<td></td>
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<td></td>
<td>Female HD 3</td>
<td>5.77 ± 1.31</td>
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Table 5-1 Summary of H3K9ac modification associated with BDNF exons.  
Values indicate enhancement ratios ± SEM.
<table>
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<tr>
<th>Target</th>
<th>Overall effects</th>
<th>ChIP enhancement ratios</th>
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<td>Group</td>
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<td>BDNF exon I</td>
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<td>$F_{(1,15)} = 1.604$ ( p = 0.229 )</td>
<td>Male HD</td>
</tr>
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<td>$F_{(1,15)} = 1.785$ ( p = 0.206 )</td>
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</tr>
<tr>
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<td>Female HD</td>
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<td>BDNF exon II</td>
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<td>Male WT</td>
</tr>
<tr>
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<td>$F_{(1,15)} = 8.045$ ( p = 0.015 )</td>
<td>Male HD</td>
</tr>
<tr>
<td></td>
<td>$F_{(1,15)} = 1.758$ ( p = 0.210 )</td>
<td>Female WT</td>
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<tr>
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<td>Female HD</td>
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<td>BDNF exon III</td>
<td>$F_{(1,13)} = 0.203$ ( p = 0.662 )</td>
<td>Male WT</td>
</tr>
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<td>$F_{(1,13)} = 1.888$ ( p = 0.199 )</td>
<td>Male HD</td>
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<tr>
<td></td>
<td>$F_{(1,13)} = 3.780$ ( p = 0.081 )</td>
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<tr>
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<td>Female HD</td>
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<td>BDNF exon V</td>
<td>$F_{(1,15)} = 0.0313$ ( p = 0.867 )</td>
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<td>$F_{(1,15)} = 0.404$ ( p = 0.537 )</td>
<td>Male HD</td>
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<td></td>
<td>$F_{(1,15)} = 3.931$ ( p = 0.071 )</td>
<td>Female WT</td>
</tr>
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<td>Female HD</td>
</tr>
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</table>

Table 5-2 Summary of H3K4me modification associated with BDNF exons.

*Values indicate enhancement ratios $\pm$ SEM.*
5.4. Discussion

The main purpose of this study was to examine the expression patterns and epigenetic regulation of BDNF exon-specific transcripts in the hippocampus in relation to the display of depression-related behaviour by female HD mice at 12-weeks of age. Using real-time PCR, total BDNF gene expression as denoted by levels of BDNF coding exon was found to be reduced in the hippocampus of both male and female HD mice. This is the earliest age at which a deficit in BDNF expression has been described in the HD hippocampus and supports previously published findings of reduced BDNF mRNA levels in 20-week old R6/1 HD mice (sexes combined) (Pang et al., 2006).

The nomenclature of the BDNF gene has recently been reviewed and updated. It was initially thought that the rodent gene sequence only contained four 5’ non-coding exons (Timmusk et al., 1993) and several of those have been implicated in the regulation of the effects of stress-response (Marmigere et al., 2003; Nair et al., 2006), antidepressant drugs (Dwivedi et al., 2006), diurnal rhythms (Berchtold et al., 1999) and neuronal signaling (Sathanoori et al., 2004). It has now been shown that there are as many as six or seven 5’ non-coding exons (Bora et al., 2005; Aid et al., 2007). As a result of this new information, further investigations will have to be undertaken to gain a better understanding of the biological contributions of each exon-specific transcript.

Here, a more detailed examination of the expression levels of the individual exon-specific transcripts contributing to total BDNF expression was undertaken as it would lead to a better understanding of the regulation of BDNF expression in the hippocampus. Only one previous study has attempted to describe the changes in levels of BDNF-exon specific mRNA in the HD brain. Zuccato et al (2005) described a loss of BDNF exon II and III (IV in the present study) transcripts in the cerebral cortex of R6/2 transgenic HD mice which correlated with the progressive onset of disease symptoms (Zuccato et al., 2005). However, that study was statistically underpowered as it only utilized 2-3 controls and 2-4 HD samples the low sample numbers made it impossible to differentiate between male
and female expression levels. The results of that study are potentially confounded by any sex differences in BDNF gene expression especially since it has emerged that BDNF mRNA levels are greater in the female than male rodent cortex (Bland et al., 2005). Hence, a study of BDNF gene expression in the HD and wild-type mouse brain that examined males and females separately was required.

The specific region of interest was the hippocampus as modulation of hippocampal BDNF levels is believed to mediate depression-related behaviours in rodents (Siuciak et al., 1997; Shirayama et al., 2002; Hoshaw et al., 2005). This chapter identified differences in the levels of BDNF exon-specific transcripts between the male and female hippocampus. Total BDNF mRNA levels determined by measuring BDNF coding exon expression did not differ significantly between male and female wild-type mice. Subsequent analyses of exon-specific transcript levels revealed that while there were also no sex-differences in the expression of exon I, II, IV and V transcripts, BDNF exon III transcripts are expressed at a greater level in the female than male hippocampus.

Similarly, despite significant reductions in total BDNF mRNA levels in the hippocampus of male and female HD mice, the extent of this down-regulation was not different between both sexes (40% for males, 54% for females). Further investigation revealed that unlike in the wild-types, the only exon-specific transcripts reduced in both the male and female HD hippocampus were the exon I and V-containing transcripts. BDNF transcripts containing exons II, III and IV were found to be down-regulated in only female HD mice and the wild-type sex-difference in exon III expression did not exist within the HD group. As mentioned above, the recent review of the BDNF gene structure has led to a reshuffle of the nomenclature of the BDNF gene sequence and the functional details of many of these newly described exon transcripts have yet to be determined. Therefore, it is difficult to fully comprehend the functional consequences of these sex-specific expression differences in the HD brain and how they might contribute to female-specific HD behavioural phenotype described in the previous chapters. One possible explanation for these female-specific molecular changes would be the presence of estrogen which has
been shown to regulate BDNF mRNA and protein expression in the different sub-regions of the hippocampus (Gibbs, 1999; Franklin and Perrot-Sinal, 2006).

In addition to the sex-differences in BDNF transcript expression, sex-specific responses to being housed under environmentally enriching conditions were observed in both wild-type and HD mice. Enriched male wild-type mice were found to have increased total BDNF gene expression compared to standard-housed controls but there was no effect of enrichment on BDNF expression in the female wild-type hippocampus. It appears that the main contributors to the male-only increase in BDNF expression are exon IV and V transcripts which were significantly up-regulated. In contrast, while not significantly impacting on total expression levels, environmental enrichment resulted in a significant down-regulation of BDNF exon III transcripts in the female wild-type hippocampus. However, these environment-mediated changes in BDNF expression in wild-type mice were not found to exist in the HD group. Environmental enrichment did not lead to an up-regulation or a rescue of the BDNF expression deficit in the hippocampus of male and female HD mice. Unlike male wild-type mice, enriched male HD mice did not respond with an up-regulation of exon IV and V transcripts. Interestingly, while enriched female HD mice did not exhibit a significant down-regulation of exon III transcripts like the wild-types, they responded with a significant up-regulation of exon IV transcripts.

The regulation of BDNF exon III and IV-transcripts has been suggested to be involved in depression-related behaviour in mice based on a study of a social defeat model of depression (Tsankova et al., 2006). In that same study, it was suggested that the effectiveness of a chronic antidepressant drug treatment in alleviating depressive behaviour was directly correlated with an up-regulation of BDNF exon III and IV-transcripts. However, a major flaw of that study was the presentation of combined transcript expression without identifying the behavioural and drug effects on exon III and IV transcripts separately. Here, while both transcripts were reduced in the female but not male HD hippocampus, only exon IV expression was up-regulated by environmental enrichment in association with a rescue of depression-related behaviour. Hence, it is
highly likely that the expression of BDNF exon IV transcripts in the hippocampus is a key molecular mediator of depression-related behaviour in mice.

The significance and specificity of this female HD-specific up-regulation of BDNF exon IV transcript levels by environmental enrichment becomes even more striking when compared with the molecular effects of increased physical activity (voluntary wheel-running) (Appendix II). As shown in Chapter 2, voluntary wheel-running also corrects the depressive-like behaviours of female HD mice on the forced-swim test while having no effect on the male HD mice. Following similar analyses of BDNF exon-specific expression in the hippocampus of wheel-running HD mice, it was found that of the different splice variants, only exon IV-containing transcripts was up-regulated by wheel-running. Furthermore, this running-induced molecular change was only evident in the female and not male HD hippocampus. Therefore, extending on the idea that BDNF exerts an ‘anti-depressive’ behavioural effect when applied exogenously to the hippocampus (Shirayama et al., 2002; Thakker-Varia et al., 2007), it can be concluded based on convincing molecular evidence that modulation of depressive behaviour by environmental modifiers is mediated by expression of exon-IV containing BDNF transcripts.

The exact mechanisms leading to the repression of BDNF gene expression in the HD brain have yet to be fully determined. One initial proposal was that BDNF gene transcription was impaired in the HD brain due to abnormal interactions with the repressor element-1 transcription factor/neuron restrictive silencer factor (REST/NRSF) which would normally act to disinhibit the repressor element-1/neuron-restrictive silencer element (RE1/NRSE) within the BDNF promoter II region (associated with CpG isld3 in this study) thus preventing an increase in BDNF transcription (Zuccato et al., 2003). This was supported by the subsequent finding that in addition to BDNF, the expression of other neuronal genes regulated by REST/NRSE was also reduced in the HD brain (Zuccato et al., 2007).
However, gene expression can also be modulated by epigenetic events such as DNA methylation and modification of core histone proteins. Recently, it was reported that DNA methylation of the BDNF gene is increased in the brains of patients with psychiatric illnesses (Mill et al., 2008). Epigenetic abnormalities have been demonstrated in the HD brain (Igarashi et al., 2003; Sadri-Vakili and Cha, 2006); however at the time of the present study, no information was available regarding the epigenetic status of the BDNF gene sequence in HD. The findings described in this chapter indicate that the decrease in expression of BDNF exon-specific transcripts is not due to increased transcriptional repression since there was no increase in methylation of CpG isld3. It was interesting to discover that the CpG island (isld4) associated with the BDNF coding exon was highly methylated, suggesting a high level of transcriptional regulation and that BDNF is not expressed basally but would require a strong activation signal. What constitutes that signal remains to be determined. In addition, despite finding no differences in the methylation patterns along the BDNF sequence in the HD and wild-type brains, given that BDNF expression (Dennis and Levitt, 2005) and neuronal activity (Nelson et al., 2008) can be regulated by dynamic methylation patterning, further work will be required to determine whether the plasticity of DNA methylation is as flexible in the HD brain as it is in the wild-type brain.

While abnormal histone modifications such as decreased histone H3K9ac acetylation concurrent with increased histone methylation have been reported in late-stage R6/2 HD brains (Stack et al., 2007), abnormal histone modifications specifically around the BDNF promoter regions have also been linked to pathophysiology of depression (Fuchikami et al., 2008). Counter to the initial hypothesis and expectations of finding hypoacetylated histones associated with BDNF as had been demonstrated with other genes, no differences in the active histone marks (H3K9ac and H3K4me) were detected. That result implies that abnormal epigenetic marks observed in HD brains are a later-occurring pathological event secondary to other molecular disruptions and do not contribute directly to the depression-related behaviours developed by the female HD mice.
Interesting, there was a strong indication that histones associated with β-tubulin were hypo-acetylated indicating that this event might be contributing to the pathology of HD. There have been reports of altered cytoskeletal components including tubulin during the early stages of the disease (Diprospero et al., 2004) and that hypo-acetylation of α-tubulin is partly involved in the impairment of vesicular trafficking of BDNF in the HD brain which is reversible by histone deacetylase inhibitors (Dompierre et al., 2007). It is presently not known whether the hypo-acetylation of tubulin impacts the level of gene and protein expression. However, the htt protein (both wild-type and mutant) is known to bind directly to microtubules via direct interaction with β-tubulin (Tukamoto et al., 1997; Hoffner et al., 2002). Hence, a significant down-regulation of β-tubulin expression would result in a reduction in the number of htt protein-interaction sites along microtubules. The immediate consequence of that reduction in the number of htt-β-tubulin interactions is a disruption of vesicular trafficking since vesicles are transported along microtubules while bound to huntingtin-associated protein-1 (HAP-1) which in turn binds to huntingtin in a glutamine-repeat length-dependant manner (Li et al., 1995; Block-Galarza et al., 1997; Engelender et al., 1997).

The absence of any significant differences in histone modifications around the BDNF gene sequence makes it a less probable cause of the depressive-like phenotype which was proposed to be the underlying mechanism in another mouse model of depression (Tsankova et al., 2006). It can also be concluded that the disruption of BDNF gene expression in HD is not due to altered histone modification which has been shown to affect the expression of other genes in the disease (Sadri-Vakili et al., 2007). It is worth noting that while this study has focused on gene expression changes in the hippocampus, the prefrontal cortex is also implicated in the pathology of depression (Rajkowska, 2000) where several histone modifications around the individual BDNF promoter regions are associated with anxiety disorders (Bredy et al., 2007). As such, an analysis of histone modification in the HD cortex will be required before it can be concluded that altered histone modification of BDNF is absolutely uninvolved in HD-related depression. Further work will be also be required to explore other possibilities to account for the
disruption of BDNF expression such as the disruption of the transcriptional machinery due abnormal interaction with the mutant huntingtin protein.

It is unclear why there is redundancy within the BDNF gene which has multiple non-coding exons involved in the translation of a common protein. However, several studies have uncovered a role for the different exons in encoding temporal and spatial information. Exons I, II and III (cluster 1) are only expressed postnatal and transiently up-regulated following neuronal signaling. In contrast, exons IV and V (cluster 2) are present from the embryonic stages and are involved in development and maturation of neuronal networks. It can be hypothesized that cluster 1 is involved in the immediate-early response to neuronal activation by environmental stimuli while cluster 2 mediates the changes required for dendritic maturation and long-term changes to neuronal networks in response to those stimuli. There are also similarities between the corresponding exons of the two clusters. Exons I and IV are both up-regulated in the soma following activation of voltage-sensitive calcium channels but exon IV expression persists longer than exon I. Expression of exons II and V is mediated by activation of subunit 2A-containing NMDA receptors (Chen et al., 2007) and is mainly in the dendritic processes. Therefore, it appears that BDNF expression following neuronal activity involves the early transcription of exons I and IV transcripts followed by exons II and V transcripts and while the levels of exons I and IV diminish with time, the persistence of exon V is required for the maturation of synaptic connections within a given neuronal network. Based on those working assumptions, the specific changes in BDNF exon expression in HD reflects a disruption of the immediate-early signaling process and the inability to sustain that signal thereby impeding the long-term changes required to sculpt the neuronal network. Future studies addressing these issues will also be required to investigate possible sex-differences since we observed differences in exon-specific BDNF expression between male and female HD mice.
5.4. Conclusion

Repression of gene transcription resulting in reduced levels of BDNF mRNA in the HD hippocampus is not a result of altered DNA methylation or histone modification. The overall reduction of BDNF mRNA levels cannot be reversed by environmental enrichment despite an enrichment-mediated increase in the levels of BDNF exon IV-containing transcripts specific to female HD mice. Given the similar molecular and behavioural effects of environmental enrichment and wheel-running, an up-regulation of BDNF exon-IV transcript is key to the correction of the female HD behavioural phenotype. Future investigations will be required to determine the functional impact of this specific up-regulation including studies of mRNA processing, trafficking and translation.
Chapter 6 Region-specific changes in serotonin receptor expression are modulated by environmental enrichment.

6.1. Introduction

The previous chapters in this thesis have identified differences in environmental enrichment-stimulated changes in hippocampal gene expression of brain-derived neurotrophic factor (BDNF) in relation to the correction of the depressive behaviours unique to female HD mice by environmental enrichment. The work described in this chapter is an examination of further changes in gene expression in the HD brain that might contribute to the development of depression in HD.

Robust alterations in gene expression occur in the HD brain and are reproduced in various in vivo models of HD including the R6/1 HD transgenic mouse model (Luthi-Carter et al., 2000; Luthi-Carter et al., 2002b; Hodges et al., 2006; Kuhn et al., 2007). Most studies to date have primarily examined the striatum, cortex and cerebellum as these have been the primary regions of interest of this disease (Iannicola et al., 2000; Luthi-Carter et al., 2003; Obrietan and Hoyt, 2004). The changes in gene expression in the hippocampus are less described but are as important since this brain region is implicated in the cognitive and psychiatric aspects of this disease.

Altered gene expression is also detected in depression. Gene profiling studies performed on prefrontal cortical tissue from post mortem brains of depressed subjects have uncovered altered expression of genes involved in cell proliferation, stress response and gene transcription (Kang et al., 2007; Tochigi et al., 2008). Similar findings have also been reported in studies using the rodent chronic mild stress model of depression (Bergstrom et al., 2007; Koh et al., 2007; Orsetti et al., 2008). Given that a dysregulation of gene expression appears to be intrinsic to both HD and depression, further work is required to determine whether some of the earliest changes in gene expression occurring
in the HD brain contribute to the increased susceptibility of HD patients to develop depression.

The previous chapter had explored the changes in BDNF gene expression in the HD hippocampus, a change which has been shown to disrupt serotonin (5-HT) receptor function (Hensler et al., 2007). Depression has been proposed to constitute a disruption of neurotrophin imbalance impacting on serotonergic neurons (Duman et al., 1997; Altar, 1999). Furthermore, BDNF regulates various aspects of serotonergic neuronal function including neurogenesis, differentiation, survival and synaptic plasticity (Eaton et al., 1995; Mamounas et al., 1995; Siuciak et al., 1996). Conversely, increased serotonergic transmission has also been proposed as the underlying anti-depressant effects of BDNF (Siuciak et al., 1997). In turn, stimulation of serotonergic signaling increases BDNF expression (Deltheil et al., 2008). Altered interactions between the BDNF and serotonin-mediated signaling pathways have therefore been proposed to be central to the development of mood disorders and each has been independently implicated in psychiatric conditions such as depression (discussed by Martinowich et al., 2003).

As discussed in Chapter 3, the sex-specific behavioural profile of the R6/1 HD transgenic mouse model is similar to the behavioural phenotype observed in several mouse models used to investigate the role of the various 5-HT receptors and the serotonin transporter (5-HTT) in depression (Mayorga et al., 2001; Holmes et al., 2002; Lira et al., 2003; Jones and Lucki, 2005; Renoir et al., 2008). The remarkable level of specificity and similarity (behavioural differences in the females but not males on the forced-swim and tail-suspension tests) indicates a possibility that a dysregulation of the serotonergic signaling system in the HD brain due to altered gene expression of the serotonin receptors might be a major contributor to the depressive behaviours of the female HD mice.

It has been established that serotonin metabolism is altered in HD. Post mortem studies found increased 5-HT levels in the basal ganglia and cortex but not hippocampus of post
mortem brains (Kish et al., 1987; Reynolds and Pearson, 1987) despite normal levels of serotonin in the blood of HD patients (Christofides et al., 2006). 5-HTT binding has also been found to be reduced in the basal ganglia of late-stage HD brains (Waeber and Palacios, 1989; Steward et al., 1993; Castro et al., 1998). There have been few studies of the serotoninergic signaling system in in vivo HD models. In contrast to the patient-based studies, 5-HT levels are reduced in the brains of transgenic HD mice (Reynolds et al., 1999). However, the one consistent finding is that of reduced 5-HT receptor 1A binding in the brains of R6/2 HD mice (Yohrling et al., 2002).

The present study examined the expression patterns of several 5-HT receptors and 5-HTT in the hippocampus and the cortex of female and male R6/1 HD mice. The primary hypothesis was that depressive-like behaviours of female HD mice are associated with altered gene expression of specific 5-HT receptors. Environmental enrichment selectively up-regulates 5-HT receptor expression (Hellemans et al., 2005) so the effects of enrichment on 5-HT receptor expression was also investigated in association with the enrichment-mediated rescue of the female behavioural phenotype.

The aims of this chapter were:

5. To describe the expression of serotonin 1A (htr1A), 1B (htr1B), 2A (htr2A) and 2C (htr2C) receptors in the hippocampus of male and female wild-type and HD mice.

6. To investigate the effect of environmental enrichment on hippocampal expression of the genes mentioned in Aim 1.

7. To describe the expression of the genes mentioned in Aim 1 in male and female wild-type and HD cortex.

8. To investigate the effect of environmental enrichment on cortical expression of the genes mentioned in Aim 1.

9. To characterise gene expression of the serotonin transporter (SerT) in the hippocampus and cortex of HD and wild-type brains, as well as following environmental enrichment.
6.2. Materials and Methods

8 week old HD and wild-type mice were housed under standard-housed or environmental enrichment conditions (see Chapter 2.2, Figures 2-2B, C) for 4 weeks. At 12 weeks of age, mice were killed by cervical dislocation, brains removed then dissected for cortical and hippocampal regions. These were snap frozen in liquid nitrogen and stored at -80°C until further use. Frozen tissue was disrupted using a sonicator prior to RNA isolation using the Qiagen RNA extraction kits. Cortical RNA was isolated using the RNeasy Midi kit while hippocampal RNA was isolated with the RNeasy Mini kit according to the manufacturer’s instructions. Extracted RNA was analysed for elute concentration and RNA quality using a bioanalyser and this service was provided by the Australian Genome Research Facility (AGRF). 1µg of RNA was reverse transcribed into cDNA using the Applied Biosystems Taqman reverse transcription kit. Appropriate controls were included in all reactions. Primer pairs specific for htr1A, htr1B, htr2A, htr2C and SerT were designed and optimized prior to commencement of real-time PCR analyses (See Chapter 2.12, Table 2-1). SYBR-green real-time PCR reactions were carried out on an ABI 7500 Realtime PCR System. The house-keeping gene of choice was cyclophilin and all samples were run in triplicate. Fold change was calculated using the $2^{-\Delta\Delta Ct}$ method and normalized to the mean fold-change of the respective wild-type groups. 2-way ANOVA was used to compared genotype and sex differences with the level of statistical significance set at $\alpha = 0.05$. 
6.3. Results

6.3.1. Sex differences in serotonin receptor expression in the HD and wild-type hippocampus

mRNA levels of the following serotonin receptors in the hippocampus of 12-week old standard-housed HD and wild-type mice were quantified using real-time PCR: serotonin 1A receptor (htr1A), serotonin 1B receptor (htr1B), serotonin 2A receptor (htr2A), serotonin 2C receptor (htr2C). The expression of the serotonin transporter (SerT) was also examined. All results are presented relative to male wild-type expression levels.

6.3.1.1. Sex differences in htr1A and htr1B mRNA levels in the hippocampus

There was no overall sex difference (F\(_{(1,19)} = 2.608, p = 0.126\)) but there was a strong genotype effect on hippocampal expression of htr1A (F\(_{(1,19)} = 20.785, p < 0.001\)). There was no significant sex-genotype interaction (F\(_{(1,19)} = 0.0345, p = 0.855\)). Male (-33%; p = 0.004) and female (-42%; p = 0.007) HD mice had significantly reduced htr1A expression in the hippocampus compared to their respective WT groups (Fig. 6-1A). There were no sex differences within the wild-type (p = 0.221) and HD (p = 0.327) groups.

In comparison, there were significant sex (F\(_{(1,18)} = 9.155; p = 0.009\)) and genotype (F\(_{(1,18)} = 22.129; p < 0.001\)) effects on hippocampal htr1B expression. The sex-genotype interaction was not significant (F\(_{(1,18)} = 0.0144; p = 0.906\)). Levels of htr1B were significantly decreased in the hippocampus of male (-50%; p = 0.004) and female (-77%; p = 0.005) HD mice (Fig. 6-1B). Female HD mice had significantly less htr1B mRNA than male HD mice (-27%, p = 0.037) and there was a similar trend in the wild-type group (-27%, p = 0.064).
Figure 6-1 Expression of htr1A and htr1B in the hippocampus.

(A) HD mice (Male: n = 5; Female: n = 5) had reduced htr1A expression in the hippocampus compared to wild-type mice (Male: n = 5; Female: n = 5). (B) Levels of htr1B mRNA are also reduced in HD mice (Male: n = 5; Female: n = 5) and htr1B expression was significantly less in female than male HD mice. Asterisks indicate post-hoc Bonferroni’s t-test: *: p < 0.05.
6.3.1.2. Sex differences in htr2A and htr2C mRNA levels in the hippocampus

There was a distinct difference in the expression patterns of htr2A and htr2C in the hippocampus. There was a trend towards a sex difference in the expression of htr2A ($F_{(1,17)} = 3.494, p = 0.083$) together with a significant genotype difference ($F_{(1,17)} = 19.250, p < 0.001$) but no significant sex-genotype interaction ($F_{(1,17)} = 1.936, p = 0.186$) (Fig. 6-2A). Both male (-44%; $p = 0.041$) and female (-99%; $p = 0.002$) HD mice had reduced htr2A expression in the hippocampus than their respective WT groups. Female wild-type mice had greater htr2A mRNA levels (+70%) in the hippocampus than male wild-type mice ($p = 0.037$) but no sex-difference existed within the HD group.

There were no differences in hippocampal expression of htr2C. There were no significant effects of sex ($F_{(1,19)} = 0.622; p = 0.442$) or genotype ($F_{(1,19)} = 0.122; p = 0.732$) on htr2C mRNA levels (Fig. 6-2B). There was also no significant sex-genotype interaction ($F_{(1,19)} = 0.0180; p = 0.895$).
Figure 6-2 Expression of htr2A and htr2C in the hippocampus.

(A) HD mice (Male: n = 5; Female: n = 4) had reduced htr2A mRNA levels. Female wild-type mice (n = 4) had significantly greater htr2A expression than male wild-types (n = 4) but this sex difference was not detected in the HD group. (B) Expression of htr2C was similar between the sexes and genotypes. Asterisks indicate post-hoc Bonferroni’s t-test: *: p < 0.05.
6.3.1.3. Sex differences in SerT mRNA levels in the hippocampus

No statistically significant differences in SerT mRNA levels were detected in the HD hippocampus. There were no overall differences attributable to the different sexes ($F_{(1,19)} = 0.329; \ p = 0.574$) or genotypes ($F_{(1,19)} = 2.750; \ p = 0.117$) (Fig. 6-3). There was no significant sex-genotype interaction ($F_{(1,19)} = 0.645, \ p = 0.434$).

![SerT expression in the hippocampus](image)

**Figure 6-3 Expression of serotonin transporter (SerT) in the hippocampus.**

No statistically significant differences were detected between hippocampal expression of SerT in the four groups of mice.
6.3.2. Effect of environmental enrichment on serotonin receptor expression in the hippocampus

Having found some sex differences in hippocampal htr1B and htr2A expression that correlated with the behavioural differences of male and female, as well as wild-type and HD mice, the effect of environmental enrichment on expression was investigated to determine whether the normalization of female HD behaviour during the forced-swim test (described in Chapter 4) could be correlated with a change in expression of a specific serotonin receptor.

6.3.2.1. Environmental modulation of htr1A mRNA levels in the hippocampus

In the female group, overall hippocampal expression of htr1A was significantly influenced by genotype ($F_{(1,18)} = 96.729; p < 0.001$) and the environment ($F_{(1,18)} = 17.885; p < 0.001$). There was no significant genotype-environment interaction ($F_{(1,18)} = 1.511; p = 0.238$). As expected, htr1A expression was reduced in standard-housed female HD mice ($n = 5, -65\%, p < 0.001$) compared to standard-housed wild-type levels ($n = 5$) (Fig. 6-4A). Environmental enrichment significantly increased htr1A mRNA levels in wild-type mice ($n = 4, +22\%, p = 0.002$) and also in HD mice ($n = 5, +12\%, p = 0.045$). However, there was still a significant difference in htr1A expression between the enriched wild-type and HD groups ($p < 0.001$).

There was also a strong genotype effect in the male group ($F_{(1,19)} = 31.774; p < 0.001$) however neither the environment effect ($F_{(1,19)} = 1.129; p = 0.304$) nor the genotype-environment interaction ($F_{(1,19)} = 2.212; p = 0.156$) were significant. Hippocampal expression of htr1A in standard-housed male HD mice ($n = 5, -33\%, p = 0.010$) was less than standard-housed wild-type mice ($n = 5$) (Fig. 6-4B). Unlike in the females, environmentally enriched male wild-type mice did not have significantly greater levels of htr1A mRNA ($n = 5, +20\%, p = 0.090$). Environmental enrichment also did not significantly alter htr1A mRNA levels in male HD mice ($n = 5, -3\%, p = 0.768$).
Figure 6-4 Effects of environmental enrichment on htr1A expression in the hippocampus.

(A) Environmental enrichment up-regulated htr1A mRNA levels in female wild-type and HD mice. (B) Enrichment did not significantly alter htr1A expression in male mice. Asterisks indicate post-hoc Bonferroni’s t-test: *: p < 0.05, ***: p < 0.001.
6.3.2.2. Environmental modulation of htr1B mRNA levels in the hippocampus

There was an overall genotype effect on hippocampal htr1B expression in female mice ($F_{(1,17)} = 77.944; p < 0.001$) as well as an effect of the environment ($F_{(1,17)} = 7.781; p = 0.014$). The genotype-environment interaction was not significant ($F_{(1,17)} = 0.0405; p = 0.843$). Levels of htr1B mRNA in standard-housed female HD mice ($n = 5, -68\%, p < 0.001$) were significantly lower than to standard-housed wild-type levels ($n = 4$) (Fig. 6-5A). Environmental enrichment did not significantly alter expression in female wild-types ($n = 4, +20\%, p = 0.104$) but enriched female HD mice ($n = 5, +18\%, p = 0.042$) had significantly greater htr1B expression than the standard-housed female HD group.

There was also a significant genotype effect observed in the male group ($F_{(1,19)} = 34.304; p < 0.001$) but unlike the female group, there was no significant effect of the environment ($F_{(1,19)} = 0.319; p = 0.580$). The genotype-environment interaction was not significant ($F_{(1,19)} = 2.020; p = 0.174$). Hippocampal expression of htr1B in standard-housed male HD mice ($n = 5, -50\%, p = 0.006$) was half that of standard-housed wild-types ($n = 5$) (Fig. 6-5B). htr1B mRNA levels in environmentally enriched male wild-type mice ($n = 5, +82\%, p < 0.001$) were significantly greater than enriched male HD mice ($n = 5$).
Figure 6-5 Effect of environmental enrichment on htr1B mRNA levels in the hippocampus.

(A) Environmentally enrichment female HD mice had significantly greater htr1B mRNA levels in the hippocampus compared to standard-housed HD mice. (B) Male HD mice had reduced levels of htr1B in the hippocampus. Environmental enrichment did not significantly alter htr1B expression in wild-type and HD mice. Asterisks indicate post-hoc Bonferroni’s t-test: *: p < 0.05, ***: p < 0.001.
6.3.2.3. Environmental modulation of htr2A mRNA levels in the hippocampus

Preliminary data from microarray analysis of gene expression in 12-week old HD mice indicates that there is differential expression of the htr2A and htr2C (Zajac & Hannan, personal communications). Therefore, it was of interest to investigate their expression patterns in relation to the development of the existing behavioural changes.

There was a strong genotype effect on htr2A mRNA expression in female mice ($F_{(1,16)} = 11.076, p = 0.005$). There was no overall environment effect ($F_{(1,16)} = 2.248, p = 0.158$) but there was a significant genotype-environment interaction ($F_{(1,16)} = 5.885, p = 0.031$). Hippocampal levels of htr2A mRNA of standard-housed HD female mice ($n = 4$, -58%, $p = 0.002$) was significantly reduced compared to standard-housed wild-type female levels ($n = 4$) (Fig. 6-6A). Surprisingly, environmentally enriched wild-type mice had significantly reduced htr2A expression ($n = 4$, -39%, $p = 0.018$). However, enrichment did not significantly alter htr2A expression in HD females ($n = 5$, $p = 0.667$).

There was also a significant genotype effect observed in the male group ($F_{(1,17)} = 11.676; p = 0.004$). However, there was no significant environment effect ($F_{(1,17)} = 0.0965; p = 0.761$) and no significant genotype-environment interaction ($F_{(1,17)} = 0.394; p = 0.541$). Htr2A expression was reduced to 56% of standard-housed wild-type levels ($n = 4$) in standard-housed HD male mice ($n = 4$, -44%, $p = 0.069$) (Fig. 6-6B). Within the environmentally enriched group, HD mice ($n = 5$, -47%, $p = 0.009$) had significantly reduced levels of htr2A compared to wild-type mice ($n = 4$).
Figure 6-6 Selective down-regulation of hippocampal htr2A expression in female wild-type mice by environmental enrichment.

(A) HD mice had reduced mRNA levels of htr2A. Expression of htr2A in female wild-type mice was significantly reduced by environmental enrichment but there was no effect on female HD mice. (B) Hippocampal expression of htr2A was reduced in HD mice. Asterisks indicate post-hoc Bonferroni’s t-test: *: p < 0.05.
6.3.2.4. Environmental modulation of htr2C mRNA levels in the hippocampus

Overall, there was no effect of genotype \( F(1,18) = 0.37; p = 0.548 \) or environment \( F(1,18) = 0.501; p = 0.490 \) within the female group (Fig. 6-7A). There was no significant genotype-environment interaction \( F(1,18) = 0.0459; p = 0.833 \). Similarly, there was also no effect of genotype \( F(1,19) = 0.00398; p = 0.950 \) or environment \( F(1,19) = 0.0609; p = 0.808 \) and no genotype-environment interaction \( F(1,19) = 0.00277; p = 0.870 \) in the male group (Fig. 6-7B).

6.3.2.5. Environmental modulation of SerT mRNA levels in the hippocampus

Overall, there was no effect of genotype \( F(1,18) = 0.340; p = 0.569 \) or environment \( F(1,18) = 0.174; p = 0.682 \) impacting on SerT mRNA levels in the hippocampus of female mice. There was no genotype-environment interaction \( F(1,18) = 0.0288; p = 0.867 \) (Fig. 6-8A).

Interestingly, there was a significant effect of genotype \( F(1,19) = 6.012; p = 0.026 \) but not of environment \( F(1,19) = 0.138; p = 0.715 \) on SerT expression in the male hippocampus (Fig. 6-8B). There was no genotype-environment interaction \( F(1,19) = 0.00210; p = 0.964 \). Standard-housed male HD mice \( (n = 5, -43\%, p = 0.108) \) had reduced SerT expression compared to standard-housed wild-type levels \( (n = 5) \). Similarly, SerT expression was also reduced in enriched HD mice \( (n = 5, -45\%, p = 0.096) \) compared to enriched wild-type levels \( (n = 5) \).
Htr2C expression in female hippocampus

A

Htr2C expression in male hippocampus

B

Figure 6-7 Environmental enrichment does not alter hippocampal levels of htr2C mRNA.

Hippocampal expression of htr2C is not altered in the female (A) and male (B) HD brain. Environmental enrichment did not alter htr2C expression in wild-type and HD mice.
Figure 6-8 Environmental enrichment does not alter SerT expression in the male and female hippocampus.

(A) Female HD mice had similar levels of SerT mRNA as wild-types. (B) There was an overall reduction of SerT expression in the hippocampus of male HD mice.
6.3.3. Effect of environmental enrichment on serotonin receptor expression in the cortex

In addition to reductions in hippocampal htr1A mRNA levels, a decrease in cortical expression and diminished 5-HT1A receptor binding is also detected in depression (Drevets et al., 1999; Lopez-Figueroa et al., 2004; Drevets et al., 2007). Hence, the investigation of serotonin receptor expression in the HD brain was extended to include the cortex.

6.3.3.1. Sex differences in htr1A and htr1B mRNA levels in the cortex

There were no overall sex-differences in cortical htr1A expression of HD and wild-type mice (F(1,18) = 0.988, p = 0.336) (Fig. 6-9A). However, there was a genotype effect (F(1,18) = 105.966, p < 0.001) as male (n = 5, -40%, p < 0.001) and female (n = 4, -39%, p < 0.001) HD mice had reduced levels of htr1A mRNA compared to their respective standard-housed wild-type groups. There was no significant sex-genotype interaction (F(1,18) = 0.509, p = 0.487).

Cortical expression of htr1B differed between the genotypes (F(1,18) = 15.144, p = 0.001) but there were no overall sex differences (F(1,18) = 0.0126, p = 0.912) (Fig. 6-9B). There was no significant sex-genotype interaction (F(1,18) = 0.00152, p = 0.969). htr1B expression in the male HD cortex (n = 5, -78%, p = 0.014) was found to be severely reduced compared to wild-type levels (n = 5). Expression levels in female HD mice were similarly reduced (n = 4, -71%, p = 0.016) compared to standard-housed female wild-types (n = 5).
Figure 6-9 Expression of htr1A and htr1B in the cortex of HD and wild-type mice.

(A) Male and female HD mice have reduced levels of htr1A in the cortex. (B) Similarly, htr1B expression in the cortex is also reduced. There were no differences in expression between male and female cortices of both genotypes. Asterisks indicate post-hoc Bonferroni’s t-test: *: p < 0.05, ***: p < 0.001.
6.3.3.2. Sex differences in htr2A and htr2C mRNA levels in the cortex

Overall, HD mice had reduced cortical htr2A expression ($F_{(1,18)} = 0.469, p = 0.504$) but there were no significant sex differences ($F_{(1,18)} = 106.497, p < 0.001$) (Fig. 6-10A). There was no sex-genotype interaction ($F_{(1, 18)} = 0.349, p = 0.564$). Both male ($n = 6, -49\%, p < 0.001$) and female HD mice ($n = 4, -44\%, p < 0.001$) had reduced htr2A expression in the cortex.

In contrast to the htr2A expression pattern, no differences in cortical htr2C expression were detected between genotypes ($F_{(1,18)} = 2.326, p = 0.148$) or sexes ($F_{(1,18)} = 2.011, p = 0.177$) (Fig. 6-10B). There was no sex-genotype interaction ($F_{(1,18)} = 0.496, p = 0.492$).
Figure 6-10 Expression of htr2A and htr2C in the cortex of HD and wild-type mice.

(A) Male and female HD mice had reduced levels of htr2A mRNA in the cortex. (B) Cortical expression of htr2C was similar between both sexes and genotypes. Asterisks indicate post-hoc Bonferroni’s t-test: ***: $p < 0.001$. 
6.3.3.3. Sex differences in SerT mRNA levels in the cortex

There was no overall sex-difference in SerT mRNA levels in the cortex ($F_{(1,18)} = 1.890, p = 0.189$) but there was a significant genotype difference ($F_{(1,18)} = 5.503, p = 0.033$) (Fig. 6-11). There was no significant sex-genotype interaction ($F_{(1,18)} = 1.964, p = 0.181$). Post hoc analysis revealed no significant difference in SerT expression between male HD ($n = 6$) and wild-type mice ($n = 4, p = 0.506$). In contrast, female HD mice ($n = 4, -59\%, p = 0.020$) had significantly less SerT mRNA levels in the cortex than female wild-types ($n = 5$).

![SerT expression in the cortex](image)

**Figure 6-11 Expression of serotonin transporter (SerT) in the cortex of HD and wild-type mice.**

Female HD mice have significantly reduced levels of SerT mRNA in the cortex compared to female wild-types. This genotype difference was not present in the male group. Asterisks indicate post-hoc Bonferroni’s t-test: *: $p < 0.05$. 
6.3.4. Environmental modulation of serotonin receptor expression in the cortex

6.3.4.1. Environmental enrichment does not alter cortical expression of htr1A

There was an overall difference in htr1A expression in the female cortex between the genotypes ($F_{(1,18)} = 37.615, p < 0.001$) but there was no effect of the environment ($F_{(1,18)} = 0.718, p = 0.410$) (Fig. 6-12A). There was no genotype-environment interaction ($F_{(1,18)} = 0.837, p = 0.375$). Standard-housed female HD mice ($n = 4$, $-41\%, p < 0.001$) had reduced cortical htr1A expression compared to standard-housed female wild-types ($n = 5$). Similarly, htr1A expression was decreased in enriched female HD mice ($n = 5$, $-30\%, p = 0.002$) compared to enriched wild-types ($n = 5$).

Similar to observations in the female cortex, htr1A expression in the male cortex significantly differed between the genotypes ($F_{(1,19)} = 88.288, p < 0.001$) but there was no effect of the environment ($F_{(1,19)} = 0.00335, p = 0.955$) (Fig. 6-12B). There was no significant genotype-environment interaction ($F_{(1,19)} = 0.00335, p = 0.955$). Levels of htr1A mRNA were reduced in standard-housed male HD mice ($n = 5$, $-44\%, p < 0.001$) compared to standard-housed wild-type levels ($n = 5$). Enriched male HD mice ($n = 5$, $-44\%, p < 0.001$) also had significantly reduced cortical expression of htr1A compared to enriched wild-type levels ($n = 5$).
Figure 6-12 Environmental enrichment does not alter htr1A expression in the male and female cortex.

(A) Female HD mice have reduced expression of htr1A. (B) Similarly, htr1A expression is also decreased in the male HD cortex. Environmental enrichment did not alter cortical levels of htr1A mRNA. Asterisks indicate post-hoc Bonferroni’s t-test: *: p < 0.05, ***: p < 0.001.
6.3.4.2. Environmental enrichment does not alter cortical expression of htr1B

There was a significant effect of genotype ($F_{(1,17)} = 8.569, p = 0.011$) but not environment ($F_{(1,17)} = 0.481, p = 0.499$) on htr1B expression in the female cortex (Fig. 6-13A). There was no significant genotype-environment interaction ($F_{(1,17)} = 0.321, p = 0.580$). htr1B expression in standard-housed female HD mice ($n = 4$, -81%, $p = 0.027$) was only 19% of standard-housed wild-type levels ($n = 5$). Enriched female HD mice ($n = 4$) had approximately double the expression of htr1B in the cortex (48% vs. 19%) compared to the standard-housed group. The difference in cortical expression of htr1B between environmentally enriched female HD ($n = 4$, -55%, $p = 0.117$) and wild-type mice ($n = 5$) was not statistically significant.

The differences in htr1B expression in the male cortex were between the genotypes ($F_{(1,18)} = 18.303, p < 0.001$) and not due to the environment ($F_{(1,18)} = 0.313, p = 0.584$) (Fig. 6-13B). There was no significant genotype-environment interaction ($F_{(1,18)} = 0.0276, p = 0.870$). htr1B mRNA levels in the cortex of male standard-housed ($n = 5$, -75%, $p = 0.006$) and environmentally enriched HD mice ($n = 5$, -82%, $p = 0.012$) were significantly reduced compared to their respective wild-type groups.
Figure 6-13 Environmental enrichment does not alter htr1B expression in the male and female cortex.

(A) Levels of htr1B mRNA were reduced in the female HD cortex. (B) htr1B expression was also significantly reduced in the male HD cortex. There was no effect of environmental enrichment on cortical htr1B expression in all groups. Asterisks indicate post-hoc Bonferroni’s t-test: *: p < 0.05.
6.3.4.3. Environmental enrichment does not alter cortical expression of htr2A

There were strong overall genotype effects on htr2A expression in the female ($F_{(1,18)} = 52.937$, $p < 0.001$) (Fig. 6-14A) and male ($F_{(1,19)} = 35.676$, $p < 0.001$) (Fig. 6-14B) cortices. On the other hand, there was no overall influence of the housing environment on cortical htr2A mRNA levels in female ($F_{(1,18)} = 1.877$, $p = 0.191$) and male mice ($F_{(1,19)} = 0.283$, $p = 0.602$).

Both standard-housed (n = 4, -46%, $p < 0.001$) and environmentally enriched (n = 5, -35%, $p < 0.001$) female HD mice had significantly reduced expression of htr2A compared to their respective wild-type groups.

htr2A expression in standard-housed male HD mice (n = 6, -49%, $p < 0.001$) was reduced to about half of standard-housed male wild-type levels (n = 4). Enriched male HD mice (n = 5, -67%, $p < 0.001$) also had significantly reduced htr2A expression compared to the enriched wild-type group (n = 5).
Figure 6-14 Environmental enrichment does not alter htr2A expression in the male and female cortex.

Cortical levels of htr2A mRNA were reduced in female (A) and male (B) HD mice. Environmental enrichment did not significantly alter htr2A expression in HD or wild-type mice. Asterisks indicate post-hoc Bonferroni’s t-test: ***: p < 0.001.
6.3.4.4. Effect of environmental enrichment on cortical expression of htr2C

The genotype ($F_{(1,18)} = 0.539, p = 0.474$) and environment ($F_{(1,18)} = 2.653, p = 0.124$) were not significant factors influencing htr2C expression in the female cortex (Fig. 6-15A). However, there was a significant genotype-environment interaction ($F_{(1,18)} = 8.283, p = 0.011$). htr2C expression of standard-housed female HD mice ($n = 4$) was 87% that of standard-housed female wild-type mice ($n = 5$). Environmental enrichment did not significantly alter cortical expression of htr2C in female wild-types (92%) but increased htr2C expression in female HD mice ($n = 5$, +26%, $p = 0.007$). There was a significant difference in htr2C expression between enriched female HD and wild-type mice ($p = 0.019$).

Expression of htr2C mRNA in the male cortex was not dependent on genotype ($F_{(1,19)} = 0.00754, p = 0.932$) or housing environment ($F_{(1,19)} = 0.0148, p = 0.905$)(Fig. 6-15B). Unlike the female group, there was no significant genotype-environment interaction ($F_{(1,19)} = 0.370, p = 0.552$). The levels of htr2C mRNA in male standard-housed HD mice (96%), environmentally enriched wild-type (89%) and HD (100%) mice were all similar to standard-housed wild-type levels.
Figure 6-15 Selective modulation of cortical expression of htr2C by environmental enrichment.

(A) Environmental enrichment increased htr2C expression in the female HD cortex but not in the wild-types. (B) Unlike in the females, enrichment had no significant effect on htr2C expression in the cortex of male mice. Asterisks indicate post-hoc Bonferroni’s t-test: *: p < 0.05.
6.3.4.5. No significant alteration of cortical SerT expression by environmental enrichment

Overall, the expression of SerT in the female cortex was significantly effected by genotype ($F_{(1,18)} = 5.828, p = 0.029$) but not by housing environment ($F_{(1,18)} = 2.594, p = 0.128$) (Fig. 6-16A). There was no significant genotype-environment interaction ($F_{(1,18)} = 0.652, p = 0.432$). Post hoc analysis revealed a significant reduction in SerT expression in standard-housed female HD mice ($n = 4, -39\%, p = 0.043$) compared to standard-housed wild-type levels ($n = 5$). Environmentally enriched female wild-type mice had a 29% reduction in cortical SerT expression. There was no difference between the levels of SerT mRNA in the cortices of environmentally enriched female HD (52%) and wild-type mice (71%, $p = 0.260$).

There was also an overall genotype effect on SerT expression in the male cortex ($F_{(1,19)} = 5.460, p = 0.033$) (Fig. 6-16B). However, there was no environmental effect ($F_{(1,19)} = 0.635, p = 0.437$) and no significant genotype-environment interaction ($F_{(1,19)} = 2.449, p = 0.137$). Post hoc analysis revealed no significant difference between SerT expression in male standard-housed HD ($n = 6, -13\%, p = 0.597$) and wild-type mice ($n = 4$). SerT expression in the enriched male wild-type cortex was 151% of standard-housed wild-type levels. Hence, there was a significant difference in SerT expression between environmentally enriched HD ($n = 5, -78\%, p = 0.013$) and wild-type mice ($n = 5$).
Figure 6-16 SerT expression in the cortex of female and male mice.

(A) Environmental enrichment did not rescue the deficit in SerT expression in the female HD cortex. (B) There was also no significant effect of environmental enrichment on SerT mRNA levels in the male HD cortex. Asterisks indicate post-hoc Bonferroni’s t-test: *: p < 0.05.
6.4. Discussion

The purpose of this study was to determine whether the depressive-like behaviours specific to female HD mice were associated with changes in serotonin receptor gene expression, in particular of serotonin 1A and 1B receptor expression. The behavioural responses of the R6/1 HD transgenic model during the pre-motor symptomatic stage on the forced-swim and tail-suspension tests (as described in Chapter 3) are similar to the behavioural phenotypes of other lines of mice with disrupted serotonergic signaling via knockout of the 5-HT$_{1A}$ and 5-HT$_{1B}$ receptors, as well as 5-HTT (serotonin transporter) (Mayorga et al., 2001; Holmes et al., 2002; Lira et al., 2003; Jones and Lucki, 2005).

Despite the similarities in depression-related behaviours, it is important to consider the effect of the different background strains onto which these different lines of mice are bred onto. As an example, 5-HTT knockout mice on a 129S6 background display reduced immobility on the tail-suspension test but have a normal baseline profile when bred onto a C57BL/6J background (Holmes et al., 2002). The 5-HT$_{1A}$ and 5-HT$_{1B}$ receptor knockout lines are on a 129/Sv background while the R6/1 transgenic line is bred onto a mixed C57BL/6J-CBA background. Therefore, the initial extrapolation of the behavioural phenotyping exercise and the hypothesis that the HD behavioural phenotype was attributable to reduced serotonin receptor gene expression in the brain required further investigation.

The work described in this chapter has confirmed that hypothesis by revealing a selective down-regulation of serotonin receptor gene expression in the cortex and hippocampus of HD mice. Expression of htr1A, htr1B and htr2A was reduced while htr2C expression remained unaffected. These findings agree with previously reports of reduced serotonin receptor binding in human HD brains (Waebber and Palacios, 1989; Steward et al., 1993; Wong et al., 1996; Castro et al., 1998) and reduced 5-HT$_{1A}$ receptor binding in the cortex and hippocampus of motor symptomatic R6/2 HD mice (Yohrling et al., 2002). It was
previously unclear whether those changes were a reflection of reduced serotonin receptor protein levels or altered receptor function. Given the reduction in gene expression described in this chapter, it appears the former scenario is more likely. Overall, there is further support for the initial hypothesis that reduced serotonin receptor expression in the HD brain contributes to the behavioural phenotype.

The reductions in serotonin receptor expression in the HD cortex and hippocampus were to similar extents so the depressive-like behaviours could not be directly attributed to a single receptor. Each receptor has been independently implicated in depression pathology. htr1A expression is reduced in the dorso-lateral prefrontal cortex and hippocampus of major depression disorder (MDD) sufferers (Lopez-Figueroa et al., 2004) together with reduced 5-HT$_{1A}$ receptor binding in the cortex (Drevets et al., 2007). The 5-HT$_{1B}$ (human homologue 5-HT$_{1D}$) regulates the release of various neurotransmitters including 5-HT, GABA and glutamate (discussed by Sari, 2004). While its exact involvement in the pathology of depression is currently unclear, it has been established that abnormal interactions of the 5-HT$_{1B}$ receptor with its adaptor protein p11 leads to the manifestation of depression-like behaviour (Svenningsson et al., 2006; Svenningsson and Greengard, 2007). It is possible that reduced expression of htr1B in the HD brain leads to altered level of p11 interaction and disturbed serotonergic, GABAergic and glutamatergic neurotransmission. Future studies would be required to investigate the extent to which 5-HT$_{1B}$ receptor mediated signaling pathways are altered in the HD brain in relation to depressive behaviours.

The 5-HT$_{2A}$ receptor gene has been implicated in the pathogenesis of suicidal behavior by a genetic association between the 5-HT$_{2A}$ C102T silent polymorphism and suicidality in patients with major depression (Du et al., 2000) although recent studies have claimed otherwise (De Luca et al., 2007). Interestingly, htr2A mRNA levels are increased in the brains of depressed suicide victims (Escriba et al., 2004) but unchanged in brains of MDD patients (Lopez-Figueroa et al., 2004) despite decreased receptor binding (Mintun et al., 2004) which suggests altered receptor function. The tendencies for suicide ideation
and frequency of suicide attempts by HD patients has yet to be clearly documented and would be interesting given the association of htr2A with suicide. It is unclear what reduced expression of htr2A in the HD brain constitutes but it could be involved in the impairment of hippocampal synaptic plasticity since the 5-HT$_{2A}$ receptor, together with the 5-HT$_{1B}$ receptor, is involved in the modulation of glutamatergic signaling in the dentate gyrus of the hippocampus (Peddie et al., 2008a, b).

A polymorphism of the serotonin transporter gene (5-HTTLPR) which influences expression of the serotonin transporter is associated with depression-related personality traits (Hoefgen et al., 2005) and 5-HTT binding is reduced in the cortex of depressed subjects (Mann et al., 2000). According to single-photon emission computed tomography (SPECT) studies, the female brain has higher 5-HTT availability in the cortex (Staley et al., 2001) and a greater reduction in that availability is detected in depressed female subjects (Staley et al., 2006). In agreement, this study found greater SerT expression in the cortex of female wild-type mice compared to males while only female HD mice had a significant reduction in SerT expression (further discussed below). Overall, it appears that the combined reduction in expression levels of genes involved in the serotonergic signaling system mediates symptoms of depression (Nutt, 2008) and the increased risk of depression in HD.

The depressive-like behaviours that were almost exclusive to female HD mice (male HD mice had altered tail-suspension test responses) could be due to sex-specific hormone differences, especially since estrogens (the group of female sex hormones) have been shown to differentially regulate serotonergic signaling in the brain (reviewed by Fink et al., 1999). Different levels of gene expression as previously reported in the human brain could also be a factor influencing behaviour. Two recent PET imaging studies of healthy subjects offer conflicting reports of serotonin receptor distribution in the male and female brain with one reporting higher 5-HT$_{1A}$ receptor and lower 5-HTT binding potentials in the female cortex (Jovanovic et al., 2008) while the other finding no sex differences (Stein et al., 2008). The different results have been attributed to the stringency of
controlling for menstrual cycling of female subjects which is critical since estrogen has been shown to modulate serotonin receptor expression in various brain regions (Zhou et al., 2002). While female HD mice in this study were not checked for estrous cycling, the low degree of variance in 5-HT receptor expression levels suggests that this did not have a significant impact on the gene expression data collected.

htr1A expression was unlikely to be contributing to the female-specific behavioural phenotype since it was similarly decreased in the cortical and hippocampal regions of male and female HD brains. In contrast, hippocampal, but not cortical, expression of htr1B was significantly less in female HD mice than in male HD mice. While expression levels were reduced in both sexes, it is possible that a more severe reduction in the female HD brain reflects expression levels decreasing beyond a minimum threshold point that leads to the development of the observed female-specific behaviours. In comparison, no differences in htr1B expression were reported in post mortem MDD brains (Lopez-Figueroa et al., 2004). Therefore, should htr1B expression be established as having a role in the development of the female HD behavioural phenotype, then this change would be specific to the HD brain and implies that the underlying pathology of HD-associated depression is different to that of MDD.

A second candidate for modulating the differences in male and female HD behaviours is the serotonin transporter. SerT expression was not significantly altered in the hippocampus of male and female HD mice. However, in the cortex, female HD mice had significantly reduced levels of SerT mRNA while male HD mice had normal (wild-type) levels. As mentioned above, cortical availability and binding of 5-HTT is reduced in depression (Mann et al., 2000; Staley et al., 2006; Oquendo et al., 2007). This specific deficit in SerT gene expression in the female HD cortex is therefore a strong candidate for mediating the female-specific depressive-like phenotype of the HD mice. However, having established the pattern of SerT gene expression in the HD brain, there are now further questions relating to 5-HTT function. 5-HTT is a key target of the selective serotonin reuptake inhibitor (SSRI) class of antidepressant drugs and the muted effects of
acute antidepressant administration on HD mice (described in Chapter 3) raises the possibility that altered transporter function contributes to these observed effects. The extent to which 5-HTT function is changing in the brains of HD patients is also unclear at present. Future studies will be required to address these uncertainties.

The one distinctive difference between the observed behavioural phenotype of the R6/1 model and the human HD population is that depression is reported in both male and female patients (E. Chiu & J. Stout, personal communications) while behavioural differences at 12 weeks of age were almost exclusive to female HD mice (male HD mice also had altered tail-suspension test responses). One interpretation of this observed difference is that carrying the HD-causing gene mutation confers a genetic susceptibility to both male and female to develop depression, especially when non-genetic, environmental influences such as negative life events are overlaid. On the other hand, positive environmental factors have also been associated with improvement of depression symptoms and the beneficial effects are clearly demonstrated in various rodents models utilizing the experimental paradigm of environmental enrichment (Chourbaji et al., 2008; Laviola et al., 2008).

Here, environmental enrichment was found to up-regulate expression of particular serotonin receptors in a region-specific manner which also corresponded with the correction of female HD behaviour on the FST, as described in Chapter 4. The only enrichment-induced changes in gene expression exclusive to female HD mice were an up-regulation of htr1B and htr2C mRNAs in the hippocampus and cortex respectively. Of the two genes, the actual significance of increased htr2C in the female HD cortex is less clear, since there was no expression deficit at baseline. Furthermore, htr2C expression does not appear to be involved in depression-related behaviour as htr2C null mutant mice exhibit a normal behavioural profile on tests of depressive behaviours (Cremers et al., 2004). Hence, any physiological significance of this enrichment-induced up-regulation of htr2C expression will require further investigation. Expression of htr1B in the hippocampus therefore stands out as the primary modulator of the depressive-like
behavioural phenotype of female HD mice. In addition to the association of reduced hippocampal htr1B expression with the display of depressive-like behaviours at baseline as discussed above, it is now apparent that the correction of female HD behaviour corresponds with greater levels of htr1B mRNA in the hippocampus. Therefore, future developments for treating HD-related depression could be focused on attempting to increase htr1B expression or facilitating 5-HT\textsubscript{1B} receptor-mediated signaling in the HD brain via a selective receptor agonist.

Environmental enrichment also led to greater hippocampal expression of htr1A in female HD mice. However a similar change was also detected in female wild-types so htr1A expression is therefore unlikely to be associated with the selective normalization of female HD FST behaviour. The enrichment-induced up-regulation of htr1A expression was region-specific since no changes were detected in the cortex. The environmental effect on htr1A expression was sex-specific as expression was unchanged in male mice of both genotypes. The enrichment effect on hippocampal htr1A expression agrees with a previous study that investigated gene expression in the rat hippocampus following thirty days of environmental enrichment which is a period of enrichment similar to the present study (Rasmuson et al., 1998). That study which examined only male rats had reported that htr2A and htr2C expression were unaltered by enrichment, which is consistent with the present results indicating no changes in htr2A and htr2C expression in male wild-type mice. However, given that enriched female wild-types had significantly reduced expression of htr2A in the hippocampus, this suggests that there is sex-dependent modulation of htr2A gene expression by environmental factors. The mechanisms underlying this sex difference are unknown and would require further investigation.

Much of this discussion has been centered on the hippocampus due to greater effects of environmental enrichment on hippocampal gene expression relative to the cortex. However, the consequence of significant reductions of serotonin receptor gene expression in the HD cortex cannot be overlooked in terms of the perceived involvement of cortical signaling in various mood disorders. The implication of reduced htr2A expression in the
The hippocampus was briefly discussed above. The 5-HT\textsubscript{2A} receptor modulates neuronal signaling by regulating glutamate release in the cortex (Wang et al., 2006; Yuen et al., 2008; Zhong et al., 2008) and a disruption of this regulatory process is associated with various psychiatric disorders (discussed by Stein et al., 2007). Similarly, the 5-HT\textsubscript{1A} and 5-HT\textsubscript{1B} receptors in the frontal cortex regulate circulating levels of extracellular serotonin and the effects of antidepressant drugs (Dawson and Nguyen, 2000; Dawson et al., 2000). Given that cortical expression of both are reduced in the HD brain, future studies would be required to determine whether serotonin metabolism in the HD brain is comparable to a non-HD brain.

It is still unclear why there is a selective down-regulation of serotonin receptor gene expression in the HD brain. Transcriptional dysregulation is well-documented in the HD brain, one example being the abnormal interaction of the mutant huntingtin protein with the transcriptional activator Sp1 (Dunah et al., 2002) leading to the down-regulation of multiple genes requiring Sp1 to facilitate gene transcription (Chen-Plotkin et al., 2006). Expression of the htr1A gene has also been shown to be regulated by Sp1 (Song et al., 2003) thereby suggesting that reduced htr1A gene expression is a consequence of disrupted Sp1-mediated transcriptional activation. Little is known at present about the exact mechanisms which control transcription of the various serotonin receptors. Pending that knowledge, the exact causes of the selective reduction in serotonin receptors in the HD brain remain to be identified.

The male and female serotonin systems clearly respond differently to environmental enrichment. It was interesting to observe that no significant environmentally-mediated changes in gene expression were detected in the male wild-type and HD brains, suggesting that the female brain is more responsive to non-genetic influences that elicit gene expression changes. An alternative interpretation is that the male brain is not as plastic as the female brain and is less susceptible to environmental influences which could be viewed as a positive characteristic if considering the impact of negative or traumatic life events and their association with depression. Similar sex-differences in the
expression of other genes have also been observed (Zajac M., personal communication) and this might be one of the reasons for the lower rates of depression in the male population. Future studies will be required to better understand the differences in affective regulation and plasticity of the female and male brain.

6.5. Conclusion

This study investigated the expression patterns of serotonin receptors in the R6/1 HD brain in conjunction with the female-specific depressive-like behavioural phenotype observed at 12 weeks of age. Reduced serotonin receptor gene expression was detected in both male and female HD brains. Female HD brains had a more severe reduction in hippocampal htr1B mRNA levels and lower cortical SerT expression which was not detected in the male HD brain, both of which contribute to the female-only depressive phenotype. Sex- and region-specific changes in htr1A, htr1B, htr2A and htr2C expression were identified in the cortex and hippocampus following environmental enrichment. Of the changes, an increase in hippocampal htr1B expression correlated with the rescue of the female HD behavioural phenotype. Overall, modification of the serotonin signaling system in the HD brain is associated with the manifestation and correction of the HD depressive-like behavioural phenotype.
Chapter 7 Summary and future directions

7.1. Using the R6/1 transgenic mouse to understand HD-related depression

Huntington’s disease (HD) and depression are complex, multi-faceted brain disorders. Each reflects an altered brain that manifests neuronal dysfunction or the pathological alterations as a diverse spectrum of symptoms. The exact pathological events that are involved in both conditions remain unknown. HD is caused by the mutation of a single gene but the precise interactions leading up to the disease state is not known. Similarly, there have been numerous discussions debating the pathological origins of a state of depression without a lone hypothesis standing out from the rest. It is easy to appreciate the complex nature of both disorders and comprehend the challenges faced in developing effective treatment therapies or the attempts to discover the all-elusive cure. Therefore, it is understandable that a combination of both would pose an even greater hurdle. As such, it is easier to continue treating the depression symptoms of HD as a separate condition than to attempt to fully understanding the factors contributing to increased co-morbidity for depression in HD.

Prior to the present study, some evidence suggested that the early pathological changes detected in the HD brain contributed to an elevated risk of depression by HD patients. The core evidence was the reduction of hippocampal volume in pre-clinical patients, a key structural change commonly observed in the brains of depressed individuals (Geuze et al., 2005). Unfortunately, due to several reasons, such as difficulty in recruiting study subjects, a large scale, longitudinal study of hippocampal structure in relation to the development of depression in HD patients has yet to be conducted. Mouse models of HD provide an excellent alternative to working on human subjects since they faithfully recapitulate all the major aspects of the human condition including the progressive development of cognitive deficits prior to the onset of motor symptoms. While the emotional status of a rodent remains a fiercely debated topic, the use of rodents and
altered behavioural responses to model human depression has persisted since there are no practical alternatives currently available. The present study has reinforced the close fidelity of disease progression in the R6/1 mouse model with the human condition by demonstrating that expression of the mutant huntingtin (htt) gene increases the genetic load and susceptibility to developing depression and this was reflected by the display of depression-related behaviours by HD mice (Figure 7-1A). The depression-related behaviours were more readily displayed by female HD mice which is consistent with the gender bias in the mainstream depression literature. The behavioural changes where detected at an age when, in the absence of a change to the volume of the hippocampus, cell proliferation was severely impaired (Figure 7-1B). This indicated that hippocampal volumetric reductions are secondary to the decrease in cell birth and neurogenesis. It also implies that multiple molecular and cellular changes already exist in the brains of HD patients by the time these changes in hippocampal volume are detected. Similar to the environmental influence on the age of onset of HD, the manifestation of depression-related behaviours benefited from, and was normalized by, wheel-running and environmental enrichment although these effects were limited to female HD mice. This study has also described the disruption of target genes implicated in depression pathology in the cortex and hippocampus of R6/1 HD mice at 12 weeks of age, which is the earliest demonstration of altered gene expression in this model to date (Figure 7-1C). A decrease in the expression of brain-derived neurotrophic factor (BDNF) and various serotonin receptors in the HD brain appears highly relevant to HD-related depression since the disrupted expression of any one of these genes forms the basis of a variety of mouse models of depression. Therefore, the concurrent disruption of BDNF and serotonin-mediated signaling due to altered gene expression may be an early molecular event that facilitates the development of depression in HD.

Collectively, these novel findings should provide the impetus for a closer examination of the psychiatric alterations, especially depression, described within the HD population to better understand the affective aspects of HD as well as to readdress the effectiveness of current therapies employed to combat depression in HD.
Figure 7-1 Enhancing the understanding of the development of depression in HD.

While cognitive deficits and motor symptoms have been demonstrated in various mouse models of HD, none have been used to investigate the psychiatric aspects of the disease. The present study has demonstrated the exhibition of depression-related behaviours in the R6/1 transgenic mouse model at a pre-motor symptomatic age (A), mirroring the progression of the human condition. This occurs while there is no change in hippocampal volume but when a dramatic deficit in cell proliferation is detectable (B). Molecular analysis has also revealed disrupted gene expression of various candidate genes already implicated in the pathology of depression and mood disorder (C).
7.2. Future directions

This study has used the R6/1 transgenic mouse model to examine the increased susceptibility of HD patients to develop depression. The molecular and cellular bases of the cognitive and motor aspects of HD have been extensively investigated; however little has been attempted to address the psychiatric symptoms. Given the challenges involved in recruiting and performing molecular analyses on preclinical HD patients, this transgenic model can now be used to explore the earliest pathological changes occurring in the HD brain that leads to the manifestation of depression during the pre-symptomatic stages of HD.

While the R6, Hdh knock-in and yeast artificial chromosome (YAC) lines have all been demonstrated to develop cognitive deficits and motor impairment, albeit at different rates of progression, it is unknown whether altered depression-related behaviours will be detectable in all mouse models of HD. It is unlikely that the R6/2 line, with its aggressive and early onset motor symptoms, will eventuate into a line that would be useful for examining a pre-motor symptomatic behavioural change because motor disability would be a major confound when evaluating the behavioural response of R6/2 mice on the various behavioural tests. Before it can be definitively concluded that the presence of the mutant huntingtin (htt) protein alone is sufficient to cause changes in the brain that result in the manifestation of depression-related behaviours, an evaluation of the behavioural responses the Hdh knock-in and YAC models on the behavioural tests used in the present study will be required.

Until recently, depression had been an often overlooked symptom of HD patients on the basis that the psychological duress of being diagnosed with a fatal neurodegenerative disorder was reasonable grounds for the development of depressed mood. Altered neuronal signaling is postulated as a cause of mood disorders, including depression, and is evident from the behavioural phenotypes of mice with altered BDNF (knock-down or conditional knock-outs) or serotonin-mediated (5-HT receptor and transporter knock-outs)
signaling. The present study has revealed that BDNF gene expression is reduced in the HD hippocampus during the early, pre-symptomatic stages, in addition to reduced mRNA levels of specific 5-HT receptors in both cortex and hippocampus. While it is known that the mutant htt protein disrupts BDNF gene transcription, the temporal development of this phenomenon is not known. A longitudinal gene expression study will be required to determine the point at which a significant disruption of BDNF gene transcription is detected in the HD brain. In addition, the exact reasons behind the disruption of BDNF gene expression are unclear. Wild-type htt protein facilitates BDNF expression via promoter II (Zuccato et al., 2001); however, as found in this study, presence of the mutant htt protein disrupts expression of a variety of exon-specific BDNF transcripts in a manner which is also sex-dependent. Therefore, a more comprehensive examination of the interaction between the mutant htt protein and the transcription factors which mediate BDNF gene transcription through other promoter sites will be necessary. Furthermore, the differences in gene expression detected in male and female HD brains require investigation since there is currently no information regarding sex-difference in gene expression in the human condition.

The genetic disruption of 5-HT$_{1A}$ and 5-HT$_{1B}$ receptor expression in mice results in altered depression-related behavioural responses (Mayorga et al., 2001; Jones and Lucki, 2005). While this study found a drastic down-regulation of htr1A and htr1B in the hippocampus and cortex of HD mice, the functional relevance of this change in gene expression is not yet understood. An initial analysis of the protein levels of the various 5-HT receptors could be undertaken which, in addition to quantifying the absolute amount of protein present, could also address possible differences between the membrane-bound and cytoplasmic fractions. Should protein levels be found to be unchanged, receptor autoradiography studies could then provide further information about the receptor binding potentials which, if altered, would also represent a change in serotonin-mediated synaptic signaling.
At the present time, it is unclear how changes in the expression of individual 5-HT receptors are contributing to the overall behavioural phenotype of the R6/1 mice. To address this, one possible experiment would be to study R6/1 mice prior to the development of deficits in 5-HT receptor gene expression (first requiring a pilot study to determine the progressive down-regulation) followed by the application of selective 5-HT receptor blockers. This might potentially lead to the identification of the pivotal 5-HT signaling pathways involved in the development of the depressive-like phenotype in this line of mice.

In addition to the 5-HT receptors, depression-related behavioural responses are also displayed by SerT knock-out mice. While SerT expression is altered in the HD brain in a sex-dependent region-specific manner, there is strong evidence that a behavioural phenotype mediated by altered 5-HT transporter expression is also strongly influenced by the background strain of the mice since only mice on a pure 129s6 background, but not C57Bl/6, show a behavioural phenotype (Holmes et al., 2002). The R6/1 line is bred on a mixed C57Bl/6-CBA background to facilitate optimal breeding and survival rates (Mangiarini et al., 1996), and future investigations will be required to determine whether the genetic background imparts variability to the development of the behavioural phenotype.

Depression is known to affect both male and female HD patients with no apparent gender bias (E.Chiu & P.Chua, personal communications). It was surprising to observe that the only behavioural test that male HD mice differed from male wild-types was the tail-suspension test. There are several possibilities which might account for this. Firstly, the behavioural phenotype could be slower to develop in male HD mice, and the detection of a behavioural difference on the tail-suspension test might be reflecting the particular sensitivity of the test. This can be addressed by re-testing male HD mice at a later age. However, similar to the point raised when discussing the R6/2 line, performing the behavioural tests when motor impairment has developed would only confound the findings. An alternative would be to employ the use of ‘stressors’ similar to the manner
by which traumatic life events are associated with increased incidence of depression. ‘Stressors’ such as chronic mild stress paradigms or social isolation are well-established experimental paradigms that induce depression-related behavioural changes. As such, it would be interesting to investigate whether male HD mice exposed to such manipulations would eventually develop altered behavioural responses on the other behavioural tests similar to the female HD mice.

The use of a working model of a disease is ultimately designed facilitate better understanding of the respective human condition under investigation. The findings of this study raise several questions regarding HD-related depression in the patient population. While it is acknowledged that depression is a common diagnosis in HD, the prevalence of depression in the HD population is uncertain as there are few records documenting the actual numbers of HD patients with depression. There have been several reports based on small numbers of HD patients but in order to gain a true perspective of this co-morbid relationship, greater efforts will be required to organize large cohort studies (ideally at an international level) which are well-controlled for sex, duration and severity of disease and medication history (amongst a list of important factors to be considered). Unfortunately, little is known about the early molecular changes occurring in the human HD brain. The ideal comparison would be between groups of depressed and non-depressed preclinical HD patients so that differences in gene expression might be uncovered that might shed some light on the development of depression in HD. However, such brains are rarely available which makes this level of analysis near impossible. As such, the HD mouse models continue to be the best tool available to study the earliest molecular changes related to HD pathology. It is hoped that ongoing studies will lead to prevention and treatment of this devastating disease.
Chapter 8 References


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Chapter 9 Appendices

Appendix I. Novelty-suppressed feeding test following modification of pre-testing food deprivation protocol. (A) Extension of feed deprivation from 24hr to 48hr with a 2hr feeding period after the first 24hr without food resulted in a consistent 15% reduction of body weight of all groups of mice. (B) Average latencies of all groups of mice to approach and commence feeding of the food pellet in the center of the test arena. (C) Average weights of food consumed by all groups of mice post-NSFT indicate no differences in hunger levels. (Du & Pang, unpublished)

Appendix II. Modulation of BDNF mRNA expression in the hippocampus of wild-type and HD mice by wheel-running. (A) Wheel-running increased total BDNF mRNA levels in the hippocampus of wild-type and HD mice. Only female HD mice responded to wheel-running by an up-regulation of BDNF gene expression while expression levels in male HD mice were unaltered. (B) There were no differences in hippocampal BDNF exon-IV transcripts in male wild-type and HD mice following wheel-running. (C) Wheel-running results in an up-regulation of BDNF exon IV transcripts in the hippocampus of female wild-type and HD mice. (Zajac & Pang, unpublished)
Appendix I

A Weight loss following food deprivation

![Graph showing weight loss for females and males.](image)

B Latency to feed

![Graph showing latency to feed for females and males.](image)

C Post-test food consumption

![Graph showing post-test food consumption for females and males.](image)
Appendix II

A  BDNF coding exon expression in hippocampus

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% SH WT mRNA levels

B  BDNF exon IV expression in male hippocampus

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% SH WT mRNA levels

C  BDNF exon IV expression in the female hippocampus

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% WT SH mRNA levels

* Indicates significant difference.
Author/s: Pang, Terence Yeow-Chwen

Title: A study of depression in Huntington's disease

Date: 2008


Persistent Link: http://hdl.handle.net/11343/35122

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