Investigating the transcriptional correlates of cognition in the heterozygous HdhQ150 mouse model of Huntington's disease

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Summary

Huntington's disease (HD) is a progressive autosomal dominant neurodegenerative disorder caused by expansion of a trinucleotide CAG repeat in the *Huntingtin* gene (*HTT*) on chromosome 4. HD symptomatology is characterised by a triad of motor, cognitive, and psychiatric disturbances, as well as transcriptional dysregulation.

The identification of *HTT* as the single causative gene in HD has led to the development of a number of animal models that are designed to recreate the molecular and behavioural phenotypes of the human disease; one such model is the HdhQ150 mouse model of HD. Investigation into the cognitive capacities of animals heterozygous for the HdhQ150 mutant allele ($Hdh^{Q150/+}$) revealed a series of novel cognitive impairments that recapitulate aspects of the cognitive phenotype observed in human HD patients; deficits in conditioning task acquisition and performance were identified in $Hdh^{Q150/+}$ mice, as were impairments in visuospatial attention, and delayed acquisition and cognitive processing of spatial discrimination (SD) learning. However, implicit learning capabilities of $Hdh^{Q150/+}$ animals were analogous to those of wild-type mice.

Microarray and reverse transcription quantitative polymerase chain reaction (RT-qPCR) investigations into the transcriptional correlates of cognition did not identify significant learning-dependent gene expression level changes in the striata of $Hdh^{Q150/+}$ or wild-type mice. Similarly, these techniques did not report significant striatal gene expression level alterations across distinct stages of SD and reversal learning operant task performance in wild-type animals. In contrast, RT-qPCR revealed significant differences in the striatal expression levels of *Adora2A*, *Arc*, *Drd2*, and *Homer1* genes between $Hdh^{Q150/+}$ and wild-type mice at 44-60 weeks of age, supporting previous evidence of abnormal expression or signalling of these genes in the HD phenotype.

Overall, this body of work contributes novel evidence that $Hdh^{Q150/+}$ animals present with molecular and cognitive phenotypes comparable to those seen in the human form of the disease.

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List of abbreviations used

°C	-	Degrees Celsius
ε	-	Estimate of sphericity
5-CSRTT	-	5-choice serial reaction time task
A	-	Acquisition of CRF
$A_{2A}R$	-	Adenosine A_{2A} receptor protein
Actb	-	Beta-actin
Adora2A	-	Adenosine A_{2A} receptor
ANOVA	-	Analysis of variance
Arc	-	Activity-regulated cytoskeleton-associated protein
BAC	-	Bacterial artificial chromosome
Bdnf	-	Brain-derived neurotrophic factor
bp	-	Base pairs
CAG	-	Cytosine-adenine-guanine
Calb2	-	Calbindin 2
cAMP	-	Cyclic adenosine monophosphate
CBP	-	CREB binding protein
CBS	-	Central Biotechnology Services
cDNA	-	Complementary DNA
CRE	-	cAMP response element
CREB	-	cAMP response element binding protein
Creb1	-	cAMP response element binding protein 1
CRF	-	Continuous reinforcement
C_t	-	Cycle threshold
DAVID	-	Database for Annotation, Visualisation and Integrated Discovery
df	-	Degrees of freedom

DNA	-	Deoxyribonucleic acid
Drd1	-	Dopamine receptor type 1
Drd2	-	Dopamine receptor type 2
DTI	-	Diffusion tensor imaging
ERK	-	Extracellular-signal regulated kinase
FDR	-	False discovery rate
fMRI	-	Functional magnetic resonance imaging
FOXO3A	-	Forkhead box O3
GABA	-	Gamma aminobutyric acid
GFAP	-	Glial fibrillary acidic protein
Glp1r	-	Glucagon-like peptide 1 receptor
GO	-	Gene ontology
GPe	-	External segment of the globus pallidus
GPi	-	Internal segment of the globus pallidus
Gpr165	-	G protein-coupled receptor 165
GTP	-	Guanosine-5'-triphosphate
H_2O	-	Water
HD	-	Huntington's disease
Hdh	-	Huntington's disease homolog
HEAT	-	Huntingtin, elongation factor 3, protein phosphatase 2A, and TOR 1
Homer1	-	Homer scaffolding protein 1
Hprt	-	Hypoxanthine guanine phosphoribosyltransferase
HSD	-	Honestly significant difference
HTT	-	Huntingtin
ITI	-	Inter-trial-interval
kb	-	Kilobases
Kcnh3	-	Potassium voltage-gated channel, subfamily H, member 3
Kcnj4	-	Potassium inwardly-rectifying channel, subfamily J, member 4

kDa	-	Kilodaltons
KO	-	Knock out
M	-	Months of age
MAPK	-	Mitogen-activated protein kinase
mHTT	-	Mutant huntingtin
min	-	Minutes
mL	-	Millilitres
MRI	-	Magnetic resonance imaging
mRNA	-	Messenger RNA
ms	-	Milliseconds
MSN	-	Medium spiny neuron
NII	-	Neuronal intranuclear inclusion
NMDA	-	N-methyl-D-aspartate
np	-	Nose-pokes
n.r.	-	Not reported
NS	-	Non-significant
OTT	-	Outside top 10 significant GO biological pathways
PCA	-	Principal component analysis
PCR	-	Polymerase chain reaction
PET	-	Positron emission topography
PFC	-	Prefrontal cortex
Prp	-	Prion promoter
Q	-	Glutamine/CAG
qPCR	-	Quantitative PCR
REST/NRSF	-	Repressor element 1-silencing transcription factor/ neuron-restrictive silencing element
RIN	-	RNA integrity number
RNA	-	Ribonucleic acid
rpm	-	Revolutions per minute

Rpph1	-	Ribonuclease P RNA component H1
RQ	-	Relative quantification
RL	-	Reversal learning
RT	-	Reverse transcription
RT-qPCR	-	Reverse transcription-qPCR
s	-	Seconds
S	-	Full SILT
S1	-	Stimulus 1
S2	-	Stimulus 2
SD	-	Spatial discrimination
S.E.M.	-	Standard error of the mean
SILT	-	Serial implicit learning task
Slc17a6	-	Solute carrier family 17, member 6
SNc	-	Substantia nigra pars compacta
Snora74a	-	Small nucleolar RNA, H/ACA box 74A
SNr	-	Substantia nigra pars reticulata
STN	-	Subthalamic nucleus
TetO	-	TetO operator
tTa	-	Tetracycline-regulated transactivator
U	-	Unhandled
Ubc	-	Ubiquitin C
UHDRS	-	Unified Huntington's Disease Rating Scale
UNG	-	Uracil-N-Glycosylase
Uprt	-	Uracil phosphoribosyltransferase
UV	-	Ultraviolet
YAC	-	Yeast artificial chromosome
Zic1	-	Zic family member 1

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Chapter 1: General introduction

1.1 Huntington's disease

1.1.1 Clinical phenotypes

Huntington's disease (HD) is a progressive neurodegenerative disorder characterised by a triad of motor, cognitive and psychiatric disturbances (Duff et al., 2007; Paulsen et al., 2008; Biglan et al., 2009; Novak and Tabrizi, 2010; Ross and Tabrizi, 2011; Wexler, 2012; Kim and Fung, 2014; Bates et al., 2015). Disease onset most commonly occurs in the fourth or fifth decades of life (Andrew et al., 1993; Snell et al., 1993; Purdon et al., 1994; Gusella and MacDonald, 2009; Novak and Tabrizi, 2010), however there is a considerable range in the age of onset and disease manifestation has been reported in patients from 2 to over 80 years of age (Andrew et al., 1993; Duyao et al., 1993; Snell et al., 1993; Wexler, 2012). The HD clinical phenotype progressively worsens until death, typically 15-20 years following symptom onset (Wexler and Res, 2004; Roos, 2010; Tabrizi et al., 2013).

1.1.1.1 Motor symptoms

A number of subtle motor symptoms, such as slowed movement and reaction time, reduced force when protruding the tongue, and oculomotor deficits, can be detectable prior to clinical onset of a motor deficit (Penney et al., 1990; Kirkwood et al., 2000; Tabrizi et al., 2009; Orth et al., 2011; Tabrizi et al., 2011; Tabrizi et al., 2012; Tabrizi et al., 2013). Recent studies suggest that accuracy in the estimation of motor symptom onset in pre-manifest HD patients can be increased by incorporating the Unified Huntington's Disease Rating Scale (UHDRS) motor and cognitive data collected upon observation into predictive models that include the patient's age and CAG repeat length (Liu et al., 2015; Long et al., 2015). The diagnosis of HD is typically made after the onset of motor symptom presentation, with examination of choreiform movements and fine motor skills, such as finger-tapping rhythm and rate, acting as useful criteria for disease diagnosis (Kirkwood et al., 2000; Walker, 2007; Tabrizi et al., 2009; Long et al., 2014). The characteristic involuntary choreiform movement that becomes progressively more severe over time during the early stages of the disease is one of the most common motor symptoms in HD (Young et al., 1986; MacDonald et al., 1993; Mahant et al., 2003; Walker, 2007; Roos, 2010). In the early stages of HD, the presence of chorea is often accompanied by incoordination, rigidity, oculomotor dysfunction, and reduced control over handwriting, while the middle stage of the disease frequently presents with more pronounced chorea, gait abnormalities, motor

impersistence, impaired speech, and decreased manual dexterity (Phillips et al., 1994; Reilmann et al., 2001; Walker, 2007; Tabrizi et al., 2009; Scahill et al., 2013; Skodda et al., 2014).

Despite chorea being prevalent and useful for diagnosis, it may not be a suitable indicator for severity of disease progression (Young et al., 1986; Mahant et al., 2003) because not all HD sufferers develop choreiform movements, or such movements may only arise transiently during their illness (Walker, 2007). Bradykinesia and dystonia can also develop in later stages of the disease, which act to make the chorea appear less prominent (Berardelli et al., 1999; Squitieri et al., 2000). The presence of rigidity and bradykinesia in more advanced stages of the disease often requires affected individuals to obtain assistance with self-care, as sufferers are often unable to walk, speak and perform everyday tasks.

1.1.1.2 Cognitive symptoms

Cognitive dysfunction often precedes motor symptom onset in HD (Diamond et al., 1992; Foroud et al., 1995; Kirkwood et al., 2000; Paulsen et al., 2001b; Paulsen et al., 2008; Tabrizi et al., 2009; Paulsen et al., 2014), with clinically pre-manifest HD patients showing impairments in emotion recognition (Gray et al., 1997; Johnson et al., 2007; Stout et al., 2011), working memory (van Walsem et al., 2010; Stout et al., 2011), visual discrimination (Lawrence et al., 1998b), and implicit learning (Kim et al., 2004; Ghilardi et al., 2008; van Asselen et al., 2012). One report found that almost 40% of pre-symptomatic HD participants met the criteria for mild cognitive impairment, which is the transitional stage between cognition and dementia (Duff et al., 2010). Cognitive processing speed progressively worsens closer to disease onset and is believed to result from frontostriatal impairments (Rothlind and Brandt, 1993; Sanchez-Pernaute et al., 2000; Snowden et al., 2002; Ho et al., 2003; Larsen et al., 2015).

Cognitive capabilities deteriorate further during the early stages of HD, with pre-clinical and early-stage HD populations showing evidence of progressive declines in executive functioning (Ho et al., 2003; Stout et al., 2012; Hart et al., 2013; Holl et al., 2013; Tabrizi et al., 2013), working memory (Tabrizi et al., 2011; Harrington et al., 2012; Stout et al., 2012), emotion recognition (Stout et al., 2012; Tabrizi et al., 2013) and attention (Harrington et al., 2012; Stout et al., 2012). Other cognitive domains impaired in HD include spatial learning and memory (Lange et al., 1995; Davis et al., 2003; Brandt et al., 2005; Pirogovsky et al., 2015), and cognitive flexibility (Lange et al., 1995; Lawrence et al., 1996; Lawrence et al., 1999; Lawrence et al., 2000; Aron et al., 2003; Tabrizi et al., 2013).

With regards to functional deficits in everyday life, an inability to independently manage finances often arises (Beglinger et al., 2010) and HD patients have displayed pathological gambling traits (De Marchi et al., 1998), which may be attributable to deficits in risky decision-making (Stout et al., 2001). Difficulties in multitasking, concentrating, and short term memory are also known to occur, as are complications in planning, initiating and organising time, thoughts and activities (Novak and Tabrizi, 2010). The ability to safely drive vehicles is also diminished in HD, as a result of both motor and cognitive deficits (Devos et al., 2014). Both pre-manifest and manifest HD patients consistently underestimate the extent of their cognitive decline (Ho et al., 2006; Williams et al., 2015), with evidence that this lack of awareness of cognitive decline may be associated with heightened disease severity (Cleret de Langavant et al., 2013). Estimating the prevalence of dementia in the HD population is difficult because there is currently no universally accepted criterion for diagnosing dementia in the disease (Paulsen, 2011), as most criteria focuses on dementia features in Alzheimer's disease (Peavy et al., 2010). Thus, the dementia diagnosis criteria used does not accurately reflect the unique deficits seen in HD.

1.1.1.3 Psychiatric symptoms

Similar to the cognitive phenotype in HD, psychiatric symptoms commonly manifest in the disease and frequently arise prior to motor symptom onset (Duff et al., 2007; Julien et al., 2007; Marshall et al., 2007; Tabrizi et al., 2009; Smith et al., 2012; Paulsen et al., 2014). Increased anxiety, apathy, depression, irritability, obsessive-compulsiveness, perseveration and suicidal ideation have all been observed prior to clinical diagnosis of HD (Duff et al., 2007; Tabrizi et al., 2009; Epping et al., 2013; Tabrizi et al., 2013; Epping et al., 2016). Unlike the cognitive and motor phenotypes of HD, the psychiatric phenotype is not necessarily progressive (Walker, 2007; Phillips et al., 2008), however, recent longitudinal studies have reported progressive psychiatric disturbances (Tabrizi et al., 2013; Paulsen et al., 2014; Epping et al., 2016) and reduced awareness of such symptoms in pre-manifest HD patients (Epping et al., 2016). Depressive symptoms in pre-manifest HD sufferers do not increase in severity with proximity to disease diagnosis and are not associated with CAG repeat length (Epping et al., 2013).

HD sufferers are known to have a higher incidence of depression than the general population (Marshall et al., 2007; Van Duijn et al., 2008) and approximately 33-69% of the HD population suffer from depression (Paulsen et al., 2005b; Van Duijn et al., 2008; Chisholm et al., 2013), which has recently been associated with changes in white matter microstructure in the HD brain (Gregory et al., 2015b). Apathy is estimated to be experienced by as many as 50-99% of patients

(Paulsen et al., 2001a; Thompson et al., 2012) while anxiety and irritability occur in estimates of 34-61% and 38-83% of patients, respectively (Craufurd et al., 2001; Kulisevsky et al., 2001; Paulsen et al., 2001a; Thompson et al., 2012). However, a recent and currently ongoing multinational observational study of HD patients in Europe reports a lower incidence of depression (12.7%), apathy (28.1%), and irritability (13.9%) than these estimates (van Duijn et al., 2014). Similar to depressive symptoms, irritability has been found to be associated with white matter microstructural changes in the HD brain, whereas no such relationship was identified for the apathy phenotype (Gregory et al., 2015b). The frequency of suicide is higher in HD patients than in the general population (Dimaio et al., 1993; Hubers et al., 2011) and the risk of HD sufferers committing suicide is estimated to be 4-8 times higher than the suicide rate in the general population (Schoenfeld et al., 1984; Farrer, 1986; Dimaio et al., 1993; Bird, 1999). The risk of suicidal ideation or attempted suicide appears to be most prominent in the HD population either during the early stages of disease manifestation or immediately prior to a formal diagnosis of the condition (Schoenfeld et al., 1984; Paulsen et al., 2005a). Suicidal ideation in the European HD population has been correlated with shorter estimated disease duration, heightened depression, anxiety, and aggression, and a previous suicide attempt (Hubers et al., 2013).

1.1.2 Molecular genetics of HD

The age of onset of HD is correlated with the genetic basis of the condition. HD is inherited in an autosomal dominant manner as the result of a polymorphic expansion of a trinucleotide cytosine-adenine-guanine (CAG) repeat on the 5' end of the *Huntingtin* gene (*HTT*) localised to chromosome 4p16.3 (MacDonald et al., 1993). CAG repeat lengths in the unaffected general population largely range from 9-35 repeats while expansions of HD sufferers have been reported between 36 and 121 repeats, with a mean repeat length of approximately 46, and age of disease onset is inversely correlated with CAG repeat length (Andrew et al., 1993; Duyao et al., 1993; Snell et al., 1993). However, CAG repeat length accounts for only approximately 50-70% of the variation in age of disease onset (Andrew et al., 1993; Rosenblatt et al., 2001; Novak and Tabrizi, 2010; Rosenblatt et al., 2012), while the remaining variance is the result of other modifying genes and environmental and familial factors (Rosenblatt et al., 2001; Chattopadhyay et al., 2003; Wexler and Res, 2004; Chattopadhyay et al., 2005; Dhaenens et al., 2009; Taherzadeh-Fard et al., 2010).

1.1.2.1 CAG repeat length and age of onset

An inverse correlation between CAG repeat length and age of disease onset has consistently been identified in the literature (Andrew et al., 1993; Duyao et al., 1993; Snell et al., 1993). However, for any CAG repeat length there is a wide range of associated ages of onset (Macmillan et al., 1993). For CAG repeat lengths of 40-50, which is the range in which 90% of the HD population lie, CAG repeat length accounts for only 40% of the variation in age of onset (Wexler, 2012). Conversely, age of disease onset has been found to be fully dependent upon the expanded CAG repeat length in combination with recently identified disease modifying genetic loci, in studies examining over 4,000 HD patients (Lee et al., 2012; Lee et al., 2015b). Specifically, genome-wide association analysis identified a locus on chromosome 15 displaying two independent effects of single-nucleotide polymorphisms that accelerate or delay disease onset by 6.1 and 1.4 years, respectively, and also identified a variant at a chromosome 8 locus that hastens onset by 1.6 years (Lee et al., 2015b). However, 73% of the variation in age of onset is accounted for by CAG repeat length in cases of juvenile HD, which is associated with much larger CAG repeat sizes (Telenius et al., 1993). The HD phenotype is believed to be fully penetrant in individuals with 40 or more CAG repeats but individuals with 36-39 repeats show decreased penetrance, where they may or may not inherit the disease (Rubinsztein et al., 1994; Rubinsztein et al., 1996). The exact mechanisms contributing to the differences in penetrance are unknown, however, much of the variance has been found to be heritable, suggesting that genetic modifiers may play a role (Dhaenens et al., 2009; Langbehn et al., 2010; Taherzadeh-Fard et al., 2010).

1.1.2.2 Juvenile HD

The manifestation of HD before 21 years of age is known as juvenile HD (van Dijk, 1985), and has been reported in 1-15% of HD case studies, with a meta-analysis estimating that juvenile HD patients comprise 4.92% of the HD population (Quarrell et al., 2012). Juvenile HD is associated with considerably longer CAG repeat lengths than the adult onset form of the disease, with juvenile cases typically presenting with 60 or more repeats (Andresen et al., 2007), although a juvenile HD patient has been identified as having a repeat expansion as low as 48 (Ribai et al., 2007). Similar to adult onset HD, juvenile cases often present with cognitive and psychiatric disturbances prior to the emergence of motor abnormalities (Duesterhus et al., 2004). These disturbances can include depression, anxiety, isolation, aggression, general changes in conduct and personality, and a decline in school performance (Rasmussen et al., 2000; Duesterhus et al., 2004; Geevasinga et al., 2006). The movement disorder in juvenile cases of HD primarily consists of bradykinesia, dystonia and rigidity, and does not usually present with the chorea

characteristic of adult onset HD (Rasmussen et al., 2000; Ribai et al., 2007; Douglas et al., 2013). There is also an increased incidence of seizures, myoclonus and epilepsy in juvenile HD (Rasmussen et al., 2000; Gonzalez-Alegre and Afifi, 2006; Chuo et al., 2012; Douglas et al., 2013), and the juvenile condition may also have a more rapid and severe rate of progression than adult onset HD (van Dijk, 1985).

1.1.3 Neuropathology

1.1.3.1 Gross neuropathology

Post-mortem examination and *in vivo* imaging techniques of HD brains have produced substantial evidence for the presence of widespread neuropathology in the disease (Scahill et al., 2013). Cell loss and astrogliosis occur most prominently in the caudate and putamen (Lange et al., 1976; Vonsattel et al., 1985; Vonsattel and DiFiglia, 1998), and this degeneration has been found to be a function of CAG repeat length and age of death (Furtado et al., 1996; Penney et al., 1997). Atrophy is not limited to the striatum and putamen, as extensive degeneration is seen in cortical structures (Rosas et al., 2003; Rosas et al., 2005; Rosas et al., 2008), including the angular gyrus in the parietal lobe (Macdonald et al., 1997) and the motor cortices (Macdonald and Halliday, 2002), and subcortical structures, such as the hypothalamus (Kremer et al., 1991; Politis et al., 2008; Soneson et al., 2010), thalamus (Heinsen et al., 1999), brainstem (Rueb et al., 2014), globus pallidus, subthalamic nucleus, substantia nigra and hippocampus (Spargo et al., 1993; Vonsattel and DiFiglia, 1998; Rueb et al., 2014), as well as the cerebellum (Fennema-Notestine et al., 2004).

In vivo quantitative measurement of HD neurodegeneration has been made possible through the development of imaging techniques that include positron emission topography (PET), magnetic resonance imaging (MRI), functional magnetic resonance imaging (fMRI) and diffusion tensor imaging (DTI). Imaging studies have confirmed that a loss of striatal volume and subsequent increase in lateral ventricle volume occurs in the brains of HD patients (Aylward et al., 1997; Aylward et al., 2004; Tabrizi et al., 2009; Aylward et al., 2011; Tabrizi et al., 2011; Tabrizi et al., 2012; Tabrizi et al., 2013), and that this atrophy can begin up to 11 years prior to estimated symptom onset in the caudate, and 9 years prior in the putamen (Aylward et al., 2004; Paulsen et al., 2004; Kipps et al., 2005; Aylward et al., 2011; Tabrizi et al., 2012). Alongside striatal volume loss, there is also evidence of global brain atrophy (Thieben et al., 2002; Henley et al., 2009; Squitieri et al., 2009; Tabrizi et al., 2012) and cortical thinning (Rosas et al., 2002; Rosas et al., 2005; Nopoulos et al., 2010; Johnson et al., 2015); all of which have been correlated

with the clinical HD phenotype (Rosas et al., 2005; Henley et al., 2008; Rosas et al., 2008; Tabrizi et al., 2013; Johnson et al., 2015).

DTI has detected differences in white matter microstructure, in axial and radial diffusivity, for example, between pre-manifest HD, early HD and healthy brains (Gregory et al., 2015a; Odish et al., 2015), which show significant correlation with depressive and irritability symptoms in the disease (Gregory et al., 2015b). Levels of putaminal metabolites that act as neuronal integrity markers are also abnormal in pre-manifest and early HD (Sturrock et al., 2015), while elevated concentrations of iron have been identified in the caudate and putamen of early, but not pre-manifest, HD patients (Dumas et al., 2012).

1.1.3.2 Cellular pathogenesis

Gamma aminobutyric acid (GABA)-ergic medium spiny neurons (MSNs) form approximately 90-95% of striatal neurons (Gerfen, 1992a, b) and are the neuron population most vulnerable to degeneration in HD, while striatal cholinergic medium aspiny interneurons remain relatively spared until later stages of the disease (Ferrante et al., 1985; Ferrante et al., 1987). Two key subpopulations of MSNs have been defined based upon their projection targets; striatopallidal neurons project to the globus pallidus, and striatonigral neurons project to the substantia nigra. Neurodegeneration differentially occurs in distinct subsets of neurons in these MSN subpopulations throughout HD. In early HD, striatopallidal neurons that contain enkephalin and project to the external segment of the globus pallidus (GPe) present with heightened levels of cell death compared to those that contain substance P and project to the internal segment of the globus pallidus (GPi) (Reiner et al., 1988). Striatonigral cells containing substance P with afferents to the substantia nigra pars compacta (SNc) are more resistant to cell death than those that project to the substantia nigra pars reticulata (SNr) (Reiner et al., 1988). Loss of the 'indirect' striatopallidal innervation in early HD results in disinhibition of the GPe, which then causes increased inhibition of the subthalamic nucleus (STN) and subsequently causes less excitatory drive to the GPi and SNr, resulting in disinhibition of the thalamus (Figure 1.1). This causes increased excitatory innervation from the thalamus to the motor cortex and produces the choreic movements that characterise HD.

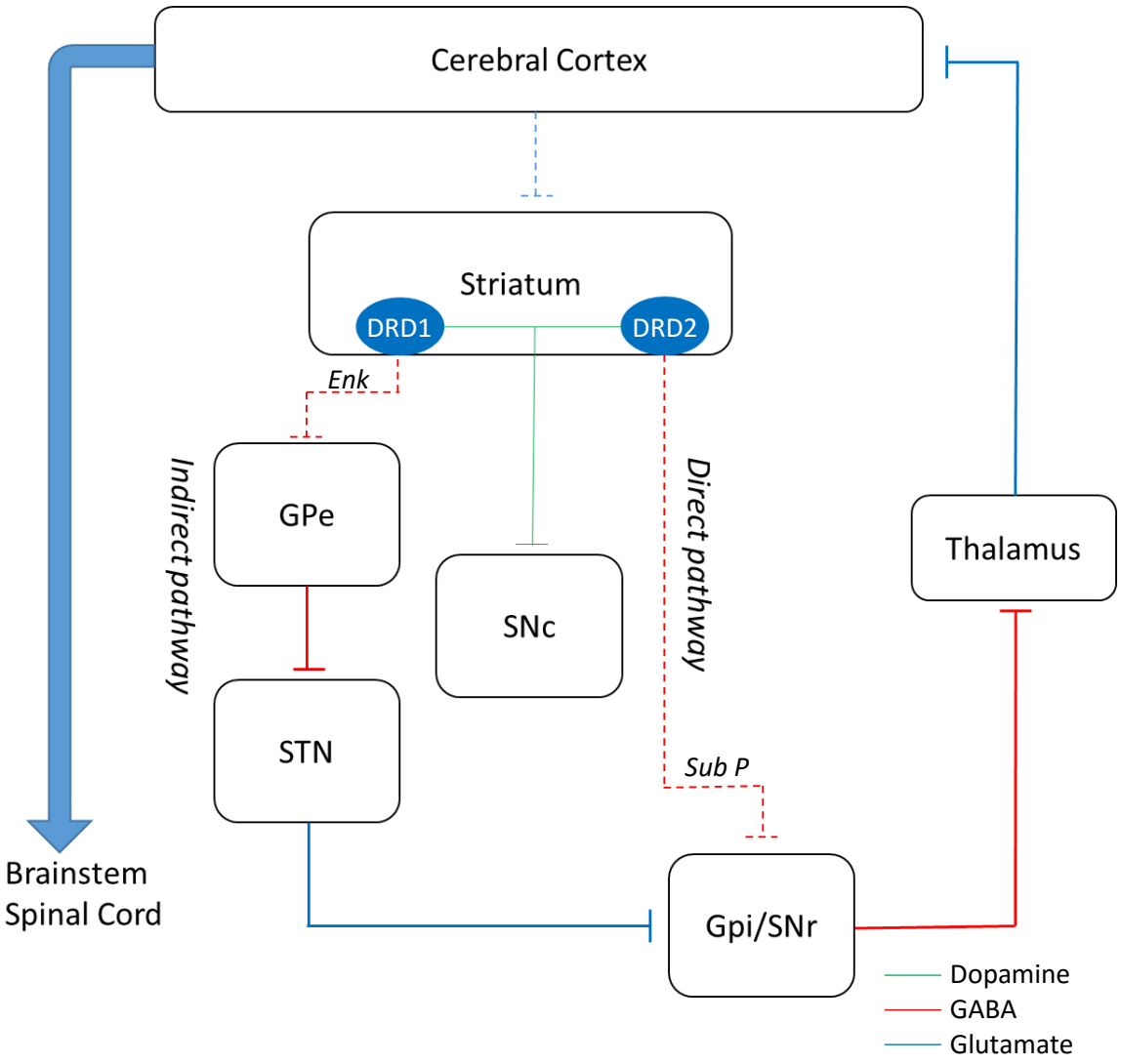


Figure 1.1. The direct and indirect pathways of the basal ganglia in HD (adapted from Alexander et al. 1990). Dashed lines represent pathways degenerated in HD. DRD1 = Dopamine receptor D1; DRD2 = Dopamine receptor D2; Enk = Enkephalin-containing neurons; Sub P = Substance P-containing neurons.

In the early stages of HD, loss of cortical volume develops prominently in posterior cortical regions, which include the middle occipital and temporal lobes, as well as the angular and supramarginal gyri (Rosas et al., 2002). As the disease progresses, cortical volume loss becomes more generalised and extends to more anterior cortical areas, causing the frontal lobes to be severely affected (Rosas et al., 2002). The cortical cells most vulnerable to degeneration are the pyramidal neurons of the middle to deep cortical layers (layers III, V and VI) (Cudkowicz and Kowall, 1990; Hedreen et al., 1991; Macdonald and Halliday, 2002). Cortical cells primarily produce brain derived neurotrophic factor (BDNF) and transport it to the striatum in an axonal anterograde manner through corticostriatal afferents (Altar et al., 1997). Degeneration of

cortical cells and corticostriatal afferents during HD pathology therefore reduces the amount of BDNF available in the striatum and may contribute to the striatal cell death found in the disease (Zuccato and Cattaneo, 2007), with evidence that HD results in decreased cortical BDNF production (Zuccato et al., 2001) and loss of BDNF transport activity, causing a loss of neurotrophic support and neuronal toxicity (Gauthier et al., 2004).

Loss of cortical interneurons has been associated with symptom heterogeneity in HD; patients with severe motor symptoms display significant loss of calbindin interneurons in the primary motor cortex, which is not seen in patients with a dominant mood phenotype (Kim et al., 2014). Conversely, HD patients with major mood disorder showed significant loss of calbindin, calretinin, and parvalbumin interneurons in the anterior cingulate cortex, while the aforementioned patients with dominant motor symptoms presented with no interneuron loss in this area. This suggests that region-specific degeneration of particular cortical interneurons may play a key role in the neural basis of different HD phenotypes.

Dendritic alterations also occur in HD, with disease progression producing a diphasic pattern of dendrite modification (Ferrante et al., 1991). Cases of HD with moderate severity predominantly presented with the initial proliferative changes in MSNs, whereby the number and size of dendrite spines were increased, which may reflect an early compensatory mechanism designed to remodel degenerating contacts (Ferrante et al., 1991). By contrast, patients with severe grades of HD pathology primarily presented with degenerative alterations to MSNs, such as dendritic swelling and discernible spine loss (Ferrante et al., 1991).

1.1.3.3 Molecular pathogenesis

Ubiquitin- and Huntingtin protein (HTT)-rich neuronal intranuclear inclusions (NIIs) have been identified in HD patients (DiFiglia et al., 1997; Becher et al., 1998; Gutekunst et al., 1999) and several mouse models of the disease (Davies et al., 1997; Lin et al., 2001; Bayram-Weston et al., 2012c, a, b; Bayram-Weston et al., 2012d). In HD patients, NIIs are present in MSNs of the striatum but not in neurons of the globus pallidus or cerebellum, while neurons with NIIs have been detected in all cortical layers (DiFiglia et al., 1997). NII-containing cortical neurons are more commonly found in juvenile HD patients, where they comprise 38-52% of neurons, than in adult onset sufferers, where only 3-6% of cortical neurons contain NIIs (DiFiglia et al., 1997). The presence of NIIs before the onset of behavioural symptoms and substantial neuronal death (Davies et al., 1997; Ordway et al., 1997), in combination with the correlation between NII presence and neuropathological severity (Davies et al., 1997; DiFiglia et al., 1997), lead to the

proposal that NIIs are causative in HD pathology, with recent evidence suggesting that NIIs may act to prevent clearance of mutant HTT (mHTT) and thereby enhance cellular toxicity (Tsvetkov et al., 2013). Conversely, an opposing theory is that NIIs may act as a cellular mechanism to protect neurons from mHTT-induced cell death (Saudou et al., 1998; Gutekunst et al., 1999; Kuemmerle et al., 1999; Arrasate et al., 2004). This has been proposed as a result of evidence that exposing mHTT-transfected striatal neurons to conditions that suppress the formation of NIIs leads to increased levels of cell death (Saudou et al., 1998), whereas NII formation reduces both the level of mHTT within the neuron and the risk of cell death (Arrasate et al., 2004). Further support for the theory that protein aggregation formation does not correspond to disease severity comes from evidence that NIIs are predominantly observed in the striatal cholinergic interneurons spared by degeneration in HD, while few aggregates are detected in the MSNs vulnerable to mHTT toxicity (Kuemmerle et al., 1999). Similarly, almost 50% (49.2%) of the mHTT toxicity resistant striatal population were found to contain smaller neuropil and cytoplasmic inclusions, in comparison to only 4% of the MSN population (Kuemmerle et al., 1999). Dystrophic neurites are known to arise in an uneven distribution through cortical layers V and VI in HD brains, and are present at a higher incidence in adult onset cases compared to juvenile patients (DiFiglia et al., 1997; Sapp et al., 1997).

1.1.4 Epidemiology and inheritance

Despite occurring worldwide, HD exhibits significant geographical differences in its prevalence, similar to the differential prevalence of spinocerebellar ataxias (a group of diseases also caused by CAG repeat expansions) identified between Japanese and Caucasian populations (Takano et al., 1998). For example, the prevalence of HD in individuals aged 21 or over has been recently been estimated at between 4.00 to 12.30 per 100,000 individuals in the United Kingdom (Hoppitt et al., 2011; Evans et al., 2013), approximately 2.12 per 100,000 individuals in Finland (Sipila et al., 2015), and 13.7 per 100,000 individuals in the general population of Canada (Fisher and Hayden, 2014). The general prevalence of HD in Caucasian populations is estimated at 5.70 per 100,000 individuals, which is much higher than the 0.40 per 100,000 individuals incidence rate reported in Asia (Pringsheim et al., 2012), while the incidence of HD in sub-Saharan African populations is approximately 3.50 per 100,000 individuals (Lekoubou et al., 2014). The differences in prevalence for HD between European and East-Asian populations has been suggested to arise as a result of geographical differences in *HTT* haplotypes that influence CAG-tract instability (Warby et al., 2009; Warby et al., 2011). A set of 22 single nucleotide polymorphisms that constitute a single haplogroup, where inheritance of the haplogroup has a

predisposing influence on CAG instability in *HTT*, has been found to be significantly enriched in HD patients of European origin (>95%) but is absent in the East Asian populations of Japan and China (Warby et al., 2009). In East-Asian populations, the majority of HD chromosomes are associated with an alternate haplogroup, and both East-Asian and European populations share a similar low level of HD on this alternate haplogroup (Warby et al., 2011). This evidence suggests that CAG expansion bias may occur in different geographical populations because of genetic *cis*-elements within the haplotype that influence CAG instability in *HTT*, potentially through different mutational mechanisms for the different haplogroups. This hypothesis is supported by recent evidence suggesting that a single ancestral chromosome contributes to 55% of the European HD population, and that the CAG tract instability in this population may be the result of variants produced by *de novo* mutations, simple recombination, structural alteration, gene conversion or double recombination within the ancient haplotype (Lee et al., 2015a).

Carrying a CAG repeat of greater than 39 repeats within *HTT* appears to produce complete penetrance of the HD phenotype (Walker, 2007). A lower CAG-tract range of 36-39 repeats causes the HD phenotype to show reduced penetrance, with a number of individuals carrying CAG repeats within this range being reported to live unaffected by HD up to 95 years of age (Goldberg et al., 1993; Rubinsztein et al., 1996) whilst others carrying CAG repeats within this range display the HD phenotype (Duyao et al., 1993; Kremer et al., 1994; Rubinsztein et al., 1996). There are also reports of individuals displaying HD phenotypes with CAG repeat lengths as low as 31-34 CAGs (Andrich et al., 2008; Groen et al., 2010). In these cases, known phenocopies have been dismissed but post-mortem neuropathological examinations have not yet been possible as a means of confirming HD diagnoses. Taken together, these cases suggest that, within this reduced penetrance repeat size range of 36-39 CAGs, HD penetrance may be moderated by additional factors, and consequently that these factors may also influence the variation in age of onset observed at all CAG repeat lengths and contribute to the heightened disease severity exhibited in the presence of longer repeat lengths.

The expanded CAG repeat within *HTT* is known to be unstable (MacDonald et al., 1993), particularly during parental transmission, where approximately 80% of inherited alleles display alterations in CAG repeat length (Duyao et al., 1993). These alterations in CAG repeat length exist in the form of both expansions and contractions of repeat length, and occur in transmissions from parents of either sex, although the largest expansions to *HTT* CAG repeat length predominantly arise from paternal transmissions (Derooij et al., 1993; Duyao et al., 1993; Zuhlke et al., 1993a; Kremer et al., 1995; Norremolle et al., 1995; Ranen et al., 1995). This paternal transmission-derived CAG repeat length expansion is believed to arise during spermatogenesis, as the CAG repeat lengths of HD patients display a large degree of mosaicism

between spermatocytes (Macdonald et al., 1993; Zuhlke et al., 1993a; Telenius et al., 1995), and because repeat lengths much larger than those found in patient lymphocytes have been observed in these cells (Duyao et al., 1993; Macdonald et al., 1993; Wheeler et al., 2007).

The prevalent CAG expansion event arising during parental transmission of *HTT* has contributed to the occurrence of 'anticipation' in HD families whereby offspring inheriting the HD phenotype tend to have a lower age of onset in comparison to the affected parent (Ridley et al., 1988). Paternal transmission of the HD allele is believed to produce a stronger anticipation effect and therefore an earlier age of onset in offspring (Ridley et al., 1988; Roos et al., 1991; Snell et al., 1993; Ranen et al., 1995). This paternal transmission anticipation effect is highlighted in a cohort of HD families where paternally transmitted cases of HD presented an age of onset that is on average 9.11 years earlier than in the parent, while maternally transmitted cases displayed a mean difference of only 2.75 years (Snell et al., 1993). An investigation in a cohort of juvenile HD patients of Italian origin did not replicate this paternal transmission anticipation effect, despite reporting larger intergenerational CAG repeat length changes in patients with paternally transmitted HD in comparison to counterparts inheriting the maternal mutation (Cannella et al., 2004). Nonetheless, this supports the suggestion that CAG repeat length is not the sole determinant of age of onset in HD.

The higher risk of CAG repeat length expansion during spermatogenesis compared to oogenesis (Zuhlke et al., 1993a) causes both juvenile and sporadic forms of HD to be more highly associated with paternally inherited cases of HD, rather than cases arising from maternal transmission of the HD allele (Ridley et al., 1988; Derooij et al., 1993; Goldberg et al., 1993; Telenius et al., 1993; Ranen et al., 1995; Cannella et al., 2004). As mentioned in section 1.1.1.5, juvenile HD is associated with longer CAG repeat lengths than adult onset cases of the disease (MacDonald et al., 1993; Andresen et al., 2007), and shows high frequency of paternal inheritance, with approximately 70-80% of cases being paternally transmitted (Telenius et al., 1993; Cannella et al., 2004); the frequency of paternal inheritance rises to 90% in cases where children display disease onset under 10 years of age (Telenius et al., 1993). Sporadic cases of HD, where offspring inherit an allele containing a CAG repeat expanded to a fully penetrant length from an unaffected parent, are estimated to comprise as many as 10% of HD cases (Almqvist et al., 2001; Falush et al., 2001), and have also displayed patterns of paternal inheritance (Derooij et al., 1993; Goldberg et al., 1993; Zuhlke et al., 1993b); multiple cases of sporadic HD have been reported where the unaffected father carries an intermediate allele containing 27-35 CAG repeats, or a reduced penetrance allele consisting of 36-39 CAG repeats, that has expanded to fully penetrant repeat sizes of 42-46 CAGs in offspring (Derooij et al., 1993; Goldberg et al., 1993; Zuhlke et al., 1993b), as a result of meiotic variability in CAG repeat lengths (Zuhlke et al., 1993b).

Conversely, the majority of European HD patients share the same haplotype, of which two variants are associated with CAG repeat length instability (Warby et al., 2009; Warby et al., 2011). Thus, these 'HD-haplotypes' are more susceptible to CAG repeat length expansion and account for many sporadic cases of HD (Myers et al., 1993; Goldberg et al., 1995; Warby et al., 2011).

1.1.5 Current treatment and therapeutics

Despite HD being a monogenic disease with a known gene and gene product being identified over 20 years ago (MacDonald et al., 1993), there is no known cure for the disease at the time of writing. Nevertheless, there are a number of pharmacological agents currently in use that aim to alleviate disease symptom severity. Hyperkinetic movements, such as chorea, dystonia and myoclonus, are the motor symptoms typically targeted for pharmacological intervention in HD (Shannon and Fraint, 2015); the antidopaminergic agent tetrabenazine is currently the only approved medication for treating chorea in HD in the United States (Venuto et al., 2012; Shannon and Fraint, 2015), whilst European experts prefer the use of the antipsychotics olanzapine and risperidone (Burgunder et al., 2011), and these antipsychotics also show potential to alleviate the dystonia and Parkinsonism that may arise in patients (Venuto et al., 2012). Antiparkinsonian medications such as levodopa and amantadine can also be used to treat Parkinsonism in HD patients (Frank, 2014). To alleviate myoclonus, a number of anti-epileptic drugs can be prescribed, such as valproic acid and clonazepam (Adam and Jankovic, 2008). Selective serotonin reuptake inhibitors, serotonin-noradrenaline reuptake inhibitors, and both tricyclic and atypical antipsychotics are frequently utilised in the treatment of the psychiatric disturbances seen in HD patients, such as depression, anxiety and irritability, despite little evidence from clinical trials on the usefulness of these drugs in effectively alleviating these symptoms in HD (Venuto et al., 2012; Frank, 2014). Treating the cognitive decline displayed in HD is particularly challenging, with no pharmacological intervention currently available (Venuto et al., 2012; Frank, 2014; Shannon and Fraint, 2015).

1.2 HTT

1.2.1 Gene expression, alternative splicing and somatic mosaicism

HTT is extensively expressed throughout the body, with particular enrichment in the brain and testes (Li et al., 1993; Strong et al., 1993). Within the brain, *HTT* expression is widespread,

though it is not enriched within the striatum, the primary site of disease pathology, and displays greater enrichment in neurons compared with glial cells (Li et al., 1993; Strong et al., 1993; Landwehrmeyer et al., 1995), with the corticostriatal projection pyramidal neurons of cortical layers III and V showing strongest *HTT* neuronal expression (Fusco et al., 1999). Peripheral expression of *HTT* is generally accepted to be ubiquitous, occurring in tissues such as testis, lung, spleen, liver, heart and kidney (Li et al., 1993; Strong et al., 1993).

In the human transcriptome, the majority of multi-exon genes are alternatively spliced (Pan et al., 2008), which is the process by which deoxyribonucleic acid (DNA) encoding a single gene can generate multiple messenger ribonucleic acid (mRNA) transcripts, and thus functionally different proteins, through the inclusion or exclusion of specific exons (Kim et al., 2008a). *HTT* contains 67 exons known to produce two major mRNA transcripts dependent upon differential polyadenylation sites on the 3' end of the *HTT* mRNA; a 10.3 kb transcript that is predominantly expressed throughout peripheral tissues, and a 13.7 kb transcript with an extended untranslated region at the 3' end of the mRNA sequence, which is predominantly expressed in the brain (Lin et al., 1993). Recently, a series of novel alternatively spliced *HTT* mRNA variants have been identified in the brains of post-mortem HD patients (Sathasivam et al., 2013; Hughes et al., 2014; Labadorf and Myers, 2015; Mort et al., 2015) and mouse models of the disease (Sathasivam et al., 2013; Hughes et al., 2014), as well as in human embryonic stem cells (Ruzo et al., 2015). It is postulated that these *HTT* mRNA splice variants may give rise to distinct isoforms of the HTT protein; thus it is possible that alternative splicing of *HTT* plays an integral role in HD pathology. In line with this theory, Sathasivam *et al.* (2013) discovered the presence of a short polyadenylated *HTT* mRNA transcript, which is created by CAG repeat length-dependent aberrant splicing of exon 1 *HTT*, in the brains of all HD mouse models expressing *mHtt* or *mHTT*, as well as in fibroblast cell lines derived from HD patients and post-mortem HD brains, that is translated into an exon 1 HTT protein believed to be pathogenic in HD.

The presence of an expanded CAG repeat has also been found to alter the expression of *HTT* in lymphoblast cultures taken from heterozygote juvenile HD patients, whereby the expression of the mutant HD allele is relatively reduced in comparison to the healthy allele (Gutekunst et al., 1995; Persichetti et al., 1996). The length of the pathological CAG repeat is believed to affect the degree of reduced expression in these studies, and a similar observation has been made in expanded CAG repeat-transfected striatal rat cultures (Miller et al., 2010). However, it is not known whether this reduction in mutant *HTT* allele expression is the result of a CAG repeat length-dependent reduction in transcription, alteration of the subsequent HTT protein processing, or aberrant splicing of *HTT*, as identified by Sathasivam *et al.* (2013).

Somatic mosaicism has been identified in human HD tissues as well as in models of the disease, with evidence of somatic expansion of the CAG repeat in *HTT* in blood, brain, kidneys, liver, heart and stomach tissue (Telenius et al., 1994; Mangiarini et al., 1997; Wheeler et al., 1999; Ishiguro et al., 2001; Mollersen et al., 2010), where initially larger CAG repeats have a greater propensity for expansion than smaller CAG repeats (Wheeler et al., 1999; Cannella et al., 2009). Neural tissue displays greater CAG repeat length instability than peripheral tissues (Telenius et al., 1994; Mangiarini et al., 1997), occurring to a greater extent in neurons than glia (Shelbourne et al., 2007), and is most marked in the neural regions showing greatest neurodegeneration in HD, the striatum and cortex, while being observed to a lesser extent in the cerebellum, where neuropathology is less extensive (Telenius et al., 1994; Mangiarini et al., 1997; Ishiguro et al., 2001; Kennedy et al., 2003; Mollersen et al., 2010). Human pre-symptomatic HD carriers have been shown to exhibit greatest CAG repeat length instability in the striatum, where CAG repeat expansions have been detected that exceed 300 and 1000 CAG repeats (Kennedy et al., 2003; Shelbourne et al., 2007). By contrast, the cortex displays higher CAG repeat instability than the striatum in late stage HD brains (Shelbourne et al., 2007). These observations support the proposal that somatic CAG repeat expansion instability may be a mechanism for the progressive and cell-selective neurodegeneration that occurs in HD.

1.2.2 Protein structure, localisation and function

HTT is a protein containing 3,144 amino acids with a molecular weight of approximately 348 kDa (MacDonald et al., 1993), and this high molecular weight has so far prevented the elucidation of its structure by mass spectrometry and crystallography studies. One feature of HTT that has undergone substantial investigation is the polyglutamine tract (encoded by the CAG repeat) containing N-terminus. The first 17 amino acids of HTT's N-terminus have been shown to contain a nuclear export signal (Zheng et al., 2013) and form an α-helical structure (Atwal et al., 2007; Kim et al., 2009; Dlugosz and Trylska, 2011; Michalek et al., 2013) that modulates HTT localisation, aggregation and toxicity (Atwal et al., 2007; Rockabrand et al., 2007; Maiuri et al., 2013; Arndt et al., 2015). The structure of this α-helix can be altered by pathogenic CAG repeat expansion (Legleiter et al., 2010; Dlugosz and Trylska, 2011; Peters-Libeu et al., 2012; Vachharajani et al., 2012), mutations to amino acids 1-17 (Atwal et al., 2007), and by post-translational modifications such as phosphorylation events (Aiken et al., 2009; Atwal et al., 2011).

The polyglutamine tract begins at the 18th amino acid of the N-terminus of HTT (MacDonald et al., 1993) and forms a polar zipper structure, which is a complex that promotes transcription

factor binding, thus suggesting that HTT may play a role in regulation of transcription (Perutz et al., 1994). Expansion of the polyglutamine tract within the N-terminus is believed to contribute to mHTT aggregate formation and cellular toxicity by reducing the stability of the HTT protein, promoting N-terminal fragment aggregation, and impairing degradation of such fragments (Li and Li, 1998; Mende-Mueller et al., 2001; Landles et al., 2010; Juenemann et al., 2011; Vachharajani et al., 2012). HTT destabilisation can occur as a result of disruption of the α-helical structure of HTT amino acids 1-17; the α-helical structure of the non-pathogenic HTT N-terminus is stabilised by interactions between amino acids 1-17 and the polyproline rich COOH-terminal that follows the polyglutamine tract (Dlugosz and Trylska, 2011). The expanded polyglutamine tract in mHTT prevents these stabilising interactions, which causes 'loosening' of the α-helix and allows the N-terminus to form more aberrant associations and structures (Li et al., 2007; Dlugosz and Trylska, 2011). This mechanism of pathogenicity has been challenged, however, by evidence that CAG repeat length has no effect on murine HTT interactions in the absence of mHTT aggregation, and that it is the presence of aggregates that causes aberrant spatial distribution and solubility of HTT protein partners (Davranche et al., 2011).

The proline-rich C-terminus of HTT, found downstream of the polyglutamine tract, contains a nuclear export signal (Xia et al., 2003) and is structurally dynamic; being capable of existing in both a polyproline II helical and random coil formation (Isas et al., 2015). Downstream of the CAG repeat sequence, HTT also contains multiple huntingtin, elongation factor 3, protein phosphatase 2A, and TOR 1 (HEAT) repeat sequences, which are repeatedly occurring binding regions consisting of sequences of ~40 amino acids that promote protein-protein interactions (Andrade and Bork, 1995). This suggests that HTT may bind to a variety of proteins to perform its physiological function. The number of HEAT repeats found in HTT is disputed, with one laboratory identifying the presence of 16 HEAT repeats, arranged into four clusters, along the length of HTT (Tartari et al., 2008), while another has reported the presence of up to 36 HEAT repeats (Takano and Gusella, 2002). Also present in HTT are a number of caspase and calpain proteolytic cleavage sites that lead to the generation of a variety of fragments following HTT protein cleavage (Wellington et al., 1998; Gafni et al., 2004; Hermel et al., 2004), and the presence of a pathogenic CAG repeat length in HTT can lead to aberrant processing of the mHTT protein and cause cell toxicity (Martindale et al., 1998; Wellington et al., 2002; Juenemann et al., 2011; Ratovitski et al., 2011; El-Daher et al., 2015).

Within the cell, HTT subcellular localisation may be regulated by kinase signalling activity (Bowles et al., 2015) and the protein is associated with a variety of subcellular compartments and organelles that include dendrites, nerve terminals, axons and cell bodies, as well as the nucleus, mitochondria, microtubules, endoplasmic reticulum, Golgi complex and vesicles

(DiFiglia et al., 1995; Sharp et al., 1995; Trottier et al., 1995; Velier et al., 1998; Hilditch-Maguire et al., 2000; Hoffner et al., 2002; Kegel et al., 2002). This diversity in subcellular localisation hinders efforts to define its molecular function.

While the exact functions of HTT have yet to be elucidated, it is known to play a number of diverse molecular roles. The HTT protein is known to be integral to embryonic development because total absence of murine *Htt* results in embryonic lethality of knock-out mice before day 8.5 (Duyao et al., 1995; Nasir et al., 1995; Zeitlin et al., 1995). A role in cerebral spinal fluid homeostasis regulation has also been discovered through evidence that conditional inactivation of *Htt* in the midbrain and hindbrain of mice produces congenital hydrocephalus (Dietrich et al., 2009). There is also a large body of evidence implicating HTT in modulating transcriptional regulation (Cha et al., 1999; Kegel et al., 2002; Hodges et al., 2006; Kuhn et al., 2007; Benn et al., 2008b; Kim et al., 2008b; Futter et al., 2009), with the protein capable of up-regulating BDNF transcription (Zuccato et al., 2001) by indirectly interacting with repressor element-1 silencing transcription factor/neuron restrictive silencer factor (REST/NRSF) to repress the silencing activity of the neuron restrictive silencing element (Zuccato et al., 2003; Shimojo, 2008), while pathogenic CAG repeat lengths have been shown to disrupt this silencing activity (Zuccato et al., 2007; Ravache et al., 2010; Soldati et al., 2013) as well as cAMP-responsive element binding protein (CREB)-dependent gene transcription (Shimohata et al., 2000; Steffan et al., 2000). Recently HTT has been found to act as a scaffold protein for selective macroautophagy, thus identifying a role for the protein in protection against cellular stress (Rui et al., 2015). Alongside these roles, HTT is also associated with protein and vesicular trafficking (Velier et al., 1998; Hilditch-Maguire et al., 2000; Gauthier et al., 2004; Strehlow et al., 2007), iron homeostasis (Hilditch-Maguire et al., 2000), neuronal synaptic activity (Xu et al., 2013), and regulation of mammary stem cell division and differentiation (Elias et al., 2014).

1.3 Genetic mouse models of HD

The identification of *HTT* as the single causative gene in HD has led to the development of a number of animal models of HD that are designed to recreate the molecular and behavioural phenotypes of the human disease. Genetic manipulation in mice has allowed the effects of *Htt* CAG repeat length, protein fragment size and expression levels to be explored in physiological models of HD. This has enabled characterisation of HD pathology at the cellular, molecular and behavioural levels across the animals' lifespan, generating further understanding of the pathological effects of mHTT. Mouse and rat models of HD also allow the assessment of potential therapeutics *in vivo* and are therefore a crucial aspect of the future of HD research, as

any treatments for the disease are unlikely to reach clinical trials without *in vivo* evidence of their efficacy.

Many mouse models of HD are currently available and the model to be utilised is dependent on the requirements and objectives of the study, as different models exhibit differences in molecular and behavioural phenotypes, lifespan, and rates of disease progression. Mouse models are generally grouped depending on their genetic manipulation; transgenic models are created by insertion of either truncated or full length *HTT* indiscriminately into the genome, while 'knock-in' models are generated by insertion of a pathological length CAG repeat expansion into the mouse genome at the appropriate location on the *Htt* gene, which is expressed under the control of the endogenous mouse promoter. Inducible mouse models of HD have also been created, where a truncated *HTT* exon 1, or a chimeric *HTT*/*Htt* exon 1, is inserted into the mouse genome under the control of a tetracycline-regulated system, allowing inducible expression of *mHTT* in the presence or absence of doxycycline. Further details regarding the most commonly used mouse models of HD can be found in Table 1.1.

	Background strain	**Transgene**	**Promoter**	**CAG repeat length**	**Lifespan**	**Reference**
N-terminal transgenic models						
R6/1	CBA x C57BL/6	Exon 1 human *HTT*	Human *HTT*	116	32-40 weeks	(Mangiarini et al., 1996)
R6/2	CBA x C57BL/6	Exon 1 human *HTT*	Human *HTT*	150	10-13 weeks	(Mangiarini et al., 1996)
N171-82Q	C3H/HEJ x C57BL/6	First 171 amino acids human *HTT*	Mouse prion promoter	82	16-22 weeks	(Schilling et al., 1999; Schilling et al., 2004)
Full-length transgenic models						
YAC72	FVB/N	Human *HTT*	Human *HTT*	72	Normal	(Hodgson et al., 1999)
YAC128	FVB/N	Human *HTT*	Human *HTT*	120	Normal	(Slow et al., 2003)
BACHD	FVB/N	Human *HTT*	Human *HTT*	97	Normal	(Gray et al., 2008)
Knock-in models						
HdhQ72-80	129Sv x C57BL/6	Expanded CAG inserted in endogenous mouse *Htt*	Mouse *Htt*	72, 80	Normal	(Shelbourne et al., 1999)
HdhQ92	CD1 x 129SvEv	Endogenous mouse *Htt* with chimeric human/mouse exon 1	Mouse *Htt*	90	Normal	(Wheeler et al., 1999)
HdhQ94	C57BL/6	Endogenous mouse *Htt* with chimeric human/mouse exon 1	Mouse *Htt*	94	Normal	(Levine et al., 1999; Menalled et al., 2002)

HdhQ111	CD1 x 129SvEv	Endogenous mouse *Htt* with chimeric human/mouse exon 1	Mouse *Htt*	109	Normal	(Wheeler et al., 1999; Wheeler et al., 2002)
HdhQ140	C57BL/6	Endogenous mouse *Htt* with chimeric human/mouse exon 1	Mouse *Htt*	140	Normal	(Menalled et al., 2003)
HdhQ150	129/Ola x C57BL/J6	Expanded CAG inserted in endogenous mouse *Htt*	Mouse *Htt*	150	Normal	(Lin et al., 2001)
HdhQ200	C57BL/6J	Expanded CAG inserted in endogenous mouse *Htt*	Mouse *Htt*	200	Euthanised at 80 weeks	(Heng et al., 2010)
zQ175	C57BL/6J	Expanded CAG inserted in endogenous mouse *Htt*	Mouse *Htt*	175	Homozygotes: 90 weeks Heterozygotes: Normal	(Menalled et al., 2012)
Inducible models						
HD94	CBA x C57BL/6	Chimeric human/mouse exon 1 *HTT*	TetO + tTA	94	Normal	(Yamamoto et al., 2000)
Inducible-148Q	C57BL/6	First 171 amino acids human *HTT*	PrP + tTA	148	28-40 weeks	(Tanaka et al., 2006)

Table 1.1. Key features and lifespans of commonly used mouse models of HD. Abbreviations: TetO = TetO operator; tTa = tetracycline-regulated transactivator; Prp = prion promoter.

1.3.1 Behavioural phenotypes

Each mouse model of HD has its own unique assortment of behavioural phenotypic characteristics (summarised in Table 1.2). The R6/1, R6/2, and N171-82Q mouse models, known as the N-terminal transgenic models, develop motor irregularities at an earlier age than full length transgenic and pathological length knock-in models, while also presenting with greatly diminished life spans (Table 1.1). YAC72, YAC128 and BACHD animals are full-length transgenic models of HD, meaning they contain a full length human *HTT* transgene, and develop similar behavioural phenotypes as the N-terminal transgenic models, but with delayed onset (Table 1.2). Despite expressing CAG repeats that are much longer than those found in transgenic mouse models of HD, knock-in models tend to display less severe phenotypes than their transgenic counterparts, with delayed onset of motor symptoms and lifespans equivalent to wild-type mice (Table 1.1 and Table 1.2).

	Motor phenotypes	Cognitive phenotypes	Psychiatric phenotypes	References
N-terminal transgenic models				
R6/1	Hyperactivity [4w]; hypoactivity & nest-building impairments [6w]; balance beam deficits [8w]; irregular gait, choreiform movements, tremor [15w]; rotarod deficits [18w]	Nest-building impairments [6w]; procedural learning and spatial memory deficits [8w]; visual & reversal learning deficits [15w]; ↓ novel object exploration [22w]	↓ startle response & prepulse inhibition [8w]; ↓ fear response [12w]; ↑ clasping [14w]; ↓ anxiety [24w]	(Mangiarini et al., 1996; Naver et al., 2003; Bolivar et al., 2004; Hodges et al., 2008; Brooks et al., 2012e; Rattray et al., 2013)
R6/2	Hyperactivity [3w]; hypoactivity [4.5w]; ↓ balance beam, rotarod & swimming abilities [5w]; ↓ grip strength [7w]; ↓ rearing [8w]; irregular gait, choreiform movements, tremor [9w]; ↑ weight loss [13w]	Spatial memory deficits [3.5w]; contextual memory deficits [5w]; working memory deficits [6w]; reversal learning deficits [6.5w]; visual discrimination & response inhibition deficits [9w]	↑ clasping [9w]; ↓ anxiety, startle reactivity & prepulse inhibition [8w]	(Mangiarini et al., 1996; File et al., 1998; Carter et al., 1999; Bolivar et al., 2003; Hickey et al., 2005; Oakeshott et al., 2013; Li et al., 2015)
N171-82Q	Tremor, abnormal gait, hypokinesis, uncoordination, ↑ weight loss [10w]; rotarod deficits [13w]; hypoactivity [21w]	Spatial learning & memory deficits [12.5w]	↑ clasping [10w]; ↑ anxiety [12w]	(Schilling et al., 1999; Schilling et al., 2001; Schilling et al., 2004; Potter et al., 2010; Swarnkar et al., 2015)
Full-length transgenic models				
YAC72	Abnormal gait, choreoathetoid movements, disorientation & balance beam deficits [39w]; ↑ weight loss [52w]; hypoactivity & rotarod deficits [69.5w]	n.r.	↑ clasping [39w]	(Hodgson et al., 1999; Seo et al., 2008)
YAC128	↑ weight gain [8w]; hyperactivity [13w]; rotarod deficits [17-26w]; balance beam deficits [34.5w]; hypoactivity [52w]	Motor & reversal learning deficits [8.5w]; spatial learning deficits [26w]; motor learning deficits [34.5w]	↓ startle response & prepulse inhibition [52w]	(Slow et al., 2003; Van Raamsdonk et al., 2005b; Van Raamsdonk et al., 2006; Brooks et al., 2012c)
BACHD	Rotarod deficits [4w]; ↑ weight gain [8-26w]; hyperactivity [13w]; gait abnormalities [39w]; hypoactivity [52w]	Reversal learning & set-shifting deficits [39w]; impaired nest-building [40w]; novel stimuli discrimination deficits [87w]	↑ grooming [20w]; ↑ anxiety & fear response [39w]; depressive-like phenotype in forced swimming test [52w]	(Gray et al., 2008; Menalled et al., 2009; Abada et al., 2013; Farrar et al., 2014; Wang et al., 2014; Estrada-Sanchez et al., 2015)
Knock-in models				

HdhQ72-80	Rotarod deficits [17w]	n.r.	↑ aggression [13w]	(Shelbourne et al., 1999; Kennedy et al., 2003)
HdhQ92	↓ grip strength [17w]; ↑ weight loss [74w]; hypoactivity [104w]; rotarod deficits [117w]	Rule learning deficits [22w]; attention, visuomotor & spatial learning deficits [52w]; ↓ motivation [65w]	↓ startle response & prepulse inhibition [17w]	(Trueman et al., 2009; Brooks et al., 2012a; Trueman et al., 2012a; Trueman et al., 2012b)
HdhQ94	↑ rearing [8w]; hyperactivity [8w]; hypoactivity [17w]	n.r.	n.r.	(Menalled et al., 2002)
HdhQ111	Hyperactivity [4w]; ↑ weight loss [28w]; abnormal gait [104w]	Long-term spatial & recognition memory deficits [34.5w]	Sex-dependent anxio-depressive phenotype [13w]	(Wheeler et al., 2002; Menalled et al., 2009; Giralt et al., 2012; Orvoen et al., 2012)
HdhQ140	↑ rearing and hyperactivity [4w]; hypoactivity, ↓ running speed, climbing & rotarod ability [17w]; abnormal gait [52w]; tremor [87w]	Motor learning deficits [26w]	↑ anxiety & fear response [17-26w]	(Menalled et al., 2003; Dorner et al., 2007; Hickey et al., 2008)
HdhQ150	↓ grip strength [6w]; ↑ weight loss [70w]; rotarod deficits [78w]; balance beam deficits [100w]; abnormal gait [100w]	Spatial memory deficit [17w]; reversal learning deficit [26w]; extra-dimensional set-shifting deficit [104w]	↓ startle reactivity [26w]; ↓ aggression, anxiety & irritability [65w]; ↑ clasping [70w]; absent fear response [95w]; ↓ exploratory activity [100w]	(Lin et al., 2001; Brooks et al., 2006; Heng et al., 2007; Woodman et al., 2007; Brooks et al., 2012b)
HdhQ200	↑ weight loss & balance beam deficits [20w]; abnormal gait [60w]; hypoactivity, ↓ grooming & grip strength [80w]	n.r.	n.r.	(Heng et al., 2010)
zQ175	↓ grip strength [4w]; ↑ weight loss [6w]; hypoactivity & ↑ rearing [8w]; rotarod deficits [30w]; ↓ climbing [32w]	Visual discrimination, novel stimuli & reversal learning deficits [26w]; ↓ response inhibition [26w]; working memory deficits [39w]; procedural learning deficits [43.5w]	↓ motivation [32-33w]	(Heikkinen et al., 2012; Menalled et al., 2012; Oakeshott et al., 2013; Farrar et al., 2014; Menalled et al., 2014; Curtin et al., 2016)
Inducible models				
HD94	Rotarod deficit [12w]; tremor & ↓ grooming [20w]; hypoactivity [36w]	n.r.	↑ clasping [4w]	(Yamamoto et al., 2000; Martin-Aparicio et al., 2001)
Inducible-148Q	Abnormal gait, tremor, incoordination, rotarod deficits & ↑ weight loss [26w]	n.r.	n.r.	(Tanaka et al., 2006)

Table 1.2. Behavioural phenotypes of the most widely used mouse models of HD. w = weeks of age; n.r. = not reported; ↑ = increase in; ↓ = decrease in.

1.3.1.1 The HdhQ150 knock-in mouse model of HD

The HdhQ150 mouse model of HD was generated by replacing the native non-pathogenic CAG sequence of exon 1 of the endogenous mouse *Htt* locus with a pathogenic repeat length of 150 CAG residues (Lin et al., 2001). The original study on the HdhQ150 mouse model of HD identified a number of motor and psychiatric phenotypes, consisting of abnormalities in general activity, 'clasping', gait variability, and rotarod performance (Lin et al., 2001), which have since been expanded upon by further longitudinal studies (Heng et al., 2007; Woodman et al., 2007; Brooks et al., 2012b). Lin and colleagues (2001) also discovered that $Hdh^{Q150/Q150}$ mice have a lower body mass than wild-type animals at 25-30 weeks of age. This recapitulates the weight loss that is seen in human HD patients (Sanberg et al., 1981; Farrer and Yu, 1985; Stoy and McKay, 2000; Djousse et al., 2002; Robbins et al., 2006; van der Burg et al., 2009) and has since been found to represent a progressive decline in $Hdh^{Q150/Q150}$ animal bodyweight from 9-14 months of age (M) until end-stage disease at approximately 22-23M (Heng et al., 2007; Woodman et al., 2007; Brooks et al., 2012b).

With regards to motor impairments, animals heterozygous and homozygous for the mutant HdhQ150 allele ($Hdh^{Q150/+}$ and $Hdh^{Q150/Q150}$, respectively) present with gait disturbances in comparison to wild-type animals, with this phenotype occurring from 40 weeks of age in heterozygote animals and from 25 weeks of age in homozygotes (Lin et al., 2001), which suggests that gait variability onset is affected by gene dosage in HdhQ150 mice. However, Heng and colleagues (2007) only identified significant abnormalities in gait, in measures that included stride lengths and base lengths, in $Hdh^{Q150/+}$ and $Hdh^{Q150/Q150}$ mice, in comparison to wild-type animals, at 100 weeks of age. This would suggest that the gait abnormalities present in the HdhQ150 mouse model have a late-onset but are not affected by gene dosage. Despite identifying different onsets of gait abnormalities in $Hdh^{Q150/+}$ and $Hdh^{Q150/Q150}$ animals, the two studies both identify a gait impairment in the HdhQ150 mouse model of HD; it is possible that the differences observed in the two studies is a result of the differing proportions of background strains in the mouse lines used in each study. The ability to remain on a rotating rod, another measure of motor capacity, has revealed a late-onset age-related decline in the motor ability of $Hdh^{Q150/Q150}$ mice (Lin et al., 2001; Heng et al., 2007; Woodman et al., 2007; Brooks et al., 2012b). Rotarod performance and learning deficits have been identified in homozygous animals from as early as 40 weeks of age (Lin et al., 2001; Heng et al., 2007), although the majority of reports identify these motor ability impairments at 18-23M (Heng et al., 2007; Woodman et al., 2007; Brooks et al., 2012b). Conversely, $Hdh^{Q150/+}$ mice have not displayed motor performance deficits in the rotarod when examined up to 100 weeks of age (Lin et al., 2001; Heng et al., 2007), suggesting that there is a gene dosage-dependent rotarod performance impairment in the

HdhQ150 mouse model of HD. At 100 weeks of age, *Hdh*$^{Q150/+}$ and *Hdh*$^{Q150/Q150}$ animals both display impairments in motor function, in relation to limb coordination and balance as assessed using a balance beam, as shown by a greater length of time being required by both genotypes to traverse the balance beam, when compared to wild-type counterparts (Heng et al., 2007). Motor learning capacities were also examined in this task, through analysis of the times taken to traverse the beam in the first 3 trials, and were found to be impaired in 100 week old homozygotes but preserved in age-matched heterozygote animals, which is indicative of a late-onset gene dosage-dependent impairment in motor learning. The manner in which HdhQ150 animals traversed the balance beam also differed to wild-type animals, with *Hdh*$^{Q150/Q150}$ mice displaying a "hindlimb drag" motion (where the abdomen is pressed against the beam, the hindlimbs are laterally wrapped around the beam, and the forelimbs are used to drag the mouse along the beam) from 40 weeks of age. Both homozygote and heterozygote animals displayed significantly greater use of this method than wild-type animals from 100 weeks of age, which provides further support for a late-onset motor impairment in the HdhQ150 mouse model of HD. To test whether the motor impairments identified were related to loss of muscle power, Heng *et al.* (2007) performed the hanging wire test, and found that there was no significant difference in the latency to fall between wild-type animals and heterozygote or homozygote HdhQ150 mice up to 100 weeks of age. This would suggest that there is no loss of muscle power in aged HdhQ150 mice and provides further support the evidence for a late-onset motor impairment in this mouse model. However, *Hdh*$^{Q150/Q150}$ mice have been reported elsewhere to display significant deficits in grip strength from as early as 1.5M (Woodman et al., 2007) and 10M (Brooks et al., 2012b), which would suggest that weakness in grip strength is a motor phenotype in mice homozygous for the HdhQ150 mutant allele.

Lin and colleagues (2001) reported two psychiatric phenotypes in their original study of the HdhQ150 mouse model of HD. The first was a deficit in exploratory activity, where *Hdh*$^{Q150/+}$ animals display greater levels of inactivity than their wild-type counterparts from 40 weeks of age, while *Hdh*$^{Q150/Q150}$ mice show this inactivity phenotype prior to 40 weeks of age, which suggests there may be a gene dosage effect in this behavioural abnormality. However, subsequent longitudinal analyses past the 52-week time point studied by Lin *et al.* (2001) have revealed that *Hdh*$^{Q150/Q150}$ mice exhibit deficits in exploratory activity only from 100 weeks of age (Heng et al., 2007), which are not evident at 18M (Woodman et al., 2007), and that heterozygote animals display activity levels comparable to wild-type counterparts until 100 weeks of age (Heng et al., 2007). The discrepancy in activity level findings between the original HdhQ150 study and subsequent longitudinal studies is likely to be the result of the different parameters and methodologies used to study activity levels; Lin and colleagues (2001) measured inactivity in the

first 30 s of a trial, whereas automated activity cages quantifying the number of beam breaks made by animals during a 60 or 120 min session were used to assess activity by Woodman and colleagues (2007) and Heng *et al.* (2007). The second psychiatric phenotype identified by Lin and colleagues (2001) was an increase in 'clasping' when HdhQ150 mice were suspended from the tail. Increased clasping arose in both *Hdh*$^{Q150/+}$ and *Hdh*$^{Q150/Q150}$ animals, when compared to wild-type littermates, and the onset of this neurological irregularity seems to display an apparent gene dosage effect, as *Hdh*$^{Q150/+}$ and *Hdh*$^{Q150/Q150}$ mice exhibited this deficit from 40 and 25 weeks of age, respectively (Lin et al., 2001). Clasping behaviour has also been identified in *Hdh*$^{Q150/Q150}$ animals as early as 20 weeks of age and was found to occur frequently from 70 weeks of age (Heng et al., 2007). Further evidence for the presence of a psychiatric phenotype in the HdhQ150 mouse model of HD comes from reports that *Hdh*$^{Q150/Q150}$ animals display reduced startle reactivity from 6M (Brooks et al., 2012b), reduced aggression, anxiety and irritability from 15M, and an absent fear response at 22M (Woodman et al., 2007).

Cognitive deficits have also been identified in the HdhQ150 mouse model of HD, with evidence of *Hdh*$^{Q150/Q150}$ mice requiring greater lengths of time to find the platform in the Morris water maze task from as early as 4M (Brooks et al., 2012b), which is suggestive of a spatial memory impairment that recapitulates aspects of the visuospatial memory deficiencies identified in human HD patients (Lange et al., 1995; Lawrence et al., 2000; Brandt et al., 2005; Majerova et al., 2012; Pirogovsky et al., 2015). *Hdh*$^{Q150/Q150}$ animals also presented with prolonged latencies in finding the reverse platform position in the Morris water maze task from 6M (Brooks et al., 2012b), which may be indicative of a reversal learning (RL) deficit similar to those seen in HD patients (Lange et al., 1995; Lawrence et al., 1999). A deficit in extra-dimensional set-shifting has also been demonstrated in homozygous HdhQ150 animals at 24M (Brooks et al., 2006), which is comparable to the extra-dimensional set-shifting cognitive deficit observed in the human disease (Lawrence et al., 1996).

In summary, a number of motor, psychiatric and cognitive phenotypes that can be argued to be analogous to those seen in human HD patients have been identified in the HdhQ150 mouse model of HD (Table 1.2). Motor phenotypes demonstrated include an abnormal gait, deficits in rotarod and balance beam performance, and a weakness in grip strength, while psychiatric disturbances include deficits in exploratory and startle activity, as well as reductions in aggression, anxiety and irritability, but increased clasping behaviour (Lin et al., 2001; Heng et al., 2007; Woodman et al., 2007; Brooks et al., 2012b). Cognitive assessment of the HdhQ150 mouse model of HD has not been as extensive as the motor and psychiatric evaluations, however there is evidence of disturbances in spatial memory and extra-dimensional set-shifting in *Hdh*$^{Q150/Q150}$ mice (Brooks et al., 2006; Brooks et al., 2012b).

1.3.2 Neuropathology

The neuropathological profile of each mouse model of HD is unique, however there are some common characteristics (summarised in Table 1.3). In N-terminal transgenic mice, the onset of neuropathological features occurs at a similar time to the manifestation of behavioural symptoms (Table 1.2 and Table 1.3). Neuropathology in full-length transgenic animal models, on the other hand, tends to arise at a later time point than behavioural phenotypes (Table 1.2 and Table 1.3). Similar to their behavioural phenotypes, the neuropathology in knock-in mouse models of HD is generally milder than that of their transgenic counterparts, and also occurs later in the mouse life time (Table 1.2 and Table 1.3).

	Neuronal cell loss & degeneration	Inclusions & aggregates	References
N-terminal transgenic models			
R6/1	↓ brain volume [8w]; ↓ striatal, cortical & hippocampal volume [17w]	Widespread HTT nuclear and extranuclear inclusion distribution [8w]	(Davies et al., 1997; Bayram-Weston et al., 2012a; Rattray et al., 2013)
R6/2	Cortical atrophy [3w]; ↓ brain weight [4w]; striatal, hippocampal & thalamic atrophy [5w]; ↓ brain volume [8.5w]; ↓ striatal volume & cell count [13w]; ↑ GFAP immunostaining [13w]	Cortical HTT NIIs [3.5w]; striatal HTT NIIs [4.5w]; cortical & striatal ubiquitin NIIs [5-6w]; widespread inclusion distribution [8w]	(Mangiarini et al., 1996; Davies et al., 1997; Naver et al., 2003; Stack et al., 2005; Aggarwal et al., 2012)
N171-82Q	Striatal, cortical, hippocampal, amygdalar atrophy [10w]; ↑ GFAP immunostaining [13w]; striatal atrophy [17w]; ↑ striatal & cortical neuron apoptosis [20w]	Widespread distribution of HTT & ubiquitin NIIs, & neuritic aggregates [12-18w]	(Schilling et al., 1999; Yu et al., 2003; Gardian et al., 2005; Cheng et al., 2011; Aggarwal et al., 2012)
Full-length transgenic models			
YAC72	Evidence of striatal degeneration [52w]	Nuclear translocation of N-terminal HTT fragments of striatal & cortical neurons [52w]	(Hodgson et al., 1999)
YAC128	↓ brain weight & striatal & cortical volumes [39w]; ↓ striatal cell count & size [52w]; ↑ cortical GFAP immunostaining [65w]	Nuclear translocation of N-terminal HTT fragments of striatal, cortical, hippocampal & cerebellar neurons [13w]; widespread NII distribution [65w]	(Slow et al., 2003; Van Raamsdonk et al., 2005a; Bayram-Weston et al., 2012b)
BACHD	Brain atrophy, ↓ striatal & cortical volume & presence of dark degenerating striatal & cortical neurons [52w]	Striatal & cortical mHTT aggregate accumulation [52w]	(Gray et al., 2008; Wang et al., 2014)
Knock-in models			
HdhQ72-80	10-15% ↓ brain weight [13w]	Striatal neuropil aggregate accumulation [52w]; striatal nuclear mHTT aggregate accumulation [91-117w]	(Shelbourne et al., 1999; Li et al., 2000; Li et al., 2001)
HdhQ92	↓ striatal volume & cell count [65-104w]	Striatal nuclear staining of HTT [11w]; striatal nuclear translocation of HTT [22w]; widespread NII distribution [43.5w]; striatal mHTT aggregate formation [52-65w]	(Wheeler et al., 2000; Bayram-Weston et al., 2012c)
HdhQ94	↓ striatal volume & striatal neuron density [78w]	Striatal nuclear staining & microaggregate distribution [17-26w]; mHTT NIIs [78w]	(Menalled et al., 2002)
HdhQ111	↑ GFAP immunostaining [104w]	Striatal nuclear staining of HTT [6w]; striatal nuclear translocation of HTT [22w]; striatal HTT aggregate formation [43.5w]; striatal NII formation [43.5-52w]; striatal neuropil aggregate formation [74w]	(Wheeler et al., 2000; Wheeler et al., 2002)

HdhQ140	Progressive ↓ MSN cell number [52w]; ↑ cortical GFAP immunostaining [52w]; ↓ brain weight, striatal volume & striatal cell count [87-113w]; ↑ striatal GFAP immunostaining [104w]	Progressive HTT nuclear inclusion distribution [4-26w]; progressive neuropil aggregate distribution [8-26w]	(Menalled et al., 2003; Hickey et al., 2008)
HdhQ150	↑ GFAP immunostaining [52-61w]; *Hdh*$^{Q150/Q150}$ striatal cell & volume loss [26-100w]; *Hdh*$^{Q150/+}$ striatal cell loss [100w]	Striatal HTT NIIs [21-26w]; striatal ubiquitin NIIs [45w]; widespread distribution of NIIs and neuropil aggregates [52-70w]	(Lin et al., 2001; Yu et al., 2003; Tallaksen-Greene et al., 2005; Heng et al., 2007; Woodman et al., 2007; Bayram-Weston et al., 2012d)
HdhQ200	↑ autophagy markers in the brain [9w]; ↓ Purkinje cell count [50w]	Striatal & cortical cytoplasmic HTT aggregate distribution [9w]; striatal & cortical HTT NII distribution [20w]	(Heng et al., 2010; Dougherty et al., 2013)
zQ175	↓ brain, striatal & cortical volumes [13w]; ↓ striatal neuron number [19.5w]; impaired myelination of striatal neurons [52w]	Striatal diffuse nuclear staining [13w]; progressive striatal HTT NII distribution [17w]; progressive cortical HTT NII distribution & diffuse nuclear staining [35w]	(Heikkinen et al., 2012; Carty et al., 2015; Ma et al., 2015)
Inducible models			
HD94	↓ brain & striatal volume [18w]; ↑ striatal GFAP immunostaining [18w]; striatal cell loss [74w]	Striatal HTT & ubiquitin intranuclear staining & cytoplasmic aggregates [12w]	(Yamamoto et al., 2000; Martin-Aparicio et al., 2001; Diaz-Hernandez et al., 2005)
Inducible-148Q	Mild brain atrophy, ventricular enlargement & ↑ striatal & cortical GFAP immunostaining [43.5w]	Striatal, cortical, hippocampal & cerebellar HTT NIIs [43.5w]; Striatal & cortical cytoplasmic aggregates [43.5w]	(Tanaka et al., 2006)

Table 1.3. Neuropathological phenotypes of the most widely used mouse models of HD. GFAP = glial fibrillary acid protein; n.r. = not reported; w = weeks of age; ↑ = increase in; ↓ = decrease in.

1.3.2.1 The HdhQ150 knock-in mouse model of HD

Neuroanatomical analyses of post-mortem HdhQ150 mice have revealed a number of neuropathologies consistent with those found in human HD cases (Table 1.3). As discussed in Chapter 1.1.2.1, loss of striatal volume and neuron number are pathological traits in HD (Lange et al., 1976; Vonsattel et al., 1985; Aylward et al., 1997; Vonsattel and DiFiglia, 1998; Aylward et al., 2004; Tabrizi et al., 2009; Tabrizi et al., 2011; Tabrizi et al., 2012; Tabrizi et al., 2013), and are recreated in HdhQ150 animals; evidence of striatal volume loss in $Hdh^{Q150/Q150}$ mice from 6M has been described (Bayram-Weston et al., 2012d), while this phenotype has been reported to have an onset later than 70 weeks of age in another study (Heng et al., 2007). Heterozygous HdhQ150 animals, on the other hand, do not display striatal volume loss up to 100 weeks of age but present with an approximate 60% decrease in striatal neuron number at this age (Heng et al., 2007). These alterations in striatal neuron number were found to occur after 70 weeks of age, which strengthens the findings of Lin and colleagues (2001) whereby $Hdh^{Q150/+}$ animals presented with healthy striatal volumes and neuronal numbers at 52 weeks of age. An increase in striatal glial fibrillary acidic protein (GFAP), a marker for reactive gliosis and thus nervous system damage, has also identified in $Hdh^{Q150/+}$ animals at 52-61 weeks of age (Lin et al., 2001; Yu et al., 2003). This suggests that striatal neuronal dysfunction may occur as early as 52-61 weeks of age in heterozygote animals but may not result in cell loss until after 70 weeks of age, as reported by Heng and colleagues (2007). The concurrent loss of striatal neuron number and volume in $Hdh^{Q150/Q150}$ mice at 100 weeks of age (Heng et al., 2007) suggests that this genotype undergoes extensive striatal neurodegeneration. While striatal volume loss was not observed in $Hdh^{Q150/+}$ animals at this time point, a large reduction in striatal neuron number was apparent, which indicates that heterozygote HdhQ150 mice experience striatal neurodegeneration that is less extensive than their homozygote counterparts.

The presence of NIIs is also recapitulated in HdhQ150 mice, with HTT immunoreactive NIIs being identified in the striatum of homozygote mutants as early as 21 and 26 weeks of age (Woodman et al., 2007; Bayram-Weston et al., 2012d). By contrast, these NIIs have been observed in $Hdh^{Q150/+}$ mice from 40-42 weeks of age, while ubiquitin immunoreactive NIIs are detected in approximately 10% of striatal neurons by 45 weeks of age (Tallaksen-Greene et al., 2005). These HTT- and ubiquitin-containing NIIs become more widespread as time progresses (Tallaksen-Greene et al., 2005; Woodman et al., 2007; Bayram-Weston et al., 2012d), being observed throughout the dorsal striatum, nucleus accumbens and the rostral part of the piriform cortex at 52 weeks of age in $Hdh^{Q150/+}$ mice, and are also present with less frequency in layers III and IV of the somatosensory cortex, the pyramidal cell layer of the hippocampus, and the neurons of the deep nuclei and granular cell layer of the cerebellar cortex (Lin et al., 2001). At approximately

65 weeks of age, HTT immunoreactive NIIs are present throughout the brain in *Hdh*$^{Q150/Q150}$ mice (Bayram-Weston et al., 2012d) and appear in approximately 40% and 65% of striatal neurons in *Hdh*$^{Q150/+}$ and *Hdh*$^{Q150/Q150}$ animals, respectively, by 70 weeks of age, while ubiquitin immunoreactive NIIs are found throughout the striatum by 72 weeks of age (Tallaksen-Greene et al., 2005). At very late stages in life (107 weeks of age), as many as 90% of striatal neurons and 85% of layer IV primary somatosensory cortical neurons contain NIIs in heterozygous HdhQ150 animals (Tallaksen-Greene et al., 2005). Tallaksen-Green *et al.* (2005) report that HTT immunoreactive NIIs are more frequently identified than ubiquitin immunoreactive inclusions in the HdhQ150 mouse model of HD, and recently this same finding has also been reported in *Hdh*$^{Q150/Q150}$ animals (Bayram-Weston et al., 2015), and the higher frequency of NIIs identified in homozygote HdhQ150 mutant animals, when compared to heterozygote littermates, is indicative of a gene dosage effect existing in this neuropathological feature. At 22M, *Hdh*$^{Q150/Q150}$ mice also present with polyglutamine aggregate pathology, in the form of nuclear inclusions, in a wide range of peripheral tissues that include heart, liver and skeletal tissue (Moffitt et al., 2009). It is not known whether this peripheral pathology is a pathological feature of the human disease, as peripheral organs are not typically collected from post-mortem HD patients and therefore are not available for analysis. Despite this, HTT inclusions have been identified in human HD myotubules (Ciammola et al., 2006) but not in myoblasts, fibroblasts, or lymphoblasts (Sawa et al., 1999; Sathasivam et al., 2001; Ciammola et al., 2006), which does not elucidate whether peripheral polyglutamine aggregate pathology is a feature of the human condition.

As well as displaying neuronal loss and the presence of NIIs, HdhQ150 animals also show diminished levels of striatal dopamine D_1 and D_2 receptor (DRD1 and DRD2, respectively) binding sites from 70 weeks of age, which further decline at 100 weeks (Heng et al., 2007). These age-dependent decreases in striatal receptor binding sites were found to be more pronounced in *Hdh*$^{Q150/Q150}$ mice than in their *Hdh*$^{Q150/+}$ counterparts, suggesting a gene dosage effect is present in the apparent deficit in dopamine transmission and signalling. This conclusion is also supported by evidence that homozygote animals exhibited a significant reduction in striatal dopamine transporter binding sites at 100 weeks of age, while no such deficit was present in heterozygote mice.

To summarise, the HdhQ150 mouse model of HD exhibits a number of neuropathological features comparable to those seen in the human disease (Table 1.3). These phenotypes have been found to be age-dependent and include a reduction in striatal cell number and dopamine receptor binding sites, the formation of HTT and ubiquitin immunoreactive NIIs throughout the brain, and an increase in reactive gliosis (Lin et al., 2001; Tallaksen-Greene et al., 2005; Heng et al., 2007; Woodman et al., 2007; Bayram-Weston et al., 2012d; Bayram-Weston et al., 2015).

The severity of neuropathology seems to be gene dosage dependent in HdhQ150 animals, with evidence of homozygote mutant mice presenting with earlier and more severe phenotypes than their heterozygote counterparts (Tallaksen-Greene et al., 2005; Heng et al., 2007).

1.5 Gene expression in HD

There are a number of potential mechanisms of HD pathogenesis, which include vulnerability of striatal MSNs to mitochondrial dysfunction (Squitieri et al., 2006; Orr et al., 2008; Kim et al., 2010; Shirendeb et al., 2011; Shirendeb et al., 2012; Damiano et al., 2013), excitotoxicity (Leavitt et al., 2006; Fernandes et al., 2007; Heng et al., 2009; Botelho et al., 2014), and the possible toxicity of mHTT NIIs and aggregates (Davies et al., 1997; DiFiglia et al., 1997; Ordway et al., 1997; Legleiter et al., 2010; Tsvetkov et al., 2013). Aberrant transcriptional regulation has also been identified as an integral process in the manifestation of HD (Cha, 2000; Luthi-Carter and Cha, 2003; Cha, 2007; Bowles et al., 2012; Seredenina and Luthi-Carter, 2012; Kumar et al., 2014; Valor, 2015), and is detectable in pre-symptomatic patients (Dunah et al., 2002; Borovecki et al., 2005; Mastrokolias et al., 2015) and mouse models of the disease (Cha et al., 1998; Carnemolla et al., 2009; Becanovic et al., 2010).

The striatal transcriptional profiles of multiple mouse models of HD have been found to recapitulate many features of the transcriptional dysregulation observed in the caudate of post-mortem human HD patients, despite variation in CAG length, and *HTT* gene dosage and expression context between each model (Hodges et al., 2006; Kuhn et al., 2007). Striatal transcriptional profiles of human HD patients can only be determined for the end-stage of the disease because sample collection is only possible from post-mortem caudate. However, the use of cell and mouse models of the disease allows longitudinal characterisation of transcriptional dysregulation in HD, and the assessment of potentially disease-modifying factors, because of the similarity of gene expression level changes detected in human HD caudate and such models. The genes exhibiting reproducible changes in expression between HD populations and models are associated with a number of biological pathways that include neurotransmitter, neurotrophin and G-protein receptor signalling, as well as energy and lipid metabolism, transcription and chromatin remodelling (Luthi-Carter et al., 2000; Hodges et al., 2006; Kuhn et al., 2007; Seredenina and Luthi-Carter, 2012).

Decreased gene expression levels are consistently observed in models of HD, particularly in early stages of the disease (Luthi-Carter et al., 2000; Sipione et al., 2002; Kuhn et al., 2007), and is generally associated with genes involved in neurotransmitter receptor functions, synaptic

transmission, signalling pathways, neuropeptides, and calcium binding and homeostasis, while upregulated genes are largely associated with genes related to stress markers, ribonucleic acid (RNA) metabolism, and protein folding (Valor, 2015). One mechanism by which these transcriptional discrepancies are believe to arise is through loss of REST/NRSF mediated gene expression regulation (Zuccato et al., 2007; Buckley et al., 2010; Soldati et al., 2013). The pattern of transcriptional alterations in response to mHTT is likely to be complex and may not be stable over time, with evidence that striatal gene expression level profiles are altered following aggregate formation in a cell model of the disease (van Roon-Mom et al., 2008) and across the lifespan of HD mouse models (Kuhn et al., 2007; Giles et al., 2012; Bayram-Weston et al., 2015). It is important to note, however, that the dysregulation of gene expression observed is unlikely to be entirely caused by direct mHTT interference with transcriptional machinery because autocompensatory mechanisms designed to counter mHTT toxicity are likely to be present in the transcriptional profiles of HD and HD models, and the regulation of these genes may not necessarily be modulated by mHTT (Seredenina and Luthi-Carter, 2012). An example of an autocompensatory mechanism of transcriptional dysregulation in HD comes from evidence that the forkhead class O transcription factor forkhead box O3 (FOXO3a), which is activated by decreased survival signalling and/or increased cellular stress, regulates its own transcription by binding to the conserved response element in the *Foxo3a* promoter, and that levels of FOXO3a are elevated in HD (Kannike et al., 2014).

1.6 Cognition

Cognition has been defined as the mental action or process of acquiring knowledge and understanding through thought, experience, and the senses, and is a highly complex phenomenon that has received extensive research into its neural and molecular foundations.

1.6.1 Neural networks underpinning cognition

The use of neuroimaging techniques that provide high spatial resolution, such as PET, MRI and fMRI, has allowed the identification of neural networks involved in different aspects of cognition, with these complex networks comprising a number of distinct structures and regions. Cognitive ability, or intelligence, has previously been defined as "… a very general capacity that, among other things, involves the ability to reason, plan, solve problems, think abstractly, comprehend complex ideas, learn quickly, and learn from experience" (Gottfredson, 1997), which highlights the multitude of processes that encompass cognition. The parietofrontal

integration theory of intelligence emphasises the differing roles of distinct neural structures and pathways in the varied aspects of cognition. In this theory, the extrastriate cortex and fusiform gyrus are believed to be involved in intelligent behaviour because of their contribution to recognition, imagery, and elaboration of visual input (Brancucci, 2012). The parietal cortex and angular gyrus then process information captured through these pathways and potentially produce structural symbolism, abstraction, and elaboration, before frontal cortical areas interact with these parietal areas and establish a working memory network that compares different potential task responses (Brancucci, 2012). Finally, the anterior cingulate cortex intervenes, once a task response is selected, and supports response engagement and the inhibition of alternative responses (Brancucci, 2012). A review of all the neural pathways associated with cognition will not be undertaken here, instead the overlapping neural structures and pathways pertinent to both cognition and HD, as well as the experiments of this thesis, will be discussed.

The striatum forms part of a number of functional subcortico-thalamo-cortical loops, where it receives afferent projections from the dorsolateral prefrontal cortex (PFC), lateral orbitofrontal cortex, supplementary motor area, frontal eye fields and anterior cingulate areas of the cortex, amongst others, while projecting efferent neurons to a number of regions within the pallidum and substantia nigra, which then project to different areas of the thalamus, which closes the loops by projecting to the aforementioned cortical regions (Alexander et al., 1986; DeLong and Wichmann, 2007; Leh et al., 2007). Specific frontostriatal interconnections have also been identified between the striatum and the primary and premotor areas as well as the inferior and middle temporal gyrus, and striato-cerebellar interconnections are also present (Leh et al., 2007). With such diverse and extensive neural connectivity, it is unsurprising that the striatum is utilised in a variety of cognitive processes. One such cognitive function is RL, which has been found to produce co-activation of the striatum and frontal cortex in human fMRI studies (Cools et al., 2002; Kringelbach and Rolls, 2003; Hampton and O'Doherty, 2007; Ghahremani et al., 2010; Ruge and Wolfensteller, 2016) and is impaired in individuals with lesions to either region (Hornak et al., 2004; Bellebaum et al., 2008). Taken together, this evidence suggests that the frontostriatal circuitry governs RL, and thus cognitive flexibility, and this suggestion is supported by numerous lesion studies in rodents and non-human primates (Roberts et al., 1990; Roberts et al., 1992; Featherstone and McDonald, 2005; Kim and Ragozzino, 2005; Ragozzino, 2007; Brigman and Rothblat, 2008; Clarke et al., 2008; McDonald et al., 2008b; Castane et al., 2010).

Similar to the frontostriatal involvement in RL, this neural circuit has also been linked with set-shifting, which is an aspect of cognitive flexibility defined as the ability to change attention from one response set to another according to the changing goals of a task. Significant increases in

activity in the PFC and striatum have been identified in individuals performing the Wisconsin Card Sorting Task during inhibition of previously acquired stimulus-response rules and acquisition of new stimulus-response associations (Monchi et al., 2001; Lie et al., 2006). These studies suggest that the frontostriatal neural network is activated, and necessary for, the switching from one rule to another following an incorrect pairing in the Wisconsin Card Sorting Task. This is supported by evidence that striatal dopamine is released during the planning and execution of a set-shift (Monchi et al., 2006) and that using transcranial magnetic stimulation to deliver continual theta burst impulses at the left dorsolateral PFC interferes with striatal dopamine release and impairs set-shifting performance (Ko et al., 2008). Further examination of the role of frontostriatal connectivity in set-shifting has revealed that striatal activity is required until a rule is used continuously for several trials (Provost et al., 2012), suggesting that the striatum is necessary for the acquisition of a new rule in set-shifting. A similar role for the striatum has also been reported in a variety of learning paradigms, with PET and fMRI studies providing evidence for the involvement of a cortical-striatal-hippocampal network during rule learning (Poldrack et al., 1999; Toni and Passingham, 1999; Seger and Cincotta, 2005; Seger and Cincotta, 2006).

Working memory is another aspect of cognition that is believed to utilise the frontostriatal circuitry, with evidence that functional connectivity between the PFC and the striatum is increased during the performance of working memory tasks (Hampson et al., 2006) and that striatal dopamine signalling capacities are associated with working memory ability (Cools et al., 2008; Landau et al., 2009; Liang et al., 2016). Striatal lesions in rodents have also been found to significantly impair spatial working memory performance (Mair et al., 2002), which suggests that the striatum may also form part of a neural network responsible for aspects of spatial memory, a form of memory that is known to be dependent upon hippocampal activity (Morris, 1981; Silva et al., 1992b; Moser et al., 1995; Tsien et al., 1996; Maguire et al., 1997; Maguire et al., 2000; Burgess et al., 2002; Ekstrom et al., 2003). Reports of a cortical-striatal-hippocampal network necessary for novel exploratory goal-directed navigation (Floresco et al., 1997) and disrupted spatial learning in striatum lesioned mice (Pooters et al., 2016) act to support evidence for a striatal role in spatial memory.

In summary, there is substantial evidence for the necessity of neural networks in many forms of cognition, with the striatum forming part of frontostriatal and cortical-striatal-hippocampal networks associated with cognitive flexibility, in the form of rule learning, set-shifting and RL, as well as working and spatial memory. There are also a wealth of studies implicating the striatum in cognitive processes that include motivation (Kimura et al., 2003; Aarts et al., 2011; Ena et al., 2011; Shohamy, 2011), habit formation (Yin et al., 2004; Yin and Knowlton, 2006; Tang et al.,

2007; Grahn et al., 2008; Tricomi et al., 2009) and implicit learning (Rauch et al., 1997; Schendan et al., 2003; Aizenstein et al., 2004; Destrebecqz et al., 2005; Reiss et al., 2005; Bennett et al., 2011; Gheysen et al., 2011), which have not been discussed in this body of work thus far. Similarly, a range of neural structures have been associated with multiple aspects of cognition, with evidence that the hippocampus is integral to spatial memory, as previously highlighted, as well as memory formation (Scoville and Milner, 1957; Zolamorgan et al., 1986; Squire and Zolamorgan, 1991; Squire, 1992; Bliss and Collingridge, 1993; Gould et al., 1999; Shors et al., 2001) while the amygdala is known to be involved in emotion and fear memory (Davis, 1992; Phillips and Ledoux, 1992; Adolphs et al., 1994; LeDoux, 2000; Davis and Whalen, 2001; Phan et al., 2002). Thus, cognition is a diverse and complex phenomenon that requires interactions between multiple brain structures that utilise a number of connective pathways and neural networks. Furthermore, the neural structure most extensively degenerated in HD, the striatum, is associated with various aspects of cognitive function and ability.

1.6.1.1 Neural networks underpinning instrumental learning in rodents

As discussed in Chapter 1.6.1, a number of neural networks within the brain form the foundations required for different aspects of learning and memory in humans. Similar interactions between distinct neural structures and regions are necessary to a number of behavioural tasks utilised in rodent studies of cognition, some of which will be discussed here.

The continuous reinforcement (CRF) schedule for food reinforcement consists of rodents having to lever-press or “nose-poke” (np) only once in response to a stimulus in order to receive a reward, and is an important reinforcement condition in which, when food is the reward, there is primary positive reinforcement involving a natural reinforcer. The striatum has been found to be the key neural structure associated with CRF performance in rodents, with evidence that lever pressing on a CRF schedule is accompanied by increases in rat nucleus accumbens dopamine release, and that these increases in nucleus accumbens dopamine release are highly correlated with the number of responses performed (McCullough et al., 1993). This evidence suggests that a major function of dopaminergic transmission in the nucleus accumbens, and thus the striatum, may be to facilitate the instigation, or acquisition, of some forms of instrumental behaviour. This suggestion is also supported by evidence that increases in nucleus accumbens dopamine release occur during instrumental lever pressing for a food reward but not during increased levels of free food consumption (Salamone et al., 1994). Further support for the role of striatal involvement in instrumental learning comes from evidence that depleting dopamine levels in the nucleus accumbens via 6-hydroxydopamine injection produces a change in

instrumental behaviour (Cousins et al., 1993). Specifically, healthy rats would readily lever-press to receive a preferred food reward rather than consume a readily-available, but less preferred, food (lab chow), whereas nucleus accumbens dopamine depleted rats would display a significant decrease in lever pressing and instead increase their consumption of lab chow. The regional specificity of this behavioural effect of dopaminergic depletion within the nucleus accumbens on lever pressing was later found be a result of depletion within the nucleus accumbens core, as rats receiving 6-hydroxydopamine injection into the nucleus accumbens core showed the aforementioned behavioural phenotype while those with dopamine depletion in the nucleus accumbens shell displayed no such phenotype (Sokolowski and Salamone, 1998). Similarly, injection of the dopamine receptor antagonist haloperidol into the rat striatum has also been found to produce a dose-dependent decrease in the number of responses made in an instrumental learning task (Salamone et al., 1993). Dissociable roles of distinct subregions within the striatum during instrumental learning were also identified by Cousins and colleagues (1993), similar to those identified by Sokolowski and Salamone (1998), where dopamine depletion within the medial striatum had no effect on lever pressing or lab chow consumption. Conversely, ventrolateral striatal dopamine depletion decreased lever pressing but also tended to reduce the consumption of lab chow, with these rats also showing profound deficits in home-cage feeding to the extent that they required wet mash or tube feeding to maintain body weight. These studies identify the rodent striatum, and more specifically the core of the nucleus accumbens, as being integral to instrumental learning of food-rewarded behavioural operant tasks such as the CRF task, while there is a wealth of evidence that implicates the striatum and nucleus accumbens with also playing a key role in the acquisition of drug-seeking behaviour (Ito et al., 2002; Ito et al., 2004; Crespo et al., 2006; Neumaier et al., 2009; Murray et al., 2012).

The five-choice serial reaction time task (5-CSRTT) is a behavioural task used to assess visuospatial attentional and impulsivity processes in rodents (Harrison et al., 1997; Robbins, 2002; Christakou et al., 2004; Fletcher et al., 2007; Sanchez-Roige et al., 2012; Jupp et al., 2013). Lesion studies have found the 5-CSRTT to be dependent upon multiple neural structures, with deficits in the task identified following lesioning or inactivation of the cortex (Muir et al., 1996), striatum (Rogers et al., 2001), subthalamic nucleus (Baunez and Robbins, 1999), dorsal globus pallidus (Robbins et al., 1989), and pedunculopine nucleus (Inglis et al., 2001), but not the hippocampus (Kirkby and Higgins, 1998). A critical dependence of the 5-CSRTT on corticostriatal circuitry is suggested by evidence that performance in the task is impaired following quinolinic acid lesions to the medial striatum, medial PFC, peri- and post-genual anterior cingulate cortex, and parietal cortex (Muir et al., 1996; Rogers et al., 2001). Similar 5-CSRTT performance deficits have been identified by pharmacological antagonism of monoaminergic systems within the

striatum or PFC (Fletcher et al., 2007; Pezze et al., 2007; Agnoli and Carli, 2012; Agnoli et al., 2013), which builds on evidence that alterations in these corticostriatal monoaminergic systems underlie poor choice accuracy and impulsivity in rats (Puumala and Sirvio, 1998; Jupp et al., 2013). Dissociable roles of distinct regions of the striatum and cortex in instrumental learning of the 5-CSRTT have also been discovered. For example, lesioning the medial striatum of rats causes significant deficits on response accuracy, lengthened response latencies, and increases in both premature and perseverative responding, while lateral striatal lesions produce a profound inability to perform the 5-CSRTT, with very few trials being performed by lesioned animals despite an absence of motor or motivational deficits (Rogers et al., 2001). Thus, this study suggests that the medial and lateral striatum are implicated in, or responsible for, different aspects of continuous visuospatial attentional task performance. Similarly, increasing the attentional load in the 5-CSRTT, by shortening stimulus presentation and presenting white noise immediately prior to the visual target, has been found to produce significantly lengthened response latencies in medial PFC lesioned and antero-dorsal lesioned animals (Muir et al., 1996). This suggests that these animals traded speed for accuracy in the task, and thus that the medial PFC and antero-dorsal cortex may play roles in assisting impulsivity. The idea that accuracy and impulsivity in the 5-CSRTT can be functionally dissociated is further supported by reports from the same study whereby rats with anterior cingulate cortex lesions displayed significant increases in impulsive responding, suggesting that the anterior cingulate cortex may play a role in suppressing impulsivity, which is different to the potential role identified for the medial PFC and antero-dorsal cortex. The evidence described here highlights how the neural processes of attention and impulsivity in rodents, as examined by the 5-CSRTT, require a number of structures within the brain, and that there is a clear necessity of functional corticostriatal circuitry signalling for these cognitive domains.

The serial implicit learning task (SILT) is a “9-hole” operant box based task developed to allow investigation into rodent visuospatial attentional domains and implicit learning, consisting of a predictable light sequence being embedded amongst other unpredictable light sequences (see Chapter 3.2.4.4 for full description of SILT methodology). Implicit learning is demonstrated in the task by animals responding with greater accuracy and/or speed to the predictable sequence than to unpredictable sequences. Neuroimaging studies in humans have identified a striatal and cortical basis of implicit learning (Rauch et al., 1997; Honda et al., 1998; Schendan et al., 2003; Aizenstein et al., 2004; Bennett et al., 2011), while there is also evidence of wider neural pathway involvement in this form of cognition (Schendan et al., 2003; Gheysen et al., 2010; Schendan et al., 2013). Based upon these findings, implicit learning in the SILT in rodents was hypothesised to have a corticostriatal basis, however, quinolinic acid lesions to the striatum or

to the premotor or supplementary motor areas of the cortex do not produce implicit learning deficits in the SILT, despite generating general deficits in performance of the task (Trueman et al., 2005; Brooks et al., 2007; Jay and Dunnett, 2007; Brooks and Dunnett, 2009). These studies suggest that implicit learning in rodents, as examined in the SILT, differs from implicit learning in humans because it appears to be independent of the corticostriatal circuitry. The neural basis for the utilisation of predictable information in the SILT has, at this moment in time, not yet been elucidated.

The neural structure integral to spatial learning and memory, as briefly discussed in Chapter 1.6.1, is the hippocampus, with a vast number of studies reporting deficits in spatial learning and memory tasks such as the Morris water maze, T-maze, Y-maze, and contextual fear conditioning following hippocampal lesions or disruption of hippocampal transcriptional regulation in rodents (Grant et al., 1992; McHugh et al., 1996; Tsien et al., 1996; Guzowski and McGaugh, 1997; Frankland et al., 1998; Guzowski et al., 2000; Pittenger et al., 2002; Saxe et al., 2006; Dupret et al., 2008; Sekeres et al., 2010). It is not only the hippocampus that plays a role in spatial learning and memory, however, as there is evidence that lesions of the rat dorsomedial or dorsolateral striatum are capable of disrupting spatial discrimination (SD) acquisition (McDonald et al., 2006; McDonald et al., 2008a), while combined lesions of cortices areas are capable of impairing contextual discrimination (Burwell et al., 2004). Thus, it seems that the neural basis of SD may involve the recruitment of a cortical-striatal-hippocampal pathway, as briefly discussed in Chapter 1.6.1.

Cognitive flexibility requires the ability to learn new rules and to be able to utilise these in place of previously learnt rules when the situation requires it. Reversal learning (RL), therefore, is an integral aspect of cognitive flexibility and has received investigation into its neural basis in humans, non-human primates, and rodents. Strong evidence of corticostriatal involvement in RL has been reported from such studies, with lesions to the PFC producing deficits in this form of cognition in non-human primates (Roberts et al., 1990; Roberts et al., 1992) and rodents (Kim and Ragozzino, 2005; Ragozzino, 2007; Brigman and Rothblat, 2008), while lesioning the dorsomedial striatum has also caused RL deficits in non-human primates (Clarke et al., 2008) and rats (Ragozzino, 2007; McDonald et al., 2008b; Castane et al., 2010). A large volume of molecular data also supports the presence of rodent RL being dependent upon corticostriatal circuitry. For example, selective striatal inactivation of adenosine A_{2A} receptors (A2ARs) is capable of enhancing RL capabilities (Wei et al., 2011), while *dopamine receptor D2* (*Drd2*) expression levels in the PFC have been found to inversely correlate with RL ability (Laughlin et al., 2011), and inactivation of *Drd2*, or antagonism of its protein (DRD2), in the striatum impairs RL ability (Kruzich et al., 2006; Desteno and Schmauss, 2009). *In vivo* electrophysiological

recordings have also identified corticostriatal circuits in the mouse that govern RL in an operant visual discrimination task, where the dorsal striatum is increasingly activated with the original choice learning, whereas prefrontal regions show increased activation during reversal of the learned choice (Brigman et al., 2013). Brigman and colleagues (2013) also found that corticostriatal or striatal deletion of the *Grin2b* gene, which encodes the N-Methyl-D-aspartate (NMDA)-type receptor subunit GluN2B, or dorsal striatum-restricted antagonism of GluN2B, impaired original choice learning, while cortical *Grin2b* deletion or orbitofrontal cortex GluN2B antagonism both impaired RL capabilities. Taken together, these studies identify the corticostriatal circuitry as the key neural pathway responsible for RL in non-human primates and rodents.

1.6.2 The role of gene expression changes in cognition

Investigations into the molecular mechanisms of cognition have demonstrated the importance of multiple signalling cascades in learning and memory storage that are dependent upon regulation of gene expression. Gene knockout studies in rodents have been particularly useful in evaluating the behavioural outcome resulting from altered transcription of genes of interest. *CREB* is one of a number of genes that has been found to be associated with memory, through such gene knockout studies. Development of a *Creb* knockout mouse, which lacks two isoforms of the CREB protein (Hummler et al., 1994), generated evidence that the CREB-cAMP response elements (CRE) signalling pathway is required for hippocampus-dependent long-term memory; *Creb* knockout mice exhibit deficits in contextual fear conditioning, cued fear conditioning, spatial memory and the social transmission of food preferences when tested 24 hours, but not 30 minutes, after training (Bourtchuladze et al., 1994). CREB-CRE signalling is induced by long-term potentiation in the hippocampus (Impey et al., 1996) and during contextual learning (Impey et al., 1998), which strengthens the suggestion that this signalling cascade plays a role in learning and memory. CREB is also known to be necessary for both amygdala- (Josselyn et al., 2004) and striatum-dependent forms of memory (Pittenger et al., 2006; Brightwell et al., 2008), suggesting that the CREB-CRE signalling pathway has diverse learning and memory functionality. CREB binding protein (CBP), a transcriptional co-activator of CREB (Kwok et al., 1994), has also been heavily implicated in learning and memory, with evidence that reduced *Cbp* transcription and CBP function results in a variety of learning and memory deficits (Alarcon et al., 2004; Wood et al., 2005) as well as transcriptional dysregulation of CREB target genes (Wood et al., 2006; Barrett et al., 2011). Taken together, these studies reveal that regulation of gene expression in

the CREB-CRE signalling cascade is integral for various aspects of learning and memory, and that disruption of this signalling pathway can be detrimental to cognition.

As previously mentioned, the transcription of a number of genes have been associated with cognitive processes, which strengthens the theory that gene expression is an important mediator of cognition. Another example is BDNF signalling, which has been linked to learning and memory due to evidence that hippocampal *BDNF* mRNA expression is induced during spatial learning (Hall et al., 2000; Mizuno et al., 2000) while animals with disrupted BDNF signalling display impairments in spatial learning (Linnarsson et al., 1997; Mizuno et al., 2000) and memory consolidation (Lee et al., 2004). An effect of impaired BDNF signalling on cognition has also been reported in humans, where individuals with a mutation in *BDNF* that causes decreased BDNF secretion present with abnormal hippocampal activation and episodic memory deficits (Egan et al., 2003). The extracellular-signal regulated kinase (ERK)/mitogen-activated protein kinase (MAPK) signalling cascade provides further evidence for the importance of gene expression in cognition. ERK is activated in the hippocampus during associative learning and is necessary for contextual fear conditioning and spatial learning (Atkins et al., 1998), whilst dysregulation of the ras/ERK pathway is associated with learning and memory deficits similar to those observed in neurofibromatosis type 1 (Silva et al., 1997; Costa et al., 2002), a disorder characterised by the presence of cognitive deficits (North, 2000; Hyman et al., 2005; Champion et al., 2014). Learning and memory abnormalities have also been identified as a result of transcriptional dysregulation of genes that encode protein kinase A (Abel et al., 1997), DRD2 (Desteno and Schmauss, 2009; Jocham et al., 2009), methyl-CpG-binding protein 2 (Collins et al., 2004; Na et al., 2012), ephrin-A2 (Arnall et al., 2010), neuronal nitric oxide synthase (Kelley et al., 2009), metabotropic glutamate receptor 1 (Aiba et al., 1994a; Aiba et al., 1994b), neurabin (Wu et al., 2008), and transcription factor 4 (Sweatt, 2013), as well as numerous other proteins.

To summarise, transcriptional regulation of a variety of genes has been shown to be instrumental in multiple cognitive processes. Thus, cognition is dependent upon healthy regulation of gene expression, and transcriptional disruption has been found to result in diverse learning and memory deficits.

1.6.2.1 Altered cognition and gene expression levels in HD patients

As noted in Chapter 1.5, neural transcriptional profiles of human HD patients are most frequently determined for the end-stage of the disease because sample collection is only possible from post-mortem tissue, which makes correlating human HD cognitive deficits (see

Chapter 1.1.1.2) with gene expression abnormalities very difficult. That being said, there is evidence that post-mortem neural tissue from HD patients exhibits abnormal expression levels of a number of genes known to be involved in cognitive processes while vast transcriptional differences have also been identified in areas of the brain associated with cognition (Hodges et al., 2006). For example, a reduction in cortical BDNF mRNA and protein levels has been found in post-mortem HD patients (Zuccato et al., 2001; Zuccato et al., 2008), and blood serum BDNF levels are also reduced in living HD sufferers (Ciammola et al., 2007). Reductions in striatal expression of CREB signalling genes have also been identified in post-mortem HD brains (Cui et al., 2006; Chaturvedi et al., 2012; Jeong et al., 2012), as have similar reductions in mRNA expression levels of cannabinoid receptor 1 (Hodges et al., 2006), DRD1 (Augood et al., 1997; Hodges et al., 2006), and DRD2 (Augood et al., 1997). Although transcriptional dysregulation of these genes in human HD patients has not been directly attributed to the deficits in cognition seen in the disease, it is speculated that such changes in gene expression and gene expression levels may result in said cognitive deficits because of the roles that transcription can play in learning and memory, and thus cognition (see Chapter 1.6.2).

1.6.2.2 Altered cognition and gene expression levels in mouse models of HD

The various mouse models of HD present with an array of cognitive phenotypes (Table 1.2), some of which have been found to correlate with transcriptional abnormalities. An example of such a correlation is shown where R6/1 mice display decreased exploration activity in a novel object recognition task, and this behavioural phenotype was found to correlate with altered expression of genes associated with cell signalling and ion channels (Hodges et al., 2008). The severity of cognitive deficits in R6/1 mice has also been found to be modulated by BDNF signalling, with cognitive impairment occurring earlier in the lifespan of BDNF diminished R6/1 animals than in unaltered R6/1 mutant mice (Giralt et al., 2009). Giralt and colleagues (2009) also discovered that downstream BDNF signalling was impaired in the hippocampus of R6/1 animals and BDNF diminished R6/1 counterparts, suggesting that dysregulation of this BDNF cascade is involved in the learning impairment seen in R6/1 mice. BDNF signalling has been further implicated in cognitive dysfunction in HD mouse models from evidence that delivery of BDNF is capable of ameliorating long-term memory deficits in HdhQ140 mice (Simmons et al., 2009).

In the R6/2 mouse model, a combination drug therapy has been found to improve spatial navigation and RL deficits while also reversing a number of gene expression irregularities, which suggests that the drug-induced improvement in cognitive function may be mediated by the

changes to the transcriptional profile (Morton et al., 2005). Long-term spatial and recognition memory impairments have been associated with reduced hippocampal expression of CBP and decreased levels of histone H3 acetylation in heterozygous HdhQ111 mice (Giralt et al., 2012). Interestingly, there was also a significant reduction in the expression levels of CREB/CBP target genes that are related to memory, such as *activity-regulated cytoskeleton-associated protein* (*Arc*) and *c-fos*, and these transcriptional abnormalities were rescued by administration of a histone acetylase inhibitor, as were the recognition memory impairments. This study suggests that cognitive dysfunction in HdhQ111 mice may be caused by disruption to CBP mediated transcriptional and epigenetic regulation.

Recent evidence suggests that overexpression of the *adenosine A2A receptor* (*Adora2A*) gene may contribute to learning and memory impairments seen in HD mouse models, as knockout of the *Adora2A* gene in R6/2 mice was found to prevent working memory deficits in the 8-arm radial maze task from 6 weeks of age (Li et al., 2015). Conversely, cognitive deficits in the R6/2 and zQ175 mouse models of HD have shown no improvement following depletion of the *tissue transglutaminase 2* gene, which is otherwise over-expressed in HD patients and mouse models, suggesting that the abnormal transcriptional regulation of this gene in HD is not responsible for the cognitive deficits observed (Menalled et al., 2014).

The studies discussed here provide evidence for a link between transcriptional dysregulation and cognitive impairment in a number of mouse models of HD, however further investigation is required to elucidate the molecular and behavioural interactions that exist across the diverse array of cognitive phenotypes found in each mouse model of the disease.

1.6.2.3 Altered cognition and transcription in the HdhQ150 mouse model of HD

Of the cognitive impairments identified in HdhQ150 animals (Table 1.2 and Chapter 1.3.1.1), only one has been associated with transcriptional changes in signalling pathways; decreased ability in a RL aspect of the Morris water maze in $Hdh^{Q150/Q150}$ mice was correlated with reduced expression of sets of histone genes related to chromatin regulation, including nucleosome assembly (Giles et al., 2012). Thus, it is likely that these chromatin-related changes impact learning and memory in HdhQ150 mice, contributing to the RL deficits observed. The initial spatial learning impairment reported in the Morris water maze task significantly correlated with abnormal expression levels in 90 genes, however the behavioural/gene expression level interaction failed to reach significance in subsequent pathway analyses. While there are multiple studies providing evidence that transcriptional changes can correlate with cognitive ability in

human HD patients and various mouse models of the disease (see Chapters 1.6.2.1 and 1.6.2.2), relatively little is known about such interactions in the HdhQ150 mouse model of HD, and further investigation into the transcriptional basis of cognition in this mouse model is required.

1.7 Aims of this work

The aims of the experimental work in this thesis were to assess cognitive capabilities in the heterozygous HdhQ150 mouse model of HD, to investigate gene expression level changes with respect to learning, and to investigate whether any potential behavioural dysfunction was correlated with an abnormal transcriptional profile within the striatum. *Hdh*$^{Q150/+}$ mice were chosen for examination because they provide a comparable representation of the genetic profile of HD patients, as the majority of sufferers are heterozygous for the mutant allele (Alonso et al., 2002), although the repeat length found in this mouse model is much greater than that found in human cases of the disease. Transcriptional changes were investigated in the striatum because this neural structure is the primary site of pathology in HD (Lange et al., 1976; Vonsattel et al., 1985; Vonsattel and DiFiglia, 1998), displays the greatest number and magnitude of differentially expressed mRNAs within the HD brain (Hodges et al., 2006), and is associated with various forms of cognition (see section 1.6.1).

The specific aims of this thesis were to:

- Assess implicit learning capabilities and striatal transcriptional profiles of *Hdh*$^{Q150/+}$ mice using the serial implicit learning task (SILT), and whether the degree of learning undertaken or the age of mice contribute to striatal gene expression levels (Chapter 3).
- Examine the striatal transcriptional profile of wild-type animals across distinct stages of an operant-based spatial discrimination (SD) and RL task as a means of identifying learning dependent gene expression level changes within the striatum (Chapter 4).
- Assess SD performance and striatal transcription levels of the target genes identified in Chapter 4, in *Hdh*$^{Q150/+}$ mice (Chapter 5).

Chapter 2: Methods

2.1 Animals

2.1.1 HdhQ150 line

HdhQ150 mice were bred in-house on the original 129/Ola x C57BL6/J background (Lin et al., 2001). HdhQ150 mice contain an expanded CAG tract of 150 repeats within the endogenous mouse *Htt* exon 1 sequence. This line was initially generated using a two-step gene targeting strategy utilising embryonic stem cells where the endogenous mouse *Htt* exon 1 sequence was replaced with an *hypoxanthine guanine phosphoribosyltransferase* (*Hprt*) minigene and the *Hprt* minigene then exchanged with a CAG-expanded mouse *Htt* exon 1 sequence (Lin et al., 2001). This method of construct generation results in the *Htt* exon 1 sequence 3' to the CAG tract encoding a mouse polyglutamine expanse, and an entirely mouse *Htt* locus, which is unlike the knock-in mice generated by Wheeler and MacDonald (Wheeler et al., 1999) or Zeitlin (Levine et al., 1999; Menalled et al., 2003) that encode a human exon 1 sequence within the mouse *Htt* locus. Timed mating was performed by pairing heterozygote HdhQ150 male and female animals at 4pm, and leaving overnight until 9am the next morning, when they were then separated and presence or absence of a vaginal plug was observed. Female $Hdh^{Q150/+}$ and $Hdh^{+/+}$ mice were used in experimental chapter 3; male $Hdh^{+/+}$ mice were used in experimental chapter 4; and male $Hdh^{Q150/+}$ and $Hdh^{+/+}$ mice were used in experimental chapter 5. The CAG repeat length of $Hdh^{Q150/+}$ mice tested across all experiments ranged between 117 and 164 repeats, with a mean of 140 CAG repeats (± 1.66).

2.1.2 Animal husbandry

Mice were housed in cages in cohorts not greater than five animals per cage in non-barrier conditions as per Home Office regulations. Environmental temperature was maintained at 21°C ± 1°C on a 12-h light/dark cycle with standard food (Harlan, Oxfordshire) and water freely accessible, except where described in experimental chapters. All animals were handled for a minimum of 1 week prior to behavioural training.

For water restriction, water accessibility was gradually decreased to 3 hours per day over a five-day period, with body weight monitored daily during this period. During testing, water access remained at 3 hours a day and was provided at a regular time after each task session. Body weight was recorded at weekly intervals during testing, ensuring that animals did not fall below 85% of their normal adult body weight.

2.1.3 Genotyping

Tail biopsy from 3 week old animals was shipped on dry ice to Laragen Inc. (California, USA) for genotyping by reverse transcription-quantitative polymerase chain reaction (RT-qPCR) using probe-based Taqman® assays (Life Technologies Corporation, California, USA).

2.1.4 Dissections

Animals were culled by the Schedule 1 method of cervical dislocation, as per Home Office regulations, followed by post-mortem decapitation and brain removal from the skull. Hemispheres were separated by a cut in the sagittal plane down the longitudinal fissure and through the corpus callosum. Unless otherwise stated, the following structures were then isolated from both hemispheres: striatum, prefrontal cortex, cerebellum, hippocampus and motor cortex. To expose the striatum, a coronal cut was made at the posterior of the lateral ventricle and the striatum then removed from the anterior brain segment. The anterior brain segment was then turned dorsal side upwards and prefrontal cortex tissue isolated. Motor cortex tissue was isolated from the superior surface of the posterior brain segment. The hippocampus was exposed by removing the overlaying motor cortex and surrounding tissue. The cerebellum was taken from the posterior of the brain. Each structure was snap frozen in liquid nitrogen and stored at -80°C for later analysis.

2.2 Behavioural training

2.2.1 Apparatus

2.2.1.1 Rotarod

Two accelerating mouse rotarods (model numbers: 7650 and 47600; Ugo Basile, Italy) were used to assess motor ability and co-ordination. Both apparatus consisted of a rotating rod 30 mm in diameter, with five separated chambers of 57 mm in width and rod elevation of 160. The Rota-Rod 7650 (Fig. 2.1A) was used in the initial habituation session (see Chapter 5.2.3.1) while Rota-Rod 47600 (Fig. 2.1B) was used in the subsequent test sessions (see Chapter 5.2.3.1). Rota-Rod 7650 rotation acceleration ranged from 3 to 20 rpm over the course of a 300 s session, while Rota-Rod 47600 rotation acceleration ranged from 4 to 44 rpm over the course of a 300 s session.

See Chapter 5 for experimental details.

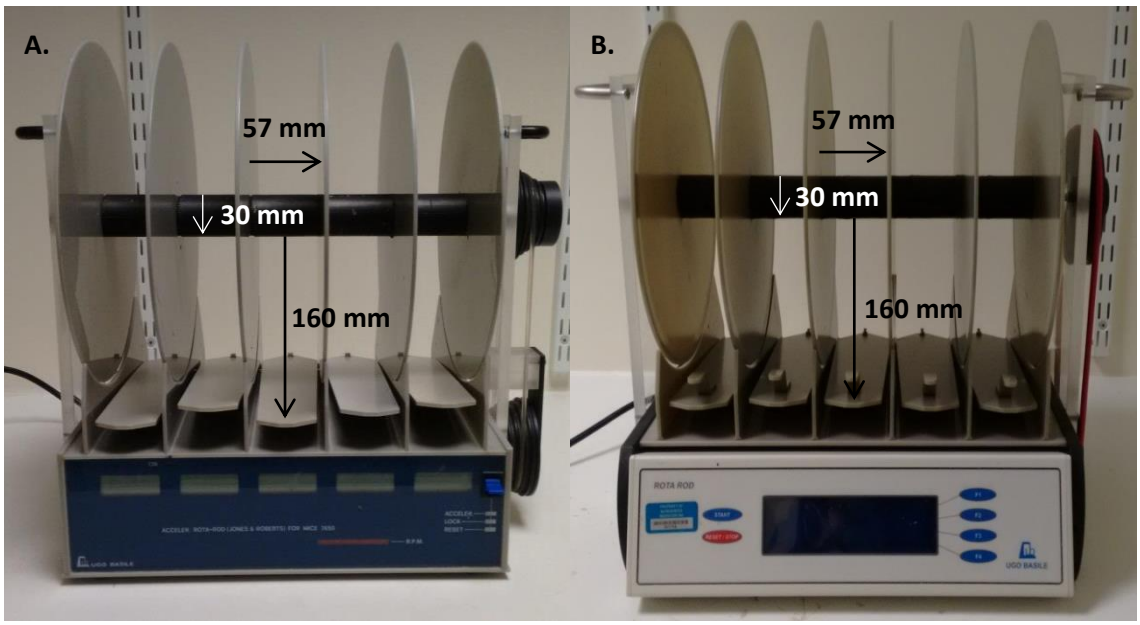


Figure 2.1. Mouse rotarod apparatus. Both Rota-Rod 7650 (A) and 47600 (B) consisted of a rotating rod 30 mm in diameter, with five separated chambers of 57 mm in width and rod elevation of 160 mm.

2.2.1.2 Operant chambers

Testing was performed in sixteen '9-hole' operant chambers (Fig. 2.2; Campden Instruments, Loughborough, UK) used in parallel. Chambers were based on a design first used in rats (Carli et al., 1983) and adapted for use in mice (Humby et al., 1999). Each chamber was constructed of stainless steel walls, perforated steel floor and a clear Perspex roof, and measured 140 mm x 130 mm x 125 mm in size. On the front inner wall of each chamber was a horizontal array of 9 holes (11 mm diameter, 2 mm apart from one another and 15 mm above floor level) along the curved front wall of the chamber, each of which housed a 2.5 Watt bulb stimulus light and a photocell beam to detect np responses. For the continuous reinforcement (CRF) task, aperture 5 (from the left side) was used, while the 5-choice serial reaction time task (5-CSRTT) and SILT both utilised apertures 1, 3, 5, 7 and 9. Three holes were used in the SD and RL experiments (numbers 3, 5 and 7 from left to right). Apertures 2, 4, 6 and 8 were capped and inaccessible throughout testing. A reward magazine was located at the rear of the chamber opposite the aperture array. For rewarding correct responses, 5 µl of Yazoo strawberry milk (Campina UK, Horsham, UK) was delivered by peristaltic pump and 0.8 mm polyethylene tubing to a small spout located in the base of the reward magazine. The entry of the mouse's head into the magazine when collecting the reward and the removal of the mouse's head from the magazine after delivery collection was detected by an infrared beam. The magazine was fitted with a light

to signal reward delivery. Two additional “house” lights, one situated on each of the side walls, were used to signal performance errors. Chambers also contained loudspeakers, although they were not utilised in behavioural testing. Each chamber was housed in a sound attenuation chamber that was fitted with a fan to keep a low constant noise and provide ventilation. The chambers were operated under online control by a PC computer and control unit programmed in the BNC Control language (Campden Instruments, Cambridge, UK).

See Chapter 3, 4 and 5 for experimental details.

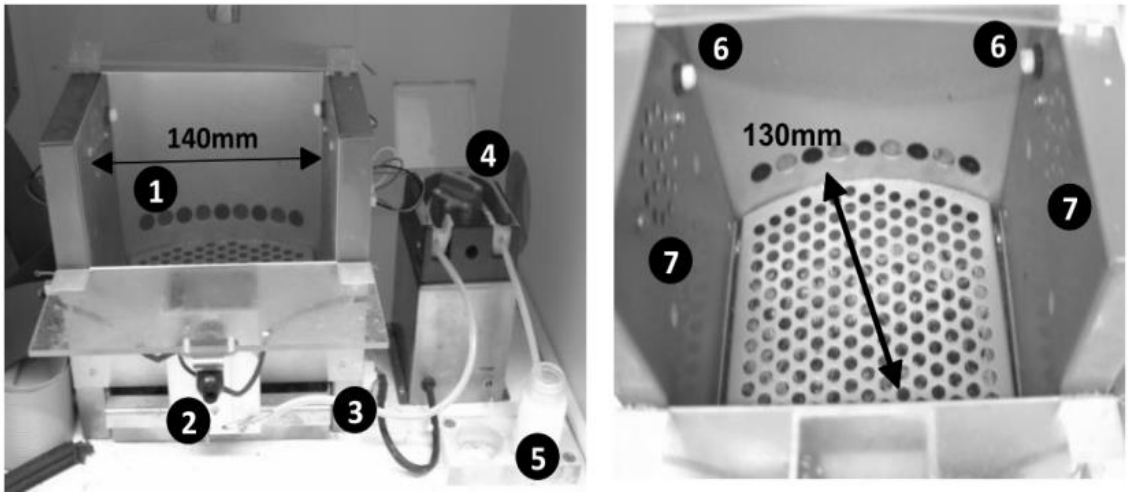


Figure 2.2. ‘9-hole’ operant chamber apparatus. 1: response array (9 apertures of 10 mm in diameter, spanned by vertical infra-red beams and each containing a small light); **2:** food magazine (containing reward light and food well); **3:** polyethylene tubing; **4:** peristaltic pump; **5:** reinforcer bottle containing Yazoo strawberry milk (Campina UK, Horsham, UK); **6:** house light; and **7:** loudspeakers.

2.3 Molecular methodology

2.3.1 Phenol/chloroform RNA extraction

For total RNA extraction, RNA was prepared from striatal tissue using TRIzol (Life Technologies Corporation, Carlsbad, California, USA) and RNeasy MinElute Cleanup Kit (Qiagen, Manchester, UK) according to manufacturers’ protocols, with minor modifications. Briefly, dissected striata were flash frozen within Lysing Matrix D tubes (MP Biomedicals, Santa Ana, California, USA) in liquid nitrogen and stored at -80°C. On ice, 1 mL of TRIzol (Life Technologies Corporation, Carlsbad, California, USA) was added to dissected striata prior to 3 pulses of 3 s homogenisation at speed 4.5 in a FastPrep FP210 homogeniser (MP Biomedicals, Santa Ana, California, USA),

with 1 min on ice between pulses. 500 µL of TRIzol Reagent (Life Technologies Corporation, Carlsbad, California, USA) was then added to the homogenate and the homogenate transferred to a 10 mL polypropylene tube before 5 min incubation at room temperature. 300 µL of 1-Bromo-3-chloropropane (Sigma-Aldrich Company Ltd, Dorset, UK) was added to samples, followed by vortexing for 15 s and 5 min incubation at room temperature.

Samples were centrifuged at 11,000 rpm for 15 min at 4°C in an Avanti J-E Centrifuge (Beckman Coulter (UK) Ltd, High Wycombe, UK) and approximately 600 µL of the upper aqueous phase transferred to a 2 mL eppendorf tube. 750 µL of 2-Propanol (Sigma-Aldrich Company Ltd, Dorset, UK) was added to the upper aqueous phase and the samples mixed by inversion before being left to precipitate at room temperature for 10 min. To pellet the RNA, samples were centrifuged at 11,000 rpm for 10 min at 4°C in Biofuge *Fresco* Heraeus centrifuge (Thermo Fisher Scientific Inc., Massachusetts, USA) and the supernatant removed. Pellets were washed in 1.5 mL of 75% ethanol (Sigma-Aldrich Company Ltd, Dorset, UK), made with nuclease-free water (Life Technologies Corporation, Carlsbad, California, USA), and samples centrifuged at 9,500 rpm for 5 min at 4°C. The supernatant was removed, pellets washed again in 1.5 mL of 75% ethanol and samples centrifuged at 9,500 rpm for 5 min at 4°C. Supernatant was then removed, samples centrifuged at 9,500 rpm for 15 s at 4°C to drain the remaining ethanol, remaining ethanol removed and RNA pellets dried in a 45°C oven for 5 min. Pellets were resuspended in 100 µL nuclease-free H_2O (Life Technologies Corporation, Carlsbad, California, USA), vortexed for 15 s, heated at 65°C for 5 min on an Eppendorf Thermomixer Comfort heating block (Eppendorf UK Ltd, Stevenage, UK), and vortexed again for 15 s.

For RNA purification, 350 µL of RLT buffer (Qiagen, Manchester, UK) was added to samples and mixed by inversion, before centrifuging at 9,500 rpm for 15 s at 22°C. Next, 250 µL of 100% ethanol (Sigma-Aldrich Company Ltd, Dorset, UK) was added to the diluted RNA and mixed thoroughly by pipetting. The samples (approximately 700 µL) were immediately applied onto a Mini-elute column placed in a 2 mL collection tube (Qiagen, Manchester, UK) and centrifuged at 10,000 rpm for 15 s at 22°C. Flow-through and collection tubes were discarded, Mini-elute columns placed into new 2 mL collection tubes, and 500 µL of Buffer RPE (Qiagen, Manchester, UK) added to the columns before centrifuging samples at 10,000 rpm for 15 s at 22°C. Flow-through and collection tubes were discarded, Mini-elute columns placed into new 2 mL collection tubes (Qiagen, Manchester, UK), and 500 µL of 80% ethanol (Sigma-Aldrich Company Ltd, Dorset, UK), made with nuclease-free water (Life Technologies Corporation, Carlsbad, California, USA), was added onto the Mini-elute columns (Qiagen, Manchester, UK). Samples were centrifuged at 10,000 rpm for 2 min at 22°C, flow-through and collection tubes discarded, Mini-elute columns placed into new 2 mL collection tubes (Qiagen, Manchester, UK), and

samples centrifuged at 13,000 rpm for 5 min at 22°C. The samples were rotated 180° in the centrifuge and centrifuged at 13,000 rpm for 1 min at 22°C. Mini-elute columns were transferred to 1.5 mL collection tubes (Qiagen, Manchester, UK) and 14 µL of nuclease-free water (Qiagen, Manchester, UK) pipetted onto the centre of the RNeasy silica-gel membrane. After 5 min, samples were centrifuged at 13,000 rpm for 1 min at 22°C. Tubes were rotated 180°C and centrifuged for 1 min at 13,000 rpm at 22°C.

To degrade any possible DNA contaminants, RNA samples were treated with TURBO DNA-*free*™ Kit (Life Technologies Corporation, Carlsbad, California, USA) according to manufacturer's instructions. Briefly, a typical reaction was a 50 µL volume consisting of nuclease-free water, 1x TURBO DNase buffer, 1 µL of TURBO DNase for up to 10 µg of RNA. The reaction mixture was incubated at 37°C for 30 min before 1x DNase Inactivation reagent was added to the reaction and thoroughly mixed. The mixture was then incubated at room temperature for 2 min before centrifugation at 10,000 rpm for 90 s. RNA was transferred into a fresh tube and stored at -80°C.

2.3.1.2 Quantification of the RNA sample

Quantity and quality of RNA was assessed by measurements of ultraviolet (UV) light absorption on a NanoVue Plus spectrophotometer (GE Healthcare Life Sciences, Buckinghamshire, UK). RNA absorbs UV light maximally at 260 nm, thus the ratio of absorbance at 260 nm and 280 nm (protein) was used to assess the RNA purity of a given RNA preparation. Pure RNA has an A260/A280 ratio of 2.1. For nucleic acid quantification, the Beer-Lambert equation is manipulated to give:

$$c = \frac{A \times e}{b}$$

Where *c* is the nucleic acid concentration in ng/µL, *A* is the absorbance in AU (for the arbitrary absorbance unites), *e* is the wavelength-dependent extinction coefficient in ng-cm/µL and *b* is the path length in cm. For nucleic acids, data are normalised to a 1 cm path length. The generally accepted extinction coefficient for RNA is 40. Use of the spectrophotometer enabled highly accurate analysis of particularly small sample volumes. Surface tension was used to hold a column of liquid sample in place while a measurement was made. 2 µL of the RNA sample was pipetted directly onto a measurement pedestal, and a measurement column was then drawn between the ends of two fibres in order to establish a measurement path. The measurement was carried out and presented on the display of the spectrophotometer.

Quality of RNA to be utilised in a microarray was further assessed by Dr. Joanne Morgan or Megan Musson of Cardiff University's Central Biotechnology Services (CBS; Cardiff, UK) using the Agilent 2100 Bioanalyzer (Agilent Technologies UK Ltd, Berkshire, UK). This system uses electrophoretic separation on microfabricated chips to separate and detect RNA samples via laser induced fluorescence detection (Schroeder et al., 2006). An electropherogram and gel-like image is then generated alongside details including sample concentration and ribosomal ratio, allowing detailed visual assessment of the quality of an RNA sample. A software algorithm generates the RNA Integrity Number (RIN) for each sample, an indication of the quality of eukaryotic total RNA that takes into account the entire electropherogram and removes individual interpretation in RNA quality control. The RIN value ranges from 1 to 10, with 1 being the most degraded profile and 10 being the most intact. RIN values of samples are provided in the relevant experimental chapters.

2.3.2 Quantitative reverse-transcription polymerase chain reaction

2.3.2.1 RNA to cDNA conversion

500 ng RNA from each sample underwent reverse transcription (RT) using a High Capacity RNA to cDNA™ kit (Life Technologies Corporation, California, USA) according to manufacturer's instructions. This concentration of RNA was determined by carrying out an RT reaction efficiency curve, which was achieved by performing the RT reaction with 250 ng, 500 ng and 1 µg RNA followed by quantitative polymerase chain reaction (qPCR) using an example primer pair. If the RT reaction is efficient, the cycle threshold (C_t) values will decrease with increased RNA load. Briefly, 500 ng of RNA was loaded into each reaction, and made up to a total volume of 9 µL with nuclease-free H_2O. This was added to 10 µL of 2X RT Buffer Mix and 1 µL of 20X RT Enzyme Mix, generating a 1X master mix used for the RT reaction (Table 2.1). Two negative controls were included; one in which the RT Enzyme Mix was substituted for nuclease-free H_2O and the other where RNA was substituted for nuclease-free H_2O. The master mix was briefly centrifuged at 10,000xg before incubation at 37°C for 60 minutes, and the reaction stopped by sample incubation at 95°C for 5 minutes. RT reactions were performed using a Bio-Rad S1000™ thermal cycler (Bio-Rad Laboratories, Inc., California, USA). Complementary DNA (cDNA) was diluted 1:50 in nuclease-free H_2O for use in PCR and stored at -20°C.

Reagent	Component Volume/Reaction (µL)
2X RT Buffer Mix	10.0
20X RT Enzyme Mix	1.0
RNA	Up to 9.0
Nuclease-free H_2O	Up to 9.0

Table 2.1. 1X master mix for RNA reverse transcription.

2.3.2.2 qPCR protocol

Similar to the RT reaction efficiency described in Chapter 2.3.2.1, efficiency of TaqMan® assays were determined by loading a serial dilution of cDNA into the RT-qPCR reaction. If the reaction is efficient, there will be a linear relationship between the concentration of cDNA loaded into the reaction and the resulting C_t value. The R^2 and slope of this relationship were then identified from the Sequence Detection Systems (SDS) Version 2.3 programme (copyright 2005, Applied Biosystems), and the PCR efficiency can be determined from these (Efficiency: ((10^(-1/slope)) - 1) x 100). For illustrative purposes, Figure 2.3 displays the efficiency data for the TaqMan® assay investigating *Dopamine receptor D2* (*Drd2*) expression levels.

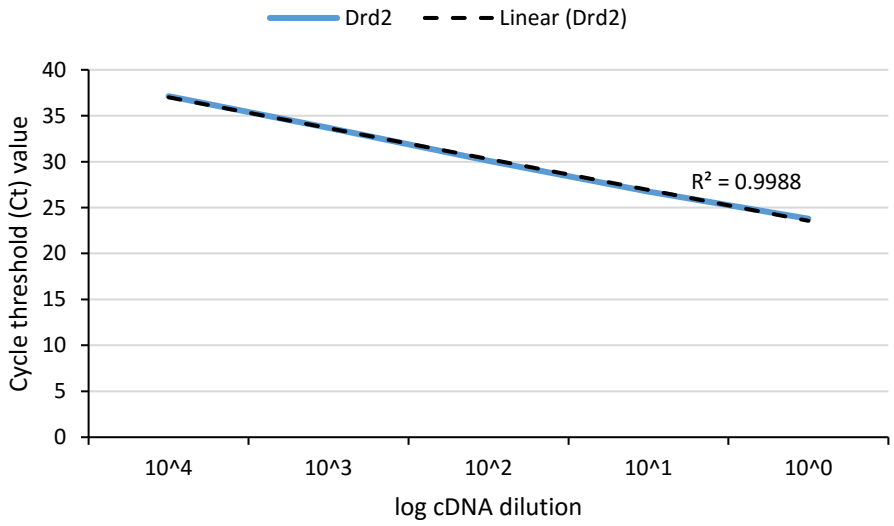


Figure 2.3. The RT-qPCR efficiency curve for the Drd2 TaqMan® assay. The R^2 value is provided in the chart area, while the slope was determined to be -3.34 by the Sequence Detection Systems (SDS) Version 2.3 programme (copyright 2005, Applied Biosystems) and efficiency calculated to be 99.43%.

For each experiment, amplification of all genes of interest and two housekeeping genes was performed in triplicate in the same qPCR run. All preparations were performed on ice. 40 ng of cDNA template was used for each reaction and made up to a total volume of 9 µL with nuclease-free H_2O. This was added to 10 µL of TaqMan® Universal Master Mix II, with Uracil-N-Glycosylase (UNG; Life Technologies Corporation, California, USA) and 1 µL of the relevant TaqMan® gene expression assay (Life Technologies Corporation, California, USA) per well, generating a 1X master mix used for the qPCR (Table 2.2).

Reagent	**Component Volume/Reaction (µL)**
2X TaqMan® Universal Master Mix II, with UNG	10.0
20X TaqMan® gene expression assay	1.0
RNA	4.0
Nuclease-free H_2O	5.0

Table 2.2. 1X qPCR master mix.

To ensure there was no contamination of samples in the 2-step process (cDNA synthesis and the qPCR protocol itself), each plate also included an RT-control (where no RT reaction was performed), a water RT control (where the RT reaction was performed, but with water replacing the RNA template), and a non-template control (where cDNA was replaced with nuclease-free H_2O).

Reactions were loaded into MicroAmp® Fast Optical 96 well plates (Life Technologies Corporation, California, USA) and sealed with MicroAmp® Optical Adhesive Film (Life Technologies Corporation, Carlsbad, California, USA) before brief centrifugation at 4,600xg using a Sorvall™ Legend™ RT centrifuge (Thermo Fisher Scientific, Massachusetts, USA). Plates were loaded into the StepOnePlus™ Real-Time PCR System (Life Technologies Corporation, California, USA) and cycling conditions were as shown in Table 2.3.

	Stage 1		Stage 2	
	UNG Activation	Polymerase Activation	PCR (40 Cycles)	
			Denature	Annealing/Extension
Temperature	50°C	95°C	95°C	60°C
Time	02:00	10:00	00:15	01:00

Table 2.3. qPCR cycling conditions.

Control and analysis of RT-qPCR protocols were performed by StepOne™ Software Version 2.2.2 (Life Technologies Corporation, California, USA).

2.3.2.3 Data analysis using the $2^{-\Delta\Delta Ct}$ method

The $2^{-\Delta\Delta Ct}$ method was used to calculate relative changes in gene expression levels determined from each RT-qPCR experiment. Derivation of the $2^{-\Delta\Delta Ct}$ equation, including assumptions, experimental design and validation tests have been previously described by (Livak and Schmittgen, 2001). In order to normalise the qPCR data for the amount of RNA that is added to a reaction, housekeeping genes are commonly used as an internal control. In this thesis, *Beta-actin* (*Actb*) and *Ubiquitin C* (*Ubc*) were selected as the reference genes because they showed no significant differences in expression in comparisons between groups, or genotypes, in the microarray performed in Chapter 3 (data not shown) and have both been reported to show stable levels of expression within the striatum of a mouse model of HD (Benn et al., 2008a). The change in expression levels of the target gene was normalised to the mean of the mean C_t values of *Actb* and *Ubc* for each individual sample. In order to minimise sampling errors, each sample was run in triplicate. The C_t values (defined by the number of cycles required for the fluorescence to cross the threshold, i.e. exceeding the background level) obtained from the StepOne™ Software Version 2.2.2 (Life Technologies Corporation, California, USA) were imported into Microsoft Excel, which enabled descriptive analysis of the data, and conversion to $2^{-\Delta\Delta Ct}$ for subsequent analysis (see (VanGuilder et al., 2008). The C_t values for the control genes (*Actb* and *Ubc*) mRNAs and the target gene mRNAs were averaged across the triplicates for each sample, prior to performance of the ΔC_t calculation. The ΔC_t value was calculated by subtracting the average C_t values of the control genes (consisting of the mean of the average C_t value of both *Actb* and *Ubc*) from the average C_t values of the target genes. Next, the subtraction of the ΔC_t values of the control gene samples from the ΔC_t values of the target gene samples yielded the $\Delta\Delta C_t$ values. The negative values of this subtraction, the - $\Delta\Delta C_t$ values, were then used as an exponent of 2, and represent in "corrected" number of cycles to threshold (Livak and

Schmittgen, 2001), but the exponent conversion is based on the fact that the reaction doubles the amount of product per cycle. These values were then combined with the values of relative change in the expression of the target gene between the comparison groups and presented as a graph.

2.3.2.4 Statistical analysis of the RT-qPCR data

The final stage of the RT-qPCR analysis was to determine the C_t value. The C_t value was derived from a log-linear plot of the PCR signal against the cycle number, which depicts the C_t value as an exponential instead of a linear term. Hence, the data were converted to a linear form using $2^{-\Delta\Delta Ct}$ (transformed data). In order to examine the statistical significance of the relevant change, independent two tailed T-tests were performed on the transformed data.

2.4 General data presentation and statistical methods

The data in this thesis is presented as mean values ± standard error of the mean (S.E.M.), calculated from the following formula:

$$\text{Standard error of the mean} = \frac{\text{standard deviation of values}}{\sqrt{\text{number of values}}}$$

All statistical analyses were performed using IBM SPSS® Statistics software, version 20 for windows (IBM United Kingdom Ltd, Hampshire, UK). The data were analysed by either 2-tailed, paired T-test, independent samples T-test, one-way analysis of variance (ANOVA) or repeated measures ANOVA, where appropriate. All statistical tests were performed with an alpha value of <0.05 regarded as significant. Interaction statistics are only reported if they reach significance, unless otherwise stated in experimental chapters. *Post hoc* pairwise comparisons were performed using Tukey's honestly significant difference (HSD) test, unless otherwise stated in experimental chapters. Repeated measures data were assessed for equality of variance using Mauchly's test of sphericity, with the Greenhouse-Geisser correction used if the assumption of Sphericity was violated and the Greenhouse-Geisser estimate of sphericity (ε) < 0.75. If the assumption of Sphericity was violated and ε > 0.75, the Huynh-Feldt correction was used. Similarly, homogeneity of variances for data was assessed using Levene's test, and the Welch test used in the event of data violating this assumption. Specific details of statistical analyses are described in the relevant experimental chapter.

Chapter 3: Age- and learning-dependent changes in the transcriptional profile of the heterozygous HdhQ150 mouse model of Huntington's disease

3.1 Introduction

Implicit learning has previously been defined as "…(learning that) occurs without concurrent awareness of what is being learned" (Shanks and Stjohn, 1994). Human neuroimaging studies have identified implicit learning as a complex process likely to utilise a number of regions in the brain, with evidence of striatal (Rauch et al., 1997; Schendan et al., 2003; Aizenstein et al., 2004; Destrebecqz et al., 2005; Reiss et al., 2005; Bennett et al., 2011; Gheysen et al., 2011), cortical (Honda et al., 1998; Schendan et al., 2003; Aizenstein et al., 2004; Bennett et al., 2011), hippocampal (Schendan et al., 2003; Gheysen et al., 2010) and medial temporal lobe (Schendan et al., 2013) involvement in the processing of implicit information. Cases of HD patients with deficits in this form of learning have been reported (Heindel et al., 1989; Knopman and Nissen, 1991; Gabrieli et al., 1997; Kim et al., 2004). Conversely, other studies have not observed such impairments (Brown et al., 2001; Schneider et al., 2010). The discrepancy in these findings may be the result of differences in methodology, as suggested by Brown and colleagues (2001), while the correlation between abnormal striatal activity, identified by fMRI, and implicit learning impairments in HD patients discovered by Kim et al. (2004) provides strong evidence that this learning deficiency can occur in cases of HD. Deficits in implicit learning have also been identified in Parkinson's disease (Wilkinson et al., 2009; Schendan et al., 2013; Gamble et al., 2014), a disorder that, similar to HD, is characterised by striatal dysfunction (Gerfen et al., 1990; Hughes et al., 1993), which supports the theory that the striatum plays a key role in implicit learning.

Motor symptom onset in HD, which is often the criteria used for diagnosis of disease onset (Kirkwood et al., 2000; Walker, 2007; Long et al., 2014), is often preceded by subtle cognitive disturbances in processes such as implicit learning (Kim et al., 2004; Ghilardi et al., 2008; van Asselen et al., 2012), semantic memory and visual discrimination (Lawrence et al., 1998b), working memory (van Walsem et al., 2010; Stout et al., 2011) and emotion recognition (Gray et al., 1997; Johnson et al., 2007; Stout et al., 2011). Over time, early-stage HD patients show a progressive decline in a number of cognitive capabilities including those related to working memory (Tabrizi et al., 2011; Harrington et al., 2012), emotion recognition (Stout et al., 2012; Tabrizi et al., 2013) and executive functioning (Stout et al., 2012; Hart et al., 2013; Tabrizi et al., 2013). Implicit learning capabilities have been shown to decline in healthy ageing, with changes in frontostriatal activity believed to contribute to this deterioration (Aizenstein et al., 2006; Bennett et al., 2011; Schendan et al., 2013). Thus, it is feasible that the frontostriatal degeneration that progresses over time in the premanifest phase of HD (Tabrizi et al., 2009;

Tabrizi et al., 2011; Tabrizi et al., 2013) may contribute to the implicit learning deficits that can arise in the disease, as suggested in an earlier imaging study of the condition (Kim et al., 2004).

Transcriptional dysregulation is hypothesised to be an integral mechanism in the pathophysiology of HD (Cha, 2000; Sugars and Rubinsztein, 2003; Thomas, 2006; Buckley et al., 2010; Bowles et al., 2012; Seredenina and Luthi-Carter, 2012), with evidence of gene expression level alterations arising in a range of neural structures (Hodges et al., 2006; Johnson et al., 2008; Neueder and Bates, 2014; Capurro et al., 2015), including the striatum (Augood et al., 1996; Norris et al., 1996; Desplats et al., 2006; Hodges et al., 2006; Kuhn et al., 2007). Similar changes in the transcriptome have been reported in a number of mouse models of HD (Luthi-Carter et al., 2000; Luthi-Carter et al., 2002; Kuhn et al., 2007; Johnson et al., 2010; Giles et al., 2012; Neueder and Bates, 2014; Bayram-Weston et al., 2015). Importantly, aberrant gene expression level profiles have been identified in individual cells, and in the absence of neurodegeneration and neurotransmission abnormalities (Hodges et al., 2006; Runne et al., 2008; Capurro et al., 2015), suggesting that the abnormal transcriptional patterns identified in HD are attributable to the intrinsic effects of mHtt and not purely the result of the neuronal death characteristic of the disease.

The SILT is a "9-hole" operant box based task developed to allow investigation into implicit learning, where a predictable light sequence is embedded amongst other unpredictable light sequences. Implicit learning is demonstrated in the task by mice responding with greater accuracy and/or speed to the predictable sequence than to unpredictable sequences. Quinolinic acid lesions to the striatum have previously been found to disrupt general SILT performance (Trueman et al., 2005), as well as acquisition and retention of the task (Brooks et al., 2007), while deficits in overall task performance have been identified in the HdhQ92 and YAC128 mouse models of HD at 4M and 6M, respectively (Trueman et al., 2007; Brooks et al., 2012d).

The HdhQ150 knock-in mouse model of HD has previously been found to present with early cognitive deficits in spatial learning and extra-dimensional set shifting that manifest prior to motor dysfunction (Brooks et al., 2006; Brooks et al., 2012b), as well as a transcriptional profile that undergoes longitudinal alterations similar to those found in human HD (Giles et al., 2012) and YAC128 mouse brain (Bayram-Weston et al., 2015). The current study aimed to utilise the SILT as a means to examine implicit learning in the HdhQ150 model of HD, and also to investigate changes in the transcriptome as a result of disease genotype, ageing and cognitive training. Ageing was examined from 3M to 6M, as this has been identified as the optimum timeframe to investigate ageing before senescence begins (Flurkey et al., 2007) and because it could be argued to act as a premanifest stage of HD in the HdhQ150 line, with previous reports of

cognitive dysfunction in the absence of motor abnormalities within this timeframe (Brooks et al., 2006; Brooks et al., 2012b). Gene expression level analyses using microarray investigation were performed across this timeframe in an attempt to identify early ageing-dependent transcriptional alterations comparable to those seen later in the lifetime of this model (Giles et al., 2012). Animals were trained to one of two stages of learning – to acquisition of a simple conditioning task or acquisition of the SILT – to elucidate whether *Hdh*$^{Q150/+}$ mice present with an abnormal capacity for acquisition of conditioning or implicit learning, and also to probe whether any gene expression level differences arise between these forms of learning using a microarray.

3.2 Methods

3.2.1 Declarative statement

Experimental design and all behavioural work, including tissue collection, in the current chapter was carried out by another party prior to me commencing my PhD program. Analysis of behavioural data and all molecular techniques and analyses, unless otherwise specified, were performed by me.

3.2.2 Animals

44 naïve female mice were used in this experimental chapter, with genotypes: *Hdh*$^{Q150/+}$ (n=23) and *Hdh*$^{+/+}$ (n=21). *Hdh*$^{Q150/+}$ CAG repeat sequences ranged from 129 to 164 CAGs (Mean: 149.50 ± 10.57). General husbandry conditions were as described in Chapter 2.1, except in regards to housing, with animals in this experiment being housed in pairs. All animals were between 7 and 9 weeks of age at the start of testing.

3.2.3 Experimental design

Each experimental cohort received different behavioural training protocols (Table 3.1). Animals in Group A and Group B had no behavioural training and were unhandled (except during routine home cage cleaning), being left in their home cages until sacrifice at 3M or 6M, respectively. Group C mice were trained to acquisition in the CRF task, before sacrifice at 3M. Group D mice received training to acquisition in CRF before being placed in operant boxes with no SILT program run and were sacrificed at 6M. Animals in Group E were trained to acquisition in CRF

prior to training in the five-choice serial reaction time task (5-CSRTT) and SILT, before being sacrificed at 6M.

Group	*Hdh*$^{+/+}$	*Hdh*$^{Q150/+}$	Mean CAG repeat length	2M	3M	6M
A	5	4	152.26 ± 6.18	Unhandled	Take brains	-
B	6	4	149.10 ± 4.43	Unhandled	Unhandled	Take brains
C	4	5	155.56 ± 2.99	Train to CRF acquisition	Take brains	-
D	3	5	141.60 ± 5.80	Train to CRF acquisition	Test boxes, no program	Take brains
E	3	5	145.46 ± 3.22	Train to CRF acquisition	Full SILT training	Take brains

Table 3.1. Table of behavioural groups, animal numbers and training paradigms. Mean CAG repeat length is presented as data ± S.E.M.

3.2.4 Behavioural training protocols

3.2.4.1 Magazine training

Mice in Groups C (n=9), D (n=8) and E (n = 8) underwent 1 day of magazine training in order to permit the mice to learn the association between magazine light illumination and food reward. The magazine light was illuminated and 150 µL of reward was delivered at the onset of the 20 min session. Upon removal of the head from the magazine, the light would extinguish for 10 s before again illuminating and 5 µL of reward delivered. This reward contingency was maintained for the remainder of the session. Following magazine training, mice began the next task in the series, the CRF task.

3.2.4.2 CRF task

CRF task training was undertaken by mice in order to teach the animals to associate the presence of an operant aperture light with the delivery of a food reward, which is a necessary prerequisite for animals to be able to perform the more complex 5-CSRTT and SILT, and was also utilised to examine the ability of mice to acquire a simple conditioning task. Mice were required to perform a single nose-poke to the light stimulus presented in the centre hole (hole 5) of the operant box in order to receive a food reward. Sessions lasted 20 min and 1 session was performed each day. When the hole 5 stimulus light was presented, a correct nose-poke caused simultaneous extinction of the stimulus light, delivery of food reward and illumination of the magazine light

(Fig. 3.1). Once the mouse collected the reward and removed its head from the magazine, the magazine light was extinguished. Following a 2 s inter-trial-interval (ITI), the next trial began through the illumination of the hole 5 light. In the event of a correct response not being made within 10 s of the hole 5 stimulus light presentation, the light stimulus was extinguished and the house lights illuminated for 5 s before a new trial began after a 2 s ITI.

Animals performed 5 days of CRF training. Following CRF acquisition, mice in Group E (n = 8) began 5-CSRTT training whilst animals in Group C (n = 9) were sacrificed and their brains taken for gene expression level analysis, and animals in Group D (n = 8) were placed in the operant boxes with no further program run until sacrifice at 6M.

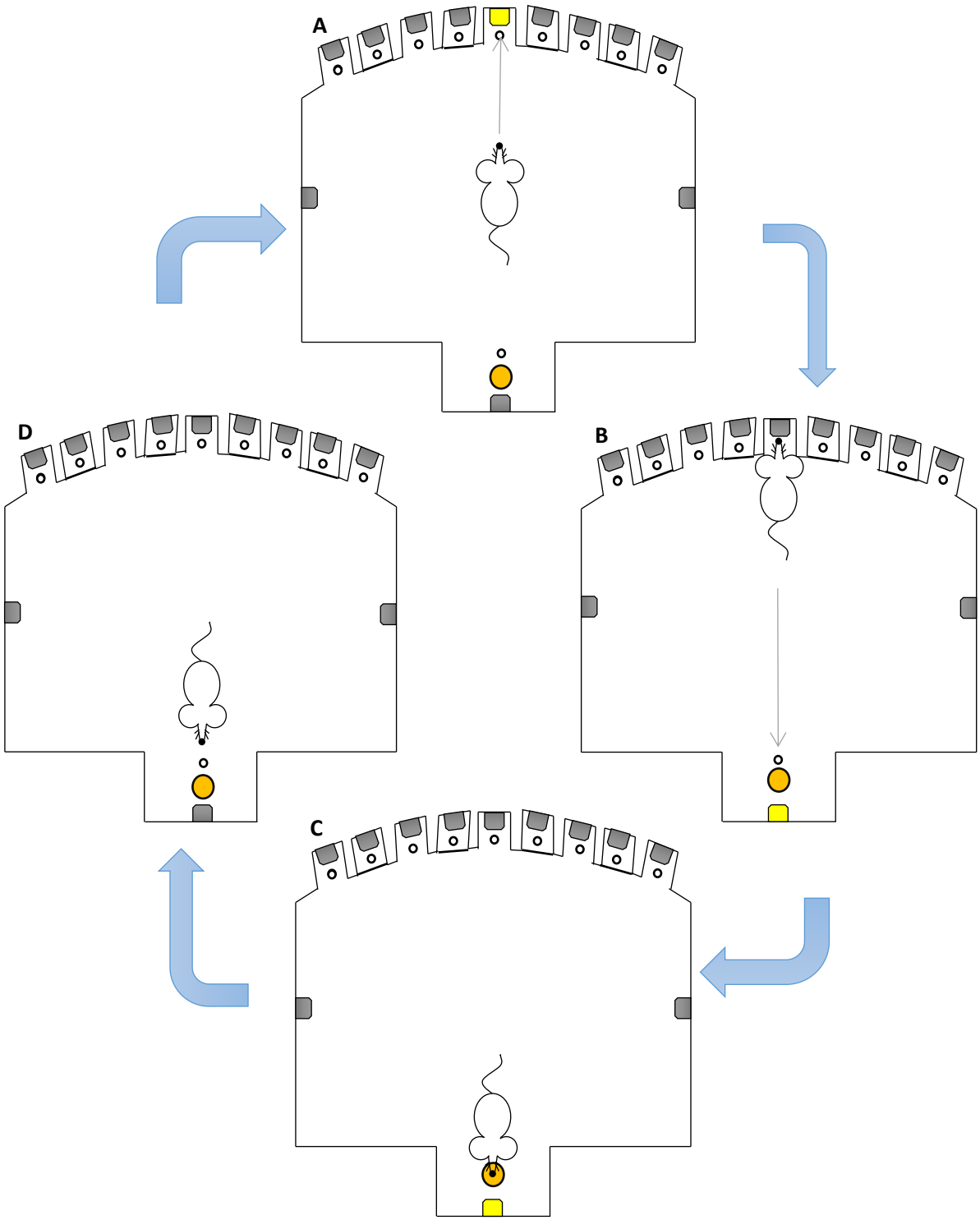


Figure 3.1. A pictorial representation of the CRF task. A light stimulus is presented in hole 5 of the operant box (A). Upon nose-poking the light stimulus, the light stimulus in hole 5 is extinguished whilst the magazine light stimulus is simultaneously illuminated (B). Once the animal has collected the food reward (C), and removed its head from the magazine, the magazine light is extinguished (D). There is a 2 s inter-trial-interval (ITI) before a new trial is commenced through the illumination of the hole 5 stimulus light (A).

3.2.4.3 5-CSRTT

5-CSRTT training is required in order to teach mice that a np into the illuminated operant aperture light (of which five apertures are available: holes 1, 3, 5, 7, and 9) will result in delivery of a food reward, which expands upon the CRF task because any of the five available stimulus lights may be illuminated in the 5-CSRTT, as opposed to the one stimulus light that is illuminated in the CRF (hole 5). The 5-CSRTT is a necessary prerequisite for animals to be able to perform the more complex SILT, which is detailed in Chapter 3.2.4.4, and also allows examination into the attentional capacities of animals. This behavioural task requires mice to make a single np into a pseudo randomly chosen illuminated hole in order to gain a reward. Stimulus hole illumination occurs pseudo randomly to ensure that each hole is illuminated an equivalent number of times during a session, therefore ensuring that a bias towards stimulus holes does not form. Sessions lasted 20 min and one session was performed each day. When a stimulus light was presented, a correct nose-poke caused simultaneous extinction of the stimulus light, delivery of food reward and illumination of the magazine light (Fig. 3.2). Once the mouse collected the reward and removed its head from the magazine, the magazine light was extinguished. Following a 2 s ITI, the next trial began through illumination of a pseudo randomly chosen hole light. The light stimulus was presented continuously until the mouse responded, and in the result of an incorrect response, the light stimulus was extinguished and the house light illuminated for 5 s before a new trial began. In each trial, any one of the five accessible holes may be illuminated. Performance on the task was measured by performance accuracy to stimulus lights, with accuracy defined as the percentage of initiated trials in which the animal makes a correct response to the presented stimulus. Mice performed 5-CSRTT training until both genotypes reached a mean accuracy level of 80% (11 sessions), before commencing SILT training.

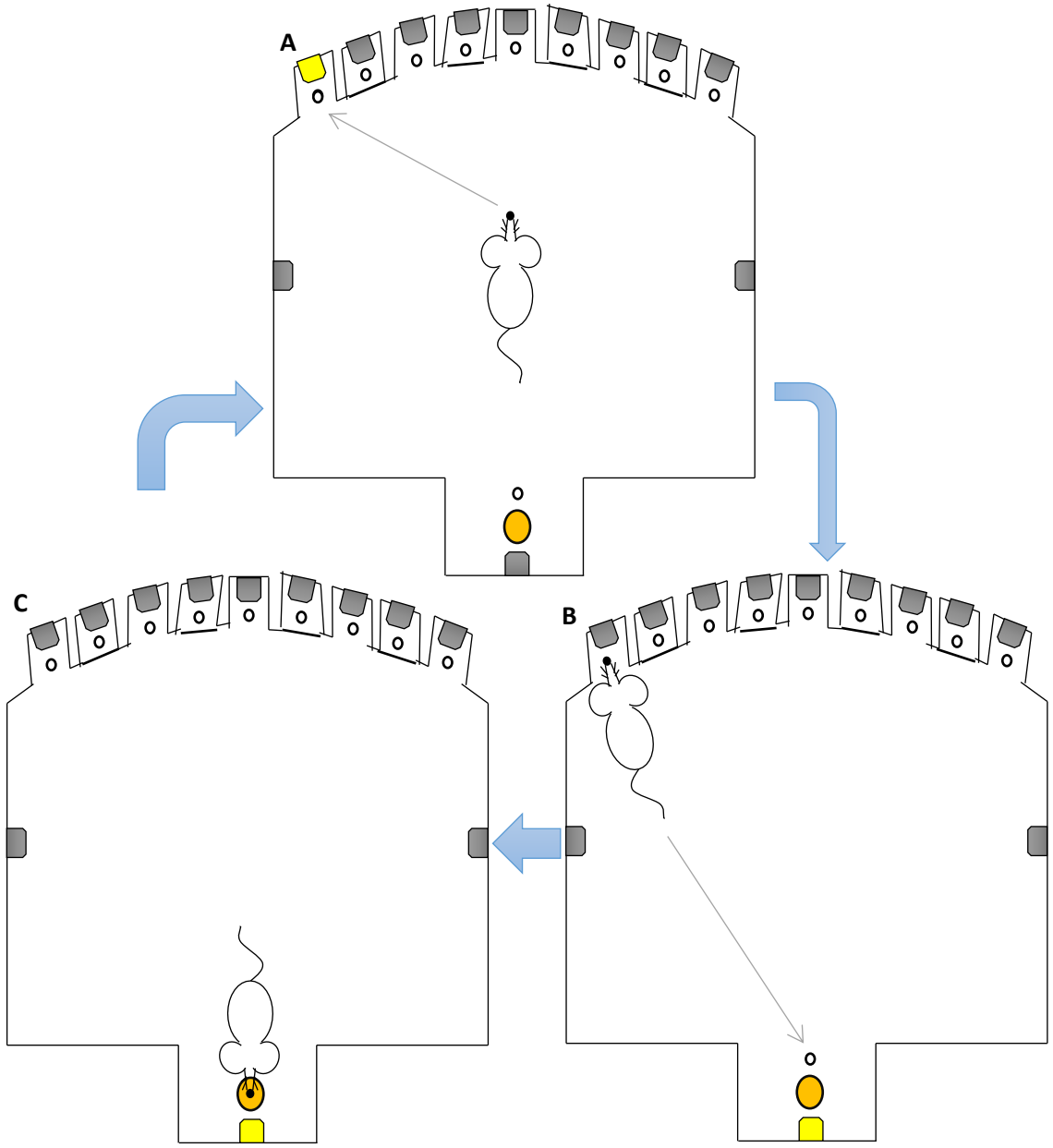


Figure 3.2. A pictorial representation of a trial in the 5-CSRTT. A trial begins when a pseudo randomly chosen stimulus light is illuminated (A). Nose-poking the correct light stimulus causes simultaneous extinction of the hole light stimulus, illumination of the reward magazine and reward delivery (B). Once the animal has collected the food reward (C), and removed its head from the magazine, the magazine light is extinguished. A 2 s ITI occurs before a new trial is commenced through the illumination of a pseudo randomly chosen stimulus light (A).

3.2.4.4 SILT

SILT sessions lasted 20 min and 1 session was performed each day. The SILT requires mice to correctly respond to 2 consecutive stimuli in order to gain a food reward. The first response, S1, is as described for the 5-CSRTT (Fig.3.2A; Fig. 3.3A). Following a correct S1 response, the S1 light would extinguish and a S2 would illuminate in a pseudo randomly chosen alternate hole (Fig. 3.3B). For the first phase of SILT training, S2 was presented continuously until the mouse responded, with a correct S2 response resulting in simultaneous extinguishing of the S2, illumination of the magazine reward light and delivery of food reward (Fig. 3.3C). During the second SILT testing phase, the S2 stimulus was presented for 2 s before being extinguished. Upon collection of food reward and subsequent removal of the head from the reward magazine, the reward magazine light is extinguished and a timer is started. After a 2 s ITI a new trial would begin by illumination of a pseudo randomly chosen S1 (Fig. 3.3A). To allow investigation into implicit learning, a single predictable 2-light sequence was embedded within the random presentations of 2 light sequences. This predictable sequence occurred when the S1 was hole 3, and the S2 presentation always followed in hole 7 (Fig. 3.4). Nose-poking an incorrect hole during S1 or S2 presentation would result in trial termination and a 5 s time-out interval (TOI) where the house lights would be illuminated prior to the onset of a new trial.

Performance on the task was measured by performance accuracy and reaction times to stimulus lights. Performance accuracy was calculated by the number of correct responses as a percentage of the total number of trials initiated. For S1 responses, accuracy and reaction times were analysed by hole (hole 1, 3, 5, 7, 9) whilst S2 responses were evaluated by the distance between the S1 and S2, referred to as the step-size (number of steps). Performance on the predictable 2-step hole 3-7 presentations were compared against that recorded on the opposite unpredictable hole 7-3 combination. Mice performed the SILT until performance was judged to have reached asymptote, which occurred following 3 days of continuous S2 training and 18 days of 2 s S2 training.

Seven error terms were examined during SILT performance, with each capable of identifying different behavioural traits. Incorrect choices to S1 or S2 consisted of a np to an unlit stimulus hole, or of a magazine entry, during S1 or S2 presentation, and are used to examine the accuracy and attention levels of mice. Incorrect S1 and S2 choice rates were calculated as percentages of the number of total responses made to S1 and S2 presentation, respectively. The rate of perseverative np to S1 was used to examine perseverative behaviour in animals and was calculated as a percentage of the total number of responses made during S1 presentation, as was the rate of magazine entries during S1 presentation. The rate of responses during ITI or TOI

was investigated in order to assess impulsivity in the mice, and was calculated as an error rate per ITI or incorrect response to S2, respectively. Not responding to the S2 stimulus within 10 s would result in a TOI, which was used as a measure of the psychomotor processing speed or attentional capacity of animals, and was calculated as a percentage of the total number of S1 responses made.

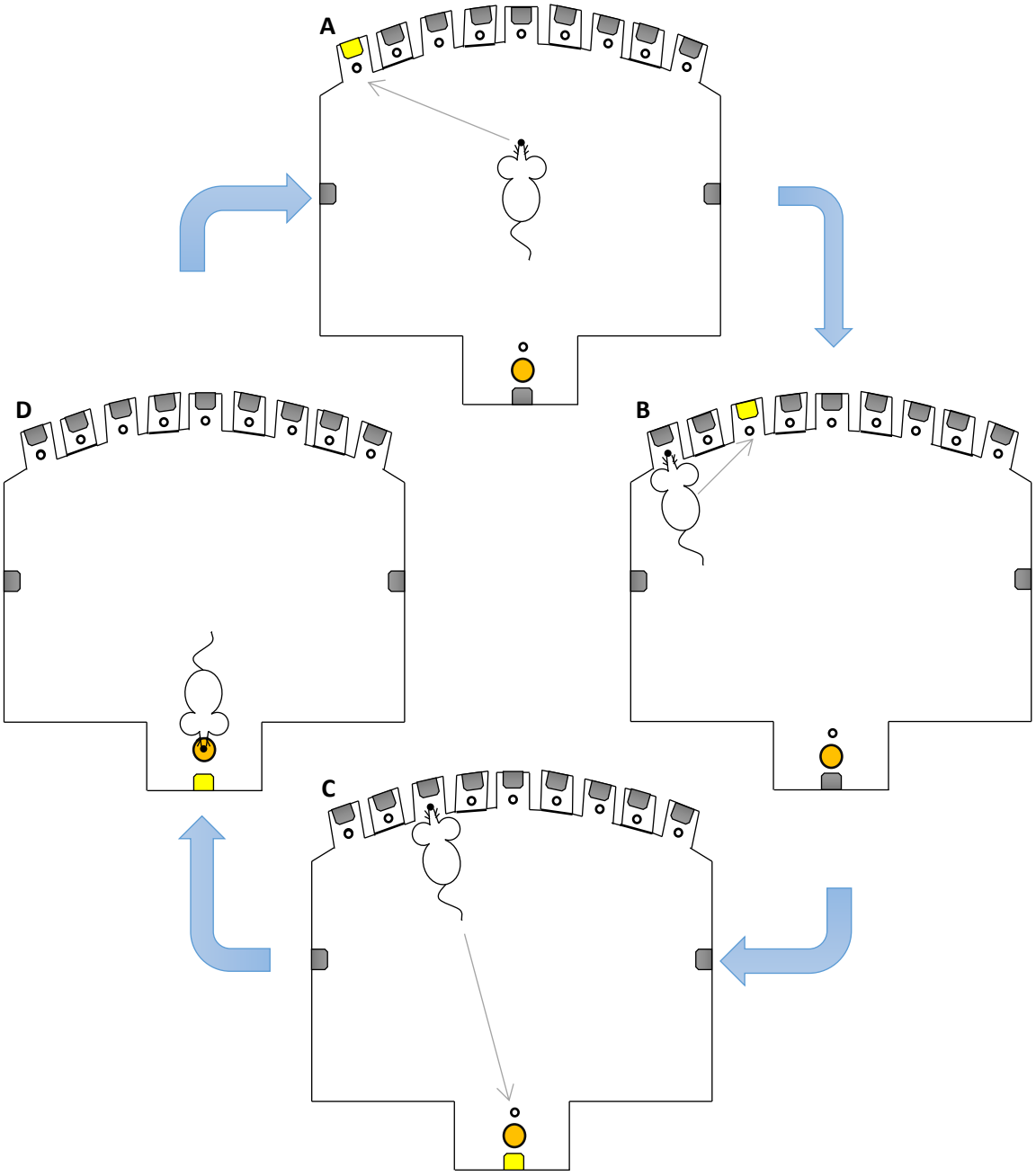


Figure 3.3. A pictorial representation of a trial in the SILT. A trial begins through illumination of a pseudo randomly chosen S1 (A). Correctly responding to S1 results in simultaneous extinction of the S1 light and illumination of a pseudo randomly chosen S2 (B). Successfully nose-poking the S2 stimulus causes illumination of the reward magazine light and food reward delivery (C). Once the animal has collected the food reward (D), and removed its head from the magazine, the magazine light is extinguished. There is a 2 s ITI before a new trial is commenced through the illumination of a randomly chosen S1 stimulus light (A).

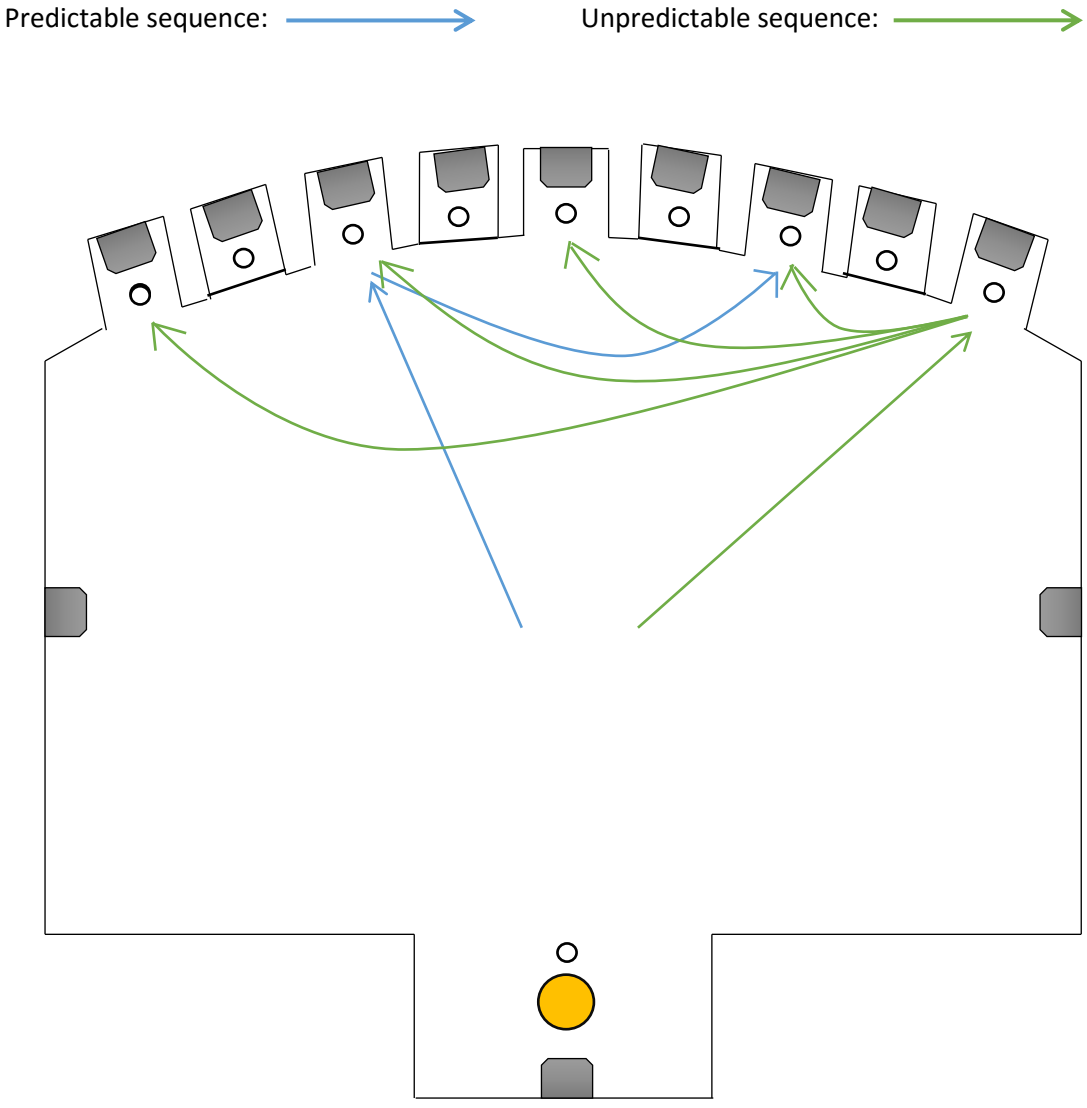


Figure 3.4. A pictorial representation of the predictable and unpredictable sequences within the SILT. The predictable sequence of stimuli begins with S1 illumination of hole 3 and is followed by S2 illumination of hole 7. In unpredictable sequences, presentation of the S1 light occurs pseudo randomly in one of holes 1, 5, 7 or 9 and followed by pseudo randomly chosen S2 presentation in any of the remaining available holes. In this unpredictable sequence example, S1 is presented in hole 9 and the subsequent S2 may occur in any of holes 1, 3, 5 or 7.

3.2.5 Molecular methodology

3.2.5.1 RNA extraction

RNA was prepared from right hemisphere striatum dissected from animals at 3M or 6M as described in Chapter 2.3.1, with RINs of RNA samples ranging from 8.1 to 9.6 (8.66 ± 0.07).

3.2.5.2 Microarray and microarray analyses

Microarray was performed by Dr. Joanne Morgan of Cardiff University's CBS (Cardiff, UK) using GeneChip® Mouse Gene 2.0 ST Array chips (http://www.affymetrix.com/catalog/131476/AFFY/Mouse+Gene+ST+Arrays#1_1; product code: 902119; Affymetrix, California, USA), and microarray gene expression level data was analysed using Partek® Genomics Suite™ Version 6.6 (Partek Incorporated, Missouri, USA).

A three-way ANOVA was performed on the microarray gene expression level data with age, genotype and learning (unhandled, acquisition of CRF or full-SILT) as variables, and also using the same variables as interaction terms in a three-way interaction. Statistical significance was taken using a false discovery rate (FDR)-corrected p-value of less than 0.05 (FDR $p < 0.05$).

3.2.5.3 Quantification of gene expression levels by RT-qPCR

Microarray gene expression level data was validated by TaqMan®-mediated (Life Technologies Corporation, Carlsbad, California, USA) RT-qPCR, as described in Chapter 2.3.2. The number of samples used for each group in the RT-qPCR is given in Table 3.2, and each reaction was performed in triplicate.

Group	Number of samples
***Hdh*$^{+/+}$ 3M U**	5
***Hdh*$^{+/+}$ 6M U**	6
***Hdh*$^{Q150/+}$ 3M U**	4
***Hdh*$^{Q150/+}$ 6M U**	4
***Hdh*$^{+/+}$ 3M A**	4
***Hdh*$^{Q150/+}$ 3M A**	5
***Hdh*$^{+/+}$ 6M A**	3
***Hdh*$^{Q150/+}$ 6M A**	5
***Hdh*$^{+/+}$ 6M S**	3
***Hdh*$^{Q150/+}$ 6M S**	5

Table 3.2. The number of samples in each group used for RT-qPCR.

Genes to be investigated were chosen based on the findings of the microarray (see Chapter *3.3.2.3.1*) and are listed in Table 3.3.

Gene name	Gene symbol	ID
Beta-actin	*Actb*	Mm00607939_s1
calbindin 2	*Calb2*	Mm00801461_m1
glucagon-like peptide 1 receptor	*Glp1r*	Mm00445292_m1
G protein-coupled receptor 165	*Gpr165*	Mm01233063_m1
potassium voltage-gated channel, subfamily H, member 3	*Kcnh3*	Mm01310207_m1
potassium inwardly-rectifying channel, subfamily J, member 4	*Kcnj4*	Mm02027786_s1
ribonuclease P RNA component H1	*Rpph1*	Mm04336066_s1
solute carrier family 17, member 6	*Slc17a6*	Mm00499876_m1
small nucleolar RNA, H/ACA box 74A	*Snora74a*	Mm04241346_s1
ubiquitin C	*Ubc*	Mm02525934_g1
uracil phosphoribosyltransferase	*Uprt*	Mm01234398_m1
zic family member 1	*Zic1*	Mm00656094_m1

Table 3.3. Identification codes for Taqman® oligonucleotide probes used in the study.

3.2.6 Statistical analyses

Statistical analyses were performed using IBM SPSS® Statistics 20 software (IBM United Kingdom Ltd, Hampshire, UK). Behavioural data for the 6M $Hdh^{Q150/+}$ and $Hdh^{+/+}$ mice are presented, with the 5-CSRTT and SILT data collapsed across the final 5 days of testing for statistical analyses. All parameters that involved repeated measures were analysed using multifactorial ANOVA, with Tukey's HSD test used for post-hoc analysis if data met the assumption of homogeneity of variances and Games Howell post-hoc test used if the data did not meet this assumption. Sphericity of data was assessed using Mauchly's test of Sphericity, with the Greenhouse-Geisser correction used if the assumption of Sphericity was violated and the Greenhouse-Geisser estimate of sphericity (ε) < 0.75. If the assumption of Sphericity was violated and $\varepsilon > 0.75$, the Huynh-Feldt correction was used. Similarly, homogeneity of variances for data was assessed using Levene's test, and the Welch test used in the event of data violating this assumption. For RT-qPCR data, specific two group comparisons were analysed by independent samples t-tests with Holm-Bonferroni sequential correction used for multiple hypothesis testing correction. A one-way ANOVA was performed on 3 group comparisons and a *post-hoc* Tukey's HSD test performed on comparisons in which a main effect was found to be significant.

3.3 Results

3.3.1 Behavioural analyses

3.3.1.1 CRF task performance

Over the five days of CRF training, both genotypes showed a trend towards an increase in the number of responses made (Fig. 3.5), suggesting that animals had learned to successfully perform the task. A repeated measures ANOVA reported no significant difference in the number of responses made between *Hdh*$^{Q150/+}$ mice and their wild-type counterparts (main effect of genotype: $F_{1,6}$ = 3.280, p = 0.120). There was no difference in the number of responses made across the 5 days of testing (main effect of day: $F_{4, 24}$ = 1.267, p = 0.310), and no significant interaction between genotype and day of testing (day*genotype: $F_{4, 24}$ = 0.217, p = 0.816).

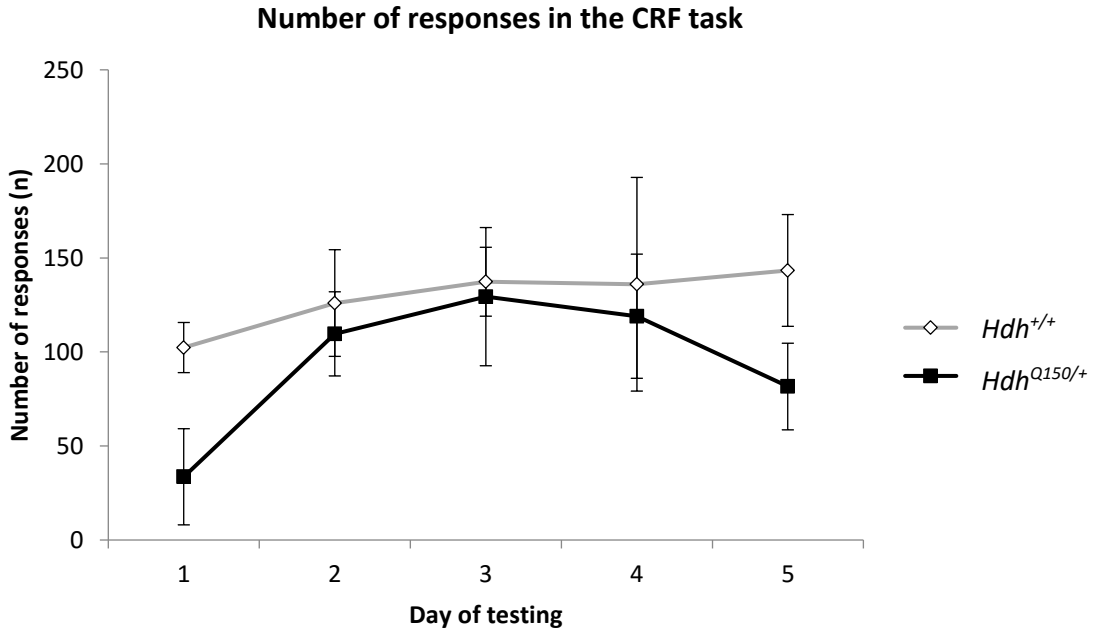


Figure 3.5. The mean number of responses made by *Hdh*$^{+/+}$ and *Hdh*$^{Q150/+}$ animals over the 5 days of training in the CRF task. There was no effect of genotype on the mean number of trials initiated by *Hdh*$^{+/+}$ and *Hdh*$^{Q150/+}$ mice over the five days of CRF training. Values are means ± S.E.M; *Hdh*$^{+/+}$: n = 3; *Hdh*$^{Q150/+}$: n = 5.

3.3.1.2 5-CSRTT performance

A significant difference in mean accuracy to S1 was identified between genotypes (main effect of genotype: $F_{1,6}$ = 7.932, p = 0.030), with $Hdh^{Q150/+}$ animals presenting with lower levels of accuracy than their wild-type counterparts at 6M (Fig. 7A). A strong effect of session was found on mean accuracy (main effect of session: $F_{4, 24}$ = 205.588, p < 0.001), and there was a significant interaction between session and genotype (genotype*session: $F_{4, 24}$ = 3.156, p = 0.032). Post-hoc tests found that mean accuracy was significantly higher in the final fourth and fifth days of 5-CSRT training compared to the first (p < 0.001), second (p < 0.001) and third (p < 0.001) days of testing (Fig. 3.6A). A difference in mean accuracy between the final fourth and fifth sessions was also reported (p < 0.001). Unlike mean accuracy, there was no significant difference in the number of trials performed between genotypes in the 5-CSRTT (main effect of genotype: $F_{1, 6}$ = 0.142, p = 0.719). There was, however, an increase in the number of trials performed across the final 5 days of testing (main effect of session: $F_{4, 24}$ = 8.300, p < 0.001), and this effect was found to be independent of genotype (genotype*session: $F_{4, 24}$ = 0.331, p = 0.855). Post-hoc analyses only found a significant increase in the number of trials performed between the first and fifth days of testing (Fig. 7B; p = 0.041).

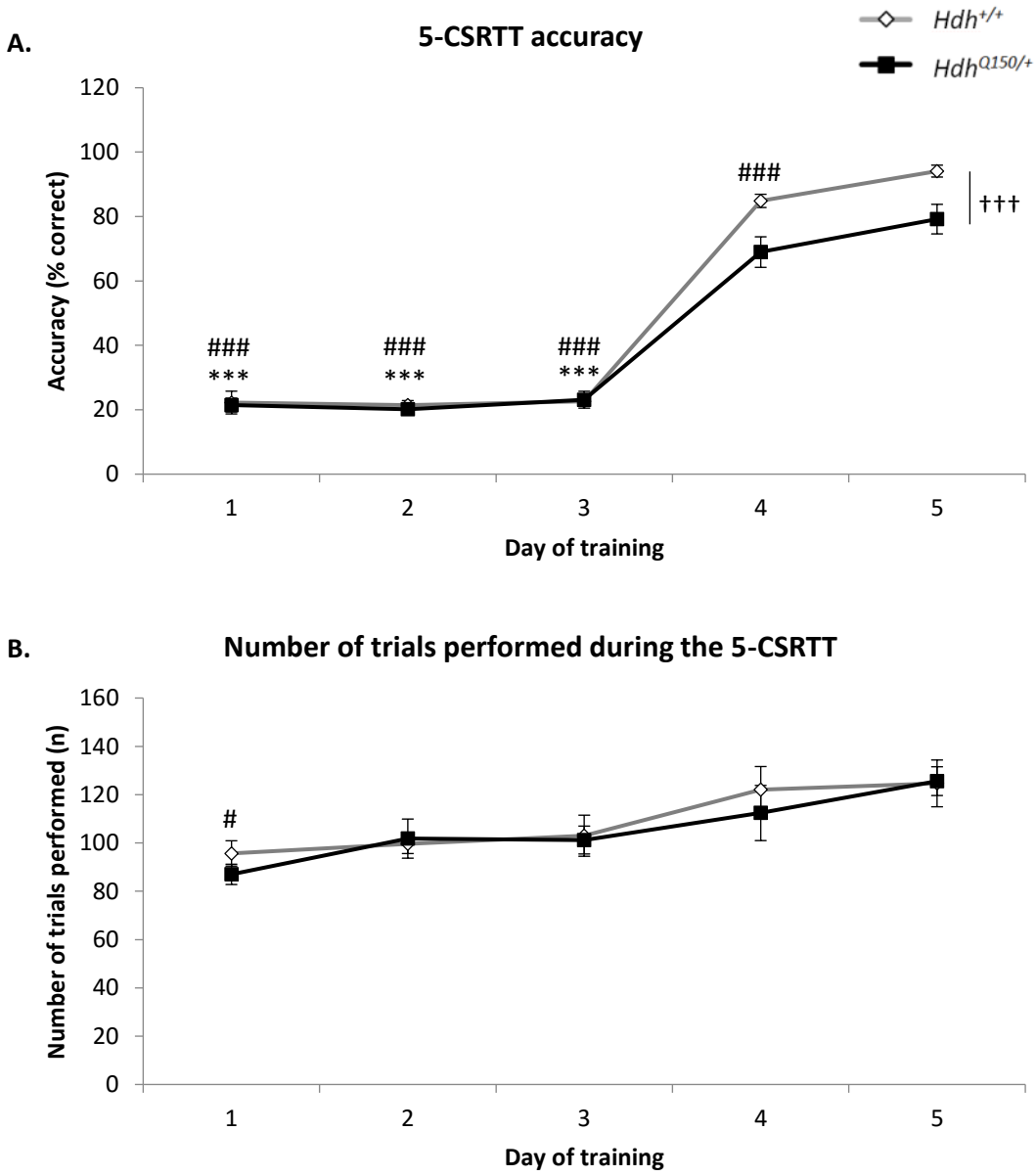


Figure 3.6. Mean accuracy and number of trials performed over the final five days of 5-CSRTT testing for *Hdh*$^{+/+}$ and *Hdh*$^{Q150/+}$ mice. Genotype had a significant effect on accuracy (A) but not the number of trials performed (B) in the 5-CSRTT. Day of training was found to significantly affect the accuracy (A) and number of trials performed (B). Values shown are means ± S.E.M.; *Hdh*$^{+/+}$: n = 3; *Hdh*$^{Q150/+}$: n = 5.

* Denotes a significant difference from day 4 # Denotes a significant difference from day 5

† Denotes a significant difference between genotypes

* $p < 0.05$ *** $p < 0.001$

3.3.1.3 SILT performance

Overall task accuracy during successive days of testing shows progressive improvement for both genotypes in accuracy to S1 and S2 (Fig. 3.7A and B), suggesting that both groups of mice were able to successfully learn the SILT.

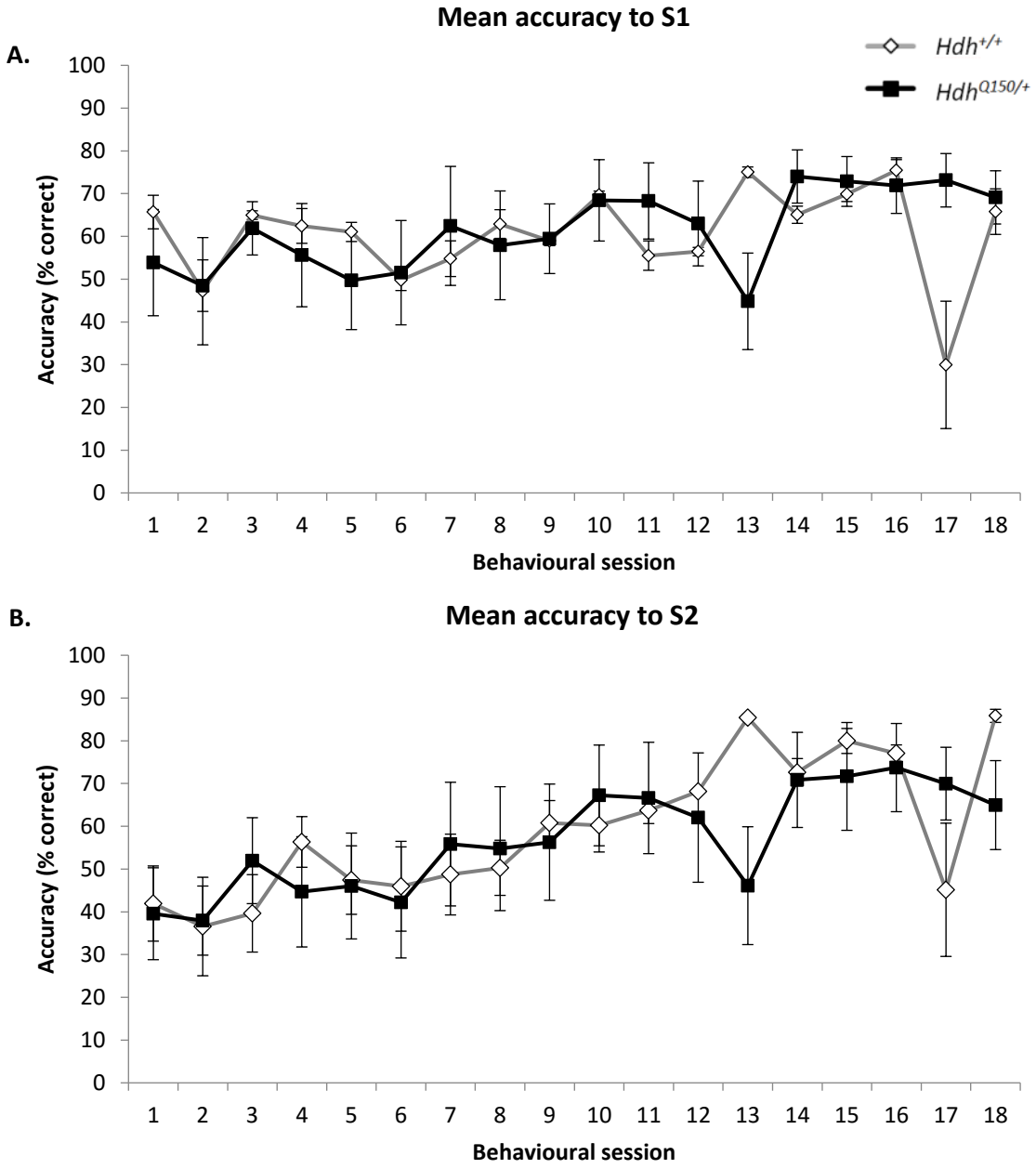


Figure 3.7. Overall task accuracy on consecutive days of training and testing on the SILT for *Hdh*$^{+/+}$ and *Hdh*$^{Q150/+}$ groups. Task accuracy is defined as the percentage of initiated trials in which the animal makes a correct response to S1 (A) or S2 (B). Values are means ± S.E.M.; *Hdh*$^{+/+}$: n = 3; *Hdh*$^{Q150/+}$: n = 5.

Examination of the accuracy and reaction times to S1 over the last 5 days of the task showed that there was no difference in performance between genotypes (main effect of genotype: $F_{1,6}$ = 1.428, p = 0.277). After subdividing the response to S1 to account for the location of the stimulus, there was significant variation in accuracy to each hole (Fig. 3.8A; main effect of hole location: $F_{4, 24}$ = 3.952, p = 0.013), independent of genotype (genotype*hole location: $F_{4, 24}$ = 0.573, p = 0.685). Tukey's HSD post-hoc test correcting for multiple testing, however, reported no significant difference in accuracy levels between any of the hole locations (p = n.s. for all comparisons). Reaction times to S1 did not significantly differ between *Hdh*$^{Q150/+}$ and *Hdh*$^{+/+}$ animals (Fig. 3.8D; main effect of genotype: $F_{1,6}$ = 0.891, p = 0.382), were not affected by hole location (Fig. 3.8D; main effect of hole location: $F_{1.557, 9.341}$ = 1.429, p = 0.280), and there was no interaction between genotype and hole location (genotype*hole location: $F_{1.557, 9.341}$ = 0.588, p = 0.534).

The response to S2 revealed no differences in accuracy between *Hdh*$^{Q150/+}$ mice and their *Hdh*$^{+/+}$ littermates (main effect of genotype: $F_{1,6}$ = 0.005, p = 0.946). Levels of accuracy to S2 showed significant variation depending on the number of steps between S1 and S2 (Fig. 3.8B; main effect of step number: $F_{1.316, 7.899}$ = 16.040, p = 0.003), and this effect was independent of genotype (genotype*step number: $F_{1.316, 7.899}$ = 0.432, p = 0.584). Post-hoc analyses reported a significant decrease in accuracy levels for S2 where S1 and S2 were 3 or 4 steps apart in comparison to where S1 and S2 were only 1 step (p = 0.038 and p = 0.036, respectively). Similar to S2 accuracy, reaction times to S2 did not significantly differ between *Hdh*$^{Q150/+}$ and *Hdh*$^{+/+}$ mice (Fig. 3.8E; main effect of genotype: $F_{1,6}$ = 0.002, p = 0.966) and the number of steps between S1 and S2 stimuli strongly influenced reaction time to S2 (Fig. 3.8E; main effect of step number: $F_{1.190, 7.139}$ = 28.558, p = 0.001). Reaction times to S2 were significantly faster when S1 and S2 were 1 step apart than when there was a distance of 2, 3 or 4 steps between the stimuli (Fig. 3.8E; p = 0.003, p = 0.003 and p < 0.001, respectively). There was no significant interaction between genotype and the number of steps between S1 and S2 causing an effect on reaction times to S2 (genotype*step number: $F_{1.190, 7.139}$ = 0.823, p = 0.416).

There was no significant difference in predictive accuracy to S2 between *Hdh*$^{Q150/+}$ and *Hdh*$^{+/+}$ mice (Fig. 3.8C; main effect of genotype: $F_{1,6}$ = 0.250, p = 0.635). Both genotypes showed improved response accuracy on predictable trials compared to unpredictable trials (Fig. 3.8C; main effect of predictability: $F_{2, 12}$ = 3.980, p = 0.047). There was no interaction between trial predictability and genotype (genotype*predictability: $F_{2, 12}$ = 0.095, p = 0.910). As with predicted accuracy to S2, there was no significant difference in reaction times to S2, based on predictability, between genotypes (Fig. 3.8F; main effect of genotype: $F_{1,6}$ = 0.194, p = 0.675). Neither genotype showed a significant difference in reaction time to predictable versus

unpredictable trials (Fig. 3.8F; main effect of predictability: $F_{1.142, 6.852} = 0.037$, $p = 0.882$), and there was no interaction between genotype and stimulus predictability (genotype*predictability: $F_{1.142, 6.852} = 0.234$, $p = 0.675$).

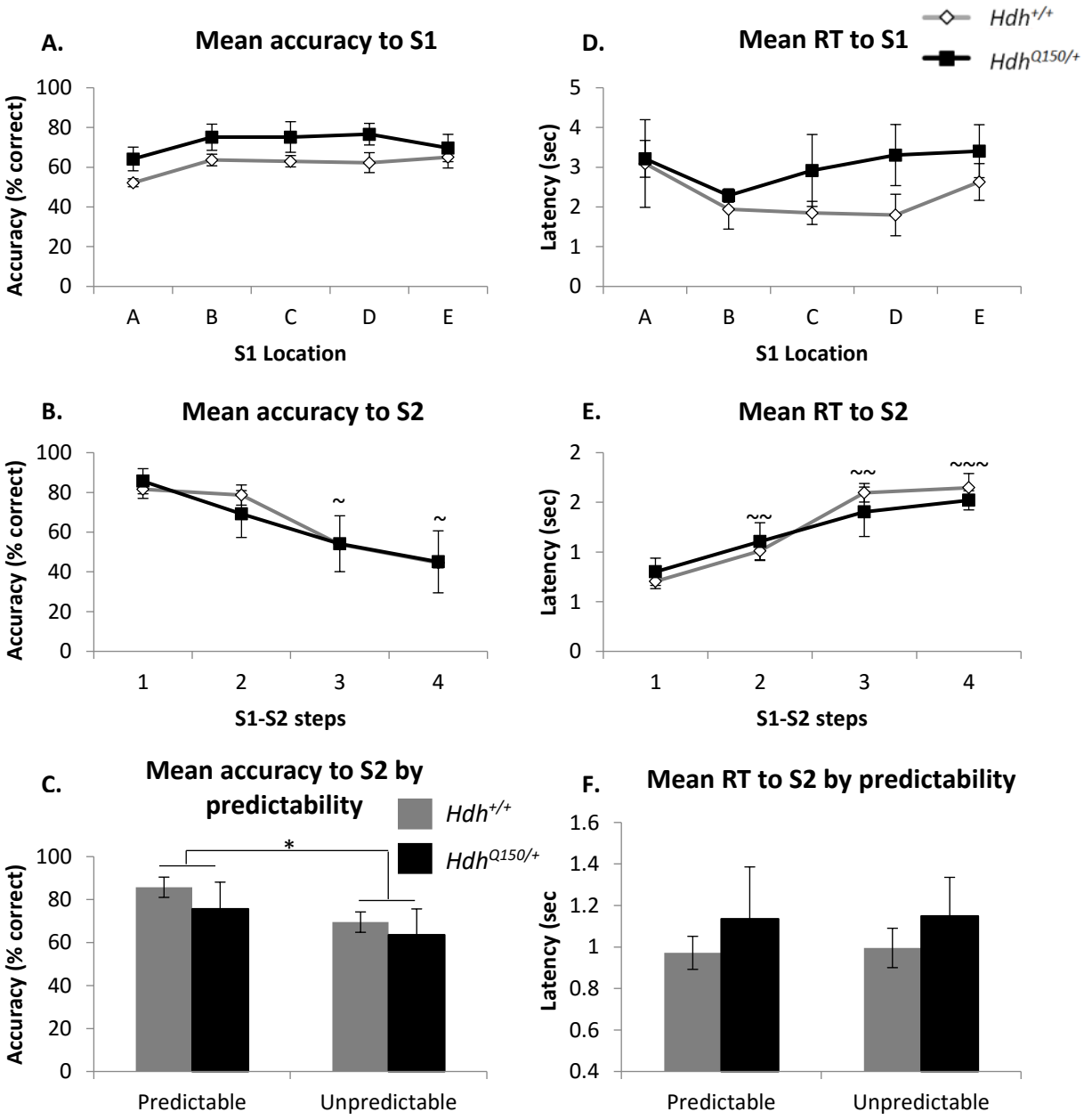


Figure 3.8. Data for the SILT collapsed over the last 5 days of testing for *Hdh*$^{+/+}$ and *Hdh*$^{Q150/+}$ mice. Panels (A-F) show accuracy and reaction times to S1 by holes (A and D), and accuracy and reaction times to S2 by steps (B and E) and predictability (C and F). Accuracy and reaction times to S1 (A and D, respectively) showed no significant difference between genotypes, independent of hole location. Similarly, no significant difference in accuracy or reaction times to S2 was found between genotypes, based on number of steps between S1 and S2 (B and E) or predictability of S2 (C and F). Values are means ± S.E.M.; *Hdh*$^{+/+}$: n = 3; *Hdh*$^{Q150/+}$: n = 5.
~ Denotes a significant difference from the 1 step distance between S1 and S2
* $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$

Seven error terms and the total number of S1 and S2 trials were analysed and summarised in Table 3.4. An independent *t*-test reported no difference in the number of S1 or S2 trials initiated by $Hdh^{+/+}$ and $Hdh^{Q150/+}$ animals ($t(6) = 0.436$, $p = 0.678$ and $t(6) = -0.437$, $p = 0.677$, respectively), and none of the seven error terms analysed showed a significant difference between genotypes (Table 3.4).

Mean number of trials initiated	***Hdh*$^{+/+}$**	***Hdh*$^{Q150/+}$**	***t*(6) =**	***p*-value**
S1 trials[a]	179.33 ± 15.88	163.80 ± 25.28	0.436	0.678
S2 trials[a]	108.80 ± 6.36	123.48 ± 24.92	-0.437	0.677
Error rates of specific error types				
Incorrect choice in S1[b]	15.66 ± 6.03	14.18 ± 5.29	0.179	0.864
Incorrect choice in S2[c]	11.11 ± 4.41	13.53 ± 5.11	-0.322	0.759
Magazine poke in S1[b]	23.12 ± 3.78	15.17 ± 3.82	1.373	0.219
Perseverative np to S1[b]	11.76 ± 5.10	12.67 ± 4.01	-0.140	0.894
Np in ITI[d]	0.63 ± 0.13	1.29 ± 0.43	-1.133	0.300
Np per TOI[e]	2.12 ± 0.97	1.01 ± 0.20	1.474	0.191
TO during S2[b]	6.29 ± 1.13	8.96 ± 2.78	-0.705	0.507

Table 3.4. Error analyses of the SILT performance of $Hdh^{Q150/+}$ mice. *t* value statistics are presented with degrees of freedom (df) of 6 unless otherwise stated. Values presented are means ± S.E.M.; $Hdh^{+/+}$: n = 3; $Hdh^{Q150/+}$: n = 5. Abbreviations: ITI, inter-trial interval; TOI, time out interval; TO, time out.

[a] Mean total counts.

[b] Error rates as a percentage of the total number of S1 trials initiated.

[c] Error rates as a percentage of the total number of S2 trials initiated.

[d] Error rates per ITI.

[e] Error rates per incorrect S2 trial.

3.3.1.4 Behavioural results summary

To summarise, no behavioural differences were evident between $Hdh^{Q150/+}$ and $Hdh^{+/+}$ mice at 6M in CRF or SILT performance. However, in the 5-CSRTT, $Hdh^{Q150/+}$ animals exhibited significantly lower levels of accuracy compared to their wild-type counterparts in the final two days of testing.

3.3.2 Gene expression level analyses

3.3.2.1 Microarray chips

This experiment used Affymetrix GeneChip® Mouse Gene 2.0 ST Array chips (see Chapter 3.2.5.2), which contain oligonucleotide probes that interrogate multiple loci on every exon of every transcript, thus allowing evaluation of whole-transcriptome gene expression levels at the gene and exon levels. Each array chip contains >698,000 probes, allowing investigation into expression levels of >33,000 genes, as well as >2,000 long intergenic non-coding RNAs.

3.3.2.2 Quality control metrics

Principal components analysis (PCA) is a mathematical technique that is useful in data reduction and allows visual estimation and clustering of data (Quackenbush, 2001), and was performed as an early exploratory analysis of global gene expression level patterns across all samples. The PCA mapping indicates that the main sources of variation in gene expression levels in this experiment are age and behavioural training, with genotype having a lesser contribution (Fig. 3.9). Examination of the PCA mapping in Figure 3.9 indicates that gene expression level patterns of untrained animals at 6M form a separate cluster from those of animals that are trained to acquisition or untrained at 3M, and also separate from those of animals that have performed any form of training at 6M.

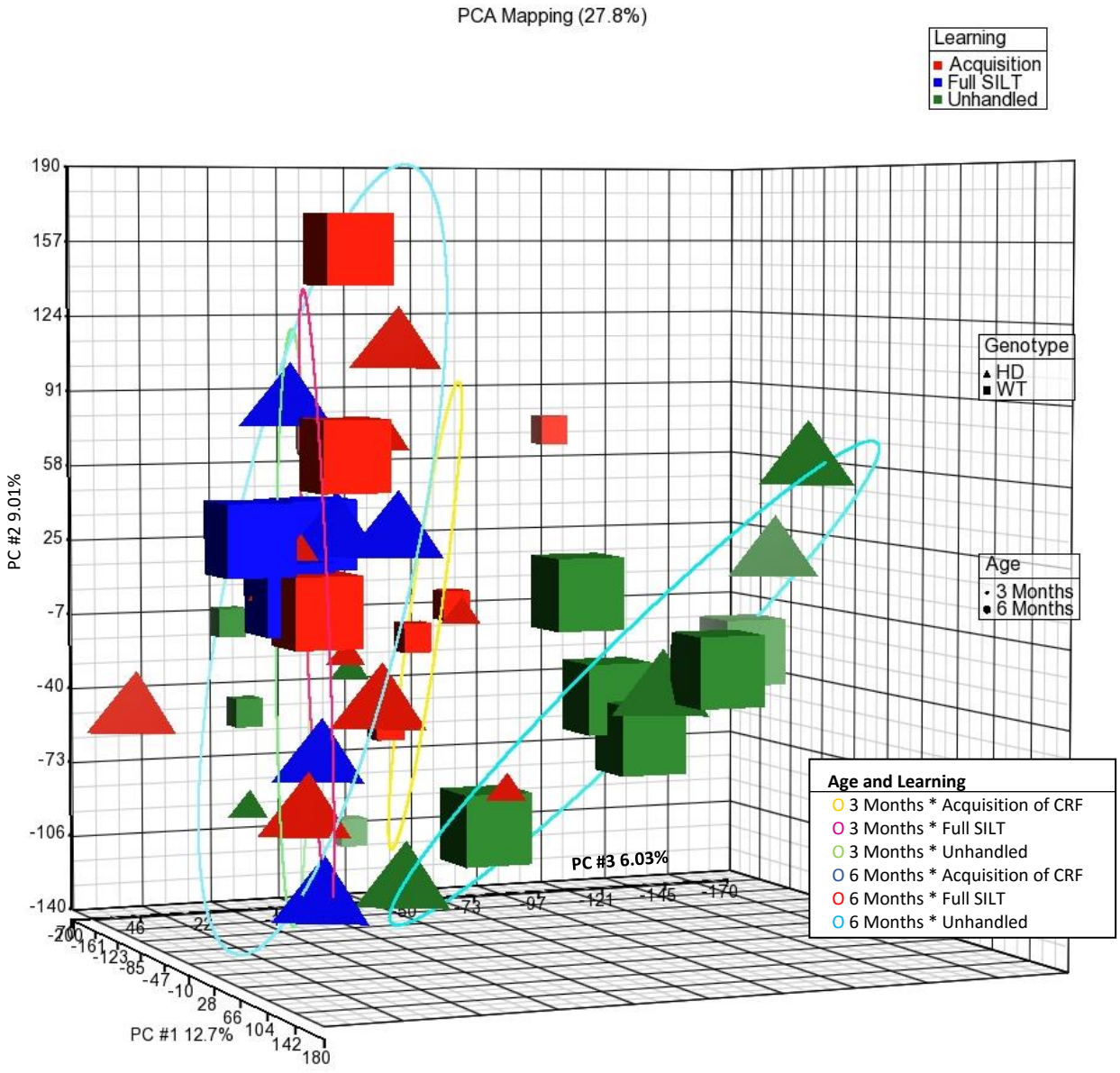


Figure 3.9. Age and training account for a greater amount of gene expression level changes than genotype. Age is the main principal component, accounting for 12.7% of gene expression level variance. The second principal component is behavioural training (untrained, acquisition or full-SILT), which accounts for 9.01% of the variance in gene expression levels, while genotype is the third principal component and accounts for only 6.03% of the variance in gene expression levels. There is a clear separation of gene expression profiles between untrained animals at 6M and all other groups. n = 3-6/group.

3.3.2.3 Differential gene expression levels

A three-way ANOVA was performed on the microarray gene expression level data with age, genotype and learning (unhandled, acquisition or full-SILT) as variables, and also using the same variables as interaction terms in a three-way interaction. Statistical significance was taken using FDR $p < 0.05$, with FDR estimation generated by Benjamini-Hochberg procedure, and no minimum magnitude of fold-change was defined when generating lists of genes showing significantly altered expression levels. Thus, gene lists were created using the parameters described in Table 3.5, with the number of genes present in each list also recorded in Table 3.5.

Briefly, the largest number of significant gene expression level changes were identified as a result of ageing in untrained wild-type animals (*Hdh*$^{+/+}$ 3M U vs *Hdh*$^{+/+}$ 6M U: n = 2,940), while untrained *Hdh*$^{Q150/+}$ mice presented with a smaller number of significant age-dependent gene expression level changes (*Hdh*$^{Q150/+}$ 3M U vs *Hdh*$^{Q150/+}$ 6M U: n = 122). In each genotype, learning-dependent gene expression level changes were identified between untrained animals and those that had undergone training to acquisition in the CRF or performed full-SILT training, and the number of gene expression level changes resulting from the behavioural paradigms were higher in *Hdh*$^{+/+}$ animals than in their *Hdh*$^{Q150/+}$ counterparts (Table 3.5). However, animals trained to acquisition of the CRF and animals that had underwent complete training and performance in the SILT displayed 0 significant gene expression level changes in both genotypes (*Hdh*$^{+/+}$ 6M A vs *Hdh*$^{+/+}$ 6M S: n = 0; *Hdh*$^{Q150/+}$ 6M A vs *Hdh*$^{Q150/+}$ 6M S: n = 0). 0 genes presented with significantly altered expression levels as a result of genotype in any of the genotype-dependent comparisons examined (Table 3.5).

Group comparison	Number of genes differentially expressed
***Hdh*$^{+/+}$ 3M U vs *Hdh*$^{Q150/+}$ 3M U**	0
***Hdh*$^{+/+}$ 3M U vs *Hdh*$^{+/+}$ 6M U**	2, 940
***Hdh*$^{+/+}$ 3M U vs *Hdh*$^{+/+}$ 3M A**	0
***Hdh*$^{Q150/+}$ 3M U vs *Hdh*$^{Q150/+}$ 6M U**	122
***Hdh*$^{Q150/+}$ 3M U vs *Hdh*$^{Q150/+}$ 3M A**	0
***Hdh*$^{+/+}$ 6M U vs *Hdh*$^{Q150/+}$ 6M U**	0
***Hdh*$^{+/+}$ 6M U vs *Hdh*$^{+/+}$ 6M A**	596
***Hdh*$^{+/+}$ 6M U vs *Hdh*$^{+/+}$ 6M S**	189
***Hdh*$^{+/+}$ 6M A vs *Hdh*$^{+/+}$ 6M S**	0
***Hdh*$^{Q150/+}$ 6M U vs *Hdh*$^{Q150/+}$ 6M A**	375
***Hdh*$^{Q150/+}$ 6M U vs *Hdh*$^{Q150/+}$ 6M S**	145
***Hdh*$^{Q150/+}$ 6M A vs *Hdh*$^{Q150/+}$ 6M S**	0
***Hdh*$^{+/+}$ 3M A vs *Hdh*$^{+/+}$ 6M A**	0
***Hdh*$^{Q150/+}$ 3M A vs *Hdh*$^{Q150/+}$ 6M A**	0
***Hdh*$^{+/+}$ 3M A vs *Hdh*$^{Q150/+}$ 3M A**	0
***Hdh*$^{+/+}$ 6M A vs *Hdh*$^{Q150/+}$ 6M A**	0
***Hdh*$^{+/+}$ 6M S vs *Hdh*$^{Q150/+}$ 6M S**	0
***Hdh*$^{+/+}$ vs. *Hdh*$^{Q150/+}$**	0
Age	450
Learning	873
Genotype	0
3M vs 6M	0
U vs A	96
U vs S	0
A vs S	0
Age*Learning*Genotype Interaction	378

Table 3.5. Microarray gene expression level group comparisons and gene list sizes. No fold change limit was applied and genes were counted as significantly differentially expressed if FDR-corrected $p < 0.05$**.** n = 3-6/group. Abbreviations: 3M, 3 months of age; 6M, 6 months of age; U, unhandled; A, trained to acquisition on the CRF; S, performed full SILT training.

Hierarchical clustering of genes based on gene expression level values derived from the lists of genes showing significantly altered expression levels between groups allows visualisation of the grouping of differentially expressed genes. Clustering based on the gene list derived from the comparison between untrained *Hdh*$^{+/+}$ animals at 3M and 6M showed strong evidence that untrained 6M old animals, of both genotypes, have a distinct gene expression level profile compared to animals that underwent training at 3M or 6M, but also to untrained animals at 3M (Fig. 3.10), supporting the findings from the PCA analysis (Fig. 3.9).

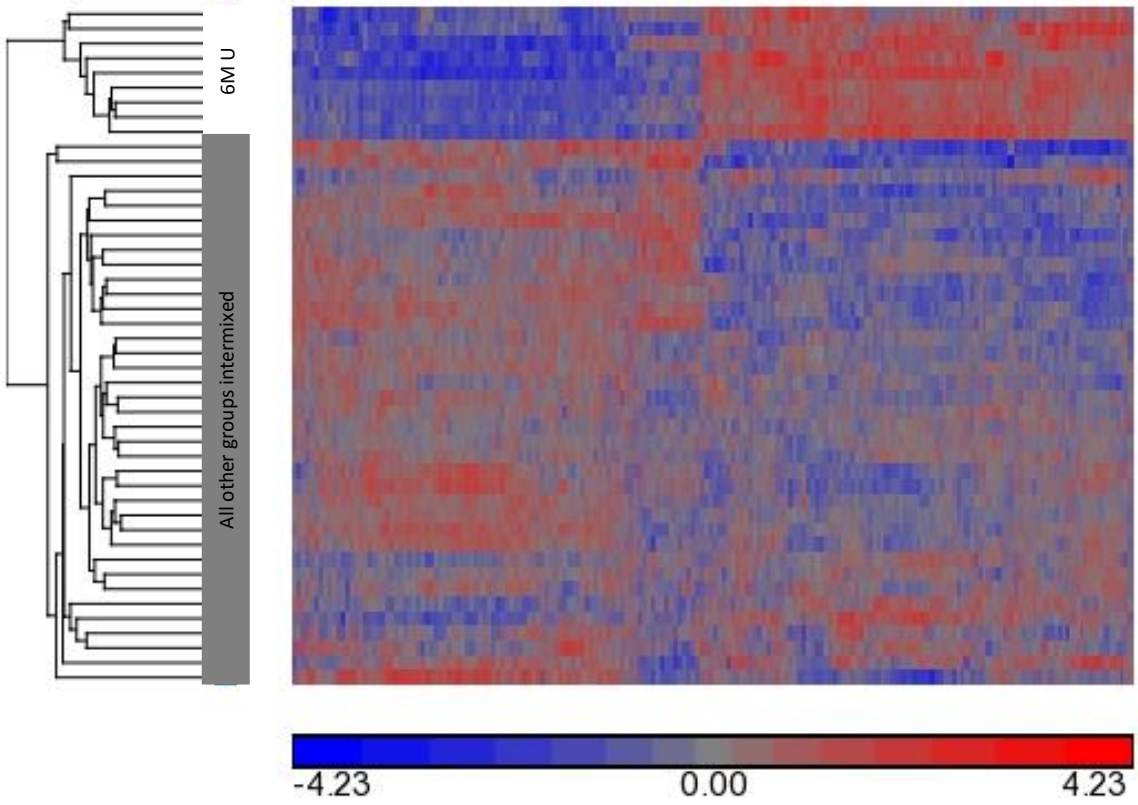


Figure 3.10. Heat map representing hierarchical clustering of genes based on gene expression level values derived from the *Hdh*$^{+/+}$ 3M U vs. *Hdh*$^{+/+}$ 6M U comparison gene list. Data was transformed whereby the mean expression value for each gene was 0, with a standard deviation of 1. Blue indicates a reduction in gene expression levels and red indicates an increase in gene expression levels, whilst grey is indicative of no change in gene expression levels. n = 3-6/group. Abbreviations: 6M, 6 months of age; U, unhandled.

3.3.2.4 Validation of microarray gene expression level results using RT-qPCR

Calbindin 2 (*Calb2*), *glucagon-like peptide 1 receptor* (*Glp1r*), *G protein-coupled receptor 165* (*Gpr165*), *potassium voltage-gated channel, subfamily H, member 3* (*Kcnh3*), *potassium inwardly-rectifying channel, subfamily J, member 4* (*Kcnj4*), *ribonuclease P RNA component H1* (*Rpph1*), *solute carrier family 17 member 6* (*Slc17a6*), *small nucleolar RNA, H/ACA box 74A* (*Snora74a*), *uracil phosphoribosyltransferase* (*Uprt*), and *zic family member 1* (*Zic1*) were genes identified as showing altered expression levels in microarray group comparisons (Table 3.6) and were chosen for RT-qPCR analysis based on the mean level of gene expression, fold-change in expression (log2) and coefficient of variation reported for the gene in any group comparison in the microarray (Table 3.6). *Actb* and *Ubc* were used as endogenous controls because they showed no significant differences in expression in comparisons between groups (data not shown) and have been reported to show stable levels of expression within the striatum of a mouse model of HD (Benn et al., 2008a).

Gene	Group Comparison	FDR *p*-value	Mean Gene Expression	Fold-Change	CV (%)
Calb2	$Hdh^{+/+}$ 3M U vs $Hdh^{+/+}$ 6M U	2.97×10^{-03}	8.31	-2.68	18.55
Glp1r	$Hdh^{+/+}$ 3M U vs $Hdh^{+/+}$ 6M U	8.40×10^{-04}	8.28	-2.88	19.15
Gpr165	$Hdh^{+/+}$ 3M U vs $Hdh^{+/+}$ 6M U	1.55×10^{-03}	8.47	-1.76	11.20
Kcnh3	$Hdh^{+/+}$ 3M U vs $Hdh^{+/+}$ 6M U	2.65×10^{-04}	9.08	+1.35	6.89
Kcnj4	$Hdh^{+/+}$ 3M U vs $Hdh^{+/+}$ 6M U	1.57×10^{-03}	8.62	+1.25	7.61
	$Hdh^{Q150/+}$ 3M U vs $Hdh^{Q150/+}$ 6M U	1.25×10^{-05}		+0.75	
	$Hdh^{Q150/+}$ 6M U vs $Hdh^{Q150/+}$ 6M A	3.56×10^{-05}		-0.66	
Rpph1	$Hdh^{Q150/+}$ 6M U vs $Hdh^{Q150/+}$ 6M S	8.50×10^{-05}	11.39	-0.60	2.72
	$Hdh^{+/+}$ 3M U vs $Hdh^{+/+}$ 6M U	2.60×10^{-04}		-0.51	
	$Hdh^{+/+}$ 6M U vs $Hdh^{+/+}$ 6M A	5.62×10^{-05}		-0.67	
	$Hdh^{+/+}$ 6M U vs $Hdh^{+/+}$ 6M S	2.54×10^{-04}		-0.55	
Slc17a6	$Hdh^{+/+}$ 3M U vs $Hdh^{+/+}$ 6M U	3.51×10^{-03}	8.02	-2.47	17.91
	$Hdh^{+/+}$ 3M U vs $Hdh^{+/+}$ 6M U	5.88×10^{-05}		+0.85	
Snora74a	$Hdh^{+/+}$ 6M U vs $Hdh^{+/+}$ 6M A	4.21×10^{-04}	7.48	-0.81	6.03
	$Hdh^{+/+}$ 6M U vs $Hdh^{+/+}$ 6M S	1.06×10^{-05}		-1.02	
	$Hdh^{Q150/+}$ 3M U vs $Hdh^{Q150/+}$ 6M U	2.45×10^{-08}		-0.78	
	$Hdh^{Q150/+}$ 6M U vs $Hdh^{Q150/+}$ 6M A	2.22×10^{-08}		+0.75	
Uprt	$Hdh^{Q150/+}$ 6M U vs $Hdh^{Q150/+}$ 6M S	5.85×10^{-08}	9.13	+0.69	3.70
	$Hdh^{+/+}$ 3M U vs $Hdh^{+/+}$ 6M U	1.73×10^{-10}		-0.83	
	$Hdh^{+/+}$ 6M U vs $Hdh^{+/+}$ 6M S	5.14×10^{-08}		+0.69	
Zic1	$Hdh^{+/+}$ 3M U vs $Hdh^{+/+}$ 6M U	1.21×10^{-03}	9.18	-1.96	11.80

Table 3.6. Microarray data for genes chosen for validation by RT-qPCR. Genes found to be exhibiting the largest mean gene expression level values and fold-changes (log2) between groups in the microarray data were chosen for validation by RT-qPCR. n = 3-6/group. Abbreviations: CV, coefficient of variation.

3.3.2.4.1 Genotype-dependent gene expression level changes

In accordance with the microarray findings, none of the genes investigated showed any differences in expression levels between genotypes in any comparison where genotype is the only variable between groups (Table 3.7).

Group comparison	***Calb2***	***Glp1r***	***Gpr165***	***Kcnh3***	***Kcnj4***
***Hdh*$^{+/+}$ 3M U vs.**	$t_{(5)}$ = -0.246	$t_{(5)}$ = -0.523	$t_{(5)}$ = -0.181	$t_{(5)}$ = 0.353	$t_{(5)}$ = 0.477
***Hdh*$^{Q150/+}$ 3M U**	p = 1.000	p = 1.000	p = 1.000	p = 1.000	p = 1.000
***Hdh*$^{+/+}$ 3M A vs.**	$t_{(7)}$ = -0.887	$t_{(7)}$ = -1.400	$t_{(7)}$ = -1.709	$t_{(7)}$ = 1.092	$t_{(7)}$ = 1.010
***Hdh*$^{Q150/+}$ 3M A**	p = 1.000	p = 1.000	p = 1.000	p = 1.000	p = 1.000
***Hdh*$^{+/+}$ 6M U vs.**	$t_{(8)}$ = -0.109	$t_{(8)}$ = 0.173	$t_{(8)}$ = 0.594	$t_{(8)}$ = -0.929	$t_{(8)}$ = -0.832
***Hdh*$^{Q150/+}$ 6M U**	p = 1.000	p = 1.000	p = 1.000	p = 1.000	p = 1.000
***Hdh*$^{+/+}$ 6M A vs.**	$t_{(6)}$ = 0.941	$t_{(6)}$ = 4.149	$t_{(6)}$ = 1.273	$t_{(6)}$ = -1.555	$t_{(4.409)}$ = -1.893
***Hdh*$^{Q150/+}$ 6M A**	p = 1.000	p = 0.060	p = 1.000	p = 0.856	p = 0.875
***Hdh*$^{+/+}$ 6M S vs.**	$t_{(6)}$ = 0.053	$t_{(6)}$ = -0.338	$t_{(6)}$ = -0.033	$t_{(6)}$ = -0.084	$t_{(6)}$ = 0.323
***Hdh*$^{Q150/+}$ 6M S**	p = 1.000	p = 1.000	p = 1.000	p = 1.000	p = 1.000
Group comparison	***Rpph1***	***Slc17a6***	***Snora74a***	***Uprt***	***Zic 1***
***Hdh*$^{+/+}$ 3M U vs.**	$t_{(5)}$ = 0.325	$t_{(5)}$ = 0.153	$t_{(5)}$ = -0.572	$t_{(5)}$ = -1.590	$t_{(5)}$ = 0.235
***Hdh*$^{Q150/+}$ 3M U**	p = 1.000	p = 1.000	p = 1.000	p = 1.000	p = 1.000
***Hdh*$^{+/+}$ 3M A vs.**	$t_{(7)}$ = -1.233	$t_{(7)}$ = -0.674	$t_{(7)}$ = -0.088	$t_{(7)}$ = -1.831	$t_{(7)}$ = -1.123
***Hdh*$^{Q150/+}$ 3M A**	p = 1.000	p = 1.000	p = 1.000	p = 1.000	p = 1.000
***Hdh*$^{+/+}$ 6M U vs.**	$t_{(3.466)}$ = 0.238	$t_{(8)}$ = -.0662	$t_{(8)}$ = -0.496	$t_{(8)}$ = 0.366	$t_{(8)}$ = 0.018
***Hdh*$^{Q150/+}$ 6M U**	p = 1.000	p = 1.000	p = 1.000	p = 1.000	p = 1.000
***Hdh*$^{+/+}$ 6M A vs.**	$t_{(6)}$ = 0.103	$t_{(6)}$ = -0.501	$t_{(6)}$ = 0765	$t_{(6)}$ = 0.856	$t_{(6)}$ = 2.026
***Hdh*$^{Q150/+}$ 6M A**	p = 1.000	p = 1.000	p = 1.000	p = 1.000	p = 0.801
***Hdh*$^{+/+}$ 6M S vs.**	$t_{(6)}$ = 1.620	$t_{(6)}$ = -0.231	$t_{(6)}$ = 0.909	$t_{(6)}$ = 1.017	$t_{(6)}$ = -0.082
***Hdh*$^{Q150/+}$ 6M S**	p = 1.000	p = 1.000	p = 1.000	p = 1.000	p = 1.000

Table 3.7. The statistical values for RT-qPCR gene expression level comparisons between genotypes using independent samples t-tests, with Holm-Bonferroni sequential correction for multiple hypothesis test correction. In the event of data violating the assumption of homogeneity of variances, as assessed by Levene's test for homogeneity of variances, results of the Welch test were used and the t-statistic value altered as a result of this. n = 3-6/group.

3.3.2.4.2 Behavioural training-dependent gene expression level changes

RT-qPCR reported striatal expression of *Rpph1* to be different between *Hdh*$^{+/+}$ mice that had undergone one of the three behavioural training protocols at 6M (Fig. 3.11A; $F_{2, 9}$ = 9.783, p = 0.006). Post-hoc analysis reported significantly lower *Rpph1* expression levels in the *Hdh*$^{+/+}$ 6M S group in comparison to *Hdh*$^{+/+}$ 6M U mice (Table 3.8b; p = 0.005), as reported in the microarray (Table 3.6). Similar to *Rpph1* expression, levels of *Snora74a* expression within the striatum were also found to be significantly altered as a result of training in 6M old *Hdh*$^{+/+}$ animals (Fig. 3.11B; $F_{2, 9}$ = 12.245, p = 0.003). CRF training resulted in significantly decreased *Snora74a* expression levels compared to untrained counterparts (Table 3.8b; p = 0.004) and the decreased expression levels resulting from full-SILT training versus an absence of training also reached significance (Table 3.8b; p = 0.016), with both comparisons also reporting significant reductions in expression levels in the microarray (Table 3.6). Statistical results for all RT-qPCR investigations examining behavioural training-dependent striatal gene expression level alterations can be found in Table 3.8a.

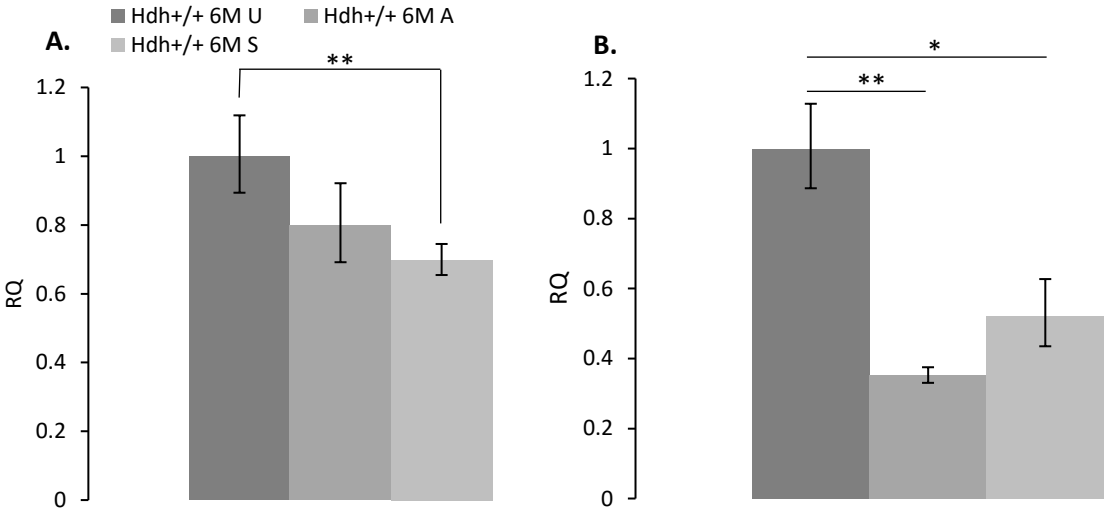


Figure 3.11. RQ values of *Rpph1* (A) and *Snora74a* (B) behaviour-dependent striatal expression levels following RT-qPCR to confirm microarray data. At 6M, full-SILT trained *Hdh*$^{+/+}$ mice show significantly reduced expression of *Rpph1* compared to untrained animals of the same genotype at 6M (A). Similarly, CRF and full-SILT training both lead to decreased expression of *Snora74a* within the striatum of 6M *Hdh*$^{+/+}$ animals, compared to their untrained counterparts (B). Data presented is mean fold change ± S.E.M.; *Hdh*$^{+/+}$ 6M A: n = 3; *Hdh*$^{+/+}$ 6M S: n = 3; *Hdh*$^{+/+}$ 6M U: n = 6.

*p < 0.05 ** p < 0.01

Group comparison	*Calb2*	*Glp1r*	*Gpr165*	*Kcnh3*	*Kcnj4*
***Hdh*$^{+/+}$ 3M U vs. *Hdh*$^{+/+}$ 3M A**	$t_{(7)}$ = -1.573 p = 1.000	$t_{(7)}$ = -0.955 p = 1.000	$t_{(7)}$ = -0.278 p = 1.000	$t_{(7)}$ = 1.252 p = 1.000	$t_{(7)}$ = 1.539 p = 1.000
***Hdh*$^{+/+}$ 6M U vs. *Hdh*$^{+/+}$ 6M A vs. *Hdh*$^{+/+}$ 6M S**	$F_{2, 9}$ = 0.038 p = 0.963	$F_{2, 9}$ = 0.282 p =0.761	$F_{2, 9}$ = 2.667 p =0.123	$F_{2, 9}$ = 0.063 p =0.939	$F_{2, 9}$ = 0.369 p =0.702
***Hdh*$^{Q150/+}$ 3M U vs. *Hdh*$^{Q150/+}$ 3M A**	$t_{(5)}$ = -1.365 p = 1.000	$t_{(5)}$ = -1.028 p = 1.000	$t_{(5)}$ = -1.592 p = 1.000	$t_{(1.201)}$ = 1.106 p = 1.000	$t_{(5)}$ = 2.473 p = 0.560
***Hdh*$^{Q150/+}$ 6M U vs. *Hdh*$^{Q150/+}$ 6M A vs. *Hdh*$^{Q150/+}$ 6M S**	$F_{2, 11}$ = 0.736 p = 0.501	$F_{2, 4.942}$ = 2.483 p = 0.179	$F_{2, 11}$ = 0.520 P = 0.608	$F_{2, 11}$ = 0.673 p = 0.530	$F_{2, 11}$ = 0.843 p = 0.456

Group comparison	*Rpph1*	*Slc17a6*	*Snora74a*	*Uprt*	*Zic 1*
***Hdh*$^{+/+}$ 3M U vs. *Hdh*$^{+/+}$ 3M A**	$t_{(7)}$ = 0.725 p = 1.000	$t_{(7)}$ = -3.479 p = 0.100	$t_{(7)}$ = -0.490 p = 1.000	$t_{(7)}$ = -0.214 p = 1.000	$t_{(7)}$ = -2.285 p = 0.504
***Hdh*$^{+/+}$ 6M U vs. *Hdh*$^{+/+}$ 6M A vs. *Hdh*$^{+/+}$ 6M S**	$F_{2, 9}$ = 9.783 p = 0.006**	$F_{2, 9}$ = 0.664 p = 0.538	$F_{2, 9}$ = 12.245 p = 0.003**	$F_{2, 9}$ = 0.083 p = 0.921	$F_{2, 9}$ = 0.637 p = 0.551
***Hdh*$^{Q150/+}$ 3M U vs. *Hdh*$^{Q150/+}$ 3M A**	$t_{(5)}$ = -0.630 p = 1.000	$t_{(5)}$ = -2.109 p = 0.801	$t_{(5)}$ = -0.004 p = 1.000	$t_{(5)}$ = 0.099 p = 1.000	$t_{(5)}$ = -1.120 p = 1.000
***Hdh*$^{Q150/+}$ 6M U vs. *Hdh*$^{Q150/+}$ 6M A vs. *Hdh*$^{Q150/+}$ 6M S**	$F_{2, 11}$ = 0.704 p = 0.515	$F_{2, 11}$ = 0.284 p = 0.758	$F_{2, 11}$ = 1.581 p = 0.249	$F_{2, 11}$ = 0.532 p = 0.602	$F_{2, 11}$ = 0.737 p = 0.501

Table 3.8a. The statistical values for RT-qPCR gene expression level comparisons between groups of animals where behavioural training was the only variable. An independent t-test with Holm-Bonferroni sequential correction was used to examine differences between specific two-group comparisons, while a one-way ANOVA was performed on 3 group comparisons and a *post-hoc* Tukey's HSD test performed on comparisons in which a main effect was found to be significant. n = 3-6/group.

Group comparison	*Rpph1*	*Snora74a*
***Hdh*$^{+/+}$ 6M U vs. *Hdh*$^{+/+}$ 6M A**	p = 0.094	p = 0.004**
***Hdh*$^{+/+}$ 6M U vs. *Hdh*$^{+/+}$ 6M S**	p = 0.005**	p = 0.016*
***Hdh*$^{+/+}$ 6M A vs. *Hdh*$^{+/+}$ 6M S**	p = 0.270	p = 0.724

Table 3.8b. The p-values of group comparisons from a *post-hoc* Tukey test for the genes in which a main effect was found to be significant in a one-way ANOVA (Table 3.8a).

3.3.2.4.3 Gene expression level changes as a result of ageing

The microarray reported a large number of genes whose altered expression levels were changed between 3M and 6M (Table 3.5), with the majority of genes to be investigated by RT-qPCR showing altered expression between the *Hdh*$^{+/+}$ 3M U and *Hdh*$^{+/+}$ 6M U groups (Table 3.6). RT-qPCR did not find significant differences in striatal expression levels of any of the genes investigated for the *Hdh*$^{+/+}$ 3M U and *Hdh*$^{+/+}$ 6M U group comparison found to show variation in the microarray (Table 3.9). However, significant differences in striatal gene expression levels that can be attributed to ageing were found between *Hdh*$^{+/+}$ 3M A and *Hdh*$^{+/+}$ 6M A animals for *Glp1r* (Fig. 3.12A; $t_{(5)}$ = -5.963, p = 0.020) and *Zic 1* (Fig. 3.12B; $t_{(5)}$ = -5.156, p = 0.036). These comparisons were not found to be significant in the microarray investigation. All other possible ageing-dependent striatal expression changes in the genes investigated by RT-qPCR were found to be non-significant (Table 3.9).

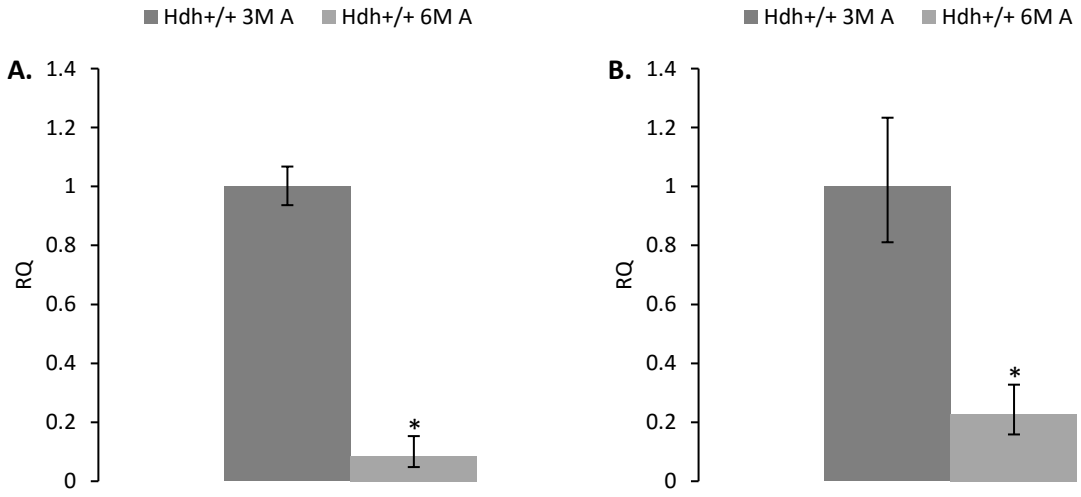


Figure 3.12. RQ values of *Glp1r* (A) and *Zic 1* (B) ageing-dependent striatal expression levels, in CRF trained *Hdh*$^{+/+}$ animals, following RT-qPCR to confirm microarray data. Both genes show significant reductions in striatal expression levels between *Hdh*$^{+/+}$ animals from 3M to 6M, neither of which were reported in the microarray (Table 3.6). Data presented is mean fold change ± S.E.M.; *Hdh*$^{+/+}$ 3M A: n = 4; *Hdh*$^{+/+}$ 6M A: n = 3.

* $p < 0.05$

Group comparison	*Calb2*	*Glp1r*	*Gpr165*	*Kcnh3*	*Kcnj4*
***Hdh*$^{+/+}$ 3M U vs. *Hdh*$^{+/+}$ 6M U**	$t_{(9)}$ = -3.500 p = 0.063	$t_{(9)}$ = -3.504 p = 0.063	$t_{(9)}$ = -3.311 p = 0.063	$t_{(9)}$ = 3.732 p = 0.050	$t_{(9)}$ = 2.972 p = 0.064
***Hdh*$^{+/+}$ 3M A vs. *Hdh*$^{+/+}$ 6M A**	$t_{(5)}$ = -1.533 p = 1.000	$t_{(5)}$ = -5.963 p = 0.020*	$t_{(5)}$ = -1.969 p = 1.000	$t_{(5)}$ = 1.567 p = 1.000	$t_{(5)}$ = 1.364 p = 1.000
***Hdh*$^{Q150/+}$ 3M U vs. *Hdh*$^{Q150/+}$ 6M U**	$t_{(4)}$ = -2.046 p = 0.990	$t_{(4)}$ = -1.688 p = 1.000	$t_{(4)}$ = -1.016 p = 1.000	$t_{(4)}$ = 0.679 p = 1.000	$t_{(4)}$ = 0.601 p = 1.000
***Hdh*$^{Q150/+}$ 3M A vs. *Hdh*$^{Q150/+}$ 6M A**	$t_{(8)}$ = 0.236 p = 1.000	$t_{(8)}$ = 0.387 p = 1.000	$t_{(8)}$ = 0.935 p = 1.000	$t_{(8)}$ = -1.020 p = 1.000	$t_{(8)}$ = -1.204 p = 1.000
Group comparison	***Rpph1***	***Slc17a6***	***Snora74a***	***Uprt***	***Zic 1***
***Hdh*$^{+/+}$ 3M U vs. *Hdh*$^{+/+}$ 6M U**	$t_{(9)}$ = 2.438 p = 0.114	$t_{(9)}$ = -3.479 p = 0.063	$t_{(9)}$ = 1.270 p = 0.472	$t_{(9)}$ = -0.447 p = 0.665	$t_{(9)}$ = -3.135 p = 0.063
***Hdh*$^{+/+}$ 3M A vs. *Hdh*$^{+/+}$ 6M A**	$t_{(5)}$ = -0.650 p = 1.000	$t_{(5)}$ = 0.695 p = 1.000	$t_{(5)}$ = -1.116 p = 1.000	$t_{(5)}$ = -0.476 p = 1.000	$t_{(5)}$ = -5.156 p = 0.036*
***Hdh*$^{Q150/+}$ 3M U vs. *Hdh*$^{Q150/+}$ 6M U**	$t_{(4)}$ = 0.638 p = 1.000	$t_{(4)}$ = -2.612 p = 0.590	$t_{(3.824)}$ = 1.792 p = 1.000	$t_{(4)}$ = 1.885 p = 1.000	$t_{(4)}$ = -1.357 p = 1.000
***Hdh*$^{Q150/+}$ 3M A vs. *Hdh*$^{Q150/+}$ 6M A**	$t_{(8)}$ = 0.476 p = 1.000	$t_{(8)}$ = 0.275 p = 1.000	$t_{(8)}$ = -0.679 p = 1.000	$t_{(8)}$ = 2.400 p = 0.430	$t_{(8)}$ = 0.627 p = 1.000

Table 3.9. The statistical values for age-dependent gene expression level comparisons examined using RT-qPCR and independent samples t-tests. Independent samples t-tests with Holm-Bonferroni sequential correction for multiple hypothesis testing corrections were performed to examine differences in gene expression levels between groups. n = 3-6/group.

3.3.2.4.3 Microarray validation by RT-qPCR summary

To summarise the findings of the RT-qPCR based validation of the microarray gene expression level data, two genes showed significant differences in levels of expression in group comparisons in the RT-qPCR analyses that were also identified in the microarray investigation. Both *Rpph1* and *Snora74a* showed significantly altered levels of mRNA transcripts in *Hdh*$^{+/+}$ mice at 6M that had undergone different levels of behavioural training in the RT-qPCR (Table 3.8a; Fig. 3.11) and microarray investigations (Table 3.6). RT-qPCR also revealed gene expression level changes that are attributable to ageing that were not identified in the microarray, where CRF trained *Hdh*$^{+/+}$ animals at 6M present with significantly decreased levels of striatal *Glp1r* and *Zic1* in comparison to their CRF trained counterparts at 3M (Table 3.9; Fig. 3.12). RT-qPCR did not find significant differences in striatal gene expression levels for the remaining group comparisons presenting with such changes in the microarray. However, the fold-changes identified in the RT-qPCR analyses did show the same trend of expression (positive or negative fold-changes) for 19 of the 21 comparisons showing such changes in the microarray (Table 3.10).

Gene	Group Comparison	Microarray Fold-Change	RT-qPCR Fold-Change	Trend Validation?
Calb2	*Hdh*$^{+/+}$ 3M U vs *Hdh*$^{+/+}$ 6M U	-2.68	-10.04	Yes
Glp1r	*Hdh*$^{+/+}$ 3M U vs *Hdh*$^{+/+}$ 6M U	-2.88	-17.04	Yes
Gpr165	*Hdh*$^{+/+}$ 3M U vs *Hdh*$^{+/+}$ 6M U	-1.76	-4.24	Yes
Kcnh3	*Hdh*$^{+/+}$ 3M U vs *Hdh*$^{+/+}$ 6M U	+1.35	+3.19	Yes
Kcnj4	*Hdh*$^{+/+}$ 3M U vs *Hdh*$^{+/+}$ 6M U	+1.25	+2.84	Yes
Rpph1	*Hdh*$^{Q150/+}$ 3M U vs *Hdh*$^{Q150/+}$ 6M U	+0.75	+1.24	Yes
	Hdh$^{Q150/+}$ 6M U vs *Hdh*$^{Q150/+}$ 6M A	-0.66	-1.29	Yes
	Hdh$^{Q150/+}$ 6M U vs *Hdh*$^{Q150/+}$ 6M S	-0.60	-1.18	Yes
	Hdh$^{+/+}$ 3M U vs *Hdh*$^{+/+}$ 6M U	-0.51	+1.25	No
	Hdh$^{+/+}$ 6M U vs *Hdh*$^{+/+}$ 6M A	-0.67	-1.25	Yes
	Hdh$^{+/+}$ 6M U vs *Hdh*$^{+/+}$ 6M S	-0.55	-1.50	Yes
Slc17a6	*Hdh*$^{+/+}$ 3M U vs *Hdh*$^{+/+}$ 6M U	-2.47	-9.51	Yes
Snora74a	*Hdh*$^{+/+}$ 3M U vs *Hdh*$^{+/+}$ 6M U	+0.85	+1.38	Yes
	Hdh$^{+/+}$ 6M U vs *Hdh*$^{+/+}$ 6M A	-0.81	-2.84	Yes
	Hdh$^{+/+}$ 6M U vs *Hdh*$^{+/+}$ 6M S	-1.02	-2.30	Yes
Uprt	*Hdh*$^{Q150/+}$ 3M U vs *Hdh*$^{Q150/+}$ 6M U	-0.78	-1.06	Yes
	Hdh$^{Q150/+}$ 6M U vs *Hdh*$^{Q150/+}$ 6M A	+0.75	+1.03	Yes
	Hdh$^{Q150/+}$ 6M U vs *Hdh*$^{Q150/+}$ 6M S	+0.69	+1.16	Yes
	Hdh$^{+/+}$ 3M U vs *Hdh*$^{+/+}$ 6M U	-0.83	-1.06	Yes
	Hdh$^{+/+}$ 6M U vs *Hdh*$^{+/+}$ 6M S	+0.69	-1.02	No
Zic1	*Hdh*$^{+/+}$ 3M U vs *Hdh*$^{+/+}$ 6M U	-1.96	-5.05	Yes

Table 3.10. A summary table for the validation of the microarray gene expression level results by RT-qPCR. The fold-changes in expression levels for the gene in each comparison for both the microarray and RT-qPCR results are presented. The gene expression level alteration trend between the microarray and RT-qPCR findings are said to be validated if the fold-changes of both investigations are in the same direction (positive or negative fold-change). n = 3-6/group.

3.3.2.5 Pathway analysis of microarray gene expression level data

Initial pathway analyses were performed on gene lists generated in the microarray (Table 3.5), the results of which are presented in Tables 3.11 and 3.12. The microarray reported 0 genes showing differences in expression levels in any comparison where genotype was the only discriminating factor (Table 3.5), thus genotype-dependent pathway analyses were not performed.

Comparison	1	2	3	4	5	6
GO Category	***Hdh*$^{+/+}$ 3M U vs *Hdh*$^{+/+}$ 6M U**	***Hdh*$^{+/+}$ 6M U vs *Hdh*$^{+/+}$ 6M A**	***Hdh*$^{+/+}$ 6M U vs *Hdh*$^{+/+}$ 6M S**	***Hdh*$^{Q150/+}$ 3M U vs *Hdh*$^{Q150/+}$ 6M U**	***Hdh*$^{Q150/+}$ 6M U vs *Hdh*$^{Q150/+}$ 6M A**	***Hdh*$^{Q150/+}$ 6M U vs *Hdh*$^{Q150/+}$ 6M S**
Protein localisation (GO:0008104)	1.61×10^{-12}	5.22×10^{-03}	NS	1.61×10^{-12}	2.23×10^{-07}	NS
Protein transport (GO:0015031)	7.04×10^{-12}	1.09×10^{-02}	NS	7.04×10^{-12}	3.28×10^{-07}	NS
Establishment of protein localisation (GO:0045184)	1.33×10^{-11}	1.69×10^{-03}	NS	1.33×10^{-11}	4.00×10^{-07}	NS
Cellular protein localisation (GO:0034613)	5.46×10^{-08}	NS	NS	5.46×10^{-08}	9.56×10^{-03}	NS
Ribonucleotide binding (GO:0032553)	7.53×10^{-05}	NS	NS	OTT	NS	NS
Intracellular transport (GO:0046907)	4.69×10^{-07}	NS	NS	1.31×10^{-07}	1.63×10^{-03}	NS
Cellular macromolecule localisation (GO:0070727)	7.60×10^{-08}	NS	NS	7.60×10^{-08}	4.76×10^{-02}	NS
Nucleotide binding (GO:0000166)	OTT	NS	NS	OTT	NS	NS
Intracellular protein transport (GO:0006886)	1.84×10^{-07}	NS	NS	1.87×10^{-07}	NS	NS
Purine nucleotide binding (GO:0017076)	1.51×10^{-06}	NS	NS	OTT	NS	NS
Vesicle-mediated transport (GO:0016192)	OTT	NS	NS	3.66×10^{-04}	9.26×10^{-05}	NS
GTP binding (GO:0005525)	OTT	NS	1.63×10^{-04}	OTT	1.03×10^{-04}	NS
Golgi apparatus (GO:0005794)	OTT	NS	NS	OTT	3.99×10^{-04}	NS
Small GTPase mediated signal transduction (GO:0007264)	1.83×10^{-07}	NS	NS	1.83×10^{-07}	NS	NS
Guanyl ribonucleotide binding (GO:0032561)	OTT	NS	2.18×10^{-04}	2.03×10^{-03}	1.52×10^{-04}	NS

Table 3.11. Comparison of the top 10 significant GO biological categories identified by DAVID pathway analysis for the gene lists listed in Table 3.5. Data are FDR p-values from a DAVID analysis (Huang et al., 2009a, b); n = 3-6/group. Abbreviations: NS = Not significant; OTT = Outside top 10 significant GO biological pathways.

Comparison	1	2	3	4	5
GO Category	***Hdh*$^{+/+}$ 3M U vs *Hdh*$^{+/+}$ 6M U & *Hdh*$^{Q150/+}$ 3M U vs *Hdh*$^{Q150/+}$ 6M U Overlap**	***Hdh*$^{+/+}$ 6M U vs *Hdh*$^{+/+}$ 6M A & *Hdh*$^{Q150/+}$ 6M U vs *Hdh*$^{Q150/+}$ 6M A Overlap**	***Hdh*$^{+/+}$ 6M U vs *Hdh*$^{+/+}$ 6M S & *Hdh*$^{Q150/+}$ 6M U vs *Hdh*$^{Q150/+}$ 6M S Overlap**	***Hdh*$^{+/+}$ 6M U vs *Hdh*$^{+/+}$ 6M A & *Hdh*$^{+/+}$ 6M U vs *Hdh*$^{+/+}$ 6M S Overlap**	***Hdh*$^{Q150/+}$ 6M U vs *Hdh*$^{Q150/+}$ 6M A & *Hdh*$^{Q150/+}$ 6M U vs *Hdh*$^{Q150/+}$ 6M S Overlap**
Protein localisation (GO:0008104)	2.62×10^{-06}	1.01×10^{-05}	NS	NS	NS
Protein transport (GO:0015031)	1.80×10^{-04}	1.15×10^{-05}	NS	NS	NS
Establishment of protein localisation (GO:0045184)	1.98×10^{-04}	2.17×10^{-05}	NS	NS	NS
Cellular protein localisation (GO:0034613)	NS	NS	NS	NS	NS
Ribonucleotide binding (GO:0032553)	NS	NS	NS	NS	NS
Intracellular transport (GO:0046907)	7.76×10^{-03}	7.52×10^{-03}	NS	NS	NS
Cellular macromolecule localisation (GO:0070727)	NS	NS	NS	NS	NS
Nucleotide binding (GO:0000166)	NS	NS	NS	NS	NS
Intracellular protein transport (GO:0006886)	NS	NS	NS	NS	NS
Purine nucleotide binding (GO:0017076)	NS	NS	NS	NS	NS
Vesicle-mediated transport (GO:0016192)	2.07×10^{-03}	5.65×10^{-04}	NS	NS	NS
GTP binding (GO:0005525)	4.10×10^{-03}	NS	NS	NS	NS
Golgi apparatus (GO:0005794)	7.42×10^{-03}	4.26×10^{-02}	NS	NS	NS
Small GTPase mediated signal transduction (GO:0007264)	4.81×10^{-02}	NS	NS	NS	NS
Guanyl ribonucleotide binding (GO:0032561)	5.03×10^{-03}	NS	NS	NS	NS

Table 3.12. Comparison of the top 10 significant GO biological categories identified by DAVID pathway analysis for overlaps between groups of interest from gene lists listed in Table 3.5. Data are FDR p-values from DAVID analysis (Huang et al., 2009a, b); n = 3-6/group. Abbreviations: NS = Not significant.

3.3.2.5.1 Behavioural training-dependent pathway analyses

Both genotypes showed alterations in three similar pathways as a result of CRF training, identified by examination of pathway group comparisons 3.11.2 and 3.11.5 (Table 3.11), which suggests that striatal protein transport and localisation, and the establishment of protein localisation, undergo change as a result of simple conditioning learning. *Hdh*$^{Q150/+}$ animals, however, also show significant gene expression level changes in pathways associated with other aspects of protein production and transport, such as in the Golgi apparatus and vesicle-mediated transport, as well as in signalling pathways that include guanosine-5'-triphosphate (GTP) binding. 170 genes show significant alterations resulting from simple conditioning learning in the striatum of both genotypes (Fig. 3.13), and are associated with cellular localisation and trafficking, including vesicle-mediated transport and Golgi apparatus activity (Table 3.12.2).

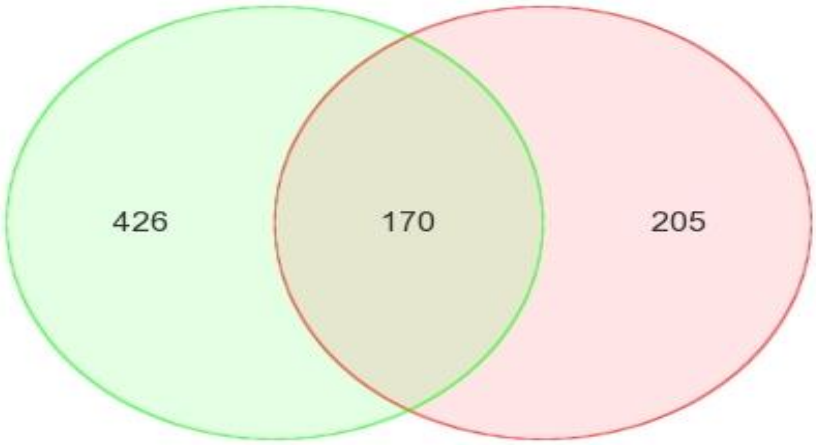
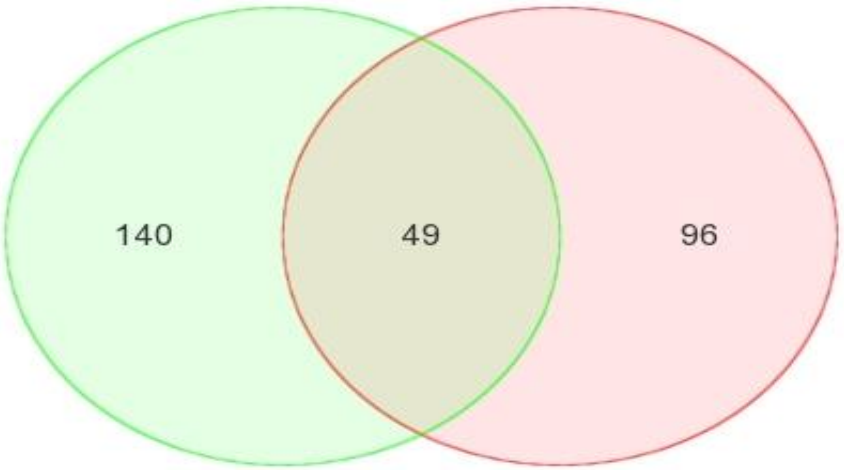


Figure 3.13. The overlap between striatal genes differentially expressed as a result of simple conditioning learning between *Hdh*$^{+/+}$ and *Hdh*$^{Q150/+}$ mice. 170 genes were found to overlap between genotypes as a result of CRF training. n = 3-6/group.

Performing a complex serial visual discrimination task produced changes in few biological pathways in each genotype (Table 3.11.3 & 3.11.6), although it is worth noting that, unlike CRF-only trained animals, *Hdh*$^{Q150/+}$ mice show significant alterations in a fewer number of GO categories than their *Hdh*$^{+/+}$ counterparts, with implicit learning altering zero GO processes in *Hdh*$^{Q150/+}$ mice (Table 3.11.6). *Hdh*$^{+/+}$ animals were found to exhibit significant differences in expression of genes associated with a smaller number of biological categories after implicit learning compared to CRF training, though both forms of learning seem to only alter gene expression level changes in specific biological processes (GTP binding, and protein localisation

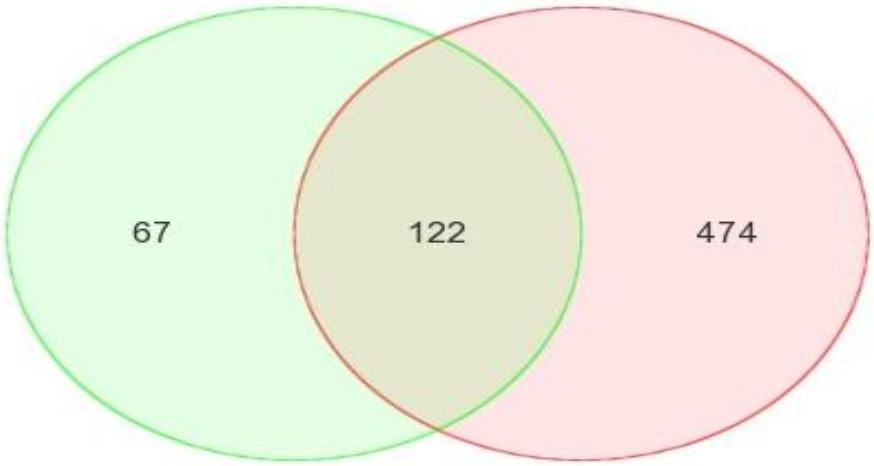
and transport, respectively). Only 49 genes were significantly altered following implicit learning in both genotypes (Fig. 3.14), and were not found to be associated with any GO biological processes showing significant alteration (Table 3.12.3).



Hdh$^{+/+}$ 6M U vs *Hdh*$^{+/+}$ 6M S (n = 189) *Hdh*$^{Q150/+}$ 6M U vs *Hdh*$^{Q150/+}$ 6M S (n = 145)

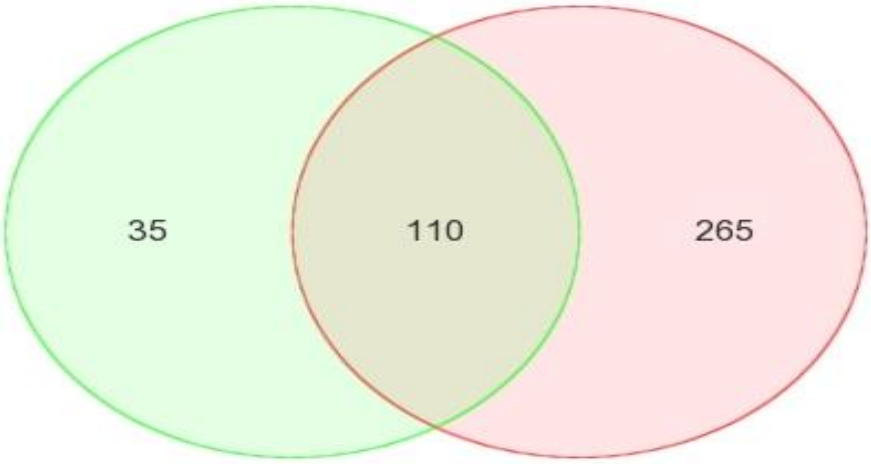
Figure 3.14. The overlap between striatal genes differentially expressed as a result of implicit learning between *Hdh*$^{+/+}$ and *Hdh*$^{Q150/+}$ mice. 49 genes were found to overlap between genotypes as a result of implicit learning. n = 3-6/group.

As a result of CRF training and implicit learning, a large proportion of genes exhibit abundant expression changes in the striatum of both genotypes (*Hdh*$^{+/+}$ n = 122, *Hdh*$^{Q150/+}$ = 110; Fig. 3.15 & Fig. 3.16). DAVID analyses reported that genes showing an overlap of altered expression between conditioning and implicit learning in *Hdh*$^{+/+}$ animals were not significantly associated with any GO biological pathways (Table 3.12.4), however they were associated with the non-GO process "acetylation" (FDR $p = 3.36 \times 10^{-02}$). Similarly, the 110 genes that underwent expression changes as a result of both conditioning and implicit learning in *Hdh*$^{Q150/+}$ animals were not significantly associated with any GO biological categories (Table 3.12.5).



Hdh$^{+/+}$ 6M U vs *Hdh*$^{+/+}$ 6M S (n = 189) *Hdh*$^{+/+}$ 6M U vs *Hdh*$^{+/+}$ 6M A (n = 596)

Figure 3.15. The overlap between striatal genes differentially expressed as a result of conditioning and implicit learning in *Hdh*$^{+/+}$ mice. 122 genes were found to overlap between learning paradigms in *Hdh*$^{+/+}$ animals. n = 3-6/group.



Hdh$^{Q150/+}$ 6M U vs *Hdh*$^{Q150/+}$ 6M S (n = 145) *Hdh*$^{Q150/+}$ 6M U vs *Hdh*$^{Q150/+}$ 6M A (n = 375)

Figure 3.16. The overlap between striatal genes differentially expressed as a result of CRF training and implicit learning in *Hdh*$^{Q150/+}$ mice. 110 genes were found to overlap between learning paradigms in *Hdh*$^{Q150/+}$ animals. n = 3-6/group.

Of the genes that underwent significant expression changes in response to, or as a result of, both conditioning and implicit learning, 40 of these genes exhibited altered expression in both genotypes (Fig. 3.17). 82 of the genes found to be associated with both types of learning presented with transcriptional changes only in *Hdh*$^{+/+}$ animals, whilst 70 only underwent significant changes in expression in *Hdh*$^{Q150/+}$ animals (Fig. 3.17). The genes altered following

acquisition of a simple conditioning task and acquisition of a complex serial visual discrimination task in both genotypes were most associated with GTP signal transduction and protein transport (data not shown), although DAVID analyses reported no significant association with any GO process. The 82 striatal mRNAs that underwent expression changes only in *Hdh*$^{+/+}$ mice after acquisition of a simple conditioning task and acquisition of a complex serial visual discrimination task were linked with structural roles, such as microtubule and tubulin binding, and protein transportation (data not shown), as defined by their GO clustering classification. However, this did not reach statistical significance. Transcriptional changes only reported in *Hdh*$^{Q150/+}$ animals were also associated with protein transportation and structural roles, and oxidoreductase activity was another pathway associated with the genes (data not shown), though again there were no significant associations with any of the GO pathways.

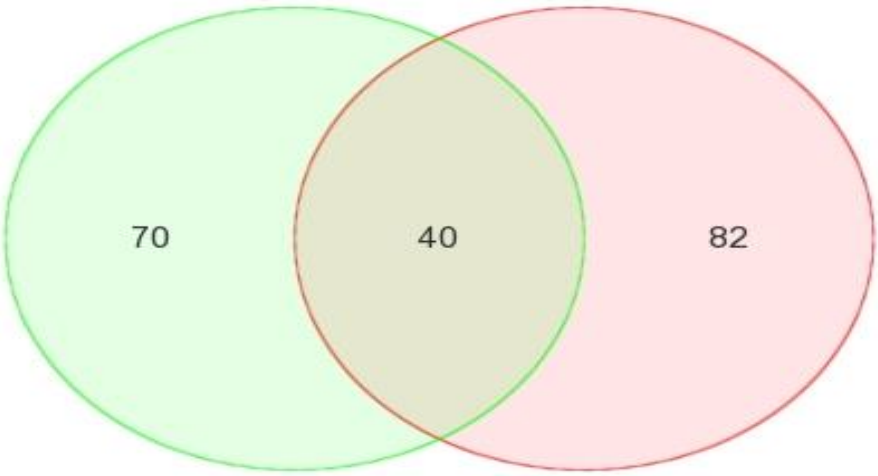
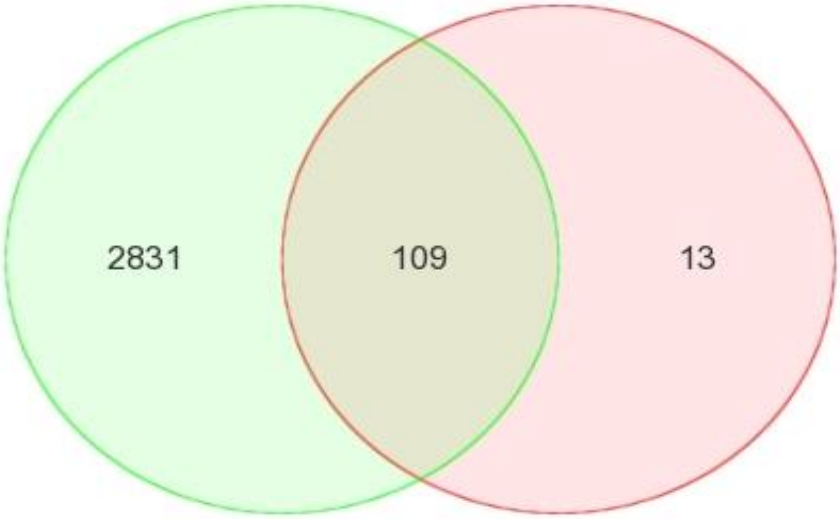


Figure 3.17. The overlap between striatal genes differentially expressed as a result of conditioning and implicit learning in both *Hdh*$^{+/+}$ and *Hdh*$^{Q150/+}$ mice. 40 genes were found to overlap between learning paradigms in both genotypes of animals. n = 3-6/group.

3.3.2.5.2 Pathway analyses of ageing group comparisons

Pathway analysis of differential gene expression level signatures between 3M and 6M show similar functions in the absence of training in the *Hdh*$^{+/+}$ and *Hdh*$^{Q150/+}$ striata (Table 3.11.1 & 3.11.4), suggesting protein localisation and transport, and GTP activity are altered in ageing. 109 genes were found to overlap between genotypes as a result of ageing in the absence of behavioural training (Fig. 3.18), and were associated with protein localisation and transport, and GTP activity (Table 3.12.1), as would be anticipated. Pathway analyses were not performed on

the other age-dependent comparisons (*Hdh*$^{+/+}$ 3M A vs *Hdh*$^{+/+}$ 6M A and *Hdh*$^{Q150/+}$ 3M A vs *Hdh*$^{Q150/+}$ 6M A) because there were 0 genes identified in the microarray as showing significantly altered expression levels in either comparison (Table 3.5).



Hdh$^{+/+}$ 3M U vs *Hdh*$^{+/+}$ 6M U (n = 2, 940) *Hdh*$^{Q150/+}$ 3M U vs *Hdh*$^{Q150/+}$ 6M U (n = 122)

Figure 3.18. The overlap between striatal genes differentially expressed as a result of ageing between *Hdh*$^{+/+}$ and *Hdh*$^{Q150/+}$ mice. 109 genes were found to overlap between genotypes as a result of ageing in the absence of behavioural training. n = 3-6/group.

3.4 Discussion

The current chapter aimed to evaluate the potential effects of the HD genotype, ageing, and different forms of learning on the striatal transcriptome of mice. No differences in behaviours tested were evident between *Hdh*$^{Q150/+}$ and *Hdh*$^{+/+}$ animals at 2M-6M in CRF or SILT performance, but *Hdh*$^{Q150/+}$ mice did present with impaired performance in the 5-CSRTT. Both genotypes were found to able to perform the SILT, with neither genotype presenting with an implicit learning deficit at 6M. The striatal gene expression level profiles of *Hdh*$^{Q150/+}$ and *Hdh*$^{+/+}$ mice did not differ at any time point or learning phase examined, using a microarray investigation, which suggests that a transcriptional phenotype is not present in the heterozygous HdhQ150 mouse model of HD at 3M or 6M. The GO biological pathways of protein transport and localisation, and GTP signalling were associated with striatal mRNAs showing significant transcriptional alterations as a result of ageing between 3M and 6M. Performance of CRF training produced alterations in common biological pathways in both genotypes, and a greater number of striatal transcriptional changes were evident following CRF training alone than as a result of CRF, 5-CSRTT and SILT performance.

3.4.1 Effects of Huntington's disease genotype on behavioural performance

No significant difference was reported between the performances of *Hdh*$^{Q150/+}$ and *Hdh*$^{+/+}$ animals in the CRF, suggesting that *Hdh*$^{Q150/+}$ mice do not present with an impairment in the acquisition of a simple conditioning task at 2M. A significant difference was reported between accuracy levels of *Hdh*$^{Q150/+}$ and *Hdh*$^{+/+}$ animals in the 5-CSRTT, a task utilised to assess visual attention (Muir et al., 1993), with *Hdh*$^{Q150/+}$ mice exhibiting lower levels of accuracy than their wild-type counterparts. The interaction of genotype and session also reported levels of significance in the 5-CSRTT, however the latency of responses to stimuli were comparable between genotypes. These findings suggest that *Hdh*$^{Q150/+}$ mice at 3M show impaired visual attentional functioning and delayed acquisition of the 5-CSRTT, phenotypes that have not previously been identified in this mouse model.

Using a probe of implicit learning (Trueman et al., 2005), the current study tested the ability of *Hdh*$^{Q150/+}$ mice to learn a complex serial visual discrimination task. There was no difference in accuracy to S1 between HD and control animals, and stimulus location did not affect accuracy to S1 for either genotype. Reaction times to S1 were also comparable between genotypes, with no significant difference between performance levels, independent of hole location. Mean accuracy and reaction times of *Hdh*$^{Q150/+}$ and *Hdh*$^{+/+}$ animals to S2 were similar, with no significant variation between genotypes while the number of steps between S1 and S2 was found to significantly affect accuracy and reaction times to S2 for both genotypes, as anticipated. Both genotypes of mice seem to exhibit the cognitive capacity for implicit learning, as demonstrated by a higher performance accuracy on predictable than unpredictable S2 trials. However, predictive accuracy to S2 did not differ significantly between genotypes, suggestive of a lack of deficit in implicit learning in the *Hdh*$^{Q150/+}$ animals. *Hdh*$^{Q150/+}$ mice were also found to perform a comparable number of S1 and S2 trials across the final 5 days of testing as their wild-type counterparts, which is indicative of healthy motivational drive and activity levels at 3M-4M, and is corroborated by previous evidence where heterozygous HdhQ150 animals did not present with an activity deficit until 40 weeks of age (Lin et al., 2001). The proportion of all types of errors made in the SILT was equivalent between genotypes and suggests that, at the age examined, *Hdh*$^{Q150/+}$ animals do not present with a phenotype of abnormal impulsivity, attention, perseveration or motor learning that have been identified in previous SILT-based studies examining striatal lesions (Trueman et al., 2005; Brooks et al., 2007) and the HdhQ92 and YAC128 mouse lines of HD (Trueman et al., 2007; Brooks et al., 2012d).

These results indicate that 6M old *Hdh*$^{Q150/+}$ mice do not present with deficits in implicit learning, similar to previous reports in striatal lesion (Trueman et al., 2005) and *Hdh*$^{Q92/Q92}$ mice (Trueman

et al., 2007). The lack of deficit in implicit learning may be due to the relatively low level of neuropathology present in our model at this age, with neuronal intranuclear inclusion formation and striatal cell loss only present from 5M and 6M, respectively (Bayram-Weston et al., 2012). Therefore, it is possible that any striatal dysfunction present in the animals at this time-point may not be severe enough to cause cognitive impairment in the SILT, and so examining the model at a later stage in the lifespan may reveal a behavioural phenotype. However, deficits in reversal and spatial learning (Brooks et al., 2012a) have previously been reported at similar ages (6M and 4M, respectively) in homozygous HdhQ150 mice. Thus, it is possible that the *Hdh*$^{Q150/+}$ animals did not present with learning deficits because of a gene-dosage effect where animals homozygous for the mutant allele show greater impairment than mice heterozygote for the mutant allele, as suggested previously (Lin et al., 2001). Previous SILT-based lesion studies have suggested that striatal dysfunction does not affect the implicit learning capabilities of rats (Jay and Dunnett, 2007) or mice (Trueman et al., 2005; Brooks et al., 2007). Thus, it is reasonable to suggest that differences in implicit learning did not arise between genotypes because the neural structure likely to have undergone the greatest degree of degeneration in the HD mouse model, the striatum, may not play a key role in implicit learning in the rodent brain, which is supported by similar findings in the HdhQ92 line (Trueman et al., 2007). Another possibility for the lack of a significant difference in performance between the two genotypes is simply that the number of animals used in the study is likely to be too low to accurately represent either population (animals that performed full-SILT training: *Hdh*$^{+/+}$: n = 3; *Hdh*$^{Q150/+}$: n = 5).

3.4.2 The effects of Huntington's disease genotype on gene expression levels

The lack of significant mRNA transcript level differences between genotypes suggests that a transcriptional phenotype does not arise in heterozygote HdhQ150 animals at 3M-6M. This may be the result of a gene-dosage effect, as *Hdh*$^{Q150/Q150}$ mice have previously been found to show abnormal gene expression profiles at 6M compared to wild-type animals (Giles et al., 2012), or may again be due to the low number of mice used in the study.

3.4.3 Effects of ageing on striatal gene expression levels

The current findings suggest that ageing results in altered expression of genes associated with protein processing and synaptic signalling, shown by G protein-associated biological processes, in the mouse striatum, similar to those noted previously in the mouse hypothalamus and cortex (Jiang et al., 2001). Ageing in laboratory mice has previously been strongly linked with

transcriptional alterations associated with immune responses (Swindell, 2009), which was not evident in our data. There are a number of factors that may contribute to the inconsistency between our data and that of Swindell (2009), such as the difference in background mouse strains used in either study. Animals on a C57BL6/J background were utilised in our study whilst multiple strains were assessed by Swindell. The age ranges examined also differed between the two studies, which is likely to contribute to the differing findings, and Swindell's report of altered immune response as a result of ageing was taken from analyses of multiple mouse tissues whereas the current study only assessed striatal transcription.

This study found that untrained 6M old *Hdh*$^{Q150/+}$ mice exhibited fewer transcriptional changes as a result of ageing compared to wild-type animals. This trend is in contrast to that seen in a previous study by Giles and colleagues (2012), where homozygote HdhQ150 animals presented with a greater number of ageing-dependent striatal transcriptional changes than their wild-type equivalents. The genetic discrepancies between our findings and the aforementioned previous findings could be an issue of gene dosage, with the current study examining heterozygous animals while homozygous animals were used by Giles and colleagues (2012), or power because of the low number of animals used in our study. Nevertheless, the trend of a substantially abnormal number of ageing-related mRNA expression changes observed in *Hdh*$^{Q150/+}$ mice may be an early indication of a genetic phenotype that arises in knock-in animals prior to a behavioural phenotype.

3.4.4 Effects of age and training on gene expression levels

The investigation into the striatal mechanisms underlying potential cognitive deficits in the *Hdh*$^{Q150/+}$ mouse model of HD uncovered a substantial difference in gene expression levels depending on whether the mice had received any task-based training. Examination of the PCA and microarray list of gene expression level changes in group comparisons suggests that the performance of learning tasks between 2M-4M appears to maintain the striatal transcription profile close to that of the 3M mice rather than that seen in their untrained 6M contemporaries. Our data are consistent with the notion that behavioural training acts to prevent or ameliorate ageing-associated gene expression level changes. Similar assertions have previously been made, whereby an exercise protocol seemed to reverse ageing-related memory decline and decreases in hippocampal histone H4 acetylation levels (Lovatel et al., 2013). However, there are limitations in the present study that may confound this conclusion: the untrained groups did not perform any learning tasks, but they also remained unhandled and were not placed in the test environment of the operant boxes. Thus, it is possible that the effects of training observed could

be a result of handling, which is likely to have multiple effects on the handled mice such as increasing fear and anxiety, both of which are known to affect neural gene expression levels (Malkani and Rosen, 2000; Hovatta et al., 2005; Coryell et al., 2007; Pantazopoulos et al., 2011; Spencer et al., 2013). The lack of exposure to the test environment of the operant box, in untrained animals, may also confound the effects of training, as extended exposure to novel environments has been found to alter gene expression levels within the striatum (Struthers et al., 2005).

3.4.5 The effects of conditioning and implicit learning on striatal gene expression levels

The current study found a number of genes showing altered expression following CRF training or implicit learning. Simple conditioning produced changes in expression of a relatively large number of genes in *Hdh*$^{+/+}$ and *Hdh*$^{Q150/+}$ animals, which were associated with protein transport in wild-type mice and mostly protein transport and GTP signalling in knock-in animals. Implicit learning following CRF and 5-CSRTT induced less transcriptional changes than CRF alone, suggesting that neural remodelling occurs early in behaviour acquisition and smaller modifications to expression need to be induced later. The mRNAs showing significant alterations following implicit learning were associated with protein transport and localisation in wild-type mice, while those showing variation in HdhQ150 animals were not significantly associated with any GO pathways. Pathway analyses did not identify significant associations with any GO biological processes in genes presenting with significant activity level changes as a result of both forms of learning.

A lower number of gene expression level changes were identified in animals that had undergone implicit learning following CRF and 5-CSRTT acquisition than those that had performed CRF alone at 2M before being sacrificed at 6M. It has been reported that extended exposure to novel environments can alter gene expression levels within the striatum (Struthers et al., 2005). This effect cannot be the cause of the lower number of genes showing altered transcription levels in SILT trained animals, however, because the 6M old acquisition trained animals, whilst only being trained at 2M, were placed in the operant boxes in the absence of SILT training until sacrifice at 6M. Thus, the 6M old CRF trained animals had the same level of operant box exposure as the SILT trained mice. This suggests that learning at 2M is capable of altering gene expression levels in a stable manner up to 6M.

3.4.6 Conclusions

This chapter aimed to assess the behavioural performance of *Hdh*$^{Q150/+}$ mice in a test of implicit learning (Trueman et al., 2005), and to assess the transcriptional responses that genotype, ageing and performing different levels of operant-based instrumental learning produce in this mouse model of HD. Behaviour was assessed using CRF, 5-CSRTT and the SILT, similar to that used previously (Trueman et al., 2005; Brooks et al., 2007; Trueman et al., 2007; Trueman et al., 2012b), but an implicit learning behavioural phenotype was not discovered in *Hdh*$^{Q150/+}$ mice at 3M-4M, seemingly dismissing the hypothesis that *Hdh*$^{Q150/+}$ mice at 3M-4M may display an implicit learning deficit. Conversely, a deficit in the 5-CSRTT was reported in *Hdh*$^{Q150/+}$ mice at 3M, which has not previously been reported, and suggests that these animals present with an impairment in visual attention and delayed acquisition of the 5-CSRTT at 3M. These conclusions require replication, due to the low number of animals that performed the tasks in the current study, and re-examination of the model using a greater number of behavioural subjects would be recommended. A microarray reported no gene expression level differences between *Hdh*$^{Q150/+}$ mice and their wild-type controls.

Inspection of the microarray PCA and gene lists suggested that the performance of cognitive tasks between 3M-6M may act to maintain the striatal transcriptional profile of 6M old animals closer to that of 3M old animals, with 6M old untrained animals displaying a transcriptional profile dissimilar to both 3M old animals and 6M old mice that had undergone CRF or SILT training. This interpretation of the data may be confounded by a number of limitations however, as the sample size of each group was low and unevenly distributed, and also because untrained animals had not received any substantial handling and were not introduced to the test environment that the trained animals had experienced, both of which have been previously found to influence neural gene expression levels (Malkani and Rosen, 2000; Hovatta et al., 2005; Struthers et al., 2005; Coryell et al., 2007; Pantazopoulos et al., 2011; Spencer et al., 2013). The microarray reported that ageing produced significant changes in striatal expression levels of mRNAs related to protein processing and synaptic transport, as has previously been identified in the mouse hypothalamus and cortex (Jiang et al., 2001).

Microarray analyses identified a number of gene expression level changes arising from performance of a simple conditioning task and a more complex serial visual discrimination task examining implicit learning, when compared to untrained animals. When individually assessing each genotype on each task, performance of both tasks resulted in alterations in biological pathways associated with protein transport and localisation, and GTP binding, although there were no significant associations with any biological processes when genetic analyses of the two

tasks were compared. As such, these biological processes may act as a generic network related to learning, in the striatum. The lack of distinction found between the striatal transcriptional components of simple conditioning and the more complex implicit learning task may be the result of a similar genetic profile being required for acquisition of both tasks.

In summary, at 2M-3M there were no differences in the performance of CRF between genotypes, and while a subtle difference arose between *Hdh*$^{Q150/+}$ and *Hdh*$^{+/+}$ mice in the 5-CSRTT this behavioural phenotype did not present itself in the SILT. No broad spectrum striatal gene expression level changes were observed in Q150 mice compared to wild-type animals at any time point or learning phase. This work identified potential generic striatal learning associated biological pathways in wild-type animals, highlighting the role of protein transport and localisation, and GTP binding in the striatum for learning.

3.4.7 Summary of key results from Chapter 3

- Identification of novel visual attention impairment and delayed acquisition of the 5-CSRTT in female *Hdh*$^{Q150/+}$ mice at 3M.
- No implicit learning deficits were identified using the SILT in female *Hdh*$^{Q150/+}$ mice at 3M-4M.
- Microarray investigation reported no genotype-dependent striatal gene expression level differences between *Hdh*$^{Q150/+}$ and wild-type littermate controls.
- Ageing from 3M-6M resulted in similar transcriptional profiles in the striata of *Hdh*$^{Q150/+}$ and wild-type littermate controls.
- A microarray reported no genetic differences between the striata of CRF and SILT trained animals.

Chapter 4: Investigating the transcriptional correlates of spatial discrimination and reversal learning in C57BL/6 mice

The investigation into age- and learning-dependent changes in the transcriptional profile of *Hdh*$^{Q150/+}$ mice in chapter 3 identified a trend in striatal transcription profiles between untrained animals and those that had undergone differing levels of behavioural training. In order to investigate transcriptional aspects of cognitive performance across learning stages in further detail, the current study examined SD and RL at distinct time points in wild-type C57BL/6 mice. Specifically, striatal gene expression levels were investigated following the first session of SD and RL training as a means of identifying neural gene expression level changes associated with early acquisition of SD and RL. Striatal gene expression levels were also examined following the final session upon which animals reached criteria in each task, in order to identify neural gene expression level changes associated with maintenance of SD and RL tasks.

4.1 Introduction

Problem solving can be broken down into its cognitive processes and these include planning, judgement, management, anticipation, decision-making and reasoning. Successful performance of these cognitive procedures is also dependent upon proficient observance of the external environment, storage and retrieval of relevant information from long-term memory, inhibition of inappropriate information, and manipulation and integration of the appropriate information. The application and management of all these mental functions is referred to as executive function, with executive function previously defined as "…a product of the co-ordinated operation of various [cognitive] processes to accomplish a particular goal in a flexible manner" (Funahashi, 2001). As suggested by this definition, an integral feature of executive function is the cognitive flexibility required to adapt to changing circumstances when making a decision and also reverse decisions based on differential conditions, and RL can be defined as a subset of cognitive flexibility.

The neural structures and pathways underpinning RL have been investigated using a number of approaches, with strong evidence for involvement of both the striatum and frontal cortex in this form of memory. For example, lesion studies in non-human primates identified that the prefrontal cortex is necessary for cognitive flexibility (Roberts et al., 1990; Roberts et al., 1992) and this finding has since been replicated in rodent lesion studies (Kim and Ragozzino, 2005; Brigman and Rothblat, 2008). Similar studies have also identified the striatum as being integral for behavioural flexibility (Featherstone and McDonald, 2005; Clarke et al., 2008; Castane et al.,

2010), while complementary roles of the prefrontal cortex and striatum in cognitive flexibility have also been proposed as a result of lesion studies (Ragozzino, 2007; McDonald et al., 2008b). This theory of frontostriatal circuitry governing behavioural flexibility is further strengthened by functional magnetic resonance imaging evidence of altered frontal cortical and striatal activity during reversal of a previously learnt rule in healthy human patients (Cools et al., 2002; Kringelbach and Rolls, 2003; Hampton and O'Doherty, 2007; Ghahremani et al., 2010). Furthermore, individuals with lesions to the frontal cortex or striatum show impairments in a variety of RL tasks (Hornak et al., 2004; Bellebaum et al., 2008), which also indicates that this form of learning may be dependent on the frontostriatal circuitry.

As the striatum and cortex are the neural structures that undergo the largest degree of degeneration in HD (Vonsattel et al., 1985; Gusella and MacDonald, 2009; Tabrizi et al., 2011; Tabrizi et al., 2013), it is unsurprising that impairments in cognitive flexibility have been reported. Such impairments in cognitive flexibility have been reported in HD sufferers, with evidence of deficits in the performance of simple RL (Lange et al., 1995; Lawrence et al., 1999), task switching (Aron et al., 2003), attentional set shifting (Lawrence et al., 1996; Lawrence et al., 1998b), and the Stroop test (Tabrizi et al., 2013). Cognitive flexibility has also been examined in mouse models of HD. One study examined the performance of homozygous HdhQ150 animals in a complex extra-dimensional set shifting task that requires mice learn to dig for a reward based upon digging medium or odour, and where a number of rule reversals occur (Brooks et al., 2006). In comparison to their wild-type counterparts, $Hdh^{Q150/Q150}$ mice exhibited deficits in extra-dimensional set shifting as well as recall impairments for compound reversal and intra-dimensional set shifting in this task. A similar extra-dimensional set shifting impairment, albeit using a different behavioural task, has also been reported in the YAC128 (Brooks et al., 2012f) and R6/1 (Harrison et al., 2013) mouse models of HD. Further evidence of cognitive inflexibility in the HdhQ150 mouse model of HD is provided by the identification of a reversal learning deficit in the Morris water maze task from 6M in homozygous mutants (Brooks et al., 2012b). More recently, cognitive inflexibility has been reported in both BACHD and zQ175 mice using a touchscreen based operant task (Farrar et al., 2014).

The neuroanatomical basis of cognitive flexibility has been extensively investigated, and the genetic mechanisms underlying RL have seen similar levels of examination in recent years. This has led to the identification of a number of genes that are believed to play a role in RL (Table 4.1). One such gene is *Dopamine receptor D2* (*Drd2*), which encodes dopamine D2 receptors (DRD2s). *Drd2* has been found to mediate RL in a number of studies, with evidence that transgenic mice lacking functional DRD2s are incapable of learning a new rule in an odour learning task (Kruzich et al., 2006) and are impaired in the reversal element of an attention set

shifting task (Desteno and Schmauss, 2009). Chronic pharmacological antagonism of DRD2s in otherwise healthy C57BL/6 mice also caused an impairment in RL of the attention set shifting task. Conversely, *Drd2* expression in the PFC has been found to inversely correlate with RL capabilities of mice, where heightened levels of *Drd2* were associated with poor RL performance (Laughlin et al., 2011). While there are contradictory findings in the studies mentioned, each study does highlight the importance of *Drd2* in RL in mice. Evidence for the role of DRD2s in human cognitive flexibility is also available, where impaired RL and abnormal striatal activity, as assessed using fMRI, was reported in individuals carrying a polymorphism in *DRD2* that causes reduced expression of striatal DRD2s (Jocham et al., 2009).

Gene name	Gene symbol	Finding	Species	Study
Adenosine A2A receptor	*Adora2A*	Selective inactivation of A2ARs in striatal neurons enhances RL.	Mouse	(Wei et al., 2011)
Activity-regulated cytoskeleton-associated protein	*Arc*	1) Striatal *Arc* mRNA expression correlates with RL performance. 2) Methamphetamine attenuates *Arc* expression in dorsal striatum and abolishes the correlation between *Arc* mRNA levels and RL performance. 3) Acute disruption of *Arc* disrupts retention of RL.	Rat	(Daberkow et al., 2007) (Daberkow et al., 2008) (Pastuzyn et al., 2012)
Brain-derived neurotrophic factor	*Bdnf*	1) Hippocampal *Bdnf* expression is induced during contextual learning. 2) Spatial learning produces an increase in hippocampal *Bdnf* levels, and BDNF mRNA and protein level reduction results in impaired spatial memory retention. 3) Disruption of BDNF exon IV-dependent transcription and reduced activity-induced BDNF expression levels cause an impairment in learning in the first day of RL training in the MWM.	Rat Rat Mouse	(Hall et al., 2000) (Mizuno et al., 2000) (Sakata et al., 2013)

cAMP response element-binding protein 1	*Creb1*	1) CREB activation is increased in the hippocampus during spatial learning. 2) Striatum-dependent response learning causes elevated levels of CREB activation in the striatum. 3) Hippocampal CREB1 is decreased in aged rats with spatial memory impairments. 4) Spatial memory deficits are associated with decreased levels of CREB activation in the hippocampus of aged rats.	Rat	(Mizuno et al., 2002) (Colombo et al., 2003) (Brightwell et al., 2004) (Porte et al., 2008)
Dopamine D2 receptor	*Drd2*	1) *Drd2* deletion impairs ability to learn new rule in odour learning task. 2) Chronic DRD2 antagonism using haloperidol impairs RL of a set-shifting task. 3) Presence of *DRD2* polymorphism associated with impaired RL ability and abnormal striatal activity. 4) *Drd2* expression in the PFC is inversely correlated with RL ability.	Mouse Mouse Human Mouse	(Kruzich et al., 2006) (Desteno and Schmauss, 2009) (Jocham et al., 2009) (Laughlin et al., 2011)
Homer scaffolding protein 1	*Homer1*	1) *Homer1* knock-out produces deficits in spatial learning and memory. 2) Hippocampal *Homer1* deletion produces a diverging phenotype on motivational behaviour in operant conditioning. 3) Striatal *Homer1a* transcription is elevated following instrumental learning.	Mouse	(Jaubert et al., 2007) (Wagner et al., 2014) (Hernandez et al., 2006)

Table 4.1. A summary of research relating to instrumental learning, spatial learning and RL for the genes examined by RT-qPCR in the current study.

A trend in striatal transcription profiles between untrained animals and those that had undergone differing levels of behavioural training in the SILT was identified in Chapter 3. Based upon this trend and the strong evidence of a cognitive flexibility deficit in HD, a genetic basis of

RL and a number of candidate genes underpinning RL, the current study aimed to examine the transcriptional profile of healthy C57BL/6 mice at different stages of SD and RL. This was designed as a preliminary study to an investigation of the biological mechanisms governing SD and RL in the *Hdh*$^{Q150/+}$ mouse model of HD. A microarray investigation was utilised in order to examine the entire transcriptional profile of the striatum of each behavioural group, while RT-qPCR was used to validate microarray data and examine the expression levels of specific mRNAs associated with SD and RL (Table 4.1). Because transcription was being investigated in the current experiment, a novel SD and RL operant task similar to that used by Farrar and colleagues (2014) was utilised that requires less motor activity and olfactory input than the behavioural tasks used in previous investigations of cognitive flexibility in HD animals (Brooks et al., 2006; Brooks et al., 2012f; Harrison et al., 2013), as locomotor activity and olfactory input are both known to influence gene expression levels (Berchtold et al., 2002; Cotman and Berchtold, 2002; Farmer et al., 2004; Irwin and Byers, 2012).

4.2 Methods

4.2.1 Subjects

Forty-eight naïve male C57BL/6J mice (Harlan, UK) were housed 4 animals per cage and animal husbandry was as described in Chapter 2.1.2. All animals were between 22 and 24 weeks-of-age at the start of testing, and were water restricted throughout testing, as described in Chapter 2.1.2.

4.2.2 Experimental design

Animals were randomly allocated into one of the following six behavioural training groups (Fig. 4.1): chronic magazine training; continuous reinforcement (CRF) until criteria (CRF maintenance); 1 day of SD training (SD acquisition); SD to criteria (SD maintenance); one day of RL (RL acquisition); and RL to criteria (RL maintenance). Each training group consisted of eight mice. All animals (n = 48) underwent an initial session of magazine training, as described in Chapter 4.2.3.1. Eight animals continued magazine training and never received any other form of behavioural training (chronic magazine training group) while the remaining mice (n = 40) underwent CRF training to criteria, as described in Chapter 4.2.3.2, following the initial magazine training session. Upon reaching criteria in the CRF task, eight animals were sacrificed (CRF maintenance) while the remaining mice (n = 32) began SD training, as described in Chapter

4.2.3.3, with eight animals performing a single session of SD training before sacrifice (SD acquisition) and the remaining mice (n = 24) performing the SD task to criteria. Once criterion was reached in the SD task, eight animals were sacrificed (SD maintenance) while the remaining mice (n = 16) performed the RL task, as described in Chapter 4.2.3.4. Eight of the remaining mice performed a single RL training session before sacrifice (RL acquisition) and the final eight animals performed the task to criteria before sacrifice (RL maintenance). Animals were sacrificed individually when reaching criteria in a task, with each individual mouse sacrificed when they reached criteria and not sacrificed as a group once the group as a unit reached criteria.

There was an instance where behavioural groups were paired to one another. Animals in the chronic magazine training group were paired to those in the CRF maintenance group whereby upon reaching criteria in CRF training, the said mouse from the CRF maintenance group, as well as a mouse from the magazine training group, would be sacrificed and tissue extracted for gene expression level analyses.

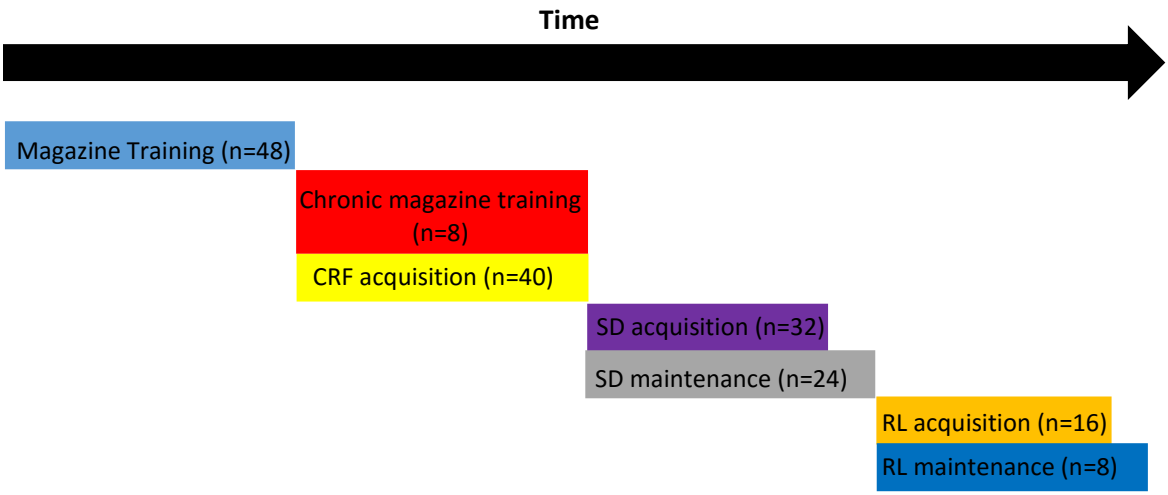


Figure 4.1. Behavioural training in the experimental design. Mice in each group underwent different levels of behavioural training.

4.2.3 Behavioural training protocols

4.2.3.1 Magazine training

Animals underwent 1 day of magazine training in order to permit the mice to learn the association between magazine light illumination and food reward. The magazine light was illuminated and 150 µL of reward was delivered at the onset of the 20 min session. Upon removal

of the head from the magazine, the light would extinguish for 10 s before again illuminating and 5 µL of reward delivered. This reward contingency was maintained for the remainder of the session.

4.2.3.2 CRF task

Mice performed CRF training as described in Chapter 3.2.4.2. Criterion in the task was set at ≥50 np in three consecutive sessions for the current study. Animals performed the task until criteria was met, at which point mice were either sacrificed and tissue taken for dissection or training begun in the SD task.

4.2.3.3 SD task

In the SD task, mice had to correctly respond to two consecutive chained stimuli to receive a reward. Sessions lasted 20 min and one session was performed each day. As with the CRF task, the hole 5 stimulus light in the operant box was illuminated and the mouse must nose-poke (np) this stimulus light. When this stimulus light was present, a correct np caused simultaneous extinction of the hole 5 stimulus light and illumination of hole 3 and hole 7 stimulus lights (Fig. 4.2). In the result of a hole 5 np not being made within 10 s of hole 5 stimulus light presentation, the light stimulus was extinguished and the house lights illuminated for 5 s before a new trial began following a 2 s ITI. Upon presentation of hole 3 and hole 7 light stimuli, mice were required to np the light stimulus of either hole 3 or hole 7. The light to be chosen was counterbalanced between animals, whereby half of the mice of each genotype were trained to np the stimulus light of hole 3 whilst the opposing half were trained to np the stimulus light of hole 7. When hole 3 and hole 7 stimulus lights were present, a correct np resulted in simultaneous hole 3 and hole 7 light stimuli extinction, magazine light illumination and food reward delivery. Once the mouse collected the reward and removed its head from the magazine, the magazine light was extinguished. Following a 2 s ITI, the next trial began through the illumination of the centre hole light. In the result of an incorrect response to hole 3 and hole 7 stimulus lights, an incorrect response was recorded, the light stimuli extinguished and the house lights illuminated for 5 s before a new trial began after a 2 s ITI.

Training performance on this task was calculated as percent accuracy (((correct choices)/(correct choices + incorrect choices)) x 100). Criterion was judged to be met when a minimum of 85% accuracy was reached in three consecutive sessions. Animals performed the

task until criteria was met, at which point mice were either sacrificed and tissue taken for dissection or they moved on to training in the RL task.

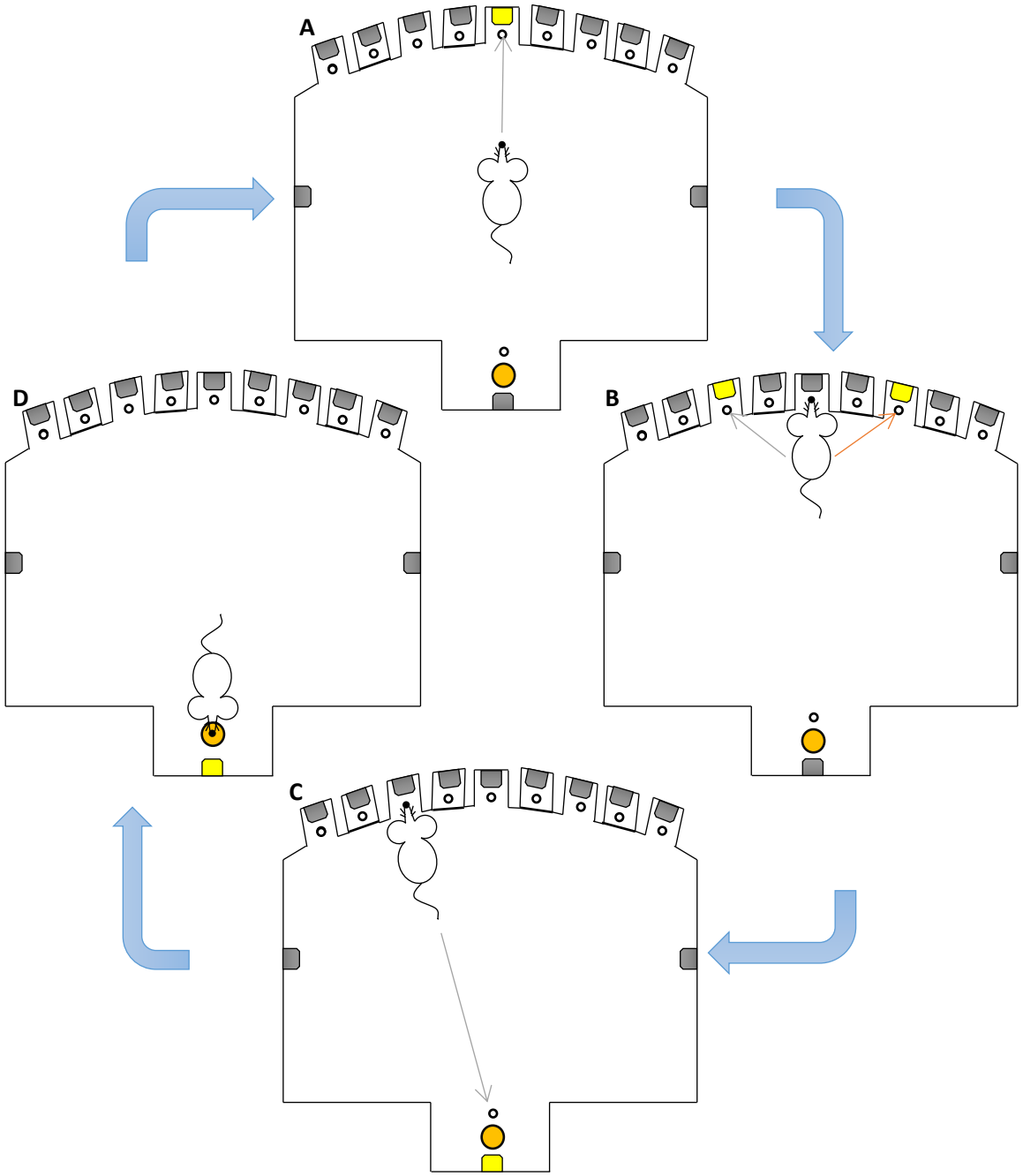


Figure 4.2. A pictorial representation of the SD task. A light stimulus is presented in hole 5 of the operant box (A). Upon nose-poking the light stimulus, the light stimulus in hole 5 is extinguished and holes 3 and 7 light stimuli are illuminated (B). The mouse then has to choose which hole to np. Nose-poking the correct hole leads to extinction of holes 3 and 7 stimulus light and illumination of the magazine light stimulus (C). Once the animal has collected the food reward (D), and removed its head from the magazine, the magazine light is extinguished. There is a 2 s inter-trial-interval (ITI) before a new trial is commenced through illumination of the hole 5 stimulus light (A).

4.2.3.4 RL task

In the RL task, mice (n = 16) had to correctly respond to two consecutive stimuli to receive a reward. Sessions lasted 20 min and one session was performed each day. The RL task was identical to the SD task described in Chapter 4.3.2.3 except that mice had to respond to the opposing stimulus light (of either hole 3 or hole 7) than that responded to in the previous SD phase. For example, if an animal had previously learnt to np the stimulus light of hole 7 when presented with holes 3 and 7 stimuli lights, the mouse would now have to np the hole 3 stimulus light in order to gain a reward.

Training performance on this task was calculated as percent accuracy (((correct choices/(correct choices + incorrect choices)) x 100). Criterion was judged to be met when a minimum of 85% accuracy was reached in three consecutive sessions. RL performance was also analysed by subdividing performance into two distinct stages based upon the percent of correct choices made in each session, specifically according to whether performance was <50% correct or ≥50% correct, as implemented by previous studies examining mouse RL (Davies et al., 2005; Brigman et al., 2008; Brigman et al., 2010; Graybeal et al., 2011). Sessions where accuracy is <50% reflects performance when perseveration to the previously learnt rule is high, while sessions where trials committed on ≥50% correct reflect performance where perseveration is relatively low and the ability to form new reinforce-stimulus associations (Davies et al., 2005; Brigman et al., 2008).

Eight mice performed one session of RL training before sacrifice and tissue extraction, forming the RL acquisition group. The remaining eight mice performed the task until criteria was met, at which point mice were either sacrificed and tissue taken (RL maintenance group). One mouse was unable to reach criterion in the RL task and was therefore removed from all analyses. Thus the RL criteria group consisted of seven animals instead of eight.

4.2.4 Molecular methodology

4.2.4.1 RNA extraction

RNA was prepared from whole striatum dissected from animals following the first session of a task or upon reaching criteria in a task, as described in Chapter 2.3.1. RINs of RNA samples ranged from 7.7 to 9.5 (8.52 ± 0.08).

4.2.4.2 Microarray and microarray analyses

Microarray was performed by Megan Musson of Cardiff University's Central Biotechnology Services (Cardiff, UK) using MouseRef-8 v2.0 Expression Beadchips (product code: BD-202-0202; Illumina Inc., California, USA), and microarray gene expression data was analysed using Partek® Genomics Suite™ Version 6.6 (Partek Incorporated, Missouri, USA).

4.2.4.3 Quantification of gene expression levels by RT-qPCR

Microarray gene expression level data was validated by TaqMan®-mediated (Life Technologies Corporation, Carlsbad, California, USA) RT-qPCR, as described in Chapter 2.3.2, and each reaction was performed in triplicate. The genes to be investigated were chosen based upon the findings of previous studies examining RL in mice, as discussed in Chapter 4.1, and are listed in Table 4.2.

Gene name	Gene symbol	ID
beta actin	*Actb*	Mm00607939_s1
adenosine A2a receptor	*Adora2A*	Mm00802075_m1
activity regulated cytoskeletal-associated protein	*Arc*	Mm01204954_g1
brain derived neurotrophic factor	*Bdnf*	Mm04230607_s1
cAMP responsive element binding protein 1	*Creb1*	Mm00501607_m1
dopamine receptor D2	*Drd2*	Mm00438545_m1
homer homolog 1	*Homer1*	Mm00516275_m1
ubiquitin C	*Ubc*	Mm02525934_g1

Table 4.2. Identification codes for Taqman® oligonucleotide probes used in the current study.

4.2.5 Statistical analyses

Statistical analyses were performed using IBM SPSS® Statistics software (Version 20; IBM United Kingdom Ltd, Hampshire, UK). Behavioural and RT-qPCR data were analysed by two-way ANOVA, with learning stage (acquisition or maintenance) and task (SD or RL) as independent variables. Specific two-group comparisons were analysed by independent samples t-tests, with Holm-Bonferroni sequential correction used for multiple hypothesis testing correction. Sphericity of data was assessed using Mauchly's test of Sphericity, with the Greenhouse-Geisser correction used if the assumption of Sphericity was violated and the Greenhouse-Geisser estimate of sphericity (ε) < 0.75. If the assumption of Sphericity was violated and $\varepsilon > 0.75$, the Huynh-Feldt

correction was used. Similarly, homogeneity of variances for data was assessed using Levene's test, and the Welch test used in the event of data violating this assumption.

4.3 Results

4.3.1 Microarray quality control metrics

PCA was performed as an early exploratory analysis of global striatal gene expression level patterns across all samples (Fig. 4.3), and the close grouping of subjects from all behavioural groups suggests that there is little variation in gene expression level patterns between behavioural groups. This indicates that neither behavioural task or learning stage significantly contribute to the striatal gene expression level patterns identified in the experiment, which is supported by the results of the gene expression level analyses reported in Table 4.3. The small percentage of contribution to the gene expression level profiles by the principal components, at only 12.7% and 8.22%, is also indicative of an absence of a causal effect of behavioural learning stage or task performance on striatal gene expression levels. Other principal components, such as RNA extraction batch, microarray processing batch, and housing cage group, were found to have no significant effect on gene expression level profiles in the PCA (data not shown).

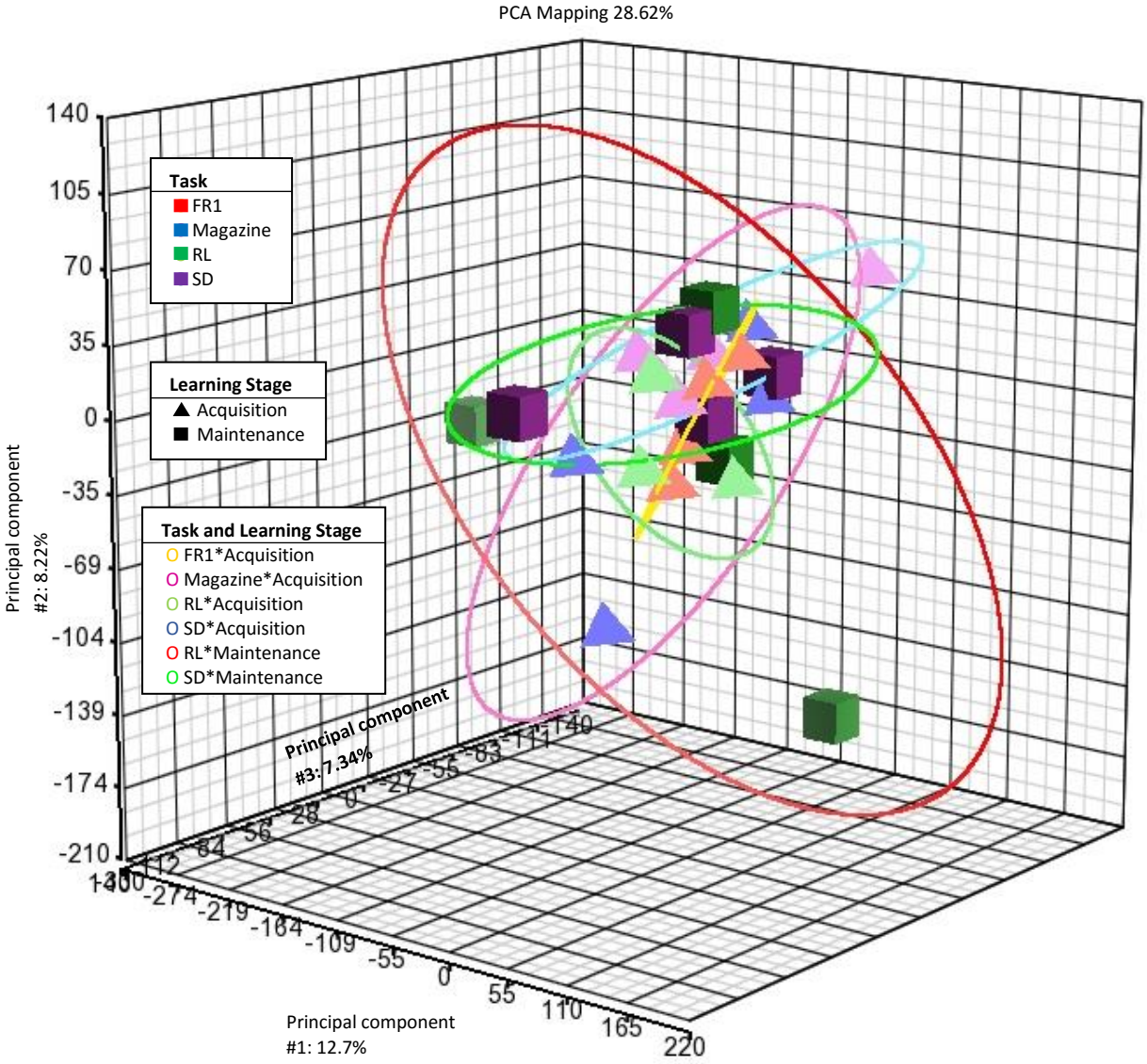


Figure 4.3. The PCA of the striatal transcriptional profiles of each behavioural group reveals no clear grouping of transcriptional profiles. Learning stage is the main principle component, which accounts for 12.7% of the variance in gene expression levels while task performance is the second principle component and accounts for 8.22% of the variance. The PCA reveals no clear separation of any group from another, suggesting the gene expression level profiles of each group show high levels of similarity. n = 4/group.

4.3.2 Microarray gene expression levels

A two-way ANOVA was performed on the microarray gene expression level data with learning stage and task performed as variables, and also using the same variables as interaction terms in a two-way interaction. Statistical significance was taken using FDR $p < 0.05$, with FDR estimation generated by Benjamini-Hochberg procedure, and no minimum magnitude of fold-change was defined when generating lists of genes showing significantly altered expression levels. This approach did not yield statistically significant changes in expression for any gene in any comparison examined (Table 4.3). Hence, pathway analyses from the microarray findings found later in the current chapter utilise microarray gene expression level results from lists of genes presenting with differential levels of expression generated using a nominal (FDR-uncorrected) p-value (Table 4.3).

Independent variable/group comparison	Number of genes differentially expressed	
	FDR<0.05	Nominal p-value
Learning stage	0	1,192
Task	0	950
Learning stage*task	0	1,140
Magazine vs CRF maintenance	0	338
SD acquisition vs SD maintenance	0	300
RL acquisition vs RL maintenance	0	674
SD acquisition vs RL acquisition	0	437
SD maintenance vs RL maintenance	0	434
SD maintenance vs RL acquisition	0	317

Table 4.3. The number of genes showing altered expression levels within the striatum as a result of the independent variables and in different group comparisons at two different significance stringencies in the microarray investigation. n = 4/group.

4.3.3 CRF task

4.3.3.1 Behaviour

Results of the CRF task revealed that all groups performed the task equivalently, in terms of the number of sessions required to reach criteria (Fig. 4.4A; main effect of group: $F_{3, 27} = 2.269$, $p = 0.103$) and the average number of responses made in the final three days of testing (Fig. 4.4B; main effect of group: $F_{3, 27} = 1.414$, $p = 0.260$).

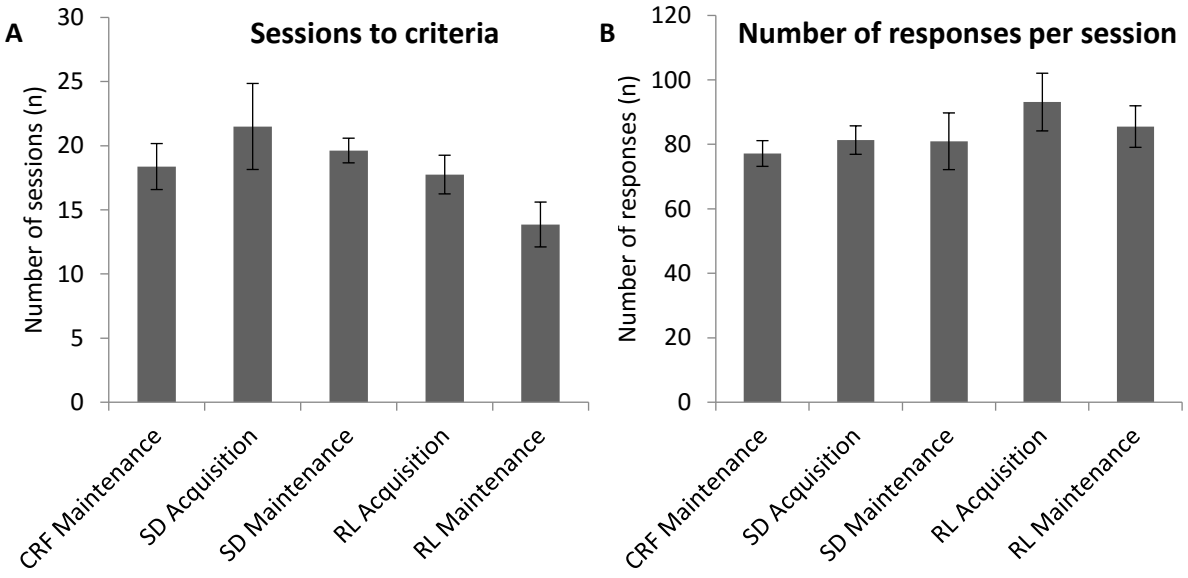


Figure 4.4. Performance of animals in the CRF task. In the CRF task, all groups took a comparable number of sessions to reach criteria (A) and performed a similar mean number of responses during the final three sessions (B). Values shown are mean ± S.E.M.; n = 7-8/group.

4.3.3.2 Validation of microarray gene expression level results and investigation of candidate gene expression levels by RT-qPCR

Creb1 was identified in the nominal p-value microarray list as being one of the genes downregulated in the striatum of animals that reached criteria in CRF, in comparison to their chronic magazine trained counterparts ($p = 0.043$). RT-qPCR was performed to validate this finding, as well as to investigate the expression levels of other genes of interest, and reported no difference in *Creb1* transcription between the two groups (Table 4.4; $t_{(6)} = 1.737$, $p = 0.665$). The remaining genes investigated by RT-qPCR also presented with comparable levels of

expression between groups (Table 4.4), despite a trend of increased gene expression levels for all genes investigated in CRF maintenance animals being identified (Fig. 4.5).

Gene investigated	$t_{(6)}$ =	p-value
Adora2A	0.804	1.000
Arc	0.207	1.000
Bdnf	$t_{(3.905)}$ = 0.875	1.000
Creb1	1.737	0.665
Drd2	1.516	0.720
Homer1	3.758	0.054

Table 4.4. The statistical values for RT-qPCR striatal expression level comparisons between magazine trained and CRF maintenance groups. A t-statistic of 6 was used, unless otherwise stated. n = 4/group.

4.3.3.3 Pathway analyses of microarray data

Microarray investigation reported 0 genes displaying significantly altered expression levels between the striata of chronic magazine training and CRF maintenance animals, the group comparison used to investigate CRF-dependent transcription alterations, at the FDR $p<0.05$ significance level (Table 4.3). Utilising the nominal p-value of 0.05 identified 338 genes presenting with significant changes in expression between chronic magazine training and CRF maintenance striata (Table 4.3). Pathway analyses revealed that these genes were associated with biological processes that include cell signalling, shown by associations with the GO pathways ion channel activity (GO:0005216; $p = 3.39 \times 10^{-02}$), substrate channel activity (GO:0022838; $p = 1.88 \times 10^{-02}$) and plasma membrane function (GO:0005886; $p = 1.26 \times 10^{-02}$), and odorant binding (GO:0005549; $p = 3.30 \times 10^{-02}$).

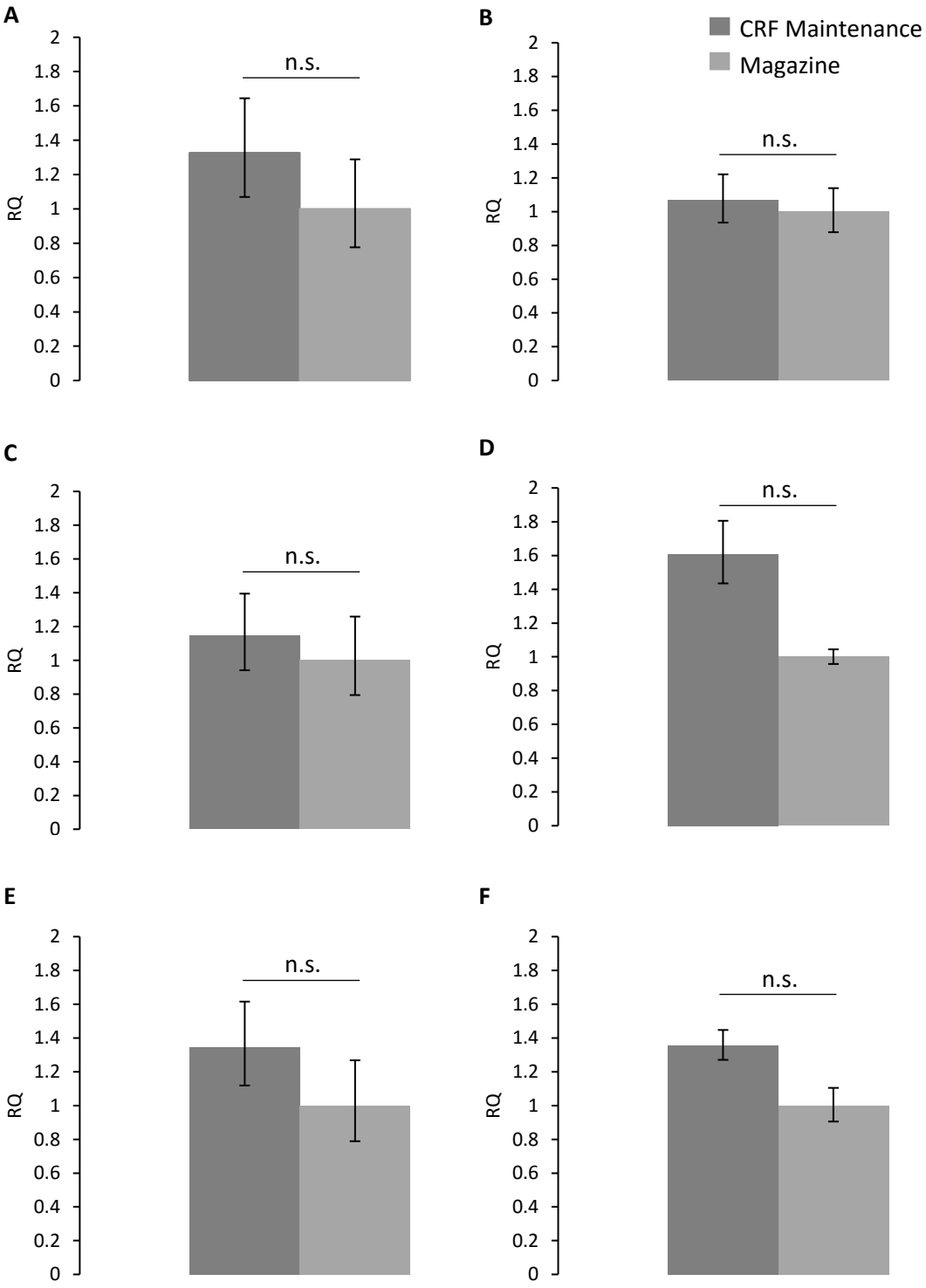


Figure 4.5. RQ value comparison of *Adora2A* (A), *Arc* (B), *Bdnf* (C), *Creb1* (D), *Drd2* (E), and *Homer1* (F) striatal expression levels between magazine trained and CRF maintenance groups following RT-qPCR to confirm microarray data. A trend of increased expression levels in the CRF maintenance group is seen in each gene examined, however all gene expression level differences between groups were found to be non-significant. Values shown are mean ± S.E.M.; n = 4/group.

n.s. = non-significant

4.3.4 SD and RL general performance

4.3.4.1 Behaviour

A two-way ANOVA examining the effect of learning stage (acquisition or maintenance) and task (SD or RL) on a number of behavioural measures was performed. Learning stage significantly affected mean accuracy to S2 (main effect of learning stage: $F_{1, 26}$ = 129.546, p < 0.001), as did the task being performed (main effect of task: $F_{1, 26}$ = 10.095, p = 0.004). A significant interaction between the effects of learning stage and task was also reported for mean accuracy to S2 (learning stage*task: $F_{1, 26}$ = 9.185, p = 0.005).

Analysis of the simple main effects found mean accuracy during RL acquisition to be considerably lower than during SD acquisition (Fig. 4.6; p < 0.001), while there was no difference in accuracy levels between maintenance of SD and RL (Fig. 4.6; p = 0.918). Independent samples t-tests were used to examine any potential differences in mean accuracy between acquisition and maintenance of either the SD or RL task, and identified a significant increase in accuracy from SD acquisition to SD maintenance (Fig. 4.6; t(7.118) = -4.559, p = 0.002) and from RL acquisition to RL maintenance (Fig. 4.6; t(6.887) = -37.329, p < 0.001).

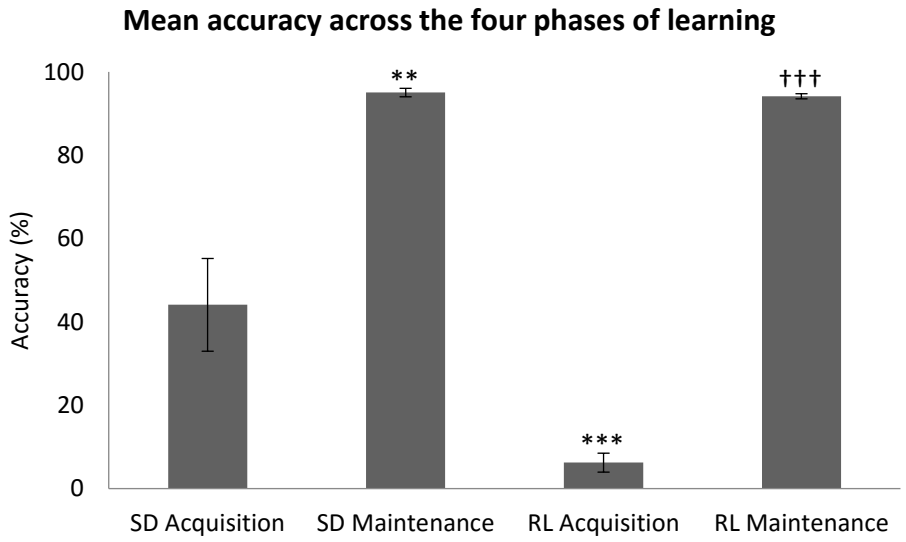


Figure 4.6. The mean percentage accuracy of animals during acquisition and maintenance of the SD and RL tasks. Mean accuracy levels during acquisition of RL were significantly lower than those during acquisition of SD, while significant increases in accuracy from SD acquisition to SD maintenance and RL acquisition to RL maintenance were also reported. Values shown are mean ± S.E.M.; n = 7-8/group.
* Denotes a significant difference from the SD Acquisition group
† Denotes a significant difference from the RL Acquisition group
** p < 0.01 *** p < 0.001

4.3.4.2 Validation of microarray gene expression level results and investigation of candidate gene expression levels by RT-qPCR

A two-way ANOVA was performed on the microarray data to examine striatal expression in the mouse genome and, at FDR p<0.05, revealed no significant changes in transcription of any gene as a result of learning stage or task, or of an interaction between learning stage and task (Table 4.3). Investigating striatal gene expression levels of specific group comparisons of interest (SD acquisition vs SD maintenance, RL acquisition vs RL maintenance, SD acquisition vs RL acquisition, SD maintenance vs RL maintenance, SD maintenance vs RL acquisition) in the microarray data also failed to identify any significant alterations, in any of the group comparisons, using FDR p<0.05 (Table 4.3). Decreasing the stringency of significance, through the use of a nominal p-value, lead to the discovery of a number of genes showing evidence of altered transcription in the striatum in all the aforementioned group comparisons (Table 4.3).

A two-way ANOVA examining striatal expression of all genes of interest, using RT-qPCR, revealed no significant effect of either learning stage or task on transcription levels (Table 4.5). Similarly, no interaction between learning stage and task was reported for any of the genes investigated (Table 4.5).

Gene of interest	Learning stage		Task		Learning stage*Task	
	$F_{1, 11}$ =	p-value	$F_{1, 11}$ =	p-value	$F_{1, 11}$ =	p-value
Adora2A	0.001	0.982	2.399	0.150	0.139	0.716
Arc	0.955	0.350	1.531	0.242	1.569	0.236
Bdnf	0.133	0.722	1.302	0.278	0.990	0.341
Creb1	0.438	0.522	0.865	0.372	1.213	0.294
Drd2	0.354	0.564	0.261	0.619	0.311	0.588
Homer1	0.167	0.690	0.817	0.385	0.295	0.598

Table 4.5. The statistical values of the two-way ANOVA examining the effect of learning stage and task on striatal gene expression levels of all genes of interest in SD acquisition and maintenance, and RL acquisition and maintenance groups. n = 4/group.

4.3.5 SD task

4.3.5.1 Behaviour

As expected, all groups that reached criteria in the SD task required a comparable number of SD sessions to reach criteria (Fig. 4.7; main effect of group: $F_{2, 20}$ = 0.574, p = 0.572) and also performed a comparable rate of below-chance perseverative errors (Fig. 4.7; main effect of group: $F_{2, 20}$ = 0.386, p = 0.684) and above-chance acquisition errors (Fig. 4.7; main effect of group: $F_{2, 20}$ = 0.824, p = 0.497).

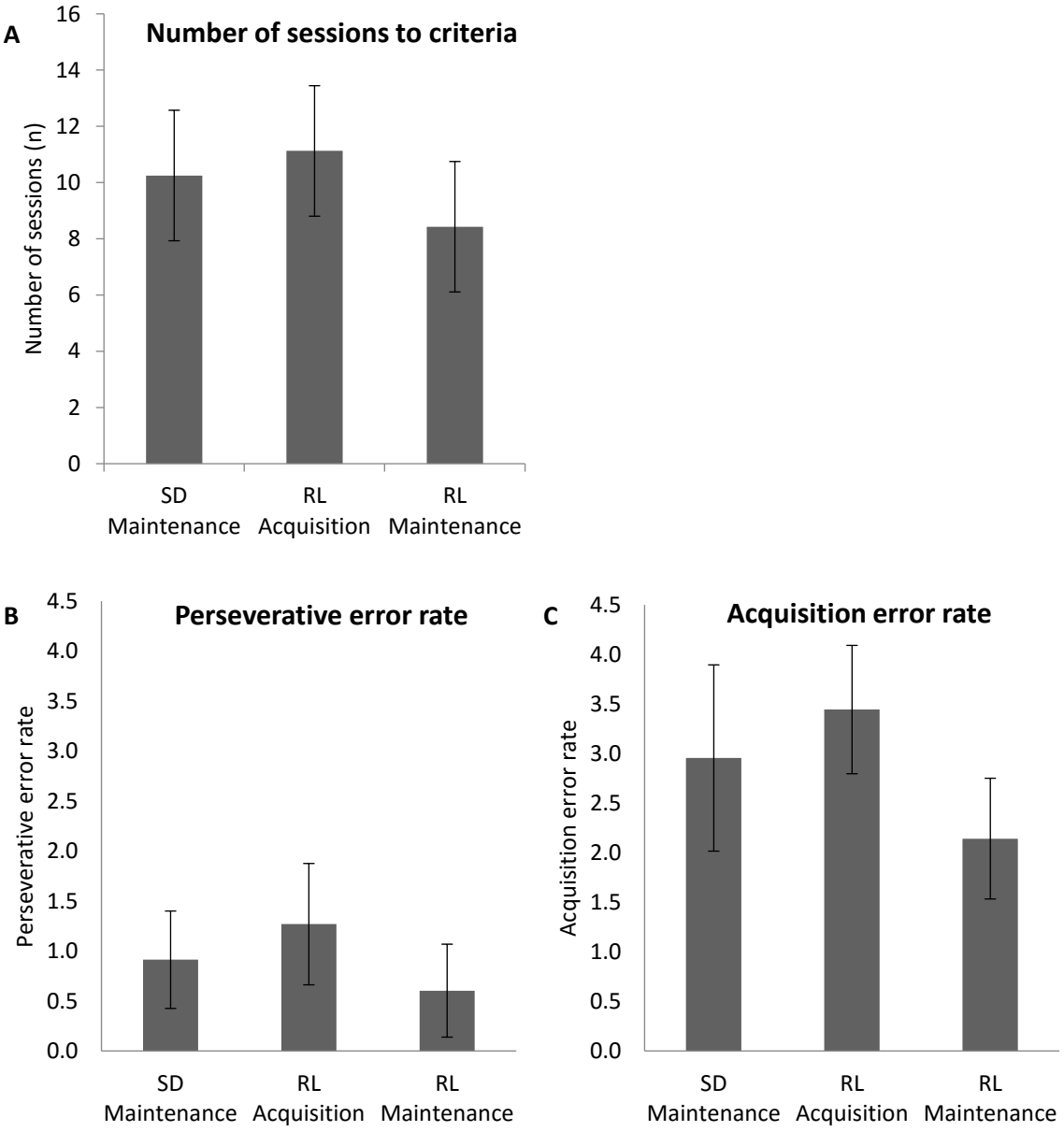


Figure 4.7. Behaviour in the SD task. All experimental groups that performed the SD task to criteria displayed comparable performance levels in the task, as shown by the number of

sessions required to reach criteria (A), and the rates of perseverative (B) and acquisition (C) errors. The data presented are mean values ± S.E.M.; n = 7-8/group.

4.3.5.2 Validation of microarray gene expression level results and investigation of candidate gene expression levels by RT-qPCR

The striatal transcriptome of CRF maintenance and SD acquisition animals were compared as a means of investigating gene expression level changes that arise as a result of early SD learning. The six genes of interest examined by RT-qPCR showed no significant differences in striatal expression levels between the CRF maintenance and SD acquisition groups (Table 4.6), despite a trend of SD acquisition animals exhibiting decreased striatal expression levels for each gene (Fig. 4.8).

Gene of interest	$t_{(6)}$ =	p-value
Adora2A	-1.361	0.756
Arc	-1.481	0.756
Bdnf	-0.410	1.000
Creb1	-2.268	0.384
Drd2	-0.522	1.000
Homer1	-2.189	0.384

Table 4.6. Statistical values from the independent-samples *t*-test examining potential differences in striatal gene expression levels between CRF maintenance and SD acquisition groups. n = 4/group.

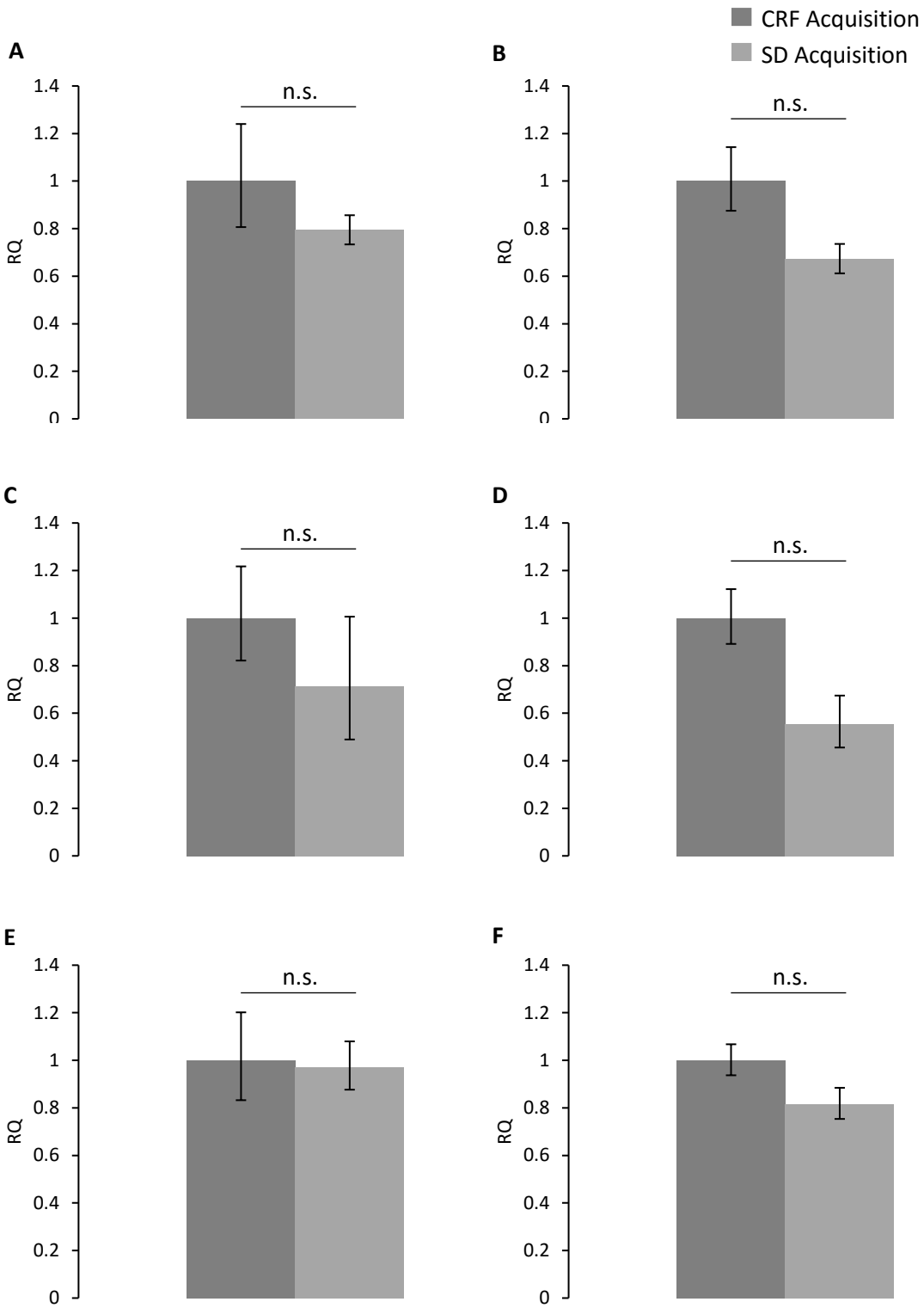


Figure 4.8. RQ value comparison of *Adora2A* (A), *Arc* (B), *Bdnf* (C), *Creb1* (D), *Drd2* (E), and *Homer1* (F) striatal expression levels between CRF maintenance and SD acquisition groups following RT-qPCR to confirm microarray data. A trend of decreased expression levels in the SD acquisition group is seen in each gene examined, however all gene expression level differences between groups were found to be non-significant. Values shown are mean ± S.E.M.; n = 4/group.

n.s. = non-significant

In order to examine the genetic differences between early and late SD learning, the transcriptional profiles of SD acquisition and SD maintenance animals were compared. The results of the RT-qPCR failed to demonstrate differences in transcription levels of any of the genes under investigation (Table 4.7), however, a general trend of increased striatal expression levels for all genes examined, with the exception of *Adora2A*, was exhibited in SD maintenance animals (Fig. 4.9).

Gene of interest	$t_{(6)}$ =	p-value
Adora2A	-0.223	1.000
Arc	2.153	0.450
Bdnf	0.391	1.000
Creb1	1.366	1.000
Drd2	0.023	1.000
Homer1	0.676	1.000

Table 4.7. Statistical values from the independent-samples *t*-test examining potential differences in striatal gene expression levels between SD acquisition and SD maintenance groups. n = 4/group.

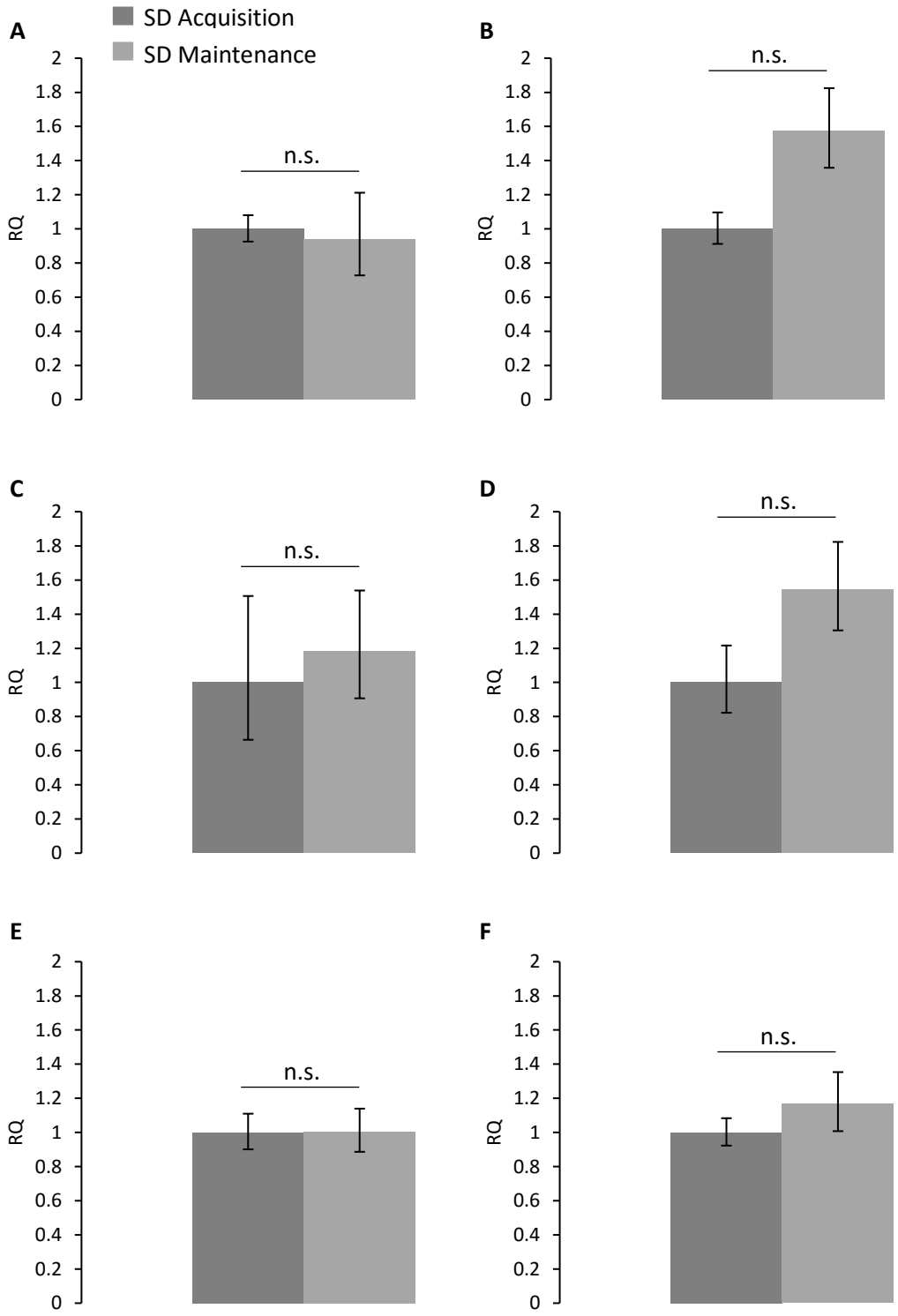


Figure 4.9. RQ value comparison of *Adora2A* (A), *Arc* (B), *Bdnf* (C), *Creb1* (D), *Drd2* (E), and *Homer1* (F) striatal expression levels between SD acquisition and SD maintenance groups following RT-qPCR to confirm microarray data. A trend of increased expression levels in the SD maintenance group is seen in each gene examined, with the exception of *Adora2A* (A), however all gene expression level differences between groups were found to be non-significant. Values shown are mean ± S.E.M.; n = 4/group.

n.s. = non-significant

4.3.5.3 Pathway analyses of microarray data

The nominal p-value microarray list identified 404 striatal mRNAs as having altered expression levels between CRF maintenance and SD acquisition mice (Table 4.3). DAVID analyses found that these genes showed enrichment with biological processes that include small GTPase mediated signal transduction (GO:0007264; $p = 3.44 \times 10^{-02}$), microtubule cytoskeleton (GO:0015630; $p = 3.58 \times 10^{-02}$) and microtubule organizing center (GO:0005815; $p = 3.55 \times 10^{-02}$).

In order to examine the genetic differences between early and late SD learning, the transcriptional profiles of SD acquisition and SD maintenance animals were compared. 300 genes showed altered expression in the striatum between animals that had undergone an initial acquisition session of SD training and those that had learnt the task to criteria, when using a nominal p-value (Table 4.3). Pathway analyses identified a number of genes being significantly associated with transport processes, such as anion and solute antiporter activity (GO:0015301; $p = 2.25 \times 10^{-02}$ and GO:0015300; $p = 2.50 \times 10^{-02}$, respectively), but also noted significant associations with transcriptional regulation, notably transcription repressor activity (GO:0016564; $p = 3.61 \times 10^{-02}$) and negative regulation of transcription from RNA polymerase II promoter (GO:0000122; $p = 4.71 \times 10^{-02}$). A number of genes presenting with altered expression were also associated with transcriptional regulation through epigenetic mechanisms, shown by an association with the GO pathway of histone-lysine N-methyltransferase activity (GO:0018024), although this association did not reach levels of significance ($p = 0.055$).

4.3.6 RL task

4.3.6.1 Behaviour

It was only possible to compare performance in the first session of RL between groups, as only the RL maintenance group advanced past this stage of the task. Mean accuracy was found to be comparable between groups in the initial RL session (Fig. 4.10A; main effect of group: $t_{(11)} = 1.276$, $p = 0.228$), as was the rates of perseverative (Fig. 4.10B; main effect of group: $t_{(11)} = -1.276$, $p = 0.228$) and acquisition (Fig. 4.10C; main effect of group: $t_{(11)} = -1.276$, $p = 0.228$) errors.

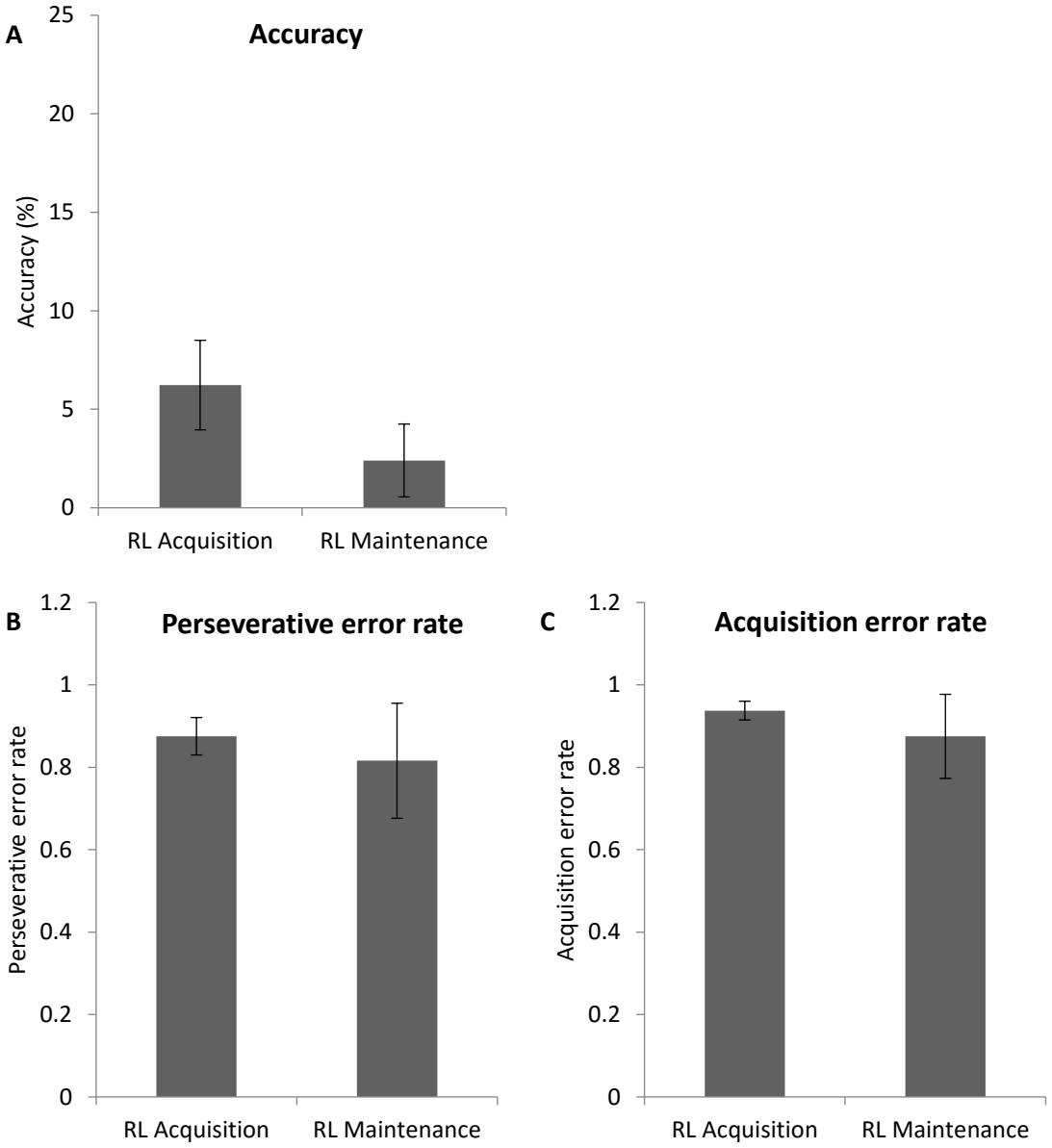


Figure 4.10. Behaviour in the initial session of the RL task. RL acquisition and maintenance animals displayed comparable performance levels in the initial session of the RL task, as shown by the mean accuracy (A), and the rates of perseverative (B) and acquisition (C) errors. The data presented are mean values ± S.E.M.; RL Acquisition: n = 8; RL Maintenance: n = 7.

Examining the number of sessions required to reach criteria in the SD and RL task by RL maintenance animals, using a paired samples t-test, revealed no significant difference between the number of sessions needed to attain criteria in the two tasks (Fig. 4.11; $t_{(6)}$ = -0.521, p = 0.621).

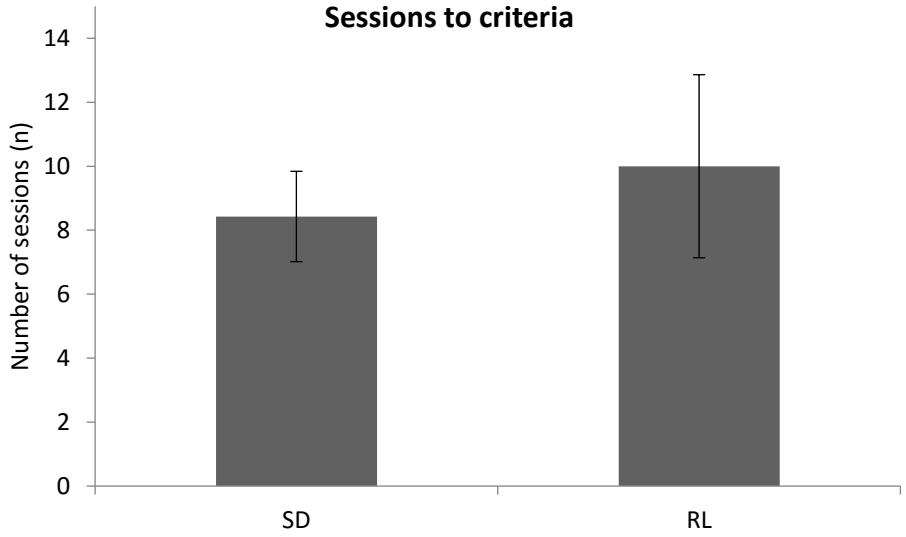


Figure 4.11. A comparison of the number of sessions required to reach criteria in the SD and RL tasks by RL maintenance animals. RL maintenance animals required a comparable number of sessions to achieve criteria in each task. The data presented are mean values ± S.E.M.; n = 7.

4.3.6.2 Validation of microarray gene expression level results and investigation of candidate gene expression levels by RT-qPCR

Expression levels of genes of interest were examined in RL acquisition and RL maintenance groups' striata using RT-qPCR and failed to identify significant variation in expression levels of any gene between groups (Table 4.8). Two of the fours genes of interest displayed a trend towards increased expression levels in RL maintenance animals (Fig. 4.12A & Fig. 4.12E) while the remaining genes investigated displayed trends of decreased expression levels in RL maintenance mice (Fig. 4.12).

Gene of interest	$t_{(5)}$ =	p-value
Adora2A	0.350	1.000
Arc	-0.153	1.000
Bdnf	-1.301	1.000
Creb1	-0.283	1.000
Drd2	1.090	1.000
Homer1	-0.96	1.000

Table 4.8. The statistical values from the independent-samples *t*-test, with Holm-Bonferroni multiple hypothesis test corrections, examining striatal gene expression levels in RL acquisition and RL maintenance mice. n = 4/group.

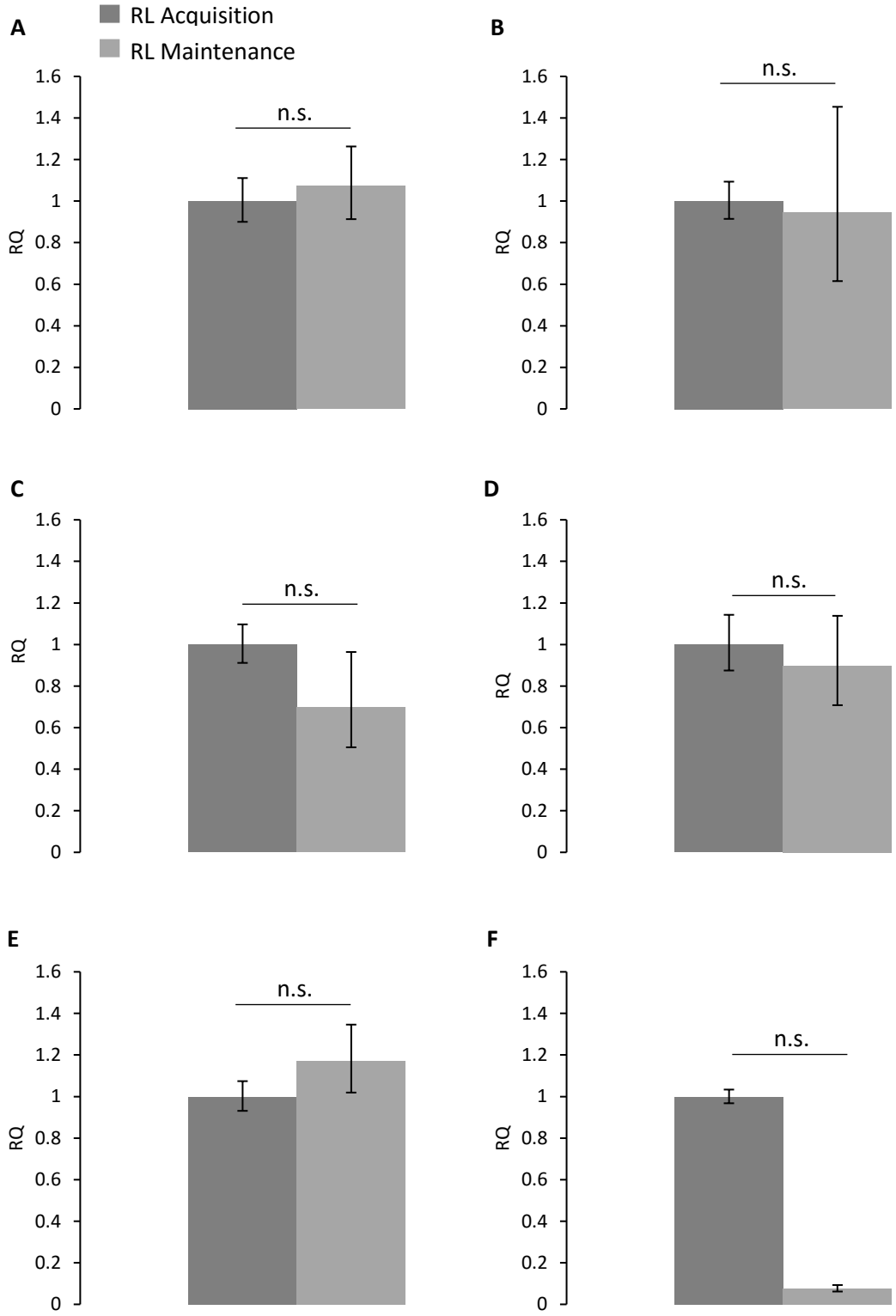


Figure 4.12. RQ value comparison of *Adora2A* (A), *Arc* (B), *Bdnf* (C), *Creb1* (D), *Drd2* (E), and *Homer1* (F) striatal expression levels between RL acquisition and RL maintenance groups following RT-qPCR to confirm microarray data. A trend of increased expression levels in the RL maintenance group is seen for *Adora2A* (A) and *Drd2* (E), while all remaining genes investigated show a trend towards decreased expression levels in these animals. However, all gene expression level differences between groups were found to be non-significant. Values shown are mean ± S.E.M.; n = 4/group.

n.s. = non-significant

The striatal expression levels of the genes of interest were examined in SD maintenance and RL acquisition animals in order to investigate the neural correlates of early RL acquisition, as well as to validate the microarray findings. Supporting the microarray findings, when taking significance at FDR $p<0.05$, examination of the RT-qPCR data for the six genes of interest revealed no alteration in striatal transcription between SD maintenance and RL acquisition groups for any gene investigated (Table 4.9). However, a trend towards an increase in gene expression levels in the striata of RL acquisition animals was identified for all genes investigated (Fig. 4.13), with the exception of *Drd2* (Fig. 4.13E).

Gene of interest	$t_{(5)}$ =	p-value
Adora2A	0.909	1.000
Arc	0.208	1.000
Bdnf	1.571	1.000
Creb1	0.218	1.000
Drd2	-0.067	1.000
Homer1	0.344	1.000

Table 4.9. Statistical values from the independent-samples *t*-test examining potential differences in striatal gene expression levels between SD maintenance and RL acquisition groups. n = 4/group.

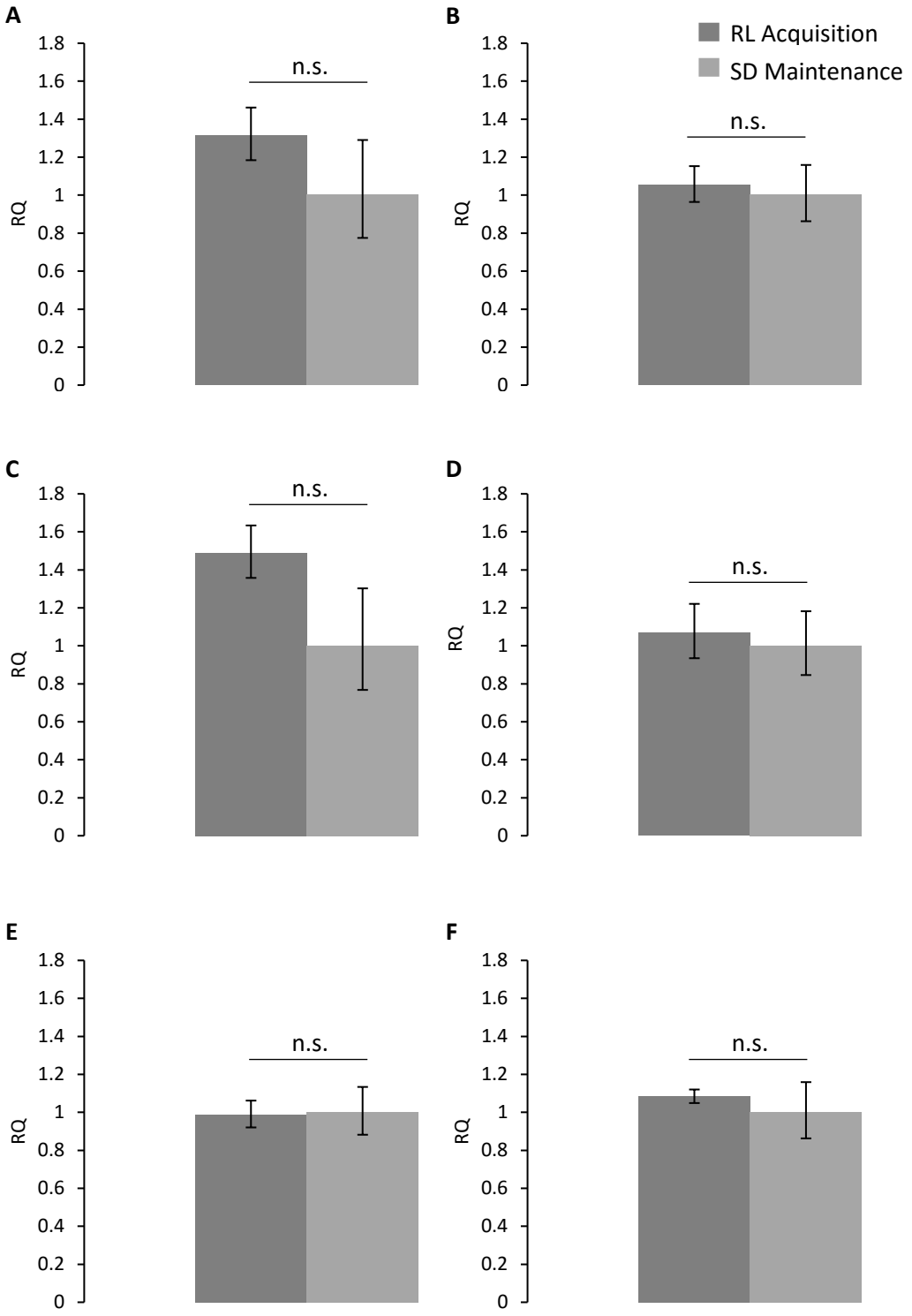


Figure 4.13. RQ value comparison of *Adora2A* (A), *Arc* (B), *Bdnf* (C), *Creb1* (D), *Drd2* (E), and *Homer1* (F) striatal expression levels between SD maintenance and RL acquisition groups following RT-qPCR to confirm microarray data. A trend of increased expression levels in the RL acquisition group is seen for all genes investigated, with the exception of *Drd2* (E). However, all gene expression level differences between groups were found to be non-significant. Values shown are mean ± S.E.M.; n = 4/group.

n.s. = non-significant

4.3.5.3 Pathway analyses of microarray data

The microarray investigation identified 674 mRNAs presenting with significant fluctuations in striatal expression levels between RL acquisition and RL maintenance animals (Table 4.3), which can be argued to correlate with the late acquisition of RL. DAVID pathway analysis reported alterations in genes significantly associated with a number of cellular signalling processes, such as plasma membrane (GO:0005886; $p = 4.97 \times 10^{-03}$), peptidyl-serine phosphorylation (GO:0018105; $p = 5.53 \times 10^{-03}$), calcium ion binding (GO:0005509; $p = 1.08 \times 10^{-02}$) and protein domain specific binding (GO:0019904; $p = 1.33 \times 10^{-02}$).

To investigate gene expression level differences that arise from early acquisition of RL, the striatal transcriptomes of SD maintenance and RL acquisition groups were also analysed. SD maintenance and RL acquisition striatal gene expression level profiles were found to exhibit variation in 317 transcripts in the microarray investigation, when using a nominal p-value for significance (Table 4.3). These mRNAs significantly associate with a multitude of biological processes, and the most highly significant were related to activation of immune response (GO:0002253; $p = 4.32 \times 10^{-04}$), regulation of fibroblast growth factor signalling pathway (GO:0040036; $p = 5.95 \times 10^{-04}$), extracellular space (GO:0005615; $p = 2.44 \times 10^{-03}$) and regulation of protein amino acid phosphorylation (GO:0001932; $p = 3.18 \times 10^{-03}$).

4.4 Discussion

Chapter 3 identified a trend in gene expression level profiles between groups that were untrained and those that had undergone different levels of behavioural training. Specifically, a greater number of transcriptional changes arose between untrained animals and those that had undergone initial CRF training in comparison to the number arising between untrained mice and those that had performed CRF, 5-CSRTT and SILT. The current study looked to identify striatal expression changes at distinct phases of SD and RL in healthy C57BL/6 animals, as a precursor to examining transcription levels during SD and RL in the HdhQ150 mouse model of HD in a later study. Behavioural results were as anticipated, with mice capable of reaching criteria in both the SD and RL task, and significant effects of learning stage and task on performance accuracy, as well as a significant interaction between learning stage and task. Surprisingly, microarray exploration of striatal transcription reported an absence of significant expression changes between any of the learning stage comparisons, when stringent multiple test corrections were utilised. RT-qPCR analyses of striatal learning-associated genes acted to verify the microarray findings by also discovering no differences in expression between any of the learning stages.

These findings suggest that striatal gene expression level changes that arise at the different stages of SD and RL in wild-type animals may not be of a high enough magnitude to be detected by a microarray investigation. There is also the possibility that the dorsal and ventral striatum may function differentially in RL, resulting in the absence of signal found in the current study.

4.4.1 CRF task

All groups that undertook CRF training performed the task equivalently, as shown by a comparable mean number of responses being made per session and an analogous mean number of sessions required to attain criteria in the task. This was anticipated, as all groups consisted of animals of the same genotype and sex, at the same age. The results of the microarray, however, were unexpected, with 0 genes presenting significant changes in striatal expression between CRF and chronic magazine trained mice when significance was taken at FDR $p<0.05$. One reason for this absence of transcriptional diversity is the possibility that the striatal gene expression level profiles of each group are comparable because the natural magnitude of gene expression level changes that occur following acquisition of CRF in wild-type animals are not high enough to be detected by microarray. When investigating the role of transcription in learning, it is common practice to use a 'bottom-up' approach to alter the genetic profile of animals prior to, or during, behavioural testing using transgenic (Silva et al., 1992b; Bourtchuladze et al., 1994; Sakimura et al., 1995; Abel et al., 1997; McClung and Nestler, 2003; Gerstein et al., 2012) or pharmacological methods (Egerton et al., 2005; Bredy and Barad, 2008; Romieu et al., 2008; Stefanko et al., 2009; Haettig et al., 2011; Dagnas et al., 2013). This approach often yields changes in transcription or behaviour, which can be linked to the gene(s) or pathway(s) under investigation. The current study used a 'top-down' approach where behaviour was examined before examination of potential genetic differences. It is possible that using an approach of this nature in wild-type animals does not lead to transcriptional changes large enough for detection by microarray, as suggested by the lack of microarray findings when using stringent multiple test corrections. Another potential reason for the lack of significant microarray findings is that examining gene expression level alterations in chronically trained animals may be too late in the learning process to identify such changes, as the variations in transcription may arise after the first few sessions of acquisition when plasticity is induced.

Pathway analyses of the genes reporting significant differences in expression in the microarray when a nominal p-value was utilised identified that these genes were highly associated with a number of cell signalling functions and odorant binding. This might imply that these biological processes are necessary for acquisition of the CRF task, and are similar to the findings of Chapter

3 where cell signalling via GTP mechanisms were altered following CRF acquisition. *Creb1*, a gene associated with learning (Brightwell et al., 2004; Tyan et al., 2008), was identified in the nominal p-value microarray list as having greater expression levels in the striatum of CRF trained animals than their magazine trained counterparts. This would suggest that striatal *Creb1* levels play a role in the acquisition of conditioning learning. However, this conclusion is contradicted by the evidence from the RT-qPCR data, which found no significant difference in *Creb1* transcription between chronic magazine and CRF trained animals.

4.4.2 SD task

The behavioural data from the SD task, in terms of accuracy, was as expected. Learning stage and task were both found to significantly affect performance levels, and an interaction between the two independent variables was also reported. The SD acquisition animals produced a mean accuracy of ≈50%, which would be the performance level anticipated in the first session of a two-choice paradigm by chance. Heightened accuracy was seen in the SD maintenance group, which was expected because animals were actively learning the task between the initial session and final three sessions. Such an increase in accuracy was also anticipated, and necessary, because SD maintenance animals were required to reach ≥85% accuracy across the final three sessions in order to reach criteria in the task. Accuracy levels dropped to the lowest point during RL acquisition, which was also anticipated based on the findings of previous RL studies (Havekes et al., 2006; Izquierdo et al., 2006; Trinh et al., 2012; Brigman et al., 2013), as animals were learning a new rule at this stage and would be expected to show high levels of perseveration to the originally learnt rule in this initial session. A final increase in performance levels occurred between RL acquisition and maintenance, which would be predicted because animals become adept at learning the new rule between the first and final three RL sessions, similar to the increase in accuracy seen between SD acquisition and SD maintenance. The number of sessions required to reach criteria in the SD task was examined in the three behavioural groups that reached criteria in this task as a means of ensuring that any gene expression level differences that may arise between groups did not simply occur because of different performance capabilities, or levels of intelligence, in the SD task. As anticipated, the three groups all required a similar number of sessions to reach criteria, and also performed a comparable ratio of perseverative and acquisition errors, which shows that mice in each group were equally capable of performing the SD task.

The microarray exploration of striatal transcription reported no significant changes in expression of any genes as a result of learning stage or task performed, and also reported no variation in

expression resulting from the interaction of learning stage and task. Direct group comparisons of interest, examining expression at different phases of SD learning, such as CRF maintenance vs SD acquisition, and SD acquisition vs SD maintenance, also failed to identify any mRNAs presenting with altered expression levels when using an FDR<0.05. The genetic components of early SD learning, examined by the CRF maintenance vs SD acquisition group comparison and using the nominal p-value gene list, were associated with biological pathways including those concerning cell structure and organisation, and also cell signalling through GTPase signal transduction. This indicates that early acquisition of a spatial learning task produces alterations in a number of aspects of cellular regulation, and supports previous findings of spatial learning-dependent alterations to cellular structure and signalling (Cavallaro et al., 2002; Burger et al., 2008; Klur et al., 2009; Paban et al., 2010). However, the genetic correlates of SD learning have also previously been examined by microarray investigation, with 19 genes displaying significant changes in expression in the rat hippocampus following SD learning in the hole-board maze task (Robles et al., 2003). The mRNAs identified by Robles et al. (2013) were associated with processes that include axonal growth and guidance, signal transduction, neurotransmitter and neuropeptide receptors and nuclear proteins; none of which were associated with SD learning in the present study. The discrepancies in biological processes associated with SD in the two studies are likely to be the result of a number of differences in experimental design, such as the contrasting species of animals, brain regions and behavioural tasks examined in either study. The time at which gene expression levels were examined following training also differed between the two studies, with the current study investigating transcription 20 min after the initial or final SD session while Robles and colleagues (2013) waited until 3 h after the third SD acquisition session, which is likely to contribute to the differing findings. It is important to note, however, that the pathway analyses discussed in the current study have been produced from gene lists created in the absence of stringent multiple test corrections and caution must therefore be taken when drawing conclusions from the work. None of the six genes investigated by RT-qPCR showed any changes in striatal expression as a result of performing a single session of SD training after reaching criteria in CRF. This suggests that striatal expression of these genes may not play an active role in the early acquisition of SD learning. It is possible that expression of these genes elsewhere in the brain may be necessary for early acquisition of the SD task. It is most likely that hippocampal transcription of these genes may alter following SD learning, based upon the wealth of evidence implicating the hippocampus as the neural region underpinning spatial learning (Silva et al., 1992b; Silva et al., 1992a; Bliss and Collingridge, 1993; McHugh et al., 1996; Tsien et al., 1996; Burgess et al., 2002) and evidence of altered hippocampal expression of multiple genes affecting, or resulting from, spatial learning (Aiba et al., 1994a; Guzowski et al., 2000; Mizuno et al., 2000; Guzowski et al., 2001; Vazdarjanova et al., 2002; Wood et al.,

2005). Hippocampal expression of one of the genes examined by RT-qPCR, *Arc*, has previously been found to correlate with learning of the hippocampal-dependent spatial Morris water maze task following just one session of training (Guzowski et al., 2001). Based on this evidence, it is possible that the SD acquisition animals of the current study may exhibit increased levels of hippocampal *Arc* transcription in comparison to CRF maintenance animals. The hippocampus was extracted from animals in the current study, so it is possible that future analysis of this tissue may reveal such changes in gene expression levels.

The genetic profile associated with late SD learning was investigated by comparing the striatal expression of SD acquisition and SD maintenance mice. When using a nominal p-value, this revealed significant changes in genes associated with cellular transport, specifically of anion and solute antiporter activity, as well as transcriptional regulation. It is well established that transcriptional regulation is associated with learning and memory (Izquierdo and Medina, 1997; Impey et al., 1998; Hall et al., 2000; Jones et al., 2001; Saura et al., 2004), which acts to strengthen the findings of the current study that the GO biological process of transcriptional regulation would be altered during acquisition of a spatial learning task. Some of the genes presenting with altered expression levels in the nominal p-value microarray list were also associated with transcriptional regulation through epigenetic mechanisms; however, this association did not reach significance. In support of this finding, and although the association did not reach levels of significance in the present study, epigenetic regulation of transcription has previously been found to play a role in learning and memory in a number of studies (Korzus et al., 2004; Guan et al., 2009; Stefanko et al., 2009; Barrett et al., 2011; McQuown et al., 2011), and it is possible that the association in question may have reached significance with a larger number of samples in each microarray comparison (n = 4 in the current study). As with early SD learning, there were no significant differences in expression levels identified for the six genes of interest examined by RT-qPCR between the SD acquisition and SD maintenance animals. This suggests that striatal transcription of the genes under investigation do not play a role in the late acquisition of a spatial learning task. It is possible that expression of these genes may influence, or be influenced by, spatial learning but that their striatal expression is not key to this form of learning, as previously suggested. Expression of the immediate early gene *Arc* within the hippocampus is known to be necessary for the consolidation of long term memory in the spatial Morris water maze task (Guzowski et al., 2000) and also correlates with learning of the task (Guzowski et al., 2001). Similarly, hippocampal expression of another immediate early gene, *Bdnf*, is also associated with hippocampus-dependent learning (Hall et al., 2000; Mizuno et al., 2000). These studies indicate that examination of transcription within the hippocampal tissue of SD acquisition and maintenance animals in the current study may yield significant differences in

expression in *Arc* and *Bdnf*. Interestingly, similar upregulation of expression of both genes have been reported in the cortex of animals following hippocampus-dependent spatial learning (Park et al., 2011), which suggests that similar changes may be identified in the PFC tissue extracted from SD maintenance animals of the current investigation, in comparison to SD acquisition counterparts. Thus examining transcription in the other extracted neural regions, particularly the hippocampus, in the future may prove worthwhile. Alternatively, the lack of evidence for altered expression of either *Arc* or *Bdnf* within the striatum following late SD learning in the current study, in combination with evidence of hippocampal and cortical changes in transcription of both genes following spatial learning (Guzowski et al., 2000; Hall et al., 2000; Mizuno et al., 2000; Guzowski et al., 2001; Park et al., 2011), argues that striatal signalling of these genes does not form part of a neural network required for spatial learning. A number of studies have linked activation of the transcription factor CREB, through phosphorylation (Gonzalez and Montminy, 1989), in the hippocampus with a role in spatial learning and memory (Mizuno et al., 2002; Brightwell et al., 2004; Porte et al., 2008; Sekeres et al., 2010; Aguiar et al., 2011; Chen et al., 2014) while long-term memory of a response strategy has previously been found to require CREB function in the dorsolateral striatum, independent of such activity in the dorsolateral hippocampus (Brightwell et al., 2008). From these studies, it was hypothesised that performance of the SD task to criterion would result in heightened levels of CREB expression in both the hippocampus and striatum. However, the data from the present study indicates that striatal *Creb1* transcription does not alter across any time point of SD learning, potentially suggestive of an absence of a role of striatal CREB signalling in SD learning and seemingly contradicting this hypothesis. The aforementioned studies that examine CREB signalling did so using Western blotting to analyse protein levels of phosphorylated CREB, the activated form of the transcription factor, while the current study, on the other hand, analysed *Creb1* transcription using RT-qPCR. The disparity in methodology between the current study and the published studies is likely to be a factor that contributes to the rejection of the CREB hypothesis outlined. By examining transcriptional levels of *Creb1*, the current study investigated the level of *Creb1* mRNA present but did not evaluate the levels of the activated protein, with the activated form of CREB being the integral factor in spatial learning and memory in the previously discussed papers. Hence, future work using Western blots to examine protein levels of CREB in the striatum and hippocampal tissue of animals in the current study would act to better test the previously outlined CREB signalling hypothesis.

4.4.3 RL task

As previously discussed, there was a clear and anticipated effect of RL on accuracy levels, with high accuracy levels achieved across the final three days of SD training dropping to highly perseverative low accuracy performance on the first day of RL training. This demonstrates that mice still had a preference for the formerly rewarded hole in the initial RL session, similar to findings of other studies examining RL (Havekes et al., 2006; Izquierdo et al., 2006; Trinh et al., 2012; Brigman et al., 2013). A subsequent rise in accuracy levels then occurred between the initial RL session and the final three sessions of RL training, as would be expected. Examining the number of sessions to reach criteria in the SD and RL tasks in the RL maintenance group revealed that mice required a similar number of sessions in order to fully acquire each task. This was somewhat unexpected, as previous studies investigating RL have reported a larger number of sessions being required to acquire the RL task than the original task (Izquierdo et al., 2006; Brigman et al., 2013). This should not lessen the validity of the task, as other studies have also reported wild-type animals requiring a comparable number of sessions to reach criteria in a RL task as the original task (Kruzich et al., 2006) or comparable levels of accuracy being attained by wild-type animals in the RL task as seen in the original task after a similar number of sessions (Havekes et al., 2006).

Unexpectedly, when using an FDR p-value<0.05 the microarray investigation failed to find any genes that presented with altered expression levels between RL acquisition and RL maintenance animals, or between SD maintenance and RL acquisition mice. This seems to suggest that either no transcriptional changes occur between any of the distinct learning phases examined in the current study, which seems unlikely because of the strong association of gene expression levels and RL identified by numerous studies (Davies et al., 2005; Izquierdo et al., 2006; Mitchell et al., 2007; Brigman et al., 2008; McDowell et al., 2010), or that the transcriptional changes that do occur were not identified as a result of the experimental design. As previously discussed, the use of a 'top-down' rather than a 'bottom-up' approach might contribute to the lack of significant changes identified in the microarray in the current study, as could the relatively small sample sizes in the microarray (n/group = 4). Another factor that may be instrumental in the lack of significant microarray findings is the method of striatal dissection. In the present study the striatum was dissected from each hemisphere as a whole entity, meaning that it was not microdissected into distinct regions. It is possible that microdissecting the striatum into dorsal and ventral regions may have yielded substantial gene expression level differences between such regions in the microarray. This might have been produced significant findings because it is known that distinct regions of the striatum play dissociable roles in learning (Reading et al., 1991; Featherstone and McDonald, 2004; Reiss et al., 2005; Atallah et al., 2007; Darvas and

Palmiter, 2010, 2011; Darvas et al., 2014; Burton et al., 2015) and previous studies have identified significant differences in RL behaviour and gene expression levels when subdividing the striatum into similar distinct regions (Hernandez et al., 2006; Daberkow et al., 2007; Graybeal et al., 2011). Thus it is plausible that by extracting the striatum as a whole, any gene expression level differences that may arise between distinct regions of the striatum could be getting lost and made unidentifiable to the microarray and RT-qPCR investigations. Therefore, microdissecting the striatum into subregions may be a worthwhile endeavour in future genetic studies. Similarly, it is possible that the lack of tissue specificity, in terms of neuron population rather than region specificity, may be responsible for the lack of gene expression level differences between groups. A previous investigation into the molecular mechanisms of RL using *in situ* hybridisation has identified performance-related striatal neuron-specific alterations in *Arc* transcription levels (Daberkow et al., 2007). Specifically, *Arc* expression was found to be heightened in the preproenkephalin-negative striatonigral cells of the dorsomedial striatum of rats that required fewer trials to attain criteria in a RL task. Thus, the results of Daberkow and colleagues (2007) suggest that examining gene expression levels in specific striatal neuron populations, as opposed to the entire neuron population of the striatum examined in the present study, may have yielded significant results. A recent paper utilised immunohistochemistry to map regional expression of the immediate early gene *c-Fos* within the brain across a number of stages of visual discrimination and RL (Brigman et al., 2013). This also indicates that examining specific neuron populations or regional patterns of transcription in the current study may have yielded significant findings. The study by Brigman and colleagues (2013) examined behaviour and gene expression levels across the early and late phases of visual discrimination and RL, similar to the current study, but also inspected gene expression levels during a 'mid' phase of RL where session performance of animals was around chance levels (50%). Evidence of altered *c-Fos* expression levels in the dorsolateral striatum were reported between the early, mid and late phases of RL. It is possible that significant gene expression level differences were not reported in the present study because the changes in transcription required for RL occur outside of the learning stages examined, and that the inclusion of a mid-stage RL group, as used by Brigman and colleagues (2013), may have identified such differences.

The microarray identified 317 genes showing altered patterns of expression between the striata of SD maintenance and RL acquisition mice, when using a nominal p-value, and this group comparison acts as an indication of striatal gene expression level changes during early acquisition of RL. The mRNAs presenting with varied transcription during this learning phase were most highly associated with immune response, fibroblast growth factor signalling, extracellular space and protein modification, which suggests that these biological processes play

a role in early acquisition of RL. To my knowledge, the current study is the first microarray investigation into the distinct phases of RL in healthy wild-type animals, making a comparison of the present pathway analysis findings with previous work impossible. RT-qPCR exploration for the six genes under investigation revealed no significant differences in levels of striatal transcripts between the SD maintenance and RL acquisition animals, suggesting that changes in striatal expression of the genes evaluated are not required for early acquisition of a RL task. *Drd2* has been strongly linked with RL, with previous evidence indicating that transgenic mice lacking DRD2s are incapable of learning a new rule in an odour discrimination task (Kruzich et al., 2006) and that DRD2 antagonism through haloperidol treatment impairs RL of an attentional set-shifting task in wild-type animals (Desteno and Schmauss, 2009). Based on these findings, one could expect to see an increase in striatal *Drd2* expression following acquisition of a RL task. It is likely that such an increase in transcription was not apparent during early acquisition of RL because the RL acquisition group of animals showed high perseverance towards the original rule during the first RL session, therefore performing poorly in the RL task. Thus, with their low proficiency in the RL task, an increase in striatal *Drd2* expression would not be expected, which was the case. Conversely, an investigation into the genetic basis of RL across 51 distinct strains of mice reported an inverse correlation between ventral mesencephalic and PFC *Drd2* expression and RL performance capabilities, where high expression of *Drd2* was correlated with poor behavioural performance (Laughlin et al., 2011). This finding suggests that differences in PFC *Drd2* expression levels should arise during early acquisition of the RL task, although this investigation has not been performed in the current study. With PFC *Drd2* transcription levels being found to inversely correlate with RL performance (Laughlin et al., 2011), the highly perseverative RL acquisition group would be expected to display heightened *Drd2* expression within the PFC, as they are not proficient in the RL task. Because of this, it may be of interest for future work to examine *Drd2* transcription within the PFC tissue extracted in the present study. It is important to note that Laughlin and colleagues (2011) did not identify a correlation between striatal *Drd2* levels and RL performance. Another gene that has been linked to RL is *Bdnf*, with a recent paper identifying an early RL acquisition impairment in a spatial memory task in mice with activity-dependent BDNF expression reduction (Sakata et al., 2013). Animals with hippocampal reduction of activity-driven BDNF expression performed significantly poorer than wild-type counterparts on the first RL session of the Morris water maze, a behavioural task assessing hippocampus-dependent spatial memory (Morris, 1981; D'Hooge and De Deyn, 2001). From this finding, one could hypothesise that mice with poor performance in the initial session of a spatially-driven RL task, such as the RL acquisition group in the current study, may present with decreased levels of *Bdnf* expression in the hippocampus compared to animals that had high accuracy in the previously learnt rule. The present study has not examined hippocampal

transcription of *Bdnf*; however, it may be of interest to do so in the future based on the hypothesis discussed.

Pathway analyses reported that the mRNAs showing altered expression within the striatum as a result of late acquisition of the RL task, when using a nominal p-value, were most highly linked with a number of cellular signalling roles. The GO biological processes associated with late RL acquisition included plasma membrane, peptidyl-serine phosphorylation, calcium ion binding and protein domain specific binding. This indicates that acquisition of a RL task is dependent upon striatal cellular signalling, and again it is difficult to assess this finding in relation to published literature because the present study is the first microarray investigation into the genetic foundations of RL in healthy wild-type mice, to my knowledge. The six genes investigated by RT-qPCR showed comparable striatal expression levels between RL acquisition and RL maintenance mice, suggesting that these striatal mRNAs do not play a role in the late acquisition of RL. Based on the finding that transgenic inactivation of A_{2A}Rs in the striatum enhanced RL in mice (Wei et al., 2011), it was hypothesised that successful acquisition of RL in the present study would produce a decrease in striatal and PFC *Adora2A* expression. This hypothesis was rejected by both the microarray and RT-qPCR findings for striatal expression levels, with RL acquisition and maintenance animals showing comparable *Adora2A* transcription intensities, and there are a number of reasons why this may be the case. As discussed previously, it is possible that the 'top-down' approach used to examine RL-dependent gene expression level differences in the current study is not capable of generating changes in transcription in wild-type animals that are large enough for detection by microarray or RT-qPCR. It is possible that changes in *Adora2A* expression may be detected in other regions of the brain, potentially in the PFC as knock-out of A_{2A}Rs in the entire forebrain also resulted in enhanced RL capabilities (Wei et al., 2011). This seems unlikely, however, as inactivation of A_{2A}Rs restricted to striatal neurons only was sufficient for the enhanced RL phenotype, suggesting that the striatal A_{2A}Rs are the cause of the RL behavioural modification and thus making it more likely that striatal expression of *Adora2A* would have altered. It is possible that the hypothesised changes in RL maintenance animals' striatal *Adora2A* expression may have occurred in RL sessions between the early and late phases of RL acquisition but prior to sacrifice. Therefore, a subsequent increase in striatal A_{2A}R protein levels may have arisen and comparison of these protein levels in the striatal tissue of RL acquisition and maintenance animals may be of future interest. Proficiency in the performance of a RL task has been found to correlate with striatal expression of the immediate early gene *Arc* (Daberkow et al., 2007) and disruption of this expression is capable of disrupting RL memory retention in rats (Pastuzyn et al., 2012). These studies suggest that *Arc* signalling is critical for RL and lead to the generation of the hypothesis that fully acquiring the RL task in the present study

would produce heightened *Arc* transcription within the striatum. However, *Arc* transcription within the striatum was statistically analogous between RL acquisition and RL maintenance mice, indicating that expression of this immediate early gene in the striatum does not correlate with RL task performance and contradicts the aforementioned findings. One potential reason for the contradictions between our findings and those previously reported is the difference in neural region specificity examined in the studies. The present study examined the striatum in its entirety while the work of Daberkow and colleagues (2007) and Pastuzyn and associates (2012) examined specific regions of the striatum, most notably the dorsomedial striatum. It is conceivable that examining transcription of the striatum in the dorsomedial and dorsolateral regions, or potentially dorsal and ventral regions because of the small size of the mouse brain, may have yielded significant differences in *Arc* expression. These hypothetical variations in expression may have arisen between subregions within the same group, dorsal versus ventral transcription profiles for example, or may have been generated between groups, with the dorsomedial striatum potentially showing altered expression between the RL acquisition and RL maintenance groups, for example, as suggested by Daberkow and colleagues (2007). The present study also examined all neuron types within the striatum, which may have led to confounding, as the original correlation between *Arc* and RL task ability was discovered not only overall in the dorsomedial striatum but also in specific preproenkephalin–negative cells within the striatum (Daberkow et al., 2007). Another variable that could contribute to the lack of correlation between the *Arc* expression findings of the current study and previous reports are the species of animal examined, with mice and rats investigated, respectively. Performance in a RL task has previously been inversely correlated with expression of *Drd2* in the PFC (Laughlin et al., 2011), which raises the possibility that the RL maintenance animals in the current study may exhibit decreased *Drd2* transcription in the PFC when compared with RL acquisition mice. This comparison has not yet been performed, but might be a worthwhile investigation in the future.

4.4.4 Conclusions

Healthy 22-24 weeks of age C57BL/6 mice were capable of performing the CRF, SD and RL tasks as anticipated, with animals able to attain criteria in all tasks and the expected differences in accuracy levels seen between behavioural groups. Unexpectedly, the microarray investigation revealed that 0 genes showed altered striatal transcriptional profiles across any of the behavioural group comparisons. This absence of significant gene expression level variation may be the result of striatal gene expression level changes that arise at the different stages of SD and RL in wild-type animals may not be of a high enough magnitude to be detected by a microarray

investigation. Conversely, a combination of the method of microdissection of the striatum, the choice of neural region examined and the behavioural stage at which transcription was evaluated could potentially contribute to the lack of significant transcriptional difference found. RT-qPCR analyses of learning and memory associated genes verified the microarray findings by reporting no significant differences in striatal expression levels between behavioural groups.

4.4.5 Summary of key results from Chapter 4

- C57BL/6 mice demonstrated the expected behavioural performance in CRF, SD and RL tasks.
- Microarray investigation reported 0 genes exhibiting significant changes in striatal expression between any of the behavioural groups.
- RT-qPCR analyses did not report significant variation in striatal expression of *Adora2A*, *Arc*, *Bdnf*, *Creb1*, *Drd2* or *Homer1* between any of the behavioural groups examined.

Chapter 5: Investigating the transcriptional correlates of spatial discrimination in the *Hdh*$^{Q150/+}$ mouse model of HD.

Chapter 4 investigated the transcriptional basis of cognitive flexibility by examining changes in striatal gene expression levels across multiple stages of SD and RL in wild-type C57BL/6 animals. A microarray and RT-qPCR investigation were unable to identify striatal gene expression level changes significantly associated with performance of SD or RL tasks in the C57BL/6 mice. A lack of striatal dissection specificity was identified as a possible cause of the absence of significant gene expression level variation in the study. The present study aimed to further probe the transcriptional correlates of SD learning by investigating SD performance capabilities and striatal expression levels, of the genes investigated by RT-qPCR in chapter 4, in the *Hdh*$^{Q150/+}$ mouse model of HD, with increased striatal dissection specificity.

5.1 Introduction

The HD phenotype displays a variety of cognitive disturbances that include impairments in executive function (Lange et al., 1995; Lawrence et al., 1996; Lawrence et al., 1999; Aron et al., 2003; Tabrizi et al., 2013), implicit learning (Heindel et al., 1989; Knopman and Nissen, 1991; Gabrieli et al., 1997; Kim et al., 2004), working memory (Tabrizi et al., 2011; Harrington et al., 2012) and emotion recognition (Stout et al., 2012; Tabrizi et al., 2013). Another cognitive process that shows disruption in HD is that of spatial learning and memory, where patients have presented with spatial location learning impairments (Brandt et al., 2005; Pirogovsky et al., 2015) and deficits in spatial memory (Lange et al., 1995; Lawrence et al., 2000; Davis et al., 2003) and perception (Soliveri et al., 2002). Similar deficits in spatial learning and memory have also been reported in mouse models of HD, with evidence of such impairments identified in HdhQ92 (Brooks et al., 2012a), HdhQ111 (Giralt et al., 2012), R6/1 (Pang et al., 2006; Smith et al., 2006; Giralt et al., 2011b; Brooks et al., 2012e), R6/2 (Lione et al., 1999; Murphy et al., 2000) and YAC128 animals (Brooks et al., 2012c). Similarly, homozygous HdhQ150 mice have previously displayed spatial learning impairments, as shown by having progressively slower improvement in response latencies as trial numbers increased in the Morris water maze, from 16 weeks of age compared to wild-type animals (Brooks et al., 2012b).

There is evidence linking gene expression level changes with spatial learning and memory capabilities, and the genes investigated by RT-qPCR in Chapter 4 of this thesis (*Adora2a*, *Arc*, *Bdnf*, *Creb1*, *Drd2* and *Homer1*) have been associated with such cognitive processes, with this evidence highlighted in Chapter 4.1 and 4.4.2. Chapter 4 examined the neural basis of SD and

cognitive flexibility in C57BL/6 mice using a microarray and RT-qPCR investigation and, surprisingly, there was no evidence of striatal transcriptional correlates of SD or RL at any of the learning stages examined (see Chapter 4.3). Despite this lack of learning-dependent changes in expression of the genes investigated, these genes are of interest in the current study because they have all previously been implicated in HD pathogenesis. For example, *Adora2A* is implicated in HD because the A_{2A}Rs produced by this gene are localised within the brain to striatopallidal MSNs that degenerate in HD (Schiffmann et al., 1991a; Schiffmann et al., 1991b) and are lost throughout the course of the disease because of this striatal neurodegeneration (Martinezmir et al., 1991; Glass et al., 2000). Similar decreases in striatal A_{2A}R levels have also been reported in the R6/1 mouse model of HD (Villar-Menendez et al., 2013). An association of *Adora2A* expression with the HD phenotype has been established within the human condition and animal models of the disease, whereby reduced levels of striatal *ADORA2A* mRNA transcripts were reported in post-mortem tissue extracted from HD patients and similar differences in *Adora2A* expression levels identified in R6/1, R6/2, $Hdh^{Q92/Q92}$ and $Hdh^{Q150/Q150}$ mice (Hodges et al., 2006; Kuhn et al., 2007). As previously mentioned, all the genes investigated in Chapter 4 and under investigation in the current study have previously been implicated in HD pathogenesis, the key findings of which have been summarised in Table 5.1.

Gene name	Gene symbol	Finding	Species	Study
Adenosine A2A receptor	*Adora2A*	1) A_{2A}Rs are expressed in striatopallidal MSNs in the striatum.	Rat	(Schiffmann et al., 1991a)
		2) Within the brain, *ADORA2A* mRNA is exclusively located in MSNs of the striatum.	Human	(Schiffmann et al., 1991b)
		3) Early HD neuropathology causes massive loss of A_{2A}R levels due to striatopallidal MSN degeneration.	Human	(Glass et al., 2000)
		4) Striatal A_{2A}R density is transiently increased in R6/2 mice at postnatal days 7-21 while *Adora2A* mRNA levels are decreased from postnatal day 21.	Mouse	(Tarditi et al., 2006)
		5) *Adora2A* expression levels are decreased in the striata of HD patients and R6/1, R6/2, $Hdh^{Q92/Q92}$ and $Hdh^{Q150/Q150}$ mice.	Human & Mouse	(Hodges et al., 2006; Kuhn et al., 2007)
		7) A_{2A}R knockout worsens motor behaviour and survival in N171-82Q mice.	Mouse	(Mievis et al., 2011)
		8) A_{2A}R levels are decreased in *R6/2* mice from 12 weeks of age.	Mouse	(Villar-Menendez et al., 2013)

		9) Hyperactivation of A_{2A}Rs and DRD1s contributes to deficits in novel object recognition, spontaneous alternation, and passive avoidance task performance in R6/1 mice.	Mouse	(Tyebji et al., 2015)
		10) Inactivation of A_{2A}Rs prevents working memory deficits in R6/2 mice.	Mouse	(Li et al., 2015)
Activity-regulated cytoskeleton-associated protein	*Arc*	1) Following Morris water maze training, *Hdh*$^{Q111/+}$ mice present with decreased expression levels of *Arc* mRNA in the hippocampus compared to wild-type animals.	Mouse	(Giralt et al., 2012)
		2) Arc localises with nuclear aggregates in R6/2 mouse brain.	Mouse	(Maheshwari et al., 2012)
Brain-derived neurotrophic factor	*Bdnf*	1) HTT-mediated *BDNF* transcription is lost in HD.	Human	(Zuccato et al., 2001)
		2) HTT-mediated upregulation of *BDNF* transcription through inhibition of the silencing activity of the neuron restrictive silencer element is lost in HD.	Human & Mouse	(Zuccato et al., 2003)
		3) HD causes loss of HTT-mediated BDNF transport along microtubules.	Human & Mouse	(Gauthier et al., 2004)
		4) Decreased BDNF levels reported in R6/2 mice, and BDNF rescue ameliorates disease phenotype.	Mouse	(Zuccato et al., 2005; Giralt et al., 2011a)
		5) Decreased BDNF levels reported in R6/1 mice, and BDNF delivery ameliorates disease phenotype.	Mouse	(Pang et al., 2006; Gharami et al., 2008)
		6) BDNF levels are reduced in the serum of HD patients.	Human	(Ciammola et al., 2007)
		7) Decreased BDNF levels reported in HdhQ92 and HdhQ111 mice, and BDNF delivery ameliorates disease phenotype.	Mouse	(Lynch et al., 2007)
		8) BDNF levels are reduced in the cortex of HD patients.	Human	(Zuccato et al., 2008)
		9) BDNF delivery ameliorates LTP and long-term memory deficits in HdhQ140 mice.	Mouse	(Simmons et al., 2009)
		10) Decreased BDNF levels reported in YAC128 mice, and BDNF overexpression ameliorates disease phenotype.	Mouse	(Xie et al., 2010)
		11) Peripheral BDNF mRNA & plasma protein levels are not reliable HD biomarkers.	Human	(Zuccato et al., 2011)
		12) Decreased BDNF levels reported in zQ175 mice.	Mouse	(Ma et al., 2015)

cAMP response element-binding protein 1	*Creb1*	1) Striatal cells derived from *Hdh*$^{Q111/Q111}$ mice exhibit abnormal *Creb1* activity.	Mouse	(Benn et al., 2008b)
		2) *CREB1* is significantly upregulated in white adipose tissue of HD patients.	Mouse	(McCourt et al., 2015)
Dopamine D2 receptor	*Drd2*	1) Decreased DRD2 binding in the striatum of asymptomatic and symptomatic HD patients.	Human	(Antonini et al., 1996)
		2) Decreased DRD2 binding in the striatum of asymptomatic HD patients.	Human	(Weeks et al., 1996)
		3) Correlation between loss of striatal DRD2 density and duration of illness in HD patients.	Human	(Ginovart et al., 1997)
		4) D1DR, D2DR and dopamine transporter density are predictive of cognitive deficits in HD.	Human	(Backman et al., 1997)
		5) Striatal *DRD2* mRNA progressively decreases with increasing HD pathology.	Human	(Augood et al., 1997)
		6) Striatal DRD2 binding levels correlate with cognitive ability in HD patients.	Human	(Lawrence et al., 1998a)
		7) Asymptomatic and symptomatic HD patients present with a progressive loss of DRD2 binding levels.	Human	(Andrews et al., 1999)
		8) Early HD neuropathology causes massive loss of DRD2 levels due to striatopallidal MSN degeneration.	Human	(Glass et al., 2000)
		9) Progressive loss of DRD2s in the striatum, and temporal and frontal cortex in HD patients.	Human	(Pavese et al., 2003)
		10) Reduced striatal *Drd2* expression levels in R6/1, R6/2, *Hdh*$^{Q92/Q92}$, *Hdh*$^{Q150/Q150}$ mice and HD patients.	Human & Mouse	(Kuhn et al., 2007)
		11) Decreased *DRD2* levels in striatal primary neuron model of HD.	Human	(Runne et al., 2008)
		12) Reduced striatal *Drd2* expression levels in YAC128 but not BACHD mice.	Mouse	(Pouladi et al., 2012)
Homer scaffolding protein 1	*Homer1*	12) Reduced striatal expression levels of *Homer1* in R6/1, R6/2, *Hdh*$^{Q92/Q92}$, *Hdh*$^{Q150/Q150}$ mice and HD patients.	Human & Mouse	(Kuhn et al., 2007)

Table 5.1. A summary of research relating to the HD phenotype for the genes examined by RT-qPCR in the current study.

The current study originally planned to examine cognitive flexibility in the *Hdh*$^{Q150/+}$ mouse model of HD but, due to the need for larger animal numbers in each group, the RL element of the study was removed in order to enable simplification of the study, allowing the study to address the learning where gene expression level changes were most likely to be evident. The current study was simplified in order to contain a larger number of animals of each genotype in two behavioural groups; untrained animals and animals trained to criteria in the SD task described in Chapter 4.2.3.3. Thus, the present study aimed to investigate the SD capacities of the heterozygous HdhQ150 mouse model of HD, if any differences in striatal expression levels arise between *Hdh*$^{Q150/+}$ and *Hdh*$^{+/+}$ animals for any of the genes discussed here, and whether potential gene expression level differences correlate with SD task performance. Based upon the previous investigations into HD pathogenesis and the genes under investigation in the current study (summarised in Table 5.1), it is anticipated that striatal expression levels of all genes investigated will be reduced in *Hdh*$^{Q150/+}$ mice. The lack of evidence for SD performance associated changes in mRNA levels for the genes under investigation in C57BL/6 mice, identified in Chapter 4, suggests that SD task performance may not correlate with expression levels of these genes in the current study. The lack of specificity of striatal dissections was raised as a potential reason for the lack of gene expression level changes identified in Chapter 4, therefore the present study aimed to address this hypothesis by microdissecting the striatum into dorsal and ventral striatal regions for RT-qPCR analyses. Increasing sample numbers and dissection specificity, compared to those performed in Chapter 4, may therefore lead to SD task performance correlating with gene expression levels. It is important to note that the investigation performed in Chapter 4 only examined gene expression levels in wild-type C57BL/6 mice, while the current study is examining transcription levels in both wild-type and *Hdh*$^{Q150/+}$ animals. Thus, it is possible that the HD genotype examined in the present study may present with SD task performance that correlates with striatal expression levels of the genes under investigation.

5.2 Methods

5.2.1 Subjects

Fifty-eight naïve male mice (genotypes: *Hdh*$^{Q150/+}$ (n = 28) and *Hdh*$^{+/+}$ littermates (n = 30) on a C57BL/6J background) were housed 1-5 animals per cage and animal husbandry was as described in Chapter 2.1.2. *Hdh*$^{Q150/+}$ CAG repeat sequences ranged from 117 to 140 CAGs (Mean: 130.83 ± 6.20). All animals were between 44 and 52 weeks-of-age at the start of testing, and were water restricted throughout testing, as described in Chapter 2.1.2.

5.2.2 Experimental design

Animals of both genotypes were randomly allocated into 1 of 2 behavioural training groups: untrained or SD. An equal number of animals of either genotype were placed in the two behavioural groups (*Hdh*$^{Q150/+}$: untrained (n = 14) and SD (n = 14); *Hdh*$^{+/+}$: untrained (n = 15) and SD (n = 15)). The motor ability of all animals (n = 58) was examined by rotarod (see Chapter 5.2.3.1) 1 week prior to operant testing. Animals that were to undergo SD training (n = 29) performed an initial session of magazine training, as described in Chapter 4.2.3.1. Following this initial magazine training, mice were trained to criteria in the CRF task as described in Chapter 5.2.3.4. Upon reaching criteria in the CRF task, animals performed the SD task until reaching criteria, as described in Chapter 5.2.3.5, before sacrifice and tissue extraction.

Individual untrained animals were paired to a SD trained counterpart of the same genotype throughout operant testing, whereby an untrained mouse would receive an equivalent number of food rewards in a session as its SD trained counterpart received. This occurred as described in Chapter 5.2.3.2, and untrained mice were sacrificed after performing the same number of operant sessions as their SD trained counterpart.

5.2.3 Behavioural training protocols

5.2.3.1 Rotarod

For the duration of testing mice had *ad libitum* access to food and water, except during the 300 s rotarod sessions where there was no access to food or water. Mice performed an initial habituation session consisting of cycles of 30 s of maintained rotation speed followed by 15 s of linear acceleration across 300 s. This habituation session was followed by sessions in which rod speed rotation accelerated linearly from 5 to 50 rpm across 300 s. Sessions were performed once a day until the motor ability of both genotypes plateaued across two consecutive sessions (five sessions). Motor ability was examined across the final two sessions. Latency to first fall was recorded in each session and was the measure of motor ability. After each fall, mice were placed back onto the rotarod until the end of the session.

5.2.3.2 Untrained food reward delivery program

Untrained animals (*Hdh*$^{Q150/+}$: n=14; *Hdh*$^{+/+}$: n=15) received no behavioural training, *per se*, but were paired to a same-genotype littermate in the SD group, whereby untrained animals received

an equivalent number of rewards in each session as their SD trained counterpart received in the previous session. Animals underwent an initial magazine training session, where 150 µL of reward was delivered at the onset of the 20 min session and upon removal of the head from the magazine, a 10 s delay would occur before another 5 µL of reward delivered. This reward contingency was maintained for the remainder of the session.

Reward delivery in subsequent operant sessions was determined by the performance of their SD counterpart. Reward delivery occurred in the absence of magazine light illumination in all sessions, to ensure that no association was made between the magazine light and food reward.

5.2.3.3 Magazine training

SD animals (*Hdh*$^{Q150/+}$: n=14; *Hdh*$^{+/+}$: n=15) underwent 1 session of magazine training in order to permit the mice to learn the association between magazine light illumination and food reward. The magazine light was illuminated and 150 µL of reward was delivered at the onset of the 20 min session. Upon removal of the head from the magazine, the light would extinguish for 10 s before again illuminating and 5 µL of reward delivered. This reward contingency was maintained for the remainder of the session.

5.2.3.4 CRF task

Mice performed CRF training as described in Chapter 3.2.4.2. Criterion in the task was set at ≥50 np in three consecutive sessions for the current study. Animals performed the task until criteria was met, at which point mice began the next phase of behavioural training; the SD task.

5.2.3.5 SD task

Mice performed SD training as described in Chapter 4.2.3.3. Training performance on this task was calculated as percent accuracy (((correct choices)/(correct choices + incorrect choices)) x 100). Criterion was judged to be met when a minimum of 85% accuracy was reached in three consecutive sessions. Seven error terms were examined during SD performance, with each capable of identifying different behavioural traits, as described in Chapter 3.2.4.4. Animals performed the task until criteria was met, at which point mice were sacrificed and tissue taken for dissection.

5.2.4 Molecular methodology

5.2.4.1 RNA extraction

RNA was prepared from dorsal and ventral striata following the protocol outlined in Chapter 2.3.1. Quantity and quality of RNA was assessed on a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific Inc., Massachusetts, USA).

5.2.4.2 Quantification of gene expression levels by RT-qPCR

Striatal RNA was converted to cDNA using a High Capacity RNA to cDNA™ kit (Life Technologies Corporation, California, USA) as outlined in Chapter 2.3.2.1 before Taqman® mediated qPCR was performed using the protocol described in Chapter 2.3.2.2. The genes to be investigated were those examined in Chapter 4, and are listed in Table 5.2.

Gene name	Gene symbol	ID
beta actin	*Actb*	Mm00607939_s1
adenosine A2A receptor	*Adora2A*	Mm00802075_m1
activity-regulated cytoskeletal-associated protein	*Arc*	Mm01204954_g1
brain derived neurotrophic factor	*Bdnf*	Mm04230607_s1
cAMP responsive element binding protein 1	*Creb1*	Mm00501607_m1
dopamine receptor D2	*Drd2*	Mm00438545_m1
homer scaffolding protein 1	*Homer1*	Mm00516275_m1
ubiquitin C	*Ubc*	Mm02525934_g1

Table 5.2. Identification codes for Taqman® oligonucleotide probes used in the current study.

5.2.5 Statistical analyses

Statistical analyses were performed using IBM SPSS® Statistics software (Version 20; IBM United Kingdom Ltd, Hampshire, UK). Behavioural was analysed by independent samples t-tests, with Holm-Bonferroni sequential correction used for multiple hypothesis testing correction. RT-qPCR data was analysed by a three-way ANOVA, with genotype ($Hdh^{+/+}$ or $Hdh^{Q150/+}$), training (untrained or SD) and striatal region (dorsal or ventral) as independent variables. Specific two-group comparisons were analysed by independent samples t-tests, with Holm-Bonferroni sequential correction used for multiple hypothesis testing correction. The assumption of

Sphericity of data was assessed using Mauchly's test of Sphericity, with the Greenhouse-Geisser correction used if the assumption of Sphericity was violated and the Greenhouse-Geisser estimate of sphericity (ε) < 0.75. If the assumption of Sphericity was violated and ε > 0.75, the Huynh-Feldt correction was used. Similarly, homogeneity of variances for data was assessed using Levene's test, and the Welch test used in the event of data violating this assumption.

5.3 Results

5.3.1 Behavioural results

5.3.1.1 Rotarod

To assess the motor capabilities of *Hdh*$^{Q150/+}$ animals, mice underwent rotarod training prior to operant behavioural training. An independent samples t-test reported no significant difference in the mean fall latency between *Hdh*$^{+/+}$ and *Hdh*$^{Q150/+}$ animals (Fig. 5.1; $t(56) = 0.069$, $p = 0.945$).

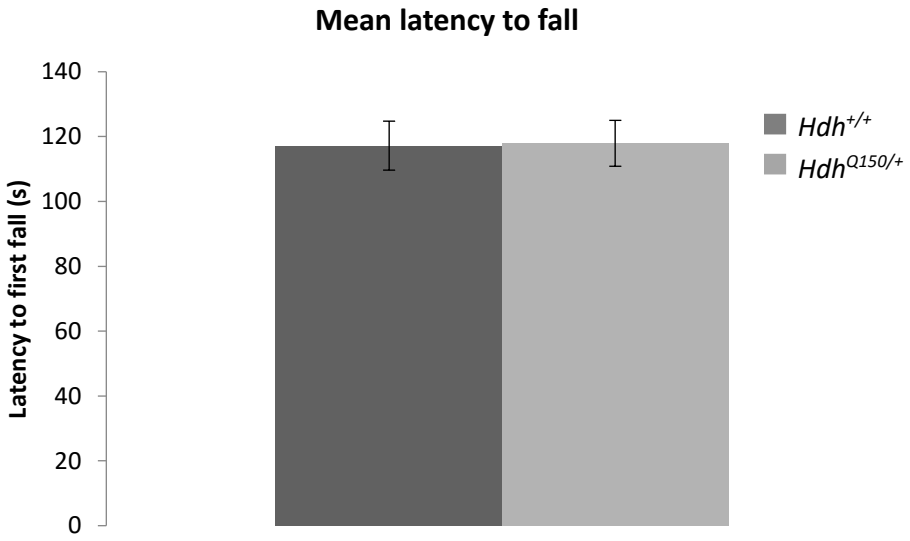


Figure 5.1. Motor ability of *Hdh*$^{+/+}$ and *Hdh*$^{Q150/+}$ animals, as assessed on the rotarod. *Hdh*$^{+/+}$ (n = 30) and *Hdh*$^{Q150/+}$ (n = 28) mice showed comparable motor ability on the rotarod prior to operant training. Values shown are mean ± S.E.M.

5.3.1.2 CRF task

Examining the mean number of sessions to criteria in the CRF task revealed that *Hdh*$^{Q150/+}$ mice required a greater number of sessions to reach the designated criteria than *Hdh*$^{+/+}$ mice (Fig.

5.2A; $t(27) = -3.301$, $p = 0.003$). In the final 3 days of CRF training, *Hdh*$^{Q150/+}$ mice performed significantly fewer np per session than their wild-type counterparts (Fig. 5.2B; $t(27) = 2.807$, $p = 0.009$). Conversely, examination of the total number of trials performed across all sessions until criteria was reached revealed no difference in performance between genotypes (Fig. 5.2C; $t(27) = -1.026$, $p = 0.314$). Averaging the total number of np made across all sessions until criteria was met identified that *Hdh*$^{Q150/+}$ mice made, on average, significantly fewer CRF responses in each session throughout the entirety of FR1 training (Fig. 5.2D; $t(27) = 3.025$, $p = 0.005$).

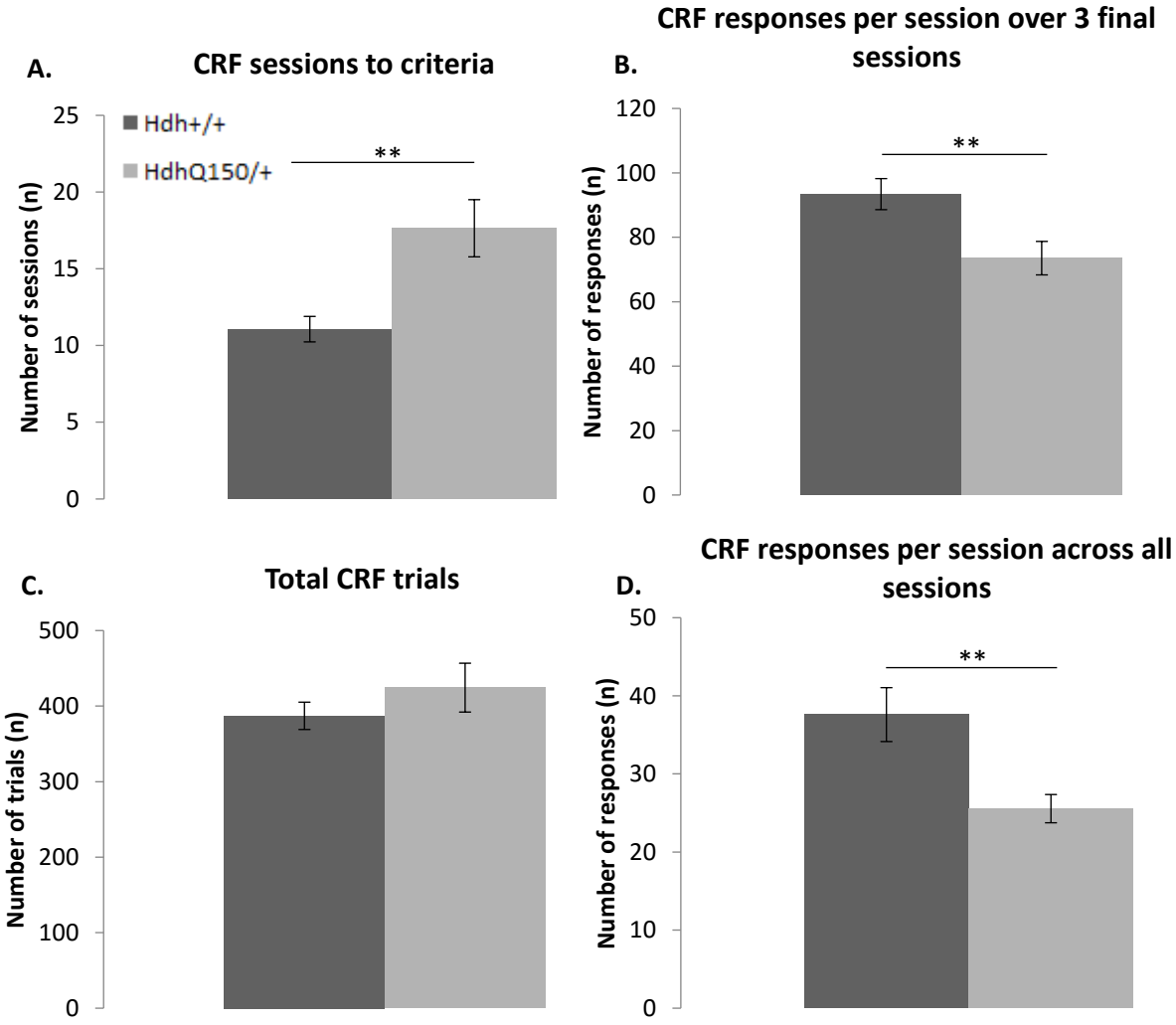


Figure 5.2. Behavioural performance in the CRF task. *Hdh*$^{Q150/+}$ (n = 14) animals took a greater number of sessions to reach criteria (A) in the CRF task, and also made fewer responses in the final 3 sessions before criteria was met than their wild-type (n = 15) counterparts (B). No difference was reported in the total number of trials performed throughout CRF training (C), while *Hdh*$^{Q150/+}$ mice made a significantly lower mean number of np across all CRF sessions (D). Values are means ± S.E.M.

** $p < 0.01$

5.3.1.3 SD task

Hdh$^{Q150/+}$ mice required a significantly greater number of sessions to reach criteria in the SD task in comparison to their wild-type counterparts (Fig. 5.3A; $t(15.235) = -2.936$, $p = 0.010$). Conversely, mean accuracy over the final 3 test sessions was comparable between genotypes (Fig. 5.3B; $t(27) = -0.902$, $p = 0.375$). Likewise, mean accuracy when examined across all sessions until criteria was met was analogous between *Hdh*$^{Q150/+}$ and *Hdh*$^{+/+}$ animals (Fig. 5.3C; $t(27) = 1.471$, $p = 0.153$).

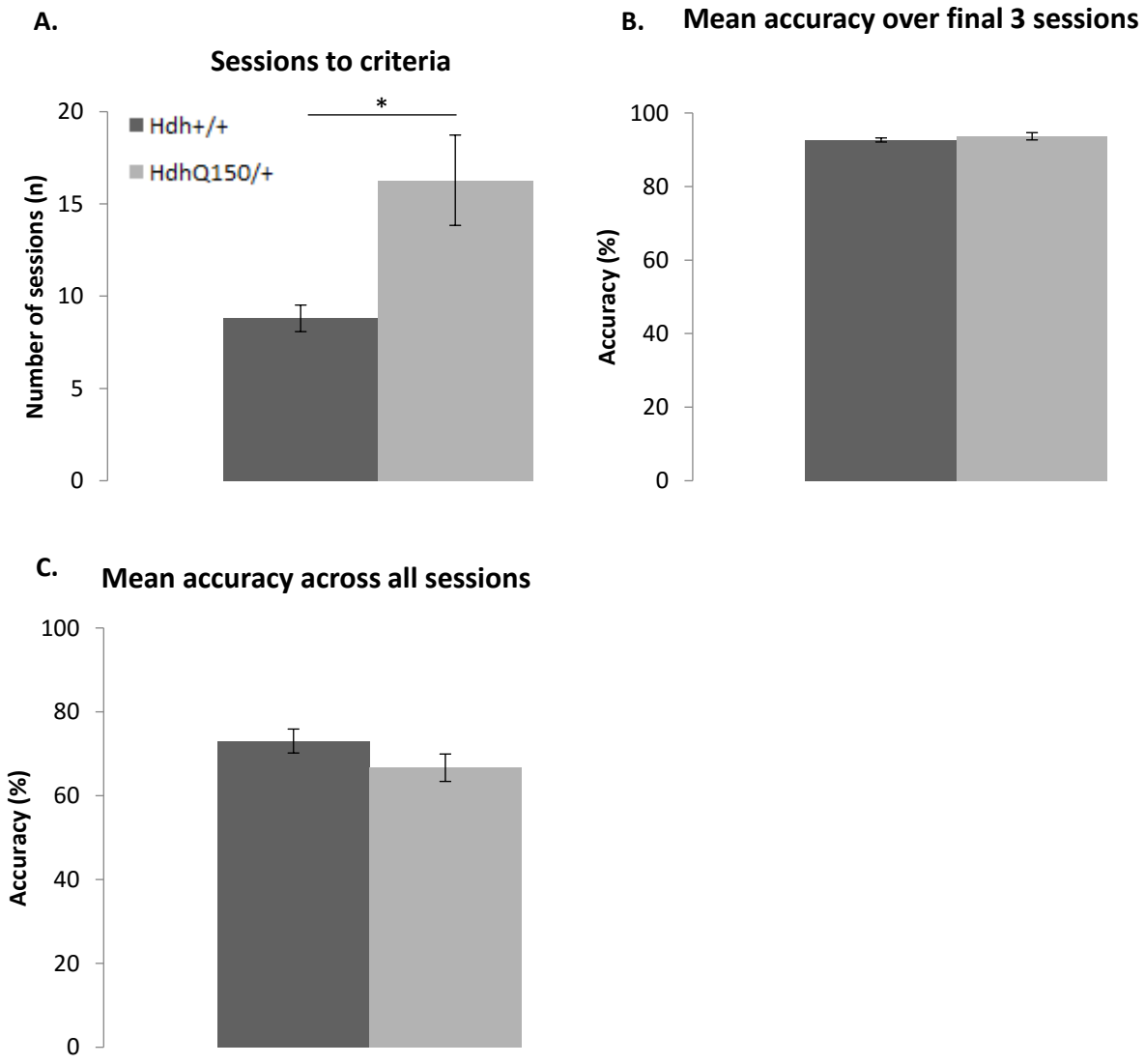


Figure 5.3. SD performance in *Hdh*$^{Q150/+}$ and *Hdh*$^{+/+}$ mice. *Hdh*$^{Q150/+}$ mice (n = 14) required a greater number of sessions to reach criteria in the SD task than *Hdh*$^{+/+}$ mice (n = 15) (A). However, the mean accuracy of *Hdh*$^{Q150/+}$ and *Hdh*$^{+/+}$ mice were equivalent when examined in the final 3 SD sessions (B) and across all SD sessions (C). Values shown are mean ± S.E.M.

* $p < 0.05$

Genotypes showed a difference in the mean number of centre hole S1 np made over the final 3 SD sessions ($t(27) = 2.363$, $p = 0.026$), with $Hdh^{Q150/+}$ mice initiating fewer S2 trials than $Hdh^{+/+}$ mice (Fig. 5.4A). On the other hand, investigating the total number of S2 trials initiated across all SD sessions revealed no difference in the total number of S1 np made between genotypes (Fig. 5.4B; $t(27) = -1.020$, $p = 0.317$). When the total number of correct S1 np were averaged across the number of sessions required to reach criteria, to give the mean number of S2 trials initiated across all SD sessions, $Hdh^{+/+}$ mice were found to initiate a greater amount of S2 trials than $Hdh^{Q150/+}$ animals (Fig. 4C; $t(19.587) = 2.822$, $p = 0.011$). The equivalent number of total S2 trial initiation across all SD sessions between genotypes (Fig. 5.4B) is therefore a result of the $Hdh^{Q150/+}$ animals performing fewer S2 trial initiations per session across a greater number of sessions, in order to reach criteria in the task, cancelling out the higher number of S2 trials initiated by $Hdh^{+/+}$ animals in fewer sessions.

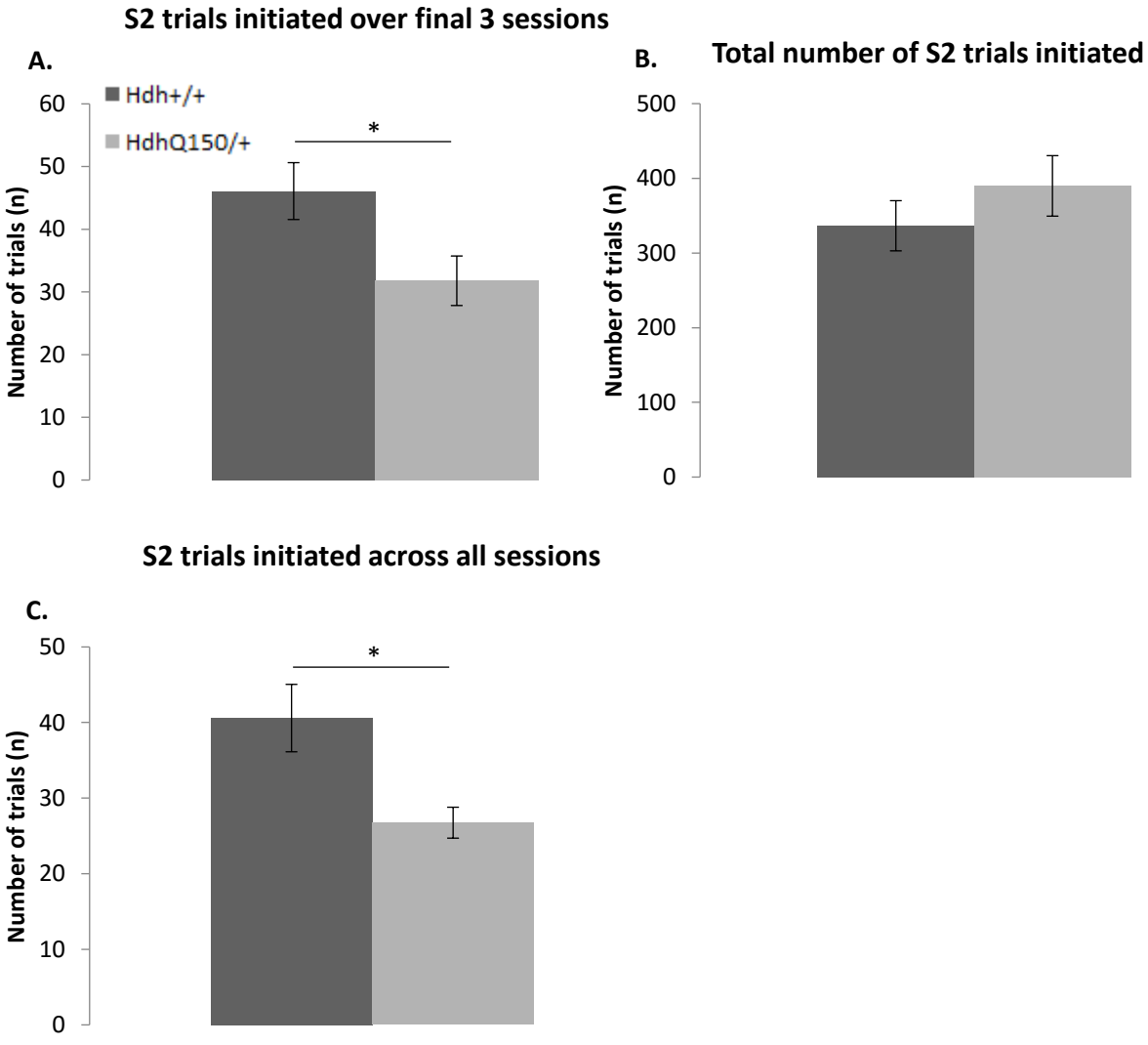


Figure 5.4. S2 trial initiation in the SD task. $Hdh^{Q150/+}$ animals (n = 14) initiated significantly fewer S2 trials over the final 3 sessions of SD training than their wild-type (n = 15) counterparts (A).

This impairment was not observed in the total number of S2 trials initiated across all SD sessions (B). However, when the mean number of S2 trials initiated per SD session, for all sessions, was examined the deficit in trial initiation was again apparent (C). Values shown are means ± S.E.M. * $p < 0.05$

An independent samples *t*-test revealed $Hdh^{Q150/+}$ subjects showed significantly delayed latencies in responding to the correct S2 stimulus (Table 5.3; $t(19.907) = -2.992$, $p = 0.007$) and S1 stimulus (Table 5.3; $t(27) = -2.932$, $p = 0.007$). However, genotypes presented with equivalent response latencies when collecting food reward (Table 5.3; $t(27) = -1.895$, $p = 0.069$).

Response latency	$Hdh^{+/+}$	$Hdh^{Q150/+}$
Correct response reaction time (s)	1.93 ± 0.32 **	3.97 ± 0.60
Correct S1 response time (s)	3.41 ± 0.16 **	4.20 ± 0.22
Reward collection latency (s)	1.26 ± 0.09	1.48 ± 0.08

Table 5.3. Response latencies of $Hdh^{Q150/+}$ and $Hdh^{+/+}$ animals during the final 3 sessions of SD training. Delayed latencies in correct responses to both S1 and S2 stimuli were reported in $Hdh^{Q150/+}$ animals (n = 14) compared to wild-type animals (n = 15), however there were no significant differences between genotypes in response times when collecting food reward. Values shown are means ± S.E.M.

** $p < 0.01$

Seven error terms were investigated and are summarised in Table 5.4. An independent *t*-test identified wild-type animals as performing a significantly greater percentage and proportion of incorrect and perseverant responses during S1 presentation, respectively (Table 5.4; $t(27) = 2.277$, $p = 0.031$ and $t(27) = 2.101$, $p = 0.045$, respectively), than $Hdh^{Q150/+}$ animals. However, during S1 presentation there was no evidence of altered error rates between genotypes in the number of incorrect magazine entries made (Table 5.4; $t(23.396) = 1.679$, $p = 0.106$). During S2 presentation $Hdh^{Q150/+}$ and $Hdh^{+/+}$ mice were found to have made an equivalent percentage of incorrect choices (Table 5.4; $t(27) = 0.917$, $p = 0.367$), and rate of incorrect magazine entries

(Table 5.4; $t(27) = -1.162$, $p = 0.255$) and perseverant responses (Table 5.4; $t(27) = -1.196$, $p = 0.242$). Both genotypes also made a comparable percentage of responses during TOIs (Table 5.4; $t(17.630) = -0.712$, $p = 0.486$).

Error rates of specific error types	*Hdh*$^{+/+}$	*Hdh*$^{Q150/+}$
Incorrect choice in S1[a]	36.18 ± 1.70 *	30.02 ± 2.13
Incorrect choice in S2[b]	7.55 ± 0.59	6.50 ± 1.01
Magazine poke in S1[c]	0.14 ± 0.02	0.11 ± 0.01
Magazine poke in S2[d]	0.13 ± 0.02	0.19 ± 0.04
Perseverant nose poke to S1[c]	0.17 ± 0.02 *	0.11 ± 0.02
Perseverant nose poke to S2[d]	0.05 ± 0.01	0.07 ± 0.01
Hole poke in TOI[e]	15.53 ± 1.80	18.79 ± 4.21

Table 5.4. Error terms of *Hdh*$^{Q150/+}$ and *Hdh*$^{+/+}$ animals during the final 3 sessions of SD training. *Hdh*$^{+/+}$ mice (n = 15) were found to produce a higher number of incorrect and perseverant responses to S1 presentation than their *Hdh*$^{Q150/+}$ (n = 14) counterparts. No other error term showed significant differences between genotypes. Values shown are means ± S.E.M.

* $p < 0.05$

[a] Error rates as a percentage of the total number of S1 trials initiated.

[b] Error rates as a percentage of the total number of S2 trials initiated.

[c] Error rates per S1 trial initiated.

[d] Error rates per S2 trial initiated.

[e] Error rates per incorrect S2 trial.

5.3.2 Molecular results

5.3.2.1 Investigation of candidate gene expression levels by RT-qPCR

A three-way ANOVA was performed on data gathered from the RT-qPCR investigating dorsal and ventral striatal gene expression levels between untrained or SD trained heterozygous HdhQ150 animals and their wild-type littermates, and is summarised in Table 5.5. *Hdh*$^{Q150/+}$ mice presented with lower striatal expression levels for *Adora2A* (main effect of genotype: $F_{1, 32} = 11.559$, $p = 0.002$), *Arc* (main effect of genotype: $F_{1, 32} = 12.576$, $p = 0.001$), *Drd2* (main effect of genotype: $F_{1, 32} = 8.525$, $p = 0.006$) and *Homer1* (main effect of genotype: $F_{1, 32} = 12.981$, $p = 0.001$) compared to their wild-type counterparts while no difference in striatal expression levels was identified between genotypes for *Bdnf* (main effect of genotype: $F_{1, 32} = 0.835$, $p = 0.368$) or *Creb1* (main effect of genotype: $F_{1, 32} = 0.805$, $p = 0.376$). Investigating specific two group

comparisons by independent samples *t*-tests revealed significantly decreased expression levels of *Homer1* in the ventral striatum of untrained $Hdh^{Q150/+}$ mice compared to the mRNA transcripts in the ventral striatum of their untrained wild-type counterparts (Fig. 5.5; $t(8) = -4.462$, $p = 0.012$). All other two group comparisons investigating genotype-dependent changes in gene expression levels failed to report significant differences between genotypes in any gene under investigation (Appendix 1.1-1.5).

Gene of interest	Genotype		Training		Striatal region	
	$F_{1, 32}$ =	p-value	$F_{1, 32}$ =	p-value	$F_{1, 32}$ =	p-value
Adora2A	11.559	0.002**	0.303	0.586	3.445	0.073
Arc	12.576	0.001**	0.051	0.822	6.553	0.015*
Bdnf	0.835	0.368	0.458	0.503	4.403	0.044*
Creb1	0.805	0.376	0.263	0.612	2.737	0.108
Drd2	8.525	0.006**	0.839	0.367	6.358	0.017*
Homer1	12.981	0.001**	0.822	0.371	0.033	0.857

Table 5.5. Statistical values of the three-way ANOVA examining potential differences in striatal gene expression levels as a result of genotype, training and striatal region from which RNA was dissected.

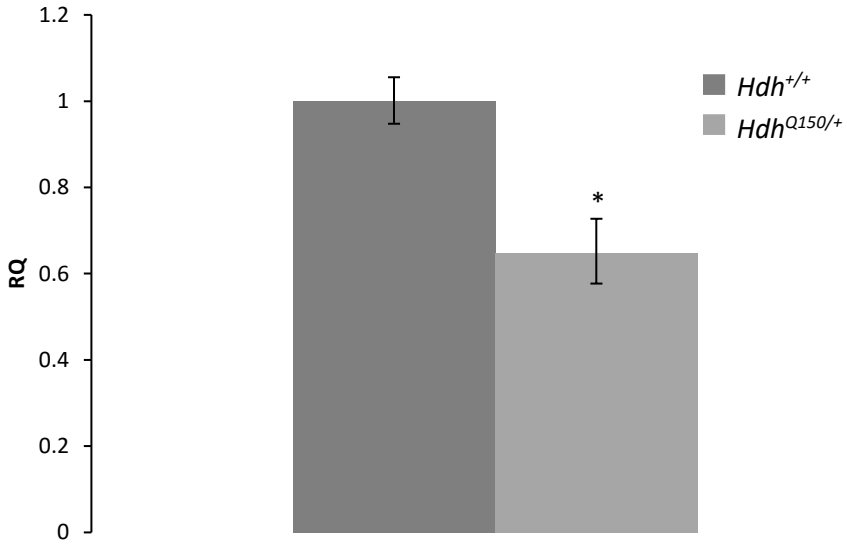


Figure 5.5. RQ values of *Homer1* genotype-dependent expression levels in the ventral striatum of untrained mice. Untrained $Hdh^{Q150/+}$ animals present with significantly decreased levels of *Homer1* transcription levels in the ventral striatum compared to untrained wild-type counterparts. Data shown is mean fold change ± S.E.M.; n = 5/group.

* $p < 0.05$

The level of training (untrained or trained to criteria in SD) undertaken by animals did not present with significant influence on striatal expression levels of any gene investigated in the three-way ANOVA (Table 5.5). Similarly, independent samples *t*-tests examining training-dependent alterations in striatal transcription levels in specific two group comparisons revealed no significant changes in expression levels of any gene in any comparison made (Appendix 1.6-1.10).

Expression levels of *Arc* and *Drd2* were elevated in the dorsal striatum in comparison to the ventral striatum (main effect of striatal region: $F_{1, 32} = 6.553$, $p = 0.015$; and main effect of striatal region: $F_{1, 32} = 6.358$, $p = 0.017$, respectively) whereas the opposite was true for *Bdnf* (main effect of striatal region: $F_{1, 32} = 4.403$, $p = 0.044$). The remaining genes investigated showed no differences in expression levels based on the striatal region investigated (Table 5.5). Examining two group comparisons where the striatal region investigated was the only variable between groups, by independent samples *t*-tests, revealed no significant differences in expression levels of any gene studied (Appendix 1.11-1.15).

A significant interaction between the effects of genotype and training was identified for *Homer1* striatal expression levels (genotype*training: $F_{1, 32} = 8.098$, $p = 0.008$), while no other interaction was found to be significant in any of the genes investigated (Appendix 1.16).

5.4 Discussion

Chapter 4 of this thesis did not identify any learning-dependent gene expression level changes in the striatum of C57BL/6 mice at several distinct stages of SD and RL. As a result of this, the design of the present study was simplified in order to examine the genotype- and learning-dependent gene expression level correlates of SD, without the RL component of Chapter 4, in $Hdh^{Q150/+}$ mice alongside the SD behavioural abilities of this mouse model. Rotarod assessment of heterozygous HdhQ150 animals and their wild-type counterparts revealed heathy motor capabilities in this mouse model of HD at 44-52 weeks of age. In the CRF task, $Hdh^{Q150/+}$ animals presented with impaired acquisition and made significantly fewer correct responses per session compared to wild-type animals. $Hdh^{Q150/+}$ animals also required a greater number of sessions to reach criteria in the SD task than $Hdh^{+/+}$ animals, suggestive of a SD acquisition impairment, but displayed equivalent levels of SD accuracy. RT-qPCR analyses revealed decreased expression levels of *Adora2A*, *Arc*, *Drd2* and *Homer1* in the striatum of $Hdh^{Q150/+}$ mice when compared to wild-type animals, which is consistent with previous investigations into gene expression level changes in HD (Table 5.1). SD task performance was found to have no effect on the expression

levels of any of the genes under investigation in the current study, in either genotype, supporting the findings of Chapter 4. mRNA levels of *Arc* and *Drd2* were found to be increased in the dorsal striatum in comparison to the ventral striatum, while expression levels of *Bdnf* were decreased in the dorsal striatum. However, dorsal and ventral striatal expression levels did not differ in any of the specific two-group comparisons made in the current study, which rejects the hypothesis proposed in Chapter 4.4; that the lack of gene expression level changes between behavioural groups was the result of a lack of specificity of striatal tissue dissection.

5.4.1 Effects of Huntington's disease genotype on behavioural performance

The rotarod assessment of the motor ability of animals reported no significant differences in the mean fall latency between genotypes. This indicates that $Hdh^{Q150/+}$ mice present with normal motor capabilities at 44-52 weeks of age, which is supported by previous evidence of equivalent motor performance in $Hdh^{Q150/+}$ and wild-type animals up to 100 weeks of age (Heng et al., 2007). This evidence suggests that any behavioural abnormalities that may arise in the $Hdh^{Q150/+}$ mice in the current study are not the result of impaired motor capabilities, as these animals presented with healthy motor capabilities prior to operant testing and a previous study has reported continuation of these normal motor abilities past the time point in the mouse lifetime at which operant testing was performed in the current study (Heng et al., 2007).

In the CRF task, $Hdh^{Q150/+}$ animals required a significantly greater number of sessions to reach criteria than their wild-type counterparts, and also made significantly fewer correct responses per session during the final 3 sessions until criteria was reached and across all CRF sessions than $Hdh^{+/+}$ mice. These findings suggest that heterozygous HdhQ150 mice present with delayed acquisition and impaired performance of a continuously reinforced response from 44-52 weeks of age, and is the first report of such a deficit in the $Hdh^{Q150/+}$ mouse model of HD, to my current knowledge. This delayed acquisition and impaired performance in the CRF task may be indicative of an impairment in cognitive abilities or potentially a motivational deficit. CRF task performance impairments were not identified in $Hdh^{Q150/+}$ animals at 26 weeks of age (see Chapter 3), which indicates that onset of this deficit occurs between 26 and 44-52 weeks of age. In order to ascertain whether these CRF task performance impairments are attributable to a motivational deficit, which can be compared to the apathy that occurs in HD (Soliveri et al., 2002; Roos, 2010; Tabrizi et al., 2013), it may be worthwhile for future studies to examine performance in the progressive ratio task. The progressive ratio task is an operant behavioural test that is frequently used to examine motivational states of animals by measuring the breakpoint at which animals stop responding in order to receive a reward (Hodos, 1961; Richardson and Roberts, 1996). Thus,

the progressive ratio task may be able to provide further insight into the potential motivational deficit identified in the *Hdh*$^{Q150/+}$ mouse model of HD, which is suggested by the impaired CRF task performance. Another possible cause of the delayed acquisition and impaired performance of the CRF task by *Hdh*$^{Q150/+}$ animals could be that this genotype display reduced levels of palatability to the food reward, thus decreasing their motivation to obtain the reward. In order to assess this theory, it may be valuable for future studies to examine lick clustering of *Hdh*$^{Q150/+}$ and *Hdh*$^{+/+}$ animals when in the presence of the food reward used in the current study (Yazoo® strawberry flavoured milkshake). Changes in the size of lick clusters, based on prescribed intra-lick intervals, have been shown to be sensitive to the consumption of more or less palatable solutions (Hsiao and Fan, 1993), suggesting that lick cluster size is the main determinant of palatability and can therefore provide an index related to hedonic components of behaviour (Davis and Smith, 1992). Thus, performing lick cluster analyses on *Hdh*$^{Q150/+}$ mice may reveal whether their abnormal CRF task acquisition and performance in the current study is the result of a decreased hedonic response to the food reward, which could result in the motivational deficit theorised earlier.

Using the mean number of sessions to criteria in the SD task as a measure of task performance identified a deficit in SD capabilities in *Hdh*$^{Q150/+}$ animals in comparison to their wild-type counterparts, as shown by *Hdh*$^{Q150/+}$ mice requiring a significantly greater number of sessions to reach criteria in the task. When examining accuracy levels between genotypes, there were no significant differences in mean accuracy across the final three SD test sessions or across all SD sessions until criteria was met between *Hdh*$^{Q150/+}$ and *Hdh*$^{+/+}$ animals. Thus, these data suggest that *Hdh*$^{Q150/+}$ mice at 46-60 weeks of age exhibit delayed acquisition of a SD task, yet they can achieve SD task performance at the level seen in wild-type animals. Homozygous HdhQ150 animals have previously been shown to present with spatial learning impairments identified using the Morris water maze, with deficits arising from 16 weeks of age (Brooks et al., 2012b). The finding of impaired spatial learning acquisition in *Hdh*$^{Q150/Q150}$ animals by Brooks and colleagues (2012b), shown by the knock-in mice presenting with progressively slower improvement in response latencies as Morris water maze trial numbers increase when compared to wild-type littermates, is comparable to the deficit in SD acquisition seen in the current study. The delayed acquisition of the SD task seen in *Hdh*$^{Q150/+}$ mice in the current study can also be argued to be comparable to the impaired initial spatial location learning previously identified in HD patients (Brandt et al., 2005; Pirogovsky et al., 2015). Conversely, the ability of *Hdh*$^{Q150/+}$ animals to perform the SD task, once acquired, at a level comparable to their wild-type littermates is not representative of the impaired spatial memory capabilities that have been seen in certain HD patient reports (Lange et al., 1995; Lawrence et al., 2000; Davis et al., 2003).

It is possible that the striatal neurodegeneration that occurs in the *Hdh*$^{Q150/+}$ mouse model of HD may not be substantial enough at the time point examined to adversely affect long term spatial memory but is capable of disrupting acquisition of such memory. This interpretation of the data is supported by neuroanatomical evidence of the presence of reactive gliosis, and ubiquitin- and Htt-positive nuclear inclusions throughout the striatum of *Hdh*$^{Q150/+}$ mice from 40 weeks of age, in the absence of striatal volume and neuronal cell loss, which occurs from approximately 100 weeks of age (Lin et al., 2001; Tallaksen-Greene et al., 2005; Heng et al., 2007).

Analysis of response latencies during the final 3 sessions of the SD task revealed further evidence of potentially decreased cognitive capabilities for SD in heterozygous HdhQ150 animals. *Hdh*$^{Q150/+}$ mice took significantly longer to correctly respond to both S1 and S2, while retrieving food rewards at comparable response latencies as *Hdh*$^{+/+}$ animals. The response latencies data strengthens the suggestion that the behavioural abnormalities observed in the *Hdh*$^{Q150/+}$ mice are a result of compromised cognitive processing, and not a result of potential motor ability deterioration as time progressed. This is suggested because one would expect the food reward latencies to also be delayed, comparably to the correct S1 and S2 delayed latencies, if impaired motor ability were the cause of the behavioural abnormalities. Therefore, because this is not the case, it is likely that the delayed S1 and S2 response latencies are an indication of slowed cognitive processing, or increased indecision, in *Hdh*$^{Q150/+}$ animals.

Examining the number of trials performed by animals in the SD task allows a comparison to be made between the levels of motivation exhibited by genotypes. *Hdh*$^{+/+}$ animals initiated a significantly greater mean number of S2 trials in the SD task, both over the final 3 SD sessions and across all SD sessions, than *Hdh*$^{Q150/+}$ animals. These data, taken with the CRF data described earlier, suggest that *Hdh*$^{Q150/+}$ mice may present with a motivational deficit at 46-60 weeks of age when compared to *Hdh*$^{+/+}$ mice of an equivalent age. This is the first report of a potential motivation abnormality in this mouse model of HD, to our current knowledge. As discussed earlier, however, detailed investigation into the hedonic responses and motivational drive of the *Hdh*$^{Q150/+}$ animals, potentially through use of lick cluster analyses and the progressive ratio task, respectively, would be required to confidently conclude whether a motivational abnormality is present in this mouse model of HD.

Hdh$^{+/+}$ mice were found to produce a higher number of perseverant and incorrect (np in hole 3 or hole 7) responses to S1 presentation than their *Hdh*$^{Q150/+}$ counterparts. These errors are often seen as a result of subjects being overzealous in their pursuit of a food reward, causing mice to either inadvertently np the S1 an excessive number of times as a means to ensure S2 occurrence, in the case of heightened perseverance, or to not np deeply enough into the S1 hole before

rushing to np S2, in the case of heightened incorrect S1 responses. Performing fewer of these perseverative and incorrect S1 responses suggests that *Hdh*$^{Q150/+}$ animals may have impaired cognition in comparison to their wild-type counterparts. The finding of decreased perseverance in the *Hdh*$^{Q150/+}$ mice is somewhat surprising, as HD patients have shown impaired performance of RL tasks as a result of heightened perseverance to the previously learnt rule (Massman et al., 1990; Lange et al., 1995; Lawrence et al., 1996; Lawrence et al., 1999). However, levels of perseveration to S1 in the SILT were comparable in wild-type and *Hdh*$^{Q150/+}$ mice in Chapter 3 of this thesis (see Chapter 3.3.1 and Table 3.4). The discrepancies between the perseveration levels of *Hdh*$^{Q150/+}$ animals in the current study and Chapter 3 may be the result of the different methodologies, or behavioural tasks, used in the two studies. It is also possible that the differences in ages of the mice may be the cause of the different findings of the two studies, with mice being 6M in Chapter 3 and 10M-14M of age in the current study. This suggests that decreased perseveration is a behavioural phenotype in *Hdh*$^{Q150/+}$ animals at 10M-14M, and that this phenotype arises sometime after 6M.

5.4.2 The effects of Huntington's disease genotype on striatal gene expression levels

A three-way ANOVA reported decreased levels of *Adora2A*, *Arc*, *Drd2* and *Homer1* mRNA transcripts in the striatum of *Hdh*$^{Q150/+}$ mice, while *Bdnf* and *Creb1* presented with equivalent levels of striatal expression as those seen in wild-type animals. *Adora2A* transcribes a G-protein-coupled receptor with preferential neural expression within the striatum, with particular localisation on the striatopallidal MSNs vulnerable to degeneration in HD (Schiffmann et al., 1991a; Schiffmann et al., 1991b). Levels of A_{2A}Rs decrease during HD (Martinezmir et al., 1991; Glass et al., 2000), although it is not known whether the decrease in A_{2A}R levels causes neurodegeneration in HD or is a side effect of such degeneration. Associations between *Adora2A* and the HD phenotype have also been identified in the R6 mouse models of HD, where protein levels of A_{2A}Rs are decreased in R6/1 animals from 12 weeks of age (Villar-Menendez et al., 2013) and R6/2 mice present with decreased *Adora2A* mRNA levels from postnatal day 21 (Tarditi et al., 2006). One study has reported decreases in striatal *Adora2A* expression in the R6/1, R6/2, *Hdh*$^{Q92/Q92}$ and *Hdh*$^{Q150/Q150}$ mouse models of HD, alongside similar reductions in *ADORA2A* in HD patients' striata (Kuhn et al., 2007). Similarly, the current study reports that *Hdh*$^{Q150/+}$ mice display significantly reduced levels of *Adora2A* expression within the striatum from 46-60 weeks of age. Expression of *Adora2A* has previously been associated with cognition (Wei et al., 2011), and the reduction in striatal *Adora2A* mRNA levels identified in the current study coincided with impaired performance in the SD task. This suggests that the decreased expression levels of

Adora2A may contribute to the cognitive impairment identified in *Hdh*$^{Q150/+}$ mice, however, specific comparisons examining *Adora2A* expression in the striatum of SD trained *Hdh*$^{Q150/+}$ and *Hdh*$^{+/+}$ animals reported no significant differences between genotypes. Recent studies present evidence that $A_{2A}R$ inactivation may actually be beneficial in cognition in HD; knockout of $A_{2A}Rs$ has been found to prevent working memory deficits in R6/2 animals (Li et al., 2015) while antagonism of $A_{2A}Rs$, in combination with DRD1 antagonism, improves cognitive ability in R6/1 mice (Tyebji et al., 2015). This indicates that despite displaying decreased *Adora2A* expression (Kuhn et al., 2007), $A_{2A}R$ hyperactivity may lead to cognitive dysfunction in HD mice. Nevertheless, the present study supports evidence from both human and rodent studies of HD that reduced expression levels of *Adora2A* is associated with HD pathogenesis.

Another candidate gene in the current study that has previously been associated with the HD phenotype is *Arc*. Hippocampal *Arc* mRNA levels have been found to be significantly lower in *Hdh*$^{Q111/+}$ mice than in wild-type animals following Morris water maze training (Giralt et al., 2012), suggesting that abnormal *Arc* expression plays a role in cognitive dysfunction in HD. This was not found to be the case in the current study, as *Arc* expression levels did not differ between *Hdh*$^{Q150/+}$ and *Hdh*$^{+/+}$ animals that had undergone SD training. Instead, *Arc* mRNA transcripts were found to be significantly reduced in *Hdh*$^{Q150/+}$ animals using a three-way ANOVA, suggesting that the HD phenotype produces a reduction in *Arc* gene expression levels from 46-60 weeks of age in the *Hdh*$^{Q150/+}$ mouse model of HD. To our current knowledge, this is the first evidence of *Arc* being associated with the phenotype of the HdhQ150 mouse model of HD.

Levels of *Drd2* mRNA were identified as being significantly reduced in the striatum of *Hdh*$^{Q150/+}$ mice when compared with *Hdh*$^{+/+}$ animals. DRD2s have been strongly implicated in the pathogenesis of HD, with numerous reports of progressive loss of striatal DRD2s and DRD2 binding in asymptomatic carriers (Antonini et al., 1996; Weeks et al., 1996; Andrews et al., 1999) and symptomatic patients (Ginovart et al., 1997; Glass et al., 2000; Pavese et al., 2003). Striatal dopaminergic neurotransmission markers, including DRD2 binding markers, have also been found to be predictive of cognitive deficits in HD patients (Backman et al., 1997; Lawrence et al., 1998a). Progressive decreases in striatal *DRD2* mRNA expression have been reported in post-mortem HD brains with increasing pathology (Augood et al., 1997), while primary neuron models of the disease have also exhibited such decreases (Runne et al., 2008). Abnormal dopaminergic gene expression levels have also been shown to translate from human cases of HD to mouse models of the disease, with evidence of reduced *Drd2* expression levels in the striatum of R6/1, R6/2, *Hdh*$^{Q92/Q92}$ and *Hdh*$^{Q150/Q150}$ animals (Kuhn et al., 2007). Similar decreases in striatal *Drd2* levels have also been identified in YAC128 mice, but were not apparent in BACHD animals (Pouladi et al., 2012). The present study supports the evidence that a reduction in dopaminergic

neurotransmission expression levels occurs in HD pathology, by providing evidence of decreased *Drd2* expression levels in the *Hdh*$^{Q150/+}$ mouse model of HD from 44-60 weeks of age.

Homer1 is a gene that produces proteins associated with a number of roles, such as regulating dendritic and axonal targeting of a metabotropic glutamate receptor (Ango et al., 2000) and addiction to cocaine (Ghasemzadeh et al., 2003; Szumlinski et al., 2004; Ary et al., 2013), and has also been implicated in spatial learning and memory (Jaubert et al., 2007; Gerstein et al., 2012; Gerstein et al., 2013). The present study reports decreased expression levels of *Homer1* in the striatum of the *Hdh*$^{Q150/+}$ mouse model of HD from 46-60 weeks of age, which suggests that abnormal *Homer1* signalling may contribute to HD phenotype. This theory is supported by previous findings of a reduction in *Homer1* mRNA transcripts in the *R6/1*, *R6/2*, *Hdh*$^{Q92/Q92}$ and *Hdh*$^{Q150/Q150}$ mouse models of HD, as well as a decrease in *HOMER1* expression levels in post-mortem striatal tissue of HD patients (Hodges et al., 2006; Kuhn et al., 2007). To further elucidate the role of *Homer1* signalling in HD, it may be of interest for future studies to examine gene expression or protein levels of the three specific *Homer1* isoforms (*Homer1a*, *Homer1b* and *Homer1c*) individually, as opposed to solely examining the gene expression levels of *Homer1* that occurred in the current study.

Neither *Bdnf* nor *Creb1* displayed differing levels of gene expression between *Hdh*$^{Q150/+}$ and *Hdh*$^{+/+}$ animals. This finding in BDNF signalling is somewhat surprising because *Bdnf* has been strongly associated with the HD phenotype. For example, wild-type HTT is known to up-regulate *BDNF* transcription by inhibiting the silencing activity of the neuron restrictive silencer element (Zuccato et al., 2003) and this up-regulatory mechanism is lost in the presence of mHTT, causing decreased production of cortical BDNF (Zuccato et al., 2001). Wild-type HTT has also been found to enhance transport of BDNF along microtubules and that this transport activity is lost in HD, resulting in loss of neurotrophic support and neuronal toxicity (Gauthier et al., 2004). Furthermore, reductions in BDNF mRNA and protein levels have been identified in the serum and cortex of human HD patients (Ciammola et al., 2007; Zuccato et al., 2008), which also strengthens the evidence that abnormal BDNF signalling is apparent in HD. A number of mouse models of HD also present with decreased BDNF levels, with overexpression or upregulation of BDNF found to ameliorate the disease phenotype in HdhQ92 and HdhQ111 (Lynch et al., 2007), HdhQ140 (Simmons et al., 2009), R6/1 (Pang et al., 2006; Gharami et al., 2008), R6/2 (Zuccato et al., 2005; Giralt et al., 2011a) and YAC128 mice (Xie et al., 2010). Based on the body of evidence presented here, one would have anticipated that *Bdnf* mRNA levels would have been decreased in the striatum of *Hdh*$^{Q150/+}$ animals but this was not found to be the case. A number of the studies mentioned here also report decreased cortical levels of *Bdnf* mRNA or protein (Zuccato et al., 2005; Gharami et al., 2008), thus examining *Bdnf* transcription or protein levels

in the PFC tissue taken from subjects in the present study may reveal similar decreases in BDNF signalling in *Hdh*$^{Q150/+}$ animals. It is also entirely possible that abnormalities in BDNF signalling may appear later in the *Hdh*$^{Q150/+}$ mouse lifespan than examined in the current study, therefore examining mRNA and protein levels of BDNF in older animals may be of interest.

As briefly highlighted, *Creb1* expression levels did not show significant variation between *Hdh*$^{Q150/+}$ mice and their wild-type counterparts. CREB1 is a transcription factor that activates transcription through recruitment of CREB binding protein (Guan et al., 2002), a protein that augments the activity of phosphorylated CREB as a means of activating transcription of cAMP-responsive genes (Chrivia et al., 1993; Kwok et al., 1994). There has only been one report of abnormal *Creb1* expression or activity in HD, to our current knowledge, which found abnormal *Creb1* activity in striatal cells derived from *Hdh*$^{Q111/Q111}$ mice (Benn et al., 2008b). Thus, the current finding of equivalent *Creb1* expression levels in *Hdh*$^{Q150/+}$ and *Hdh*$^{+/+}$ animals is not unexpected.

To summarise, the present study identified reductions in the striatal expression levels of *Adora2a*, *Arc*, *Drd2* and *Homer1* in the *Hdh*$^{Q150/+}$ mouse model of HD, which supports a large body of evidence linking these genes to HD pathogenesis (see Table 5.1). BDNF has also been strongly implicated in HD pathology by previous reports (Table 5.1), yet there were no significant differences in striatal BDNF mRNA levels between *Hdh*$^{Q150/+}$ and *Hdh*$^{+/+}$ animals in the present study. A similar equivalence in striatal expression levels between genotypes was identified for *Creb1*.

5.4.3 Effects of SD training on gene expression levels

As discussed throughout Chapter 4.4, the genes under investigation in the current study have strong associations with cognition, where decreased expression levels of *Arc* (Daberkow et al., 2007; Kelly et al., 2008; Pastuzyn et al., 2012), *Bdnf* (Mizuno et al., 2000; Sakata et al., 2013), *Creb1* (Brightwell et al., 2004; Porte et al., 2008), *Drd2* (Kruzich et al., 2006; Desteno and Schmauss, 2009; Jocham et al., 2009) and *Homer1* (Jaubert et al., 2007; Gerstein et al., 2012; Gerstein et al., 2013) have all previously been associated with impaired cognitive capabilities. Conversely, inactivation of *Adora2A* has previously been found to enhance the working memory and RL abilities of wild-type mice (Wei et al., 2011) as well as a number of cognitive abilities in the R6/1 and R6/2 mouse models of HD (Li et al., 2015; Tyebji et al., 2015). Based upon these previous reports, it was originally hypothesised in Chapter 4 that mice performing different levels of the SD and RL tasks would present with elevated levels of *Arc*, *Bdnf*, *Creb1*, *Drd2* and

Homer1 expression levels while showing decreased levels of *Adora2A* mRNA transcripts. This hypothesis was rejected in Chapter 4.4, however, with no evidence of changes in striatal expression levels of any of the genes under investigation at any stage of SD or RL training (see Chapter 4.3 and 4.4). The results of the current study support the findings of Chapter 4, with a three-way ANOVA reporting equivalent expression levels for all genes under investigation between untrained and SD trained animals. The present study strengthens the conclusions from Chapter 4 that striatal expression levels of *Adora2A*, *Arc*, *Bdnf*, *Creb1*, *Drd2* and *Homer1* do not alter as a result of SD performance. It is possible that changes in expression levels of the genes examined may occur in the hippocampus, instead of the striatum, after SD task performance, as spatial learning is known to be hippocampus-dependent (Silva et al., 1992b; Silva et al., 1992a; Bliss and Collingridge, 1993; McHugh et al., 1996; Tsien et al., 1996; Burgess et al., 2002). This theory is also supported by evidence of altered hippocampal expression of a number of genes affecting, or resulting from, spatial learning (Aiba et al., 1994a; Guzowski et al., 2000; Mizuno et al., 2000; Guzowski et al., 2001; Vazdarjanova et al., 2002; Wood et al., 2005). Thus, investigating hippocampal gene expression levels of animals in the present study may reveal SD dependent changes in mRNA transcript levels.

Based upon the findings of Chapter 4, where expression levels of the genes under investigation did not differ depending on the level of cognitive training performed, it was hypothesised that if any cognitive impairments were to be identified in $Hdh^{Q150/+}$ animals in the current study, the levels of mRNA transcripts for the genes under investigation would not differ between SD trained animals of either genotype. Cognitive impairments were identified in $Hdh^{Q150/+}$ animals, and specific two group comparisons examining striatal expression levels between SD trained $Hdh^{Q150/+}$ and $Hdh^{+/+}$ animals proved this hypothesis correct, with all genes showing equivalent levels of expression between SD trained mice of either genotype. However, a three-way ANOVA did report a significant interaction between the effects of genotype and training for *Homer1* striatal expression levels.

5.4.4 Gene expression levels in the dorsal and ventral striatum

Microdissecting the striatum into its dorsal and ventral regions, and performing subsequent RT-qPCR analyses on these specific regions, identified region specific differences in expression levels of *Arc*, *Bdnf*, and *Drd2*. Specifically, the dorsal striatum presented with increased levels of *Arc* and *Drd2* expression alongside decreased *Bdnf* expression levels, in comparison to ventral striatum tissue. In order to address the altered mRNA levels of *Drd2* identified between the ventral and dorsal striatum, it is necessary to examine striatal neuronal populations and

organisation. MSNs use GABA as a neurotransmitter (Kita and Kitai, 1988) and comprise approximately 90-95% of striatal neurons (Gerfen, 1992a, b). Two key subpopulations of MSNs have been defined based upon their projection targets; whether they project to the globus pallidus or the substantia nigra. It is known that the majority of striatopallidal neurons, the MSNs that project from the striatum to the globus pallidus (Kawaguchi et al., 1990), express enkephalin and DRD2 (Gerfen and Young, 1988; Gerfen et al., 1990; Lemoine et al., 1990; Lemoine and Bloch, 1995) while the majority of striatonigral neurons, the MSNs that project from the striatum to the substantia nigra (Kawaguchi et al., 1990) express substance P, dynorphin and DRD1 (Gerfen and Young, 1988; Gerfen et al., 1990; Lemoine and Bloch, 1995). It is believed that, across the majority of the striatum, there are approximately equal numbers of striatopallidal and striatonigral neurons and that the distribution of these neurons are intermixed throughout the striatum (Gerfen, 1992a, b). Based upon the general distribution of *Drd2* expressing striatopallidal MSNs throughout the striatum, it was anticipated that *Drd2* expression levels would not differ between the ventral and dorsal striatum. This was not the case in the current study, as the dorsal striatum presented with increased *Drd2* expression levels in comparison to the ventral striatum. This finding of striatal region-specific *Drd2* expression levels is supported by reports of DRD2 membrane expression being higher in the dorsolateral striatum than the dorsomedial striatum (Yin et al., 2009) and anatomical segregation of striatonigral and striatopallidal MSNs in the caudal region of the dorsal striatum (Gangarossa et al., 2013). While the concept of striatal region-specific *Drd2* expression level differences of the current study are supported by these reports, Gangarossa and colleagues (2013) identified a caudal region of the dorsal striatum that lacks DRD2 striatopallidal neurons. This would suggest that *Drd2* expression levels should be decreased in the dorsal striatum of the current study, but the opposite was found to be true. The different methods of investigation used in the two studies may contribute to the discrepancies of the current study and that of Gangarossa *et al.* (2013). The present study examined mRNA levels of genes of interest using RT-qPCR while Gangarossa et al. (2013) used immunofluorescence to determine protein levels and 3D reconstruction modelling of tissue slides to determine neuronal location. Thus, the different methods used to examine different molecular elements is likely to contribute to the apparent discrepancies in findings of the two studies.

The differences in *Bdnf* and *Arc* transcript levels between the dorsal and ventral regions of the striatum reported in the current study were unanticipated. This was unexpected for *Bdnf* expression because although BDNF is neurotrophic for dopaminergic neurons of the substantia nigra (Hyman et al., 1991), striatal *Bdnf* mRNA expression levels have previously been found to be very low (Hofer et al., 1990; Xie et al., 2010) because BDNF is generated in the cortex and

transported to the striatum through axonal anterograde transport in frontostriatal neurons (Altar et al., 1997). Based upon this evidence, one would expect *Bdnf* mRNA levels to be low within the striatum and also of equivalent levels within the dorsal and ventral striatum, as *Bdnf* is highly expressed in the frontal cortex and its protein transported to, and dispersed throughout, the striatum. Similarly, *Arc* induction is known to occur through N-methyl-D-aspartate (NMDA)-receptor activation, where *Arc* is transported to activated synapses and locally translated (Lyford et al., 1995; Steward et al., 1998; Bramham et al., 2008). Induction of *Arc* expression occurs within the MSNs of the striatum (Vazdarjanova et al., 2006) and in an experience-dependent manner (Guzowski et al., 1999; Vazdarjanova et al., 2002; Daberkow et al., 2007). From these studies, one would anticipate that *Arc* mRNA levels would be equivalent in the dorsal and ventral regions of the striatum in the current study because the MSNs capable of inducing *Arc* expression are believed to be evenly distributed throughout the striatum (Gerfen, 1992a, b). Based upon the evidence highlighted here, the differences reported in *Bdnf* and *Arc* expression levels between the dorsal and ventral striatum in the present study may be the result of previously unidentified differences in striatal regional expression of these genes.

Examining transcription levels in the dorsal and ventral striatum separately did not identify learning-dependent gene expression level changes in any of the genes investigated in the current study, as examined using a three-way ANOVA or independent *t*-tests on specific two-group comparisons. This acts to disprove the hypothesis presented in Chapter 4.4, that microdissection of the striatum and examination of gene expression levels in the dorsal and ventral striatum may yield cognitive based changes in mRNA levels of the genes investigated. This further suggests that striatal expression levels of *Adora2A*, *Arc*, *Bdnf*, *Creb1*, *Drd2* and *Homer1* are not altered as a result of SD performance, and play no role in SD performance capabilities. However, it is possible that alterations in transcription levels of these genes may arise at the level of individual cells, as examined using fluorescent in-situ hybridisation, following SD performance, and this was not investigated in the current study.

5.4.5 Conclusions

Male $Hdh^{Q150/+}$ mice presented with impaired acquisition and performance of the CRF task alongside a deficit in the acquisition of a SD task, in the absence of motor deficits on the Rotarod, at 44-60 weeks of age. Cognitive dysfunction in the SD task did not coincide with learning-dependent changes in the striatal mRNA levels of *Adora2A*, *Arc*, *Bdnf*, *Creb1*, *Drd2* or *Homer1*, as assessed by RT-qPCR. This is in support of the findings of Chapter 4, which reported equivalent striatal expression levels across a number of stages of SD and RL in healthy C57BL/6 mice for all

of the genes under investigation. In the present study, *Hdh*$^{Q150/+}$ mice presented with significantly decreased striatal expression levels of *Adora2A*, *Arc*, *Drd2* and *Homer1* in comparison to wild-type animals. This is in support of previous evidence linking a reduction in the expression of these genes with HD pathogenesis (Table 5.1). Conversely, *Bdnf* and *Creb1* were both found to present with comparable levels of expression in the striatum of *Hdh*$^{Q150/+}$ and *Hdh*$^{+/+}$ animals. This equivalence in *Bdnf* expression levels was unexpected because reduced BDNF signalling has previously been implicated as a potential mechanism of HD pathology (Table 5.1), although evidence that BDNF is transported from the frontal cortex to the striatum (Altar et al., 1997) suggests that it may be worthwhile to examine mRNA levels of *Bdnf* in the PFC tissue extracted from subjects in the current study. Differences between dorsal and ventral striatum expression levels of *Arc*, *Bdnf* and *Drd2* were also identified in the current study.

5.4.6 Summary of key results from Chapter 5

- At 44-60 weeks of age, *Hdh*$^{Q150/+}$ mice demonstrated impaired acquisition and performance of the CRF task, and impaired acquisition of the SD task.
- RT-qPCR investigation revealed significantly decreased striatal expression levels of *Adora2A*, *Arc*, *Drd2* and *Homer1* in *Hdh*$^{Q150/+}$ mice at 44-60 weeks of age, while *Bdnf* and *Creb1* expression levels were equivalent between *Hdh*$^{Q150/+}$ and *Hdh*$^{+/+}$ animals.
- As found in Chapter 4, RT-qPCR investigation revealed no significant variation in striatal expression of *Adora2A*, *Arc*, *Bdnf*, *Creb1*, *Drd2* or *Homer1* as a result of SD training.
- RT-qPCR analyses revealed that the dorsal striatum of animals presented with heightened *Arc* and *Drd2* mRNA levels in comparison to the ventral striatum, while *Bdnf* expression levels were decreased in the dorsal striatum.

Chapter 6: General discussion

HD is a neurodegenerative disorder that presents with an array of symptoms that fall into three broad categories: motor, cognitive, and psychiatric impairments. The discovery that a single genetic mutation is responsible for disease manifestation, and identification of the gene in question, *HTT*, has led to the generation of multiple cellular and rodent models of the condition. Transcriptional dysregulation has been identified as a pathogenic mechanism in HD (Boutell et al., 1999; Luthi-Carter et al., 2000; Zuccato et al., 2001; Kegel et al., 2002; Sipione et al., 2002; Hodges et al., 2006; Kuhn et al., 2007; Zuccato et al., 2007; Johnson et al., 2008; Johnson et al., 2010), and has been associated with cognitive impairments in both human HD patients (Augood et al., 1997; Zuccato et al., 2001; Cui et al., 2006; Hodges et al., 2006; Zuccato et al., 2008; Chaturvedi et al., 2012; Jeong et al., 2012) and mouse models of the disease (Morton et al., 2005; Hodges et al., 2008; Giralt et al., 2009; Giles et al., 2012; Giralt et al., 2012; Li et al., 2015). Based on this evidence, the primary aim of this thesis was to investigate the cognitive capabilities and striatal transcriptional profile of the heterozygous HdhQ150 mouse model of HD, a model that has undergone cellular and behavioural characterisation but has not received extensive cognitive evaluation (Table 1.2). A summary and discussion of the findings are presented here, followed by potential future directions based upon the current work.

6.1 Summary and discussion of main findings

6.1.1 Cognitive impairment in $Hdh^{Q150/+}$ mice

6.1.1.1 Early-onset visuospatial attention deficits in the 5-CSRTT

Novel impairments in visual attention and delayed acquisition of the 5-CSRTT were identified in heterozygous HdhQ150 animals at 13 weeks of age (Chapter 3). Similar visuospatial attention deficits have been reported in HD patients (Lawrence et al., 2000; Bachoud-Levi et al., 2001; Lemiere et al., 2004; Fielding et al., 2006c; Fielding et al., 2006b; Fielding et al., 2006a) and HdhQ92 mice (Trueman et al., 2009; Trueman et al., 2012a), which supports the validity of the cognitive impairment identified in the current study and suggests that this phenotype may be recapitulated in other knock-in mouse models of the disease. This cognitive impairment arises relatively early in the life of $Hdh^{Q150/+}$ animals, which is somewhat surprising because cognitive deficits have not previously been identified in the heterozygote genotype and those reported in homozygote animals manifested at a later timeframe of 17-26 weeks (Brooks et al., 2012b). A number of studies have identified apparent gene dosage effects within behavioural and neuropathological phenotypes in the HdhQ150 mouse model, where symptoms typically arise

earlier in homozygote animals compared to their heterozygote counterparts (Lin et al., 2001; Tallaksen-Greene et al., 2005; Heng et al., 2007). Thus, because of this apparent gene dosage effect and the early onset of spatial and RL deficits in homozygote HdhQ150 animals (Brooks et al., 2012b), the visuospatial attention deficit identified in *Hdh*$^{Q150/+}$ mice at 13 weeks of age in the current work was not expected this early in the lifespan. However, visuospatial attention in the 5-CSRTT has not previously been examined in homozygote HdhQ150 animals, to my current knowledge, and it is possible that deficits similar to those seen in the current study may arise earlier in *Hdh*$^{Q150/Q150}$ mice than in *Hdh*$^{Q150/+}$ counterparts. The visuospatial attention impairment identified at 13 weeks of age in the current work is unlikely to be caused by increased neuropathology in the *Hdh*$^{Q150/+}$ mice because visuospatial attention has been identified to be a neocortical process (Nobre et al., 1997; Casey et al., 2000; Beauchamp et al., 2001; Umarova et al., 2010; Kravitz et al., 2011; Bartolomeo et al., 2012) and *Hdh*$^{Q150/+}$ animals do not present with cortical pathology until 50-52 weeks of age, which is in the form of NII formation (Lin et al., 2001; Tallaksen-Greene et al., 2005). Due to the frontostriatal nature of this cognitive process, it is possible that the visuospatial attention deficits identified are a result of abnormal gene expression level changes in the *Hdh*$^{Q150/+}$ cortex. However, the experimental design of the current work does not allow further investigation of this hypothesis, as brains were removed for analyses following CRF or SILT training, but not after 5-CSRTT performance.

Although the cognitive deficit in question has previously been identified in HD patients (Lawrence et al., 2000; Bachoud-Levi et al., 2001; Lemiere et al., 2004; Fielding et al., 2006c; Fielding et al., 2006b; Fielding et al., 2006a) and another mouse model of the disease (Trueman et al., 2009; Trueman et al., 2012a), it is possible that the impairment observed in the current work is not a true reflection of the visuospatial attention ability of the *Hdh*$^{Q150/+}$ or *Hdh*$^{+/+}$ mouse populations due to the low number of animals that performed the behavioural tasks in Chapter 3 (*Hdh*$^{+/+}$: n = 3; *Hdh*$^{Q150/+}$: n = 5). Therefore, re-examination of heterozygous HdhQ150 performance in the 5-CSRTT using a greater number of behavioural subjects would be recommended in order to replicate and validate the findings of the current work. Another limitation of the findings from Chapter 3 is that female mice were utilised in behavioural tasks but were not examined for stages of oestrous cycle throughout testing, or upon the day of sacrifice for molecular analyses. This is important to note because the stage of a female mouse's oestrous cycle is able to significantly affect behaviour (Walf et al., 2009; ter Horst et al., 2013; Martini et al., 2014; Dey et al., 2015) and neural gene expression regulation (Puri et al., 2006; Sica et al., 2009; Cushman et al., 2014). Thus, it is possible that mice examined in the present study may have been at different stages of oestrous during 5-CSRTT performance and at time of sacrifice, which could have impacted 5-CSRTT performance and gene expression level profiles.

As a result of this potential limitation, male mice were used for subsequent experiments because there would be no potential impact of oestrous on behavioural and molecular analyses.

6.1.1.2 Healthy implicit learning capacity in the SILT at 14-17 weeks of age

A deficit in implicit learning was not identified in *Hdh*$^{Q150/+}$ animals at 14-17 weeks of age in the current study. This aspect of cognition has been shown to activate a variety of cortical and subcortical structures within the brain, including the striatum (Rauch et al., 1997; Honda et al., 1998; Schendan et al., 2003; Aizenstein et al., 2004; Destrebecqz et al., 2005; Reiss et al., 2005; Gheysen et al., 2010; Bennett et al., 2011; Gheysen et al., 2011; Schendan et al., 2013), and displays evidence of dysfunction in HD (Heindel et al., 1989; Knopman and Nissen, 1991; Gabrieli et al., 1997; Kim et al., 2004). As discussed with regards to the visuospatial attention investigation using the 5-CSRTT, the lack of an implicit learning deficit reported in *Hdh*$^{Q150/+}$ mice at 14-17 weeks of age could be anticipated because of the potential gene dosage effect on the behavioural phenotype in this mouse model. Similarly, a deficit in this task may not have arisen because heterozygote HdhQ150 animals do not display overt striatal pathology at this age, with neuropathology evident in the form of striatal NII formation from 40-42 weeks of age (Tallaksen-Greene et al., 2005), heightened striatal GFAP levels at 51-60 weeks of age (Lin et al., 2001; Yu et al., 2003), and a decrease in striatal neuron number at 100 weeks of age (Heng et al., 2007). Thus, the lack of neuropathology in this model at the age examined may account for the healthy implicit learning performance levels observed in the current study.

Lesion studies have suggested that striatal dysfunction does not affect the implicit learning abilities of rats (Jay and Dunnett, 2007) or mice (Trueman et al., 2005; Brooks et al., 2007) in the SILT. This could be argued to suggest that differences in implicit learning did not arise between genotypes in the current work because the neural structure likely to have undergone the greatest degree of degeneration in the *Hdh*$^{Q150/+}$ mice, the striatum, may not play a key role in SILT-based implicit learning in the rodent brain, which is supported by similar findings in the HdhQ92 line (Trueman et al., 2007). Alternatively, an implicit learning deficit may not have been observed in *Hdh*$^{Q150/+}$ animals in the current work because the behavioural task used to investigate this form of cognition may not be sensitive enough to identify such deficits. Despite being developed and utilised to assess neural mechanisms in implicit learning, the SILT has not successfully identified implicit learning deficits in mice. Cognitive deficits identified using the SILT include impairments in motor learning and visuospatial attention (Trueman et al., 2005; Brooks et al., 2007; Trueman et al., 2007, 2008; Brooks et al., 2009; Brooks et al., 2012d), however, the ability to utilise the predictable information in the task was retained in all studies,

as occurred in the current work. Thus, an apparent lack of sensitivity of the SILT task to detect implicit learning deficits may be a possible reason that such impairments were not evident in *Hdh*$^{Q150/+}$ mice in the work described here.

6.1.1.3 Mid-onset CRF task performance deficit

Hdh$^{Q150/+}$ animals displayed impaired CRF task acquisition and performance at 44-52 weeks of age (Chapter 5), which is the first report of such an impairment in the HdhQ150 mouse model of HD, to my current knowledge. This deficit was not observed in *Hdh*$^{Q150/+}$ mice at 13 weeks of age in the current work (Chapter 3), which suggests that onset of this deficit in heterozygous HdhQ150 animals occurs between 13 and 44-52 weeks of age. The validity of this conclusion is questionable, however, because of the different genders of animals used in the two experiments assessing CRF performance in *Hdh*$^{Q150/+}$ animals; Chapter 3 utilised female mice whereas Chapter 5 used male animals. Thus, it is possible that the differences in *Hdh*$^{Q150/+}$ CRF task performance identified between the time points examined resulted from sex-dependent variations in cognitive ability. A number of sex-dependent differences in cognition have been reported outside of HD mouse models (Bowman et al., 2009; Duvoisin et al., 2010; Yue et al., 2011; Breitberg et al., 2013; Jasarevic et al., 2013; Sanches et al., 2013; Suwalska and Lojko, 2014), including variation in age-related cognitive decline (Zanos et al., 2015). Similarly, gender has previously been shown to differentially affect behavioural phenotypes in a variety of HD mouse models, with sex-dependent differences observed in cognitive ability and depressive symptoms in R6/1 mice (Renoir et al., 2011; Mo et al., 2013; Mo et al., 2014), circadian dysfunction in BACHD mice (Kuljis et al., 2016), and anxiety in HdhQ140 animals (Dorner et al., 2007). Therefore, examination of CRF task performance in both male and female *Hdh*$^{Q150/+}$ mice at 13 and 44-52 weeks of age may be necessary to establish whether the deficit observed at 44-52 weeks of age results from age- or sex-dependent changes in cognitive ability.

It is also possible that the abnormal CRF task performance observed in *Hdh*$^{Q150/+}$ animals in the current work is not a cognitive deficit but a motivational deficit, which may be the result of decreased palatability to the food reward, as discussed in Chapter 5.4.1. In order to elucidate which of these behavioural phenotypes accounts for the CRF task performance deficit, examination of *Hdh*$^{Q150/+}$ mice lick cluster response to the Yazoo® strawberry flavoured milkshake reward and performance in the progressive ratio task may be required. Unpublished data from our laboratory (Brooks et al., unpublished) demonstrates that *Hdh*$^{Q150/+}$ animals display equivalent levels of palatability for sucrose pellets as their wild-type counterparts at 17-26 weeks of age (Figure 6.1), which suggests that a decreased hedonic response to the food

reward is unlikely to be responsible for the CRF task performance impairment observed in the current work. However, palatability to the Yazoo® strawberry flavoured milkshake reward used in the current study has yet to be performed in *Hdh*$^{Q150/+}$ animals.

Figure 6.1. The mean weight of sucrose pellets consumed (g/kg) by *Hdh*$^{+/+}$ and *Hdh*$^{Q150/+}$ animals (Brooks et al., unpublished). *Hdh*$^{+/+}$ (n = 13) and *Hdh*$^{Q150/+}$ (n = 11) mice consumed an equivalent weight of sucrose pellets at 17-26 weeks of age (main effect of genotype: $t(22) = 0.633$, $p = 0.533$). Values shown are mean ± S.E.M.

Conditional and optogenetic studies have demonstrated that the direct and indirect pathways of the basal ganglia (see Chapter 1.1.2.2) play opposing roles in reward and motivation; conditional knockout of striatopallidal neurons of the indirect pathway causes an increase in drug reinforcement in the conditioned place preference paradigm (Durieux et al., 2009) while activation of these neurons by optogenetic means leads to a decrease in drug reward (Lobo et al., 2010), which suggests that the indirect pathway inhibits drug reinforcement, and therefore inhibits the rewarding effect of drug-use. Conversely, optogenetic activation of striatonigral neurons of the direct pathway results in an increase in drug reinforcement (Lobo et al., 2010) while blockade of this pathway causes a decrease in drug reinforcement (Hikida et al., 2010), suggesting that activation of the direct pathway facilitates drug reinforcement, and thus reward. Based upon this evidence, and evidence that the indirect pathway first undergoes degeneration in HD (Reiner et al., 1988), one would anticipate that *Hdh*$^{Q150/+}$ animals would display a phenotype of increased palatability and motivation, which is opposite to the potential motivation phenotype observed in the current work. However, recent evidence of separate

dorsal and ventral striatonigral DRD1 neuron-dependent circuits for the metabolic and hedonic responses to sugar (Tellez et al., 2016) suggests that *Hdh*$^{Q150/+}$ animals would display healthy levels of palatability and motivation to the milkshake food reward, as DRD1 neurons of the direct striatonigral pathway are resistant to degeneration until the later stages of HD (Reiner et al., 1988). It is clear from these studies that the neuroanatomical basis of hedonic response is likely to be complex and is not yet fully understood, making it difficult to hypothesise how the striatal cell loss observed in mouse models of HD may affect motivation and hedonistic responses in the animals. Furthermore, previous reports demonstrate that *Hdh*$^{Q150/+}$ mice do not present with significant striatal neuron number loss until after 70 weeks of age (Heng et al., 2007), which is beyond the age that animals were examined in the current study, suggesting that neuron loss is unlikely to cause the potential motivational abnormality observed in *Hdh*$^{Q150/+}$ animals. However, the presence of striatal NIIs (Tallaksen-Greene et al., 2005) and heightened levels of reactive gliosis (Lin et al., 2001) have been demonstrated in *Hdh*$^{Q150/+}$ mice at the ages examined in the current work, suggesting that pathological damage to specific subpopulations of striatal neurons could account for the motivational deficits observed. Thus, examining NII formation, reactive gliosis, and gene expression levels in the distinct subpopulations of neurons in the direct and indirect pathways of the basal ganglia may be worthwhile in future studies investigating motivation in *Hdh*$^{Q150/+}$ mice, as opposed to investigating transcriptional level changes across all cell types in the striatum, which occurred in the current work.

6.1.1.4 Mid-onset SD impairments

The behavioural work of Chapter 5 revealed impaired acquisition in a test of SD at 46-60 weeks of age in heterozygous HdhQ150 animals. This was shown by *Hdh*$^{Q150/+}$ mice requiring a greater number of sessions to reach criteria in the SD task than their wild-type counterparts, while the mean accuracy levels of each genotype were equivalent across the final three SD sessions and across all SD sessions until criteria was met. Thus, *Hdh*$^{Q150/+}$ animals were capable of reaching comparable levels of accuracy as *Hdh*$^{+/+}$ mice but presented with delayed acquisition of the SD task. The identification of a delayed SD acquisition phenotype in the current work is supported by previous evidence of spatial learning impairments in the HdhQ150 mouse model of HD (Brooks et al., 2012b) and HD patients (Brandt et al., 2005; Pirogovsky et al., 2015). It is possible that the delayed acquisition phenotype observed is caused by striatal neuropathology in the *Hdh*$^{Q150/+}$ animals, with evidence of striatal NII presence (Tallaksen-Greene et al., 2005) and heightened levels of reactive gliosis (Lin et al., 2001) in this mouse model at the ages examined in the current work. This hypothesis is supported by the striatum's role in acquisition of a new

rule in a set-shifting task (Provost et al., 2012) and the activation of a cortical-striatal-hippocampal network during rule-learning (Poldrack et al., 1999; Toni and Passingham, 1999; Seger and Cincotta, 2005; Seger and Cincotta, 2006). However, the substantial evidence linking hippocampal activity with spatial learning and memory ability (Morris, 1981; Silva et al., 1992b; Moser et al., 1995; Tsien et al., 1996; Maguire et al., 1997; Maguire et al., 2000; Burgess et al., 2002; Ekstrom et al., 2003), and the lack of an interaction between aberrant striatal gene expression levels and SD task behaviour in *Hdh*$^{Q150/+}$ animals in the current study, suggests that this SD acquisition deficit may not be striatal, but hippocampal, in nature. Future analyses of hippocampal gene expression level profiles in the *Hdh*$^{Q150/+}$ animals of the current work may be of interest in order to elucidate the molecular mechanisms accountable for the SD task acquisition deficit observed.

Despite reaching comparable levels of accuracy with wild-type animals across the final 3 SD sessions until criteria was met, the response latencies to both S1 and S2 were lengthened in heterozygote HdhQ150 mice during these trials. These response impairments are unlikely to be the result of a motor deficit in *Hdh*$^{Q150/+}$ animals because the response latencies for food reward collection were comparable between genotypes, and one would anticipate that *Hdh*$^{Q150/+}$ mice would also display lengthened response latencies for reward collection if their motor capabilities were diminished. *Hdh*$^{Q150/+}$ animals did not present with a motor deficit on the rotarod task prior to operant testing, which further opposes the suggestion that a motor abnormality is responsible for the lengthened S1 and S2 response latencies observed. It is possible that a motor deficit may have arisen in the HdhQ150 animals during operant testing in the current study, as motor ability was not re-assessed following operant testing, however, the food reward response latency data discussed and previous reports of healthy rotarod performance in *Hdh*$^{Q150/+}$ mice up to 100 weeks of age (Heng et al., 2007) suggest that motor abnormalities were not present during operant testing.

The increased response latencies to S1 and S2 observed in *Hdh*$^{Q150/+}$ animals, in the likely absence of motor deficits, indicates that these mice may present with a phenotype of reduced cognitive processing speed. This phenotype can be compared to that seen in HD patients, whereby sufferers demonstrate diminished ability in tasks that assess cognitive processing, including the Stroop task (Snowden et al., 2001; Snowden et al., 2002; Tabrizi et al., 2012; Tabrizi et al., 2013), which may be attributed to the involvement of the striatum in “selection of action” in response to an action (Houk and Wise, 1995; Houk et al., 2007) and its degeneration in HD (Lange et al., 1976; Vonsattel et al., 1985; Aylward et al., 1997; Vonsattel and DiFiglia, 1998; Aylward et al., 2004; Tabrizi et al., 2009; Aylward et al., 2011; Tabrizi et al., 2011; Tabrizi et al., 2012; Tabrizi et al., 2013). Thus, the aforementioned striatal neuropathology previously

observed in *Hdh*$^{Q150/+}$ mice at the ages examined in the current work may contribute to the slowed cognitive processing described here. Although such neuropathological features were not examined in *Hdh*$^{Q150/+}$ animals in the current work because of financial and time restraints, hemispheric brain tissue of these animals is available for examination of such neuropathology in future studies. The lack of an interaction between aberrant striatal gene expression levels and SD task performance in *Hdh*$^{Q150/+}$ animals indicates that the potential cognitive processing phenotype identified in the current study is not due to abnormal striatal transcription levels of *Adora2A*, *Arc*, *Bdnf*, *Creb1*, *Drd2*, or *Homer1*. However, it is possible that striatal transcriptional dysfunction of genes that were not examined in the current work may contribute to this phenotype.

Hdh$^{Q150/+}$ animals were also found to initiate significantly fewer S2 trials in the SD task, both over the final 3 sessions until criteria was met and across all sessions performed, than their wild-type counterparts. Taken with the CRF phenotype described earlier, these data suggest that *Hdh*$^{Q150/+}$ mice may present with a motivational deficit at 46-60 weeks of age when compared to *Hdh*$^{+/+}$ mice of an equivalent age. As discussed earlier, however, detailed investigation into the hedonic responses and motivational drive of the *Hdh*$^{Q150/+}$ animals would be required to confidently conclude whether a motivational abnormality is present in this mouse model of HD.

6.1.2 Transcriptional correlates of cognition

6.1.2.1 The effects of CRF, 5-CSRTT and SILT performance on striatal transcription

The microarray investigation of Chapter 3 identified a relatively large number of genes that displayed significant expression level differences between untrained animals and those that had undergone cognitive task performance, and RT-qPCR analyses validated 19 of the 21 significant gene expression level change group comparisons investigated. CRF task performance in both *Hdh*$^{+/+}$ and *Hdh*$^{Q150/+}$ animals produced changes in transcription levels of genes that were found to be associated with protein localisation and transport, with a number of these transcripts overlapping between genotypes. Thus, CRF performance seems to result in changes expression levels of genes associated with protein transport and localisation, which is unsurprising given the evidence that performance of learning and memory tasks causes alteration to dendritic spine morphology through the synthesis, transport and localization of synaptic proteins (Martin et al., 2000; Miller et al., 2002; Sutton and Schuman, 2006; Sekino et al., 2007; Tanaka et al., 2008). Genes displaying altered levels of transcription in *Hdh*$^{Q150/+}$ animals following CRF task performance were also associated with other aspects of protein production and transport, such

as in the Golgi apparatus and vesicle-mediated transport, and signaling pathways that include GTP binding. This seems to suggest that CRF task performance, or simple conditioning, in *Hdh*$^{Q150/+}$ mice results in alterations to transcription levels of genes involved in a wider range of biological processes than occurs in wild-type animals, however, there were no significant differences in gene expression levels between genotypes reported in the microarray. Similar gene expression level changes were identified following implicit learning albeit with fewer transcripts showing altered levels of transcription, and with *Hdh*$^{Q150/+}$ animals displaying differences in expression levels of genes associated with fewer biological pathways than their wild-type counterparts. This seems to suggest that implicit learning results in alterations to transcription levels of genes involved in a narrower range of biological processes in *Hdh*$^{Q150/+}$ mice than occurs in *Hdh*$^{+/+}$ animals. However, as was found in gene expression level differences following CRF task performance, no significant differences in gene expression levels were reported between genotypes. Performing cognitive training seemed to affect ageing-dependent changes in gene expression levels, with the PCA and microarray list of gene expression level changes in group comparisons suggesting that the performance of behavioural tasks between 2M-4M appears to maintain the striatal transcription profile close to that of 3M mice rather than that seen in their untrained 6M contemporaries. This supports the theory that behavioural training acts to prevent or ameliorate gene expression level changes associated with ageing, and similar results have reported that an exercise protocol seems to reverse ageing-related memory decline and decreases in hippocampal histone H4 acetylation levels in rats (Lovatel et al., 2013).

There are methodological considerations that limit the efficacy of the gene expression level results reported in Chapter 3. One such limitation is that the untrained animals in the experiment were unhandled, other than for routine husbandry and weighing, and that these mice did not receive exposure to the behavioural training environment. This may confound the transcription level findings because we cannot confidently state that the gene expression level differences that arise between trained and untrained animals are the result of the cognitive tasks performed by the trained mice, as untrained mice did not receive the same exposure to handling or the operant boxes as their behaviourally trained counterparts. This could potentially mean that the effects of cognitive task performance observed may be a result of handling, which is likely to have multiple effects on the handled mice that may include increased fear and anxiety, both of which are known to affect neural gene expression levels (Malkani and Rosen, 2000; Hovatta et al., 2005; Coryell et al., 2007; Pantazopoulos et al., 2011; Spencer et al., 2013). Exposure to the operant boxes may also have caused the gene expression level changes observed in trained animals, as striatal transcription is susceptible to alteration by extended exposure to novel environments (Struthers et al., 2005). The lack of vaginal smear testing to

ascertain the stage of oestrous cycle in the female mice used in the gene expression level investigation in Chapter 3, as previously discussed in Chapter 6.1.1.1, also confounds the results of the microarray and RT-qPCR investigations. Because the stage of oestrous of each mouse was not known at the time of sacrifice for molecular analyses, it is not possible to ascertain whether the gene expression level differences reported between groups were the result of the cognitive training undertaken or variations in oestrous cycle. To eradicate such methodological confounds from later experiments, male mice were utilised and behavioural control groups were given equivalent exposure to the testing environments of the operant boxes as animals that received cognitive training.

6.1.2.2 The striatal transcriptional correlates of SD and RL

The microarray investigation in Chapter 4 did not identify any significant gene expression level changes in wild-type C57BL6/J animals as a result of any forms of behavioural training, and the RT-qPCR investigation confirmed this finding by reporting zero training-dependent changes in expression levels of genes previously associated with SD and/or RL (Table 4.1). Similarly, the RT-qPCR investigation in Chapter 5 reported no significant differences in expression levels of these same genes as a result of SD learning in $Hdh^{+/+}$ or $Hdh^{Q150/+}$ animals. Despite clear increases in accuracy being observed in behavioural training, an indication that the animals were able to learn, these unaltered transcriptional profiles were identified.

It was postulated that the lack of gene expression level differences identified in the microarray and RT-qPCR investigations in Chapter 4 may have been the result of an absence of striatal dissection specificity, where the striatum was extracted and examined as a whole. This was hypothesised because of evidence that distinct regions of the striatum play dissociable roles in learning (Reading et al., 1991; Featherstone and McDonald, 2004; Reiss et al., 2005; Atallah et al., 2007; Darvas and Palmiter, 2010, 2011; Darvas et al., 2014; Burton et al., 2015) and that previous studies have identified significant differences in RL behaviour and gene expression levels when subdividing the striatum into similar distinct regions (Hernandez et al., 2006; Daberkow et al., 2007; Graybeal et al., 2011). However, striata were microdissected into dorsal and ventral striatal regions for RT-qPCR analyses in Chapter 5, which did not lead to the identification of gene expression level changes associated with cognition. This therefore seems to reject the hypothesis that the lack of striatal dissection specificity in Chapter 4 was accountable for the absence of significant learning-dependent gene expression level changes. Although a role of the striatum has been identified in a potential cortical-striatal-hippocampal network for spatial learning (Floresco et al., 1997; Mair et al., 2002; Pooters et al., 2016) and in

a frontostriatal network for RL (Cools et al., 2002; Featherstone and McDonald, 2005; Ragozzino, 2007; Bellebaum et al., 2008; Clarke et al., 2008; Ghahremani et al., 2010; Ruge and Wolfensteller, 2016), the evidence presented in the current work suggests that striatal transcription may not be integral to, or altered by, performance of such learning paradigms in mice.

One potential reason for the absence of transcriptional diversity reported as a result of SD and RL is the possibility that the striatal gene expression level profiles of each group were comparable because the natural magnitude of gene expression level changes that occur following performance of these tasks in wild-type animals are not high enough to be detected by microarray. When investigating the role of transcription in learning, it is common practice to use a 'bottom-up' approach to alter the genetic profile of animals prior to, or during, behavioural testing using transgenic (Silva et al., 1992b; Bourtchuladze et al., 1994; Sakimura et al., 1995; Abel et al., 1997; McClung and Nestler, 2003; Gerstein et al., 2012) or pharmacological methods (Egerton et al., 2005; Bredy and Barad, 2008; Romieu et al., 2008; Stefanko et al., 2009; Haettig et al., 2011; Dagnas et al., 2013). This approach often yields changes in transcription or behaviour, which can be linked to the gene(s) or pathway(s) under investigation. The current study used a 'top-down' approach where behaviour was examined before examination of potential genetic differences. It is possible that using an approach of this nature in wild-type, and therefore genetically and pharmacologically unaltered, animals does not lead to transcriptional changes large enough for detection by microarray or RT-qPCR, as suggested by the lack of significant gene expression level differences using either technique. These findings also seem to indicate that striatal transcriptional regulation of the genes investigated do not play a role in SD or RL, although this conclusion is contradicted by evidence provided by multiple studies, the majority of which utilise the 'bottom-up' approach discussed (Table 4.1).

The behavioural training stage at which animals were sacrificed in Chapter 4 may be partly responsible for the lack of learning-dependent gene expression level changes observed. Mice were either sacrificed following 1 session of SD or RL training, or upon reaching criterion in these tasks, where criterion consisted of reaching ≥85% accuracy over three consecutive behavioural sessions. It is possible that gene expression level differences did not arise between animals culled at these distinct stages because of the potential temporal requirement needed for learning-dependent transcription to occur. It is possible, for example, that molecular differences were not identified between striata of animals that reached criteria in the SD and RL tasks because the gene expression level changes responsible for each form of learning (if they produce separate molecular substrates in the striatum) were arising in sessions at which the animals were improving in cognitive ability rather than when they had reached the cognitive peak of

≥85% accuracy over three consecutive sessions (Figure 6.2). Thus, examining a mid-point in cognitive ability, where accuracy levels are intermediate (at 60-80% accuracy in the SD task, for example), may have yielded significant gene expression level differences between groups (Figure 6.2). This theory is supported by evidence that the dorsolateral and posterior dorsomedial striatum of rats show differing levels of gene expression following performance of an intermediate number of instrumental learning sessions (6 sessions) when compared to performance of a minimal (3) or extended (10) number of sessions (Anna Powell thesis, Cardiff University, 2013). Differences in gene expression levels have also been identified between a 'mid-RL accuracy' group of mice and animals that had undergone 1 session of RL or had reached criteria in the task (Brigman et al., 2013), which also suggests that incorporation of an intermediate cognitive ability group in the current work may have yielded significant gene expression level differences. Incorporating this intermediate cognitive ability behavioural group based on mean accuracy, however, may have presented difficulty in establishing the criterion for intermediate accuracy.

It is also possible that performance of only 1 session of SD or RL training is insufficient to produce significant learning-dependent gene expression level changes because animals should be performing at chance level, or lower than chance level due to perseverative responding of the previously learnt rule, at these time points. This would mean that animals may not necessarily be learning the task requirements at this stage of training, which could explain the lack of significant learning-dependent transcription level differences that arise between animals that reached criteria in SD task and those that performed 1 session of RL. However, this theory seems to be contradicted by differences in gene expression levels being identified between animals that have undergone only 1 initial learning session and those that have received a greater number of learning sessions or 1 initial learning session of a new rule (Brigman et al., 2013). Conversely, early learning gene expression level changes have been evaluated following the performance of 3 initial behavioural training sessions in other published literature (Havekes et al., 2006; Hernandez et al., 2006), which may reflect anticipation of the methodological consideration raised here.

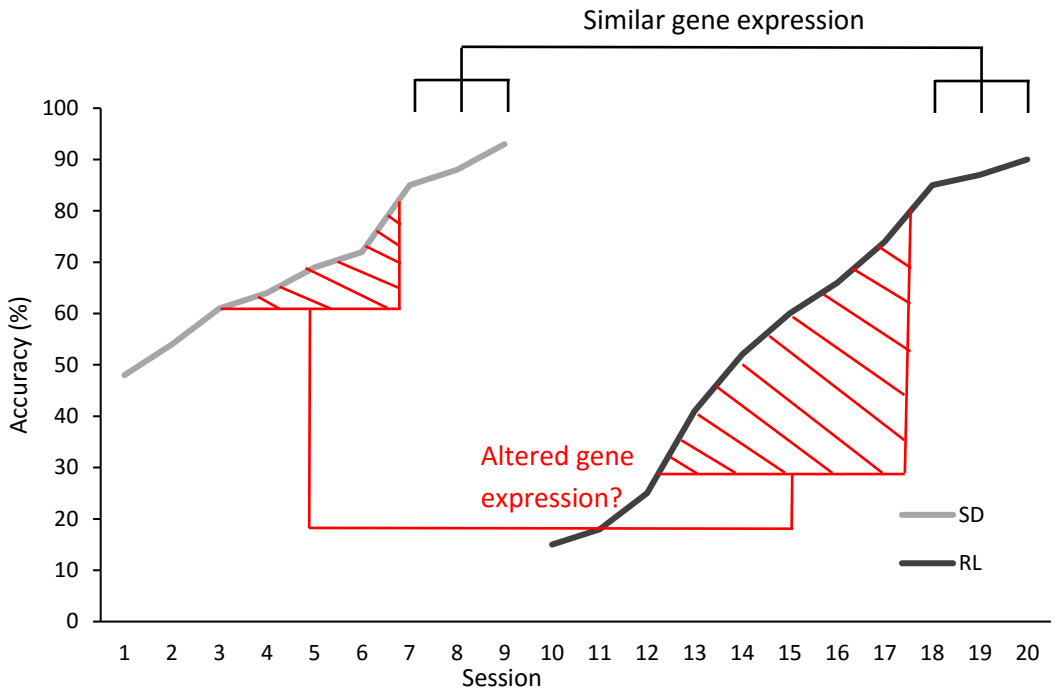


Figure 6.2. A graph of hypothetical mean accuracy data illustrating where potential gene expression level differences may have arisen during SD and RL. As learning may have already occurred by the time animals had reached 85% accuracy for 3 consecutive sessions, as suggested by the molecular findings of Chapters 4 and 5, it is hypothesised that examining gene expression levels in a group of animals at sessions in which they display an intermediate level of cognitive ability in either task would be more likely to show learning-dependent gene expression level differences.

Use of a novel behavioural task examining SD and RL may have contributed to the absence of SD- and RL-dependent gene expression level changes in the current work. Gene expression level changes dependent on these forms of learning have been investigated in rodents using behavioural tasks that include the Morris water maze (McDowell et al., 2010; Wei et al., 2011; Trinh et al., 2012; Fentress et al., 2013; Sakata et al., 2013), Y-maze (Havekes et al., 2006; Trinh et al., 2012), T-maze (Daberkow et al., 2007; Pastuzyn et al., 2012), attention-set-shifting (Egerton et al., 2005; Desteno and Schmauss, 2009), and, more recently, in touchscreen operant boxes (Izquierdo et al., 2006; Brigman et al., 2008; Brigman et al., 2009; Brigman et al., 2010; Graybeal et al., 2011; Brigman et al., 2013; Kolisnyk et al., 2013). It is possible that the likelihood of identifying SD- or RL-dependent gene expression level differences may have been higher had a well-characterised behavioural task, such as the aforementioned tasks, been used in the current work. However, with the exception of the touchscreen operant boxes, these behavioural

tasks have greater dependence on non-cognitive functions, such as motor ability or olfaction, than the tasks described in the current work, and were not utilised because these faculties have been associated with gene expression level changes themselves, which may have obscured potential mRNA level changes that resulted from altered cognition.

Other potential limitations of the current molecular work include those that have previously been discussed, which includes the fact that only striatal tissue was examined in molecular investigations. However, tissue was also extracted from a number of other brain regions, such as the hippocampus, PFC and cerebellum, meaning that molecular investigations into learning-dependent gene expression level changes in these neural structures is possible in the future. Similarly, only mRNA levels were examined in the current work, while the protein levels or localisation of the products of these transcripts were not investigated by Western blot or immunohistochemistry, for example. Investigating the protein levels of the genes examined in the current work may have contributed to the elucidation of the molecular basis of cognition studied here, as mRNA levels of a gene do not necessarily correspond with its level of protein product. However, striatal tissue, as well as tissue from the aforementioned neural regions, was extracted and remains available for such protein analyses in the future, but financial and time constraints meant that these investigations were not possible in this thesis.

6.1.3 The striatal transcriptional profile in Hdh$^{Q150/+}$ mice

The microarray and RT-qPCR investigations of Chapter 3 reported no significant differences between expression levels of the transcriptional profiles of *Hdh*$^{Q150/+}$ and *Hdh*$^{+/+}$ animals at 13 or 26 weeks of age. Significant striatal gene expression level differences that recapitulate aspects of those seen in human HD patients have previously been reported between homozygous HdhQ150 mice and wild-type counterparts at 26-96 weeks of age (Hodges et al., 2006; Kuhn et al., 2007; Giles et al., 2012; Bayram-Weston et al., 2015), which indicates that a transcriptional phenotype does arise in the HdhQ150 mouse model of HD. The evidence presented in Chapter 5 of the current work, of decreased expression levels of *Adora2A*, *Arc*, *Drd2* and *Homer1* in the striatum of *Hdh*$^{Q150/+}$ mice at 44-60 weeks of age, also provides evidence for an abnormal transcriptional phenotype, and strengthens the evidence for abnormal signaling or expression of these genes in the HD phenotype (Table 5.1). This is the first report of a transcriptional phenotype in heterozygous HdhQ150 animals, to my current knowledge, and suggests that manifestation of this phenotype may be age- or sex-dependent in *Hdh*$^{Q150/+}$ mice, as such abnormalities were not identified in younger female mice at 13 or 26 weeks of age in the current work. It is important to note that RT-qPCR analyses were not performed on the same genes in

Chapter 3 as those in Chapter 5, meaning that differences in striatal expression levels of *Adora2A*, *Arc*, *Drd2* and *Homer1* may have arisen in the female $Hdh^{Q150/+}$ mice at 13 or 26 weeks of age. However, the microarray investigation performed in Chapter 3 contradicts this suggestion, as expression levels of these genes were found to be equivalent between $Hdh^{Q150/+}$ and $Hdh^{+/+}$ animals (data not shown).

As previously discussed, the molecular findings of Chapter 3 may be limited because the stage of oestrous cycle at which each $Hdh^{Q150/+}$ mouse was present at the time of sacrifice was unknown, however this methodological consideration was corrected for Chapter 5 by using male mice. The relatively small sample sizes used in the molecular analyses (n/group = 3-6) may also be a limitation of the current work, as the analyses may be underpowered and thus may not be fully representative of the $Hdh^{Q150/+}$ mouse population; financial restraints did not allow increased sample sizes for subsequent studies. $Hdh^{Q150/+}$ mice in Chapter 5 displayed evidence of a genomic drift in CAG repeat size, whereby the mean CAG repeat observed was approximately 130 CAGs. This may also limit the conclusions made because the mutant mice had CAG repeat lengths shorter than those of the original HdhQ150 line, and thus may not be fully representative of the $Hdh^{Q150/+}$ mouse population, however, previous studies have also utilised mice displaying similar genetic drift in CAG lengths (Bayram-Weston et al., 2012d; Giles et al., 2012).

6.2 Future directions

Directions for future work could include molecular investigations, of both mRNA and protein levels, of relevant tissue extracted from animals in the current work, which in the cases of Chapters 4 and 5 would most likely involve examining PFC and hippocampal tissue, because of the aforementioned associations of the PFC with RL and the hippocampus with spatial learning and memory. Examining CRF task performance at 13 and 44-52 weeks of age in both male and female $Hdh^{Q150/+}$ mice may also be of interest, in order to elucidate whether the acquisition and performance impairments identified at 44-52 weeks of age in this genotype were age- or sex-dependent. Behavioural assessment of the hedonistic responses and motivation levels of $Hdh^{Q150/+}$ mice may also be of interest in future studies, as an attempt to reveal whether alterations to either of these psychological domains is responsible for the CRF task acquisition and performance deficits observed in these animals, or for their decreased SD task response latencies. Due to the low numbers of animals used in the SILT investigation, an attempt to replicate the visuospatial attention deficit observed in the 5-CSRTT at 13 weeks of age in $Hdh^{Q150/+}$ mice may also be worthwhile, through behavioural evaluation of a larger sample of

animals in this task. Investigating the molecular correlates of visual discrimination and RL in *Hdh*$^{Q150/+}$ mice using touchscreen-based operant boxes may also be of value, as these boxes have been implemented in a large number of studies examining these forms of learning (Izquierdo et al., 2006; Brigman et al., 2008; Brigman et al., 2009; Brigman et al., 2010; Graybeal et al., 2011; Brigman et al., 2013; Kolisnyk et al., 2013), and because investigation into RL in these animals was removed from the current work due to the lack of transcriptional correlates for this form of learning being identified in wild-type animals. These operant boxes were unavailable for use throughout the current work, however, if visual discrimination deficits, similar to the SD impairments observed, were to be identified in *Hdh*$^{Q150/+}$ animals, the conclusions of the current work would be strengthened further. Use of these touchscreen operant boxes have identified psychomotor slowing, and visual discrimination and RL deficits in the BACHD and zQ175 mouse models of HD (Farrar et al., 2014). It would be of interest to see if the use of these boxes produces similar impairments in *Hdh*$^{Q150/+}$ animals, with the results of the current behavioural work suggesting that a visual discrimination task acquisition deficit may arise.

6.3 Concluding remarks

In conclusion, the data presented in this thesis indicate that *Hdh*$^{Q150/+}$ animals display a series of novel cognitive impairments that recapitulate aspects of the cognitive phenotype observed in HD. These consist of deficits in the acquisition and performance of the CRF task, impaired visuospatial attention in the 5-CSRTT, and delayed acquisition and cognitive processing of SD learning, however, implicit learning capabilities in the SILT were found to be unaltered between *Hdh*$^{Q150/+}$ and *Hdh*$^{+/+}$ animals. Investigation into the transcriptional correlates of cognition was ultimately unsuccessful, with microarray and RT-qPCR analyses being unable to identify significant learning-dependent gene expression level changes in the striata of either *Hdh*$^{Q150/+}$ or wild-type animals following tests of conditioning, implicit learning, SD, or RL. Differences in striatal expression levels of *Adora2A*, *Arc*, *Drd2* and *Homer1* were reported between *Hdh*$^{Q150/+}$ and *Hdh*$^{+/+}$ animals at 44-60 weeks of age, which supports previous evidence of abnormal expression or signaling of these genes in the HD phenotype. Overall, this body of work contributes novel evidence that heterozygote animals of the HdhQ150 mouse model of HD present with molecular and cognitive phenotypes comparable to those seen in the human form of the disease.

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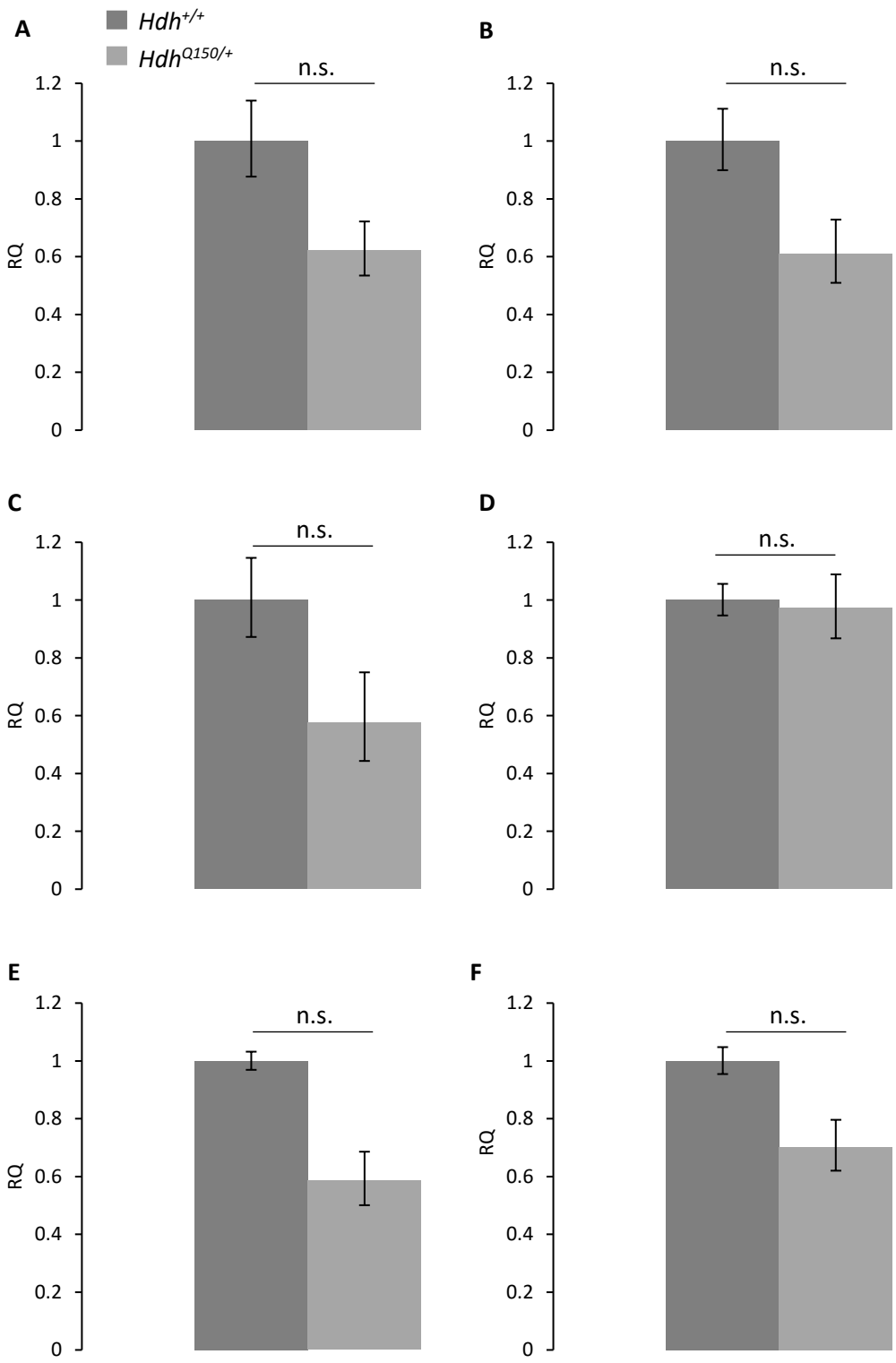
Appendix

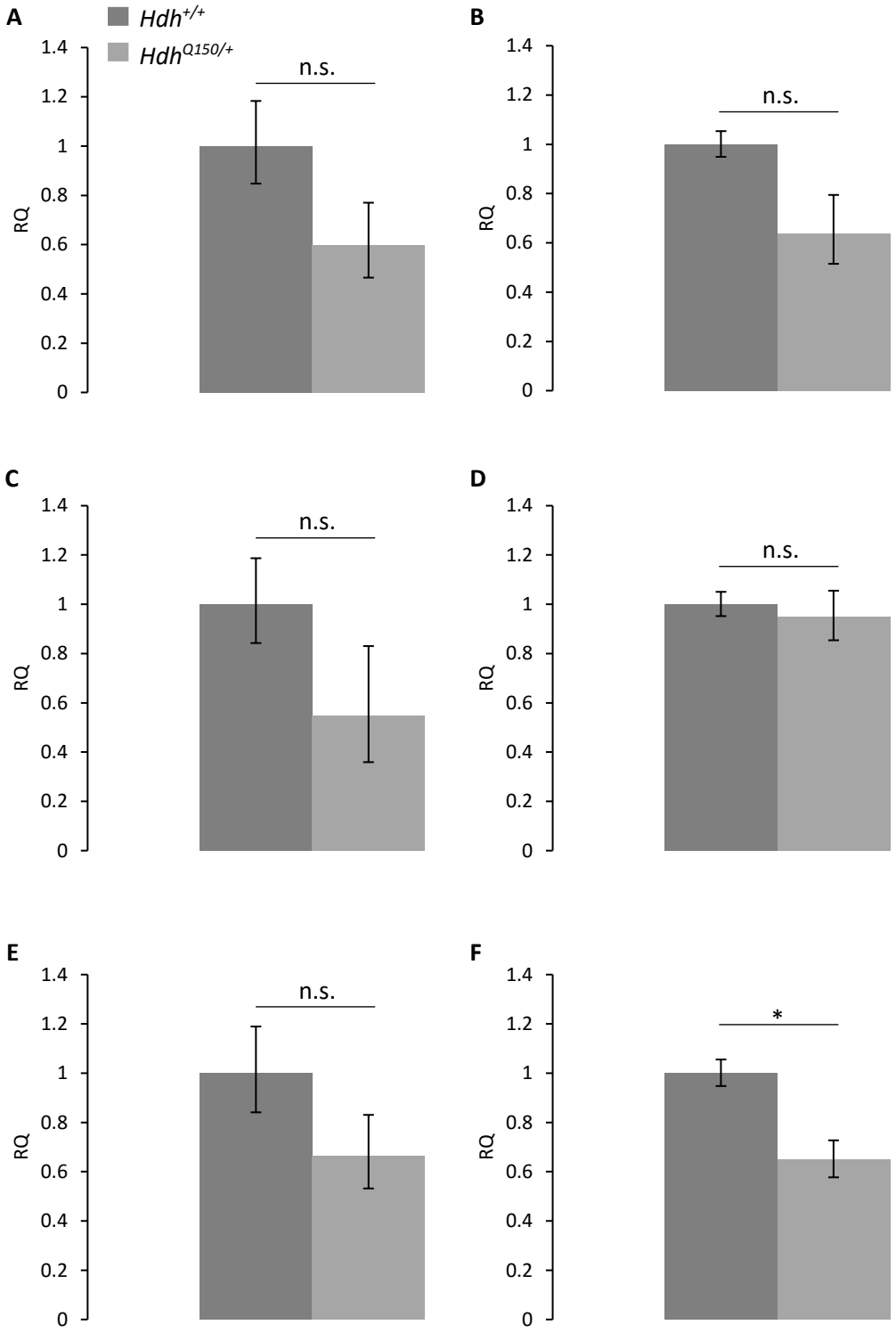
Group comparison	Gene of interest	Statistics
$Hdh^{+/+}$ untrained dorsal striatum vs $Hdh^{Q150/+}$ untrained dorsal striatum	*Adora2A*	$t(8) = -3.274$, $p = 0.066$
	Arc	$t(8) = -2.263$, $p = 0.159$
	Bdnf	$t(8) = -0.268$, $p = 1.000$
	Creb1	$t(8) = -0.438$, $p = 1.000$
	Drd2	$t(8) = -2.803$, $p = 0.115$
	Homer1	$t(4.174) = -3.007$, $p = 0.152$
$Hdh^{+/+}$ untrained ventral striatum vs $Hdh^{Q150/+}$ untrained ventral striatum	*Adora2A*	$t(8) = -1.617$, $p = 0.580$
	Arc	$t(8) = -2.431$, $p = 0.205$
	Bdnf	$t(8) = -0.875$, $p = 0.814$
	Creb1	$t(8) = -0.553$, $p = 0.814$
	Drd2	$t(8) = -1.259$, $p = 0.732$
	Homer1	$\mathbf{t(8) = -4.462, p = 0.012}$
$Hdh^{+/+}$ SD trained dorsal striatum vs $Hdh^{Q150/+}$ SD trained dorsal striatum	*Adora2A*	$t(4.720) = -0.855$, $p = 1.000$
	Arc	$t(8) = -2.094$, $p = 0.420$
	Bdnf	$t(8) = -0.107$, $p = 1.000$
	Creb1	$t(8) = 0.866$, $p = 1.000$
	Drd2	$t(5.081) = -0.261$, $p = 1.000$
	Homer1	$t(8) = -0.075$, $p = 1.000$
$Hdh^{+/+}$ SD trained ventral striatum vs $Hdh^{Q150/+}$ SD trained ventral striatum	*Adora2A*	$t(8) = -1.938$, $p = 0.445$
	Arc	$t(8) = -0.877$, $p = 1.000$
	Bdnf	$t(8) = -0.842$, $p = 1.000$
	Creb1	$t(8) = 2.190$, $p = 0.360$
	Drd2	$t(8) = -1.175$, $p = 1.000$
	Homer1	$t(8) = -0.648$, $p = 1.000$

Appendix 1.1. RT-qPCR results for genotype-dependent gene expression level changes. Specific two-group comparisons were analysed by independent samples t-tests, with Holm-Bonferroni sequential correction used for multiple hypothesis testing correction. Homogeneity of variances for data was assessed using Levene's test, and the Welch test used in the event of data violating this assumption. The test where a significant effect of genotype was identified is highlighted in bold.



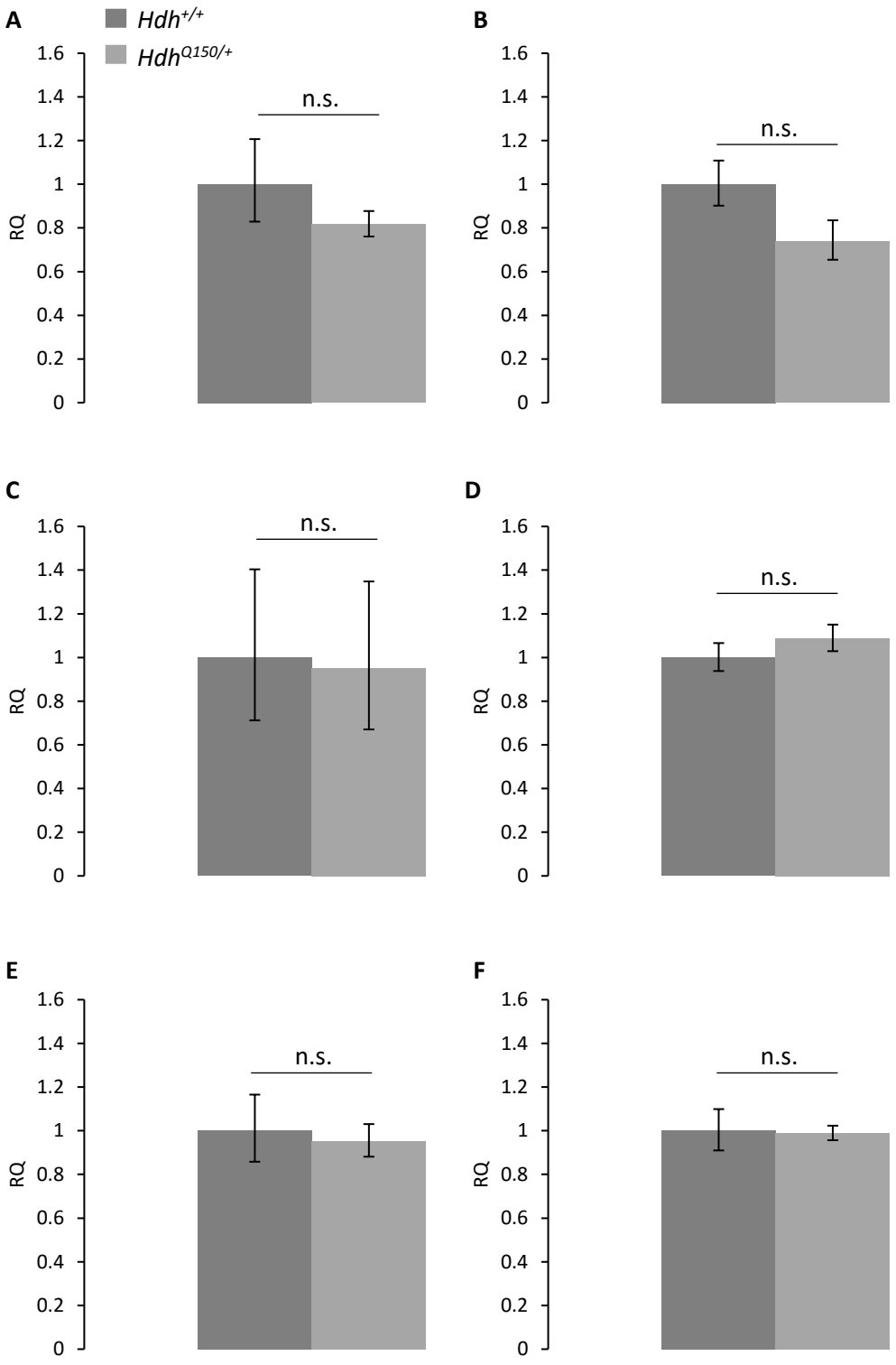
Appendix 1.2. RQ value comparison of *Adora2A* (A), *Arc* (B), *Bdnf* (C), *Creb1* (D), *Drd2* (E), and *Homer1* (F) dorsal striatal expression levels between untrained *Hdh*$^{+/+}$ and *Hdh*$^{Q150/+}$ animals following RT-qPCR. A trend of decreased expression levels in the dorsal striatum of untrained *Hdh*$^{Q150/+}$ mice is seen for all genes investigated. However, all gene expression level differences between groups were found to be non-significant (Appendix 1.1). Values shown are mean ± S.E.M.; n = 5/group.

n.s. = non-significant



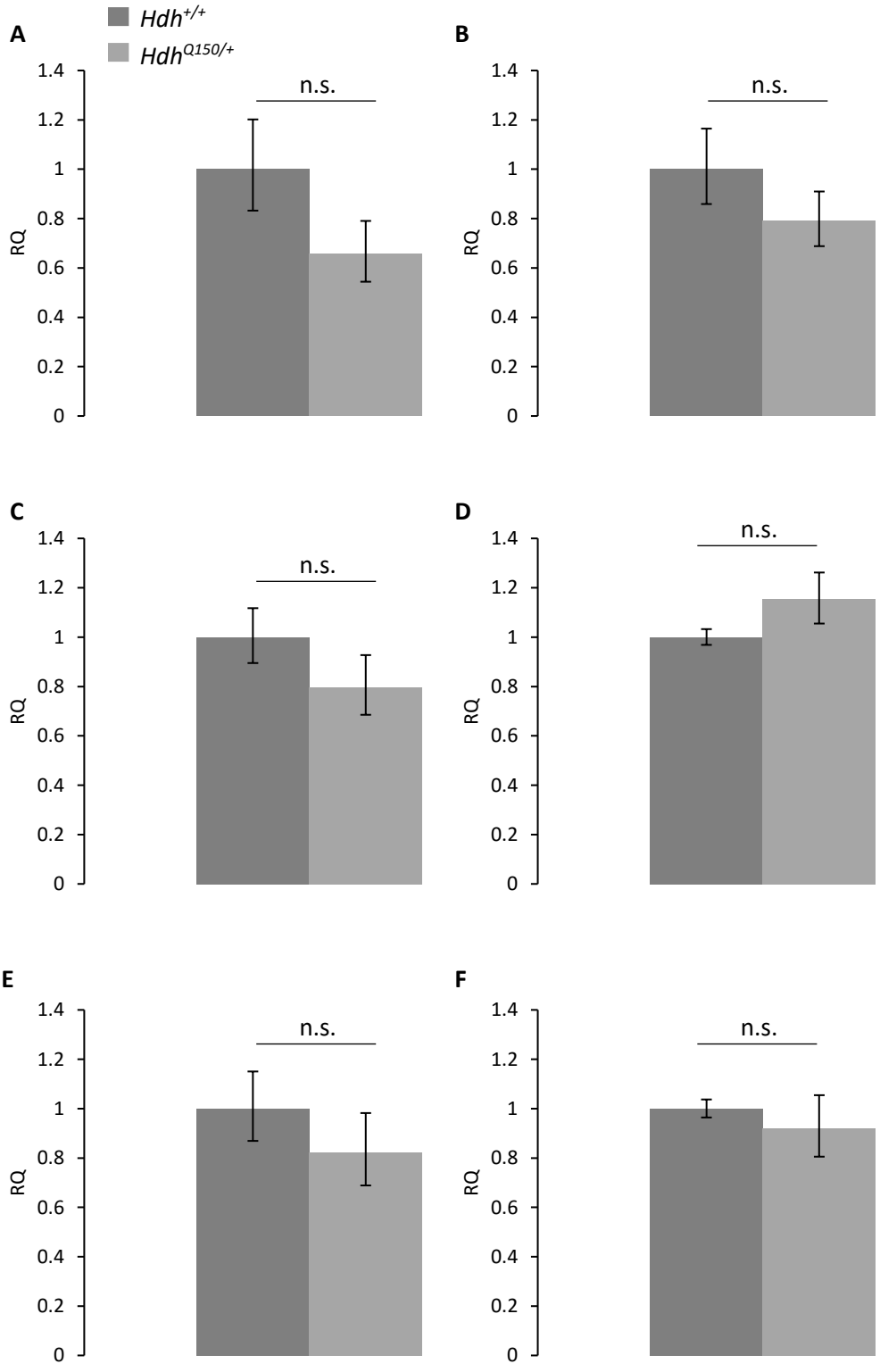
Appendix 1.3. RQ value comparison of *Adora2A* (A), *Arc* (B), *Bdnf* (C), *Creb1* (D), *Drd2* (E), and *Homer1* (F) ventral striatal expression levels between untrained $Hdh^{+/+}$ and $Hdh^{Q150/+}$ animals following RT-qPCR. A trend of decreased expression levels in the ventral striatum of untrained $Hdh^{Q150/+}$ mice is seen for all genes investigated, however, significant gene expression level differences between groups were only reported for *Homer1* (F; Appendix 1.1). Values shown are mean ± S.E.M.; n = 5/group.

* $p < 0.05$ n.s. = non-significant



Appendix 1.4. RQ value comparison of *Adora2A* (A), *Arc* (B), *Bdnf* (C), *Creb1* (D), *Drd2* (E), and *Homer1* (F) dorsal striatal expression levels between SD trained *Hdh*$^{+/+}$ and *Hdh*$^{Q150/+}$ animals following RT-qPCR. A trend of decreased expression levels in the dorsal striatum of SD trained *Hdh*$^{Q150/+}$ mice is seen for all genes investigated, with the exception of *Creb1* (D). However, all gene expression level differences between groups were found to be non-significant (Appendix 1.1). Values shown are mean ± S.E.M.; n = 5/group.

n.s. = non-significant

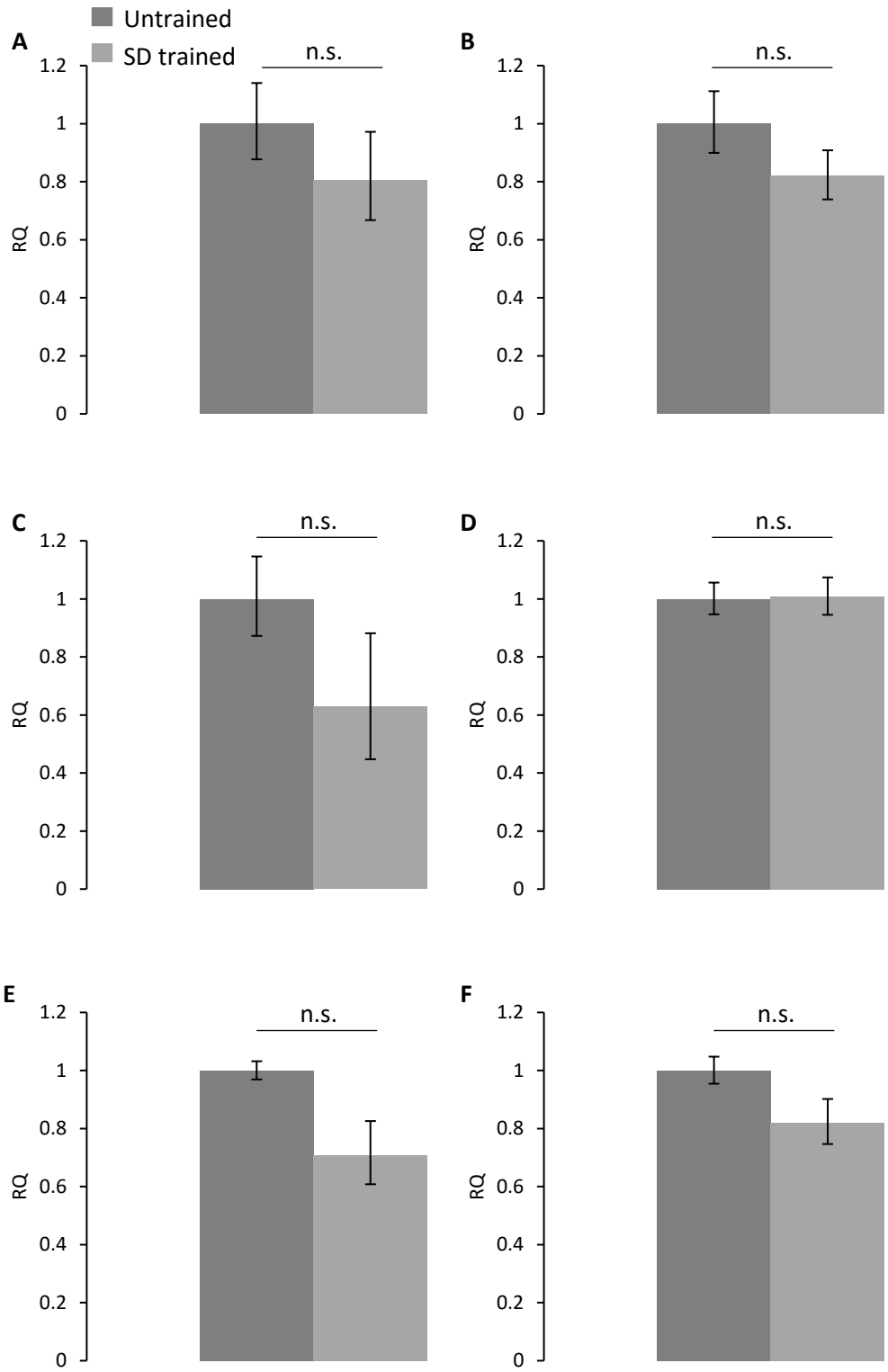


Appendix 1.5. RQ value comparison of *Adora2A* (A), *Arc* (B), *Bdnf* (C), *Creb1* (D), *Drd2* (E), and *Homer1* (F) ventral striatal expression levels between SD trained *Hdh*$^{+/+}$ and *Hdh*$^{Q150/+}$ animals following RT-qPCR. A trend of decreased expression levels in the ventral striatum of SD trained *Hdh*$^{Q150/+}$ mice is seen for all genes investigated, with the exception of *Creb1* (D). However, all gene expression level differences between groups were found to be non-significant (Appendix 1.1). Values shown are mean ± S.E.M.; n = 5/group.

n.s. = non-significant

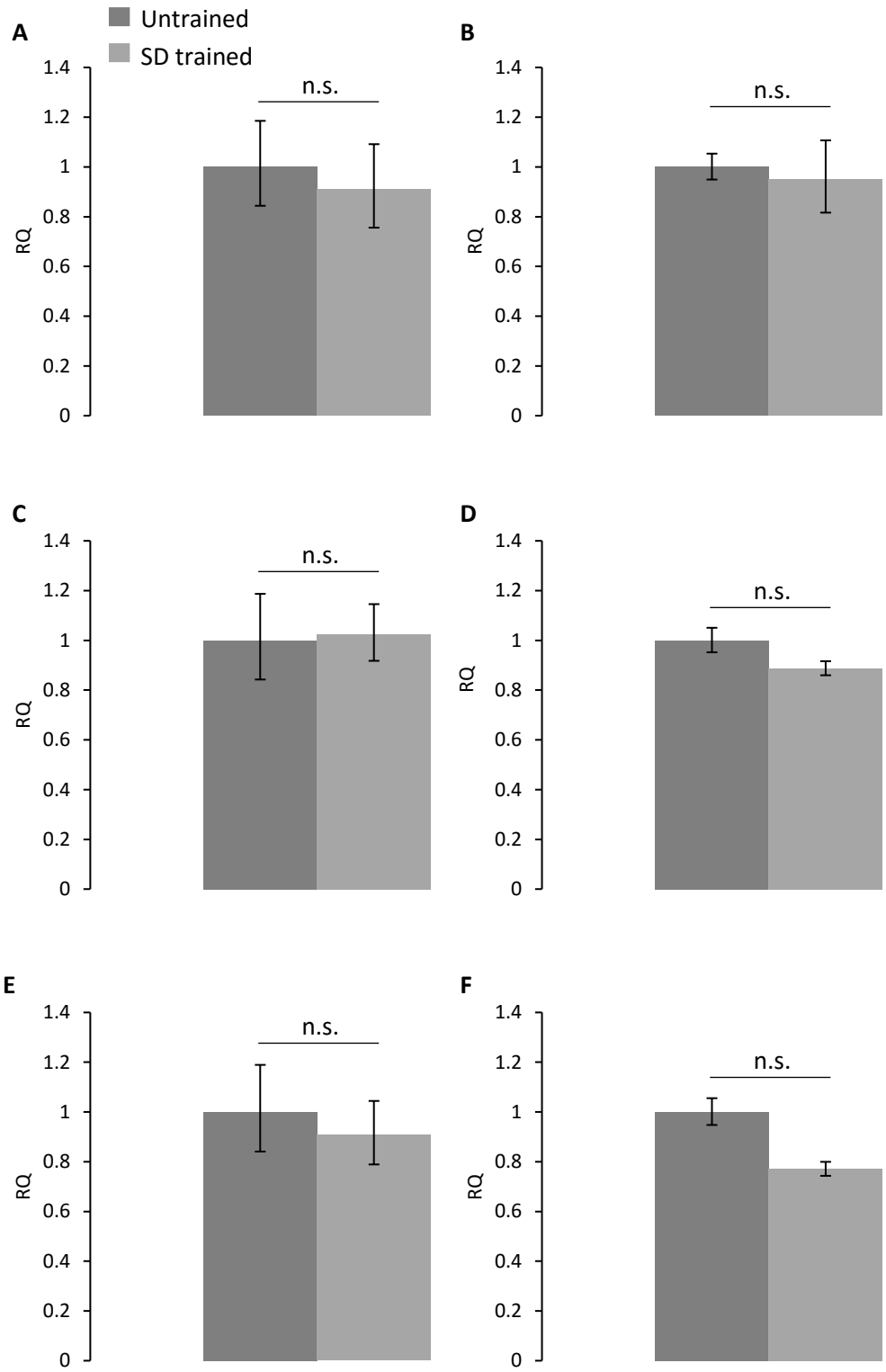
Group comparison	Gene of interest	Statistics
Hdh$^{+/+}$ untrained dorsal striatum vs *Hdh*$^{+/+}$ SD trained dorsal striatum	*Adora2A*	$t(8) = -0.842$, $p = 1.000$
	Arc	$t(8) = -1.535$, $p = 0.815$
	Bdnf	$t(8) = -0.116$, $p = 1.000$
	Creb1	$t(8) = 0.120$, $p = 1.000$
	Drd2	$t(8) = -1.889$, $p = 0.576$
	Homer1	$t(8) = -1.117$, $p = 1.000$
Hdh$^{+/+}$ untrained ventral striatum vs *Hdh*$^{+/+}$ SD trained ventral striatum	*Adora2A*	$t(8) = -0.393$, $p = 1.000$
	Arc	$t(8) = -0.268$ $p = 1.000$
	Bdnf	$t(8) = 0.763$, $p = 1.000$
	Creb1	$t(8) = -1.589$, $p = 0.755$
	Drd2	$t(8) = -0.429$, $p = 1.000$
	Homer1	$t(8) = -2.805$, $p = 0.138$
Hdh$^{Q150/+}$ untrained dorsal striatum vs *Hdh*$^{Q150/+}$ SD trained dorsal striatum	*Adora2A*	$t(8) = 0.549$, $p = 1.000$
	Arc	$t(8) = -0.028$, $p = 1.000$
	Bdnf	$t(8) = 0.074$ $p = 1.000$
	Creb1	$t(8) = 1.209$, $p = 1.000$
	Drd2	$t(8) = 1.411$, $p = 0.980$
	Homer1	$t(8) = 2.694$, $p = 0.162$
Hdh$^{Q150/+}$ untrained ventral striatum vs *Hdh*$^{Q150/+}$ SD trained ventral striatum	*Adora2A*	$t(8) = -0.019$, $p = 1.000$
	Arc	$t(8) = 0.615$, $p = 1.000$
	Bdnf	$t(6.443) = 0.948$, $p = 1.000$
	Creb1	$t(8) = 0.875$, $p = 1.000$
	Drd2	$t(8) = 0.406$, $p = 1.000$
	Homer1	$t(8) = 0.713$, $p = 1.000$

Appendix 1.6. RT-qPCR results for behavioural training-dependent gene expression level changes. Specific two-group comparisons were analysed by independent samples t-tests, with Holm-Bonferroni sequential correction used for multiple hypothesis testing correction. Homogeneity of variances for data was assessed using Levene's test, and the Welch test used in the event of data violating this assumption.



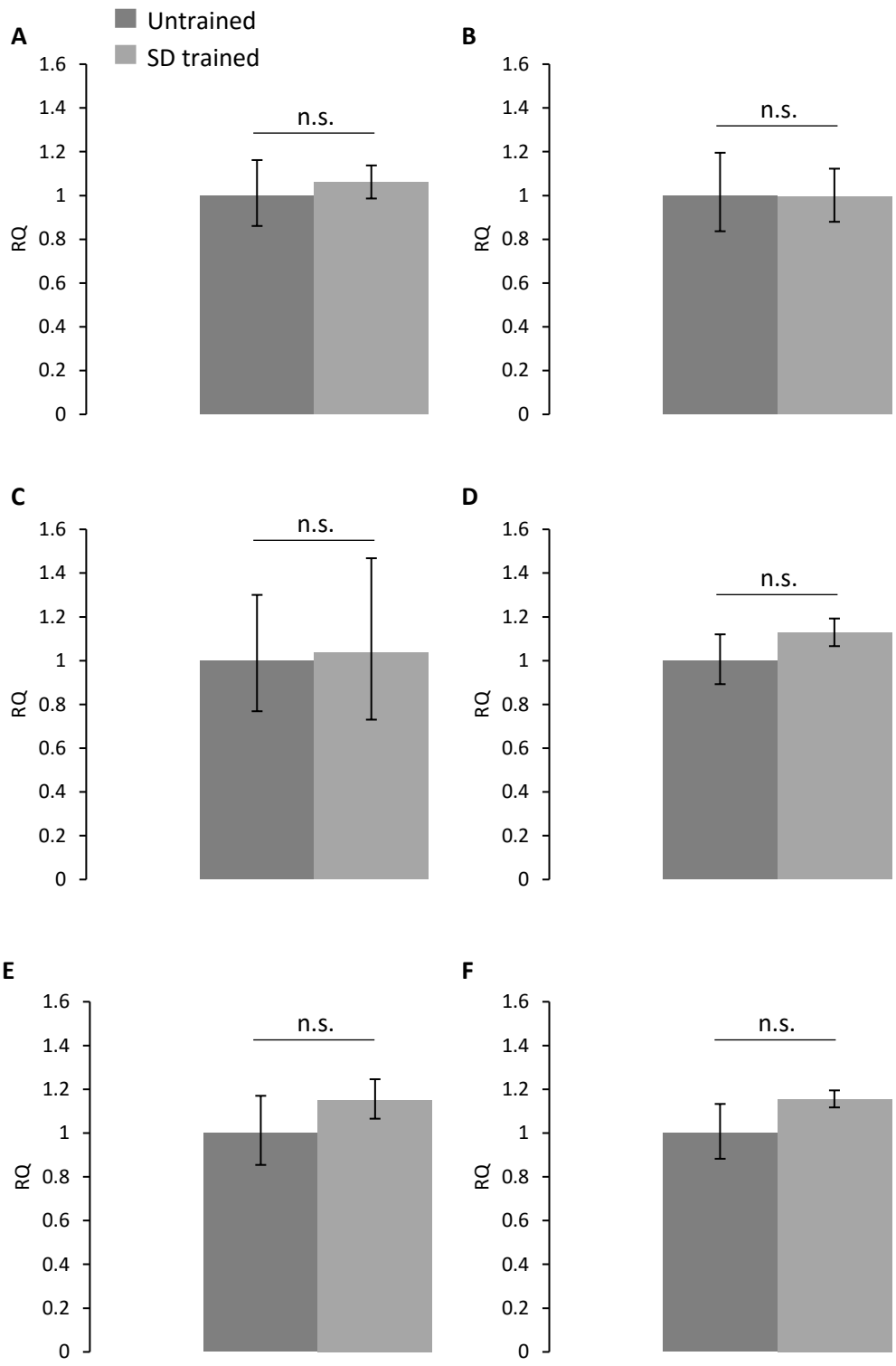
Appendix 1.7. RQ value comparison of *Adora2A* (A), *Arc* (B), *Bdnf* (C), *Creb1* (D), *Drd2* (E), and *Homer1* (F) dorsal striatal expression levels between untrained and SD trained *Hdh*$^{+/+}$ animals following RT-qPCR. A trend of decreased expression levels in the dorsal striatum of SD trained *Hdh*$^{+/+}$ mice is seen for all genes investigated, with the exception of *Creb1* (D). However, all gene expression level differences between groups were found to be non-significant (Appendix 1.6). Values shown are mean ± S.E.M.; n = 5/group.

n.s. = non-significant



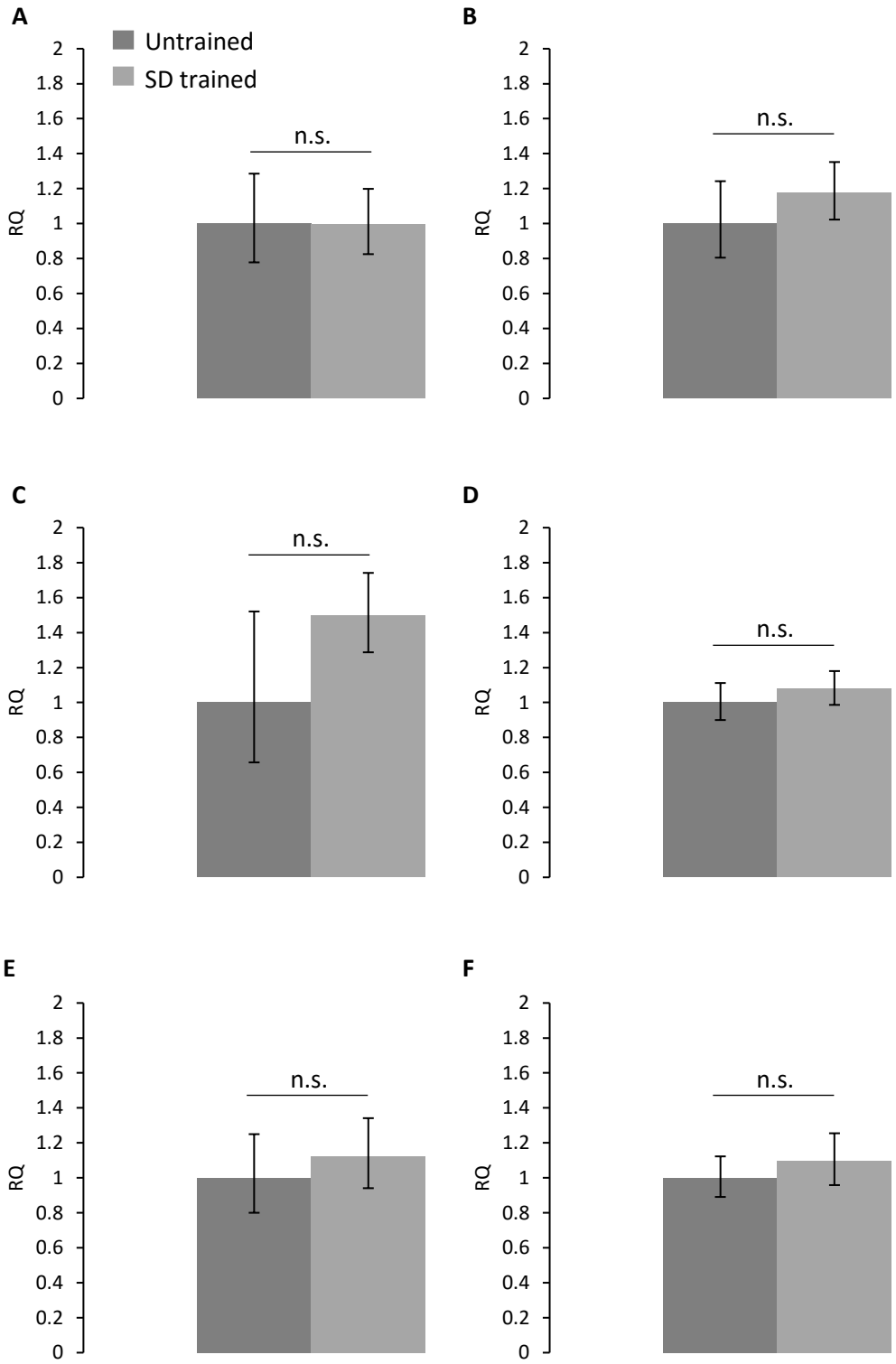
Appendix 1.8. RQ value comparison of *Adora2A* (A), *Arc* (B), *Bdnf* (C), *Creb1* (D), *Drd2* (E), and *Homer1* (F) ventral striatal expression levels between untrained and SD trained *Hdh*$^{+/+}$ animals following RT-qPCR. A trend of decreased expression levels in the ventral striatum of SD trained *Hdh*$^{+/+}$ mice is seen for all genes investigated, with the exception of *Bdnf* (C). However, all gene expression level differences between groups were found to be non-significant (Appendix 1.6). Values shown are mean ± S.E.M.; n = 5/group.

n.s. = non-significant



Appendix 1.9. RQ value comparison of *Adora2A* (A), *Arc* (B), *Bdnf* (C), *Creb1* (D), *Drd2* (E), and *Homer1* (F) dorsal striatal expression levels between untrained and SD trained $Hdh^{Q150/+}$ animals following RT-qPCR. A trend of increased expression levels in the dorsal striatum of SD trained $Hdh^{Q150/+}$ mice is seen for all genes investigated, with the exception of *Arc* (B). However, all gene expression level differences between groups were found to be non-significant (Appendix 1.6). Values shown are mean ± S.E.M.; n = 5/group.

n.s. = non-significant

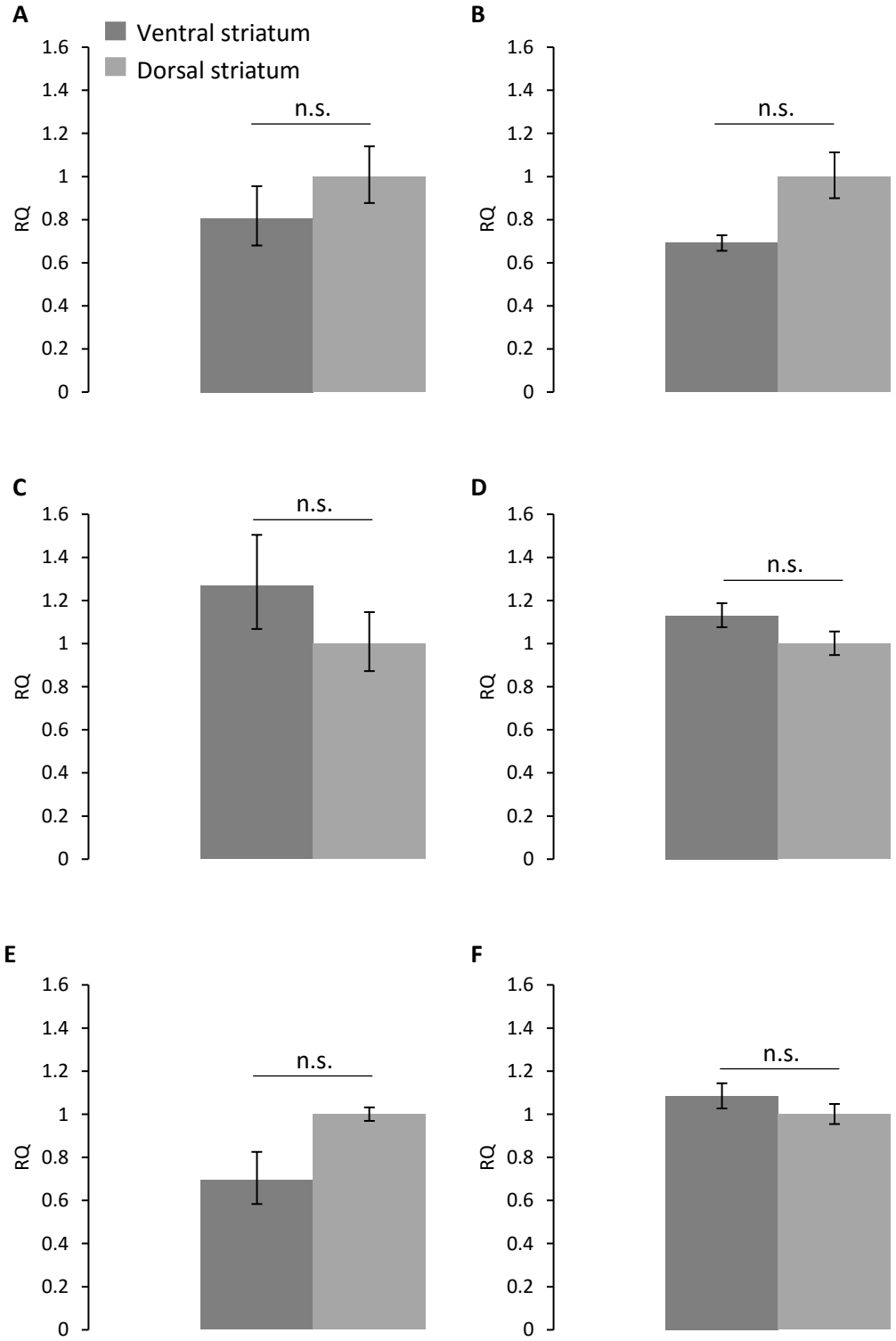


Appendix 1.10. RQ value comparison of *Adora2A* (A), *Arc* (B), *Bdnf* (C), *Creb1* (D), *Drd2* (E), and *Homer1* (F) ventral striatal expression levels between untrained and SD trained *Hdh*$^{Q150/+}$ animals following RT-qPCR. A trend of increased expression levels in the ventral striatum of SD trained *Hdh*$^{Q150/+}$ mice is seen for all genes investigated, with the exception of *Adora2A* (A). However, all gene expression level differences between groups were found to be non-significant (Appendix 1.6). Values shown are mean ± S.E.M.; n = 5/group.

n.s. = non-significant

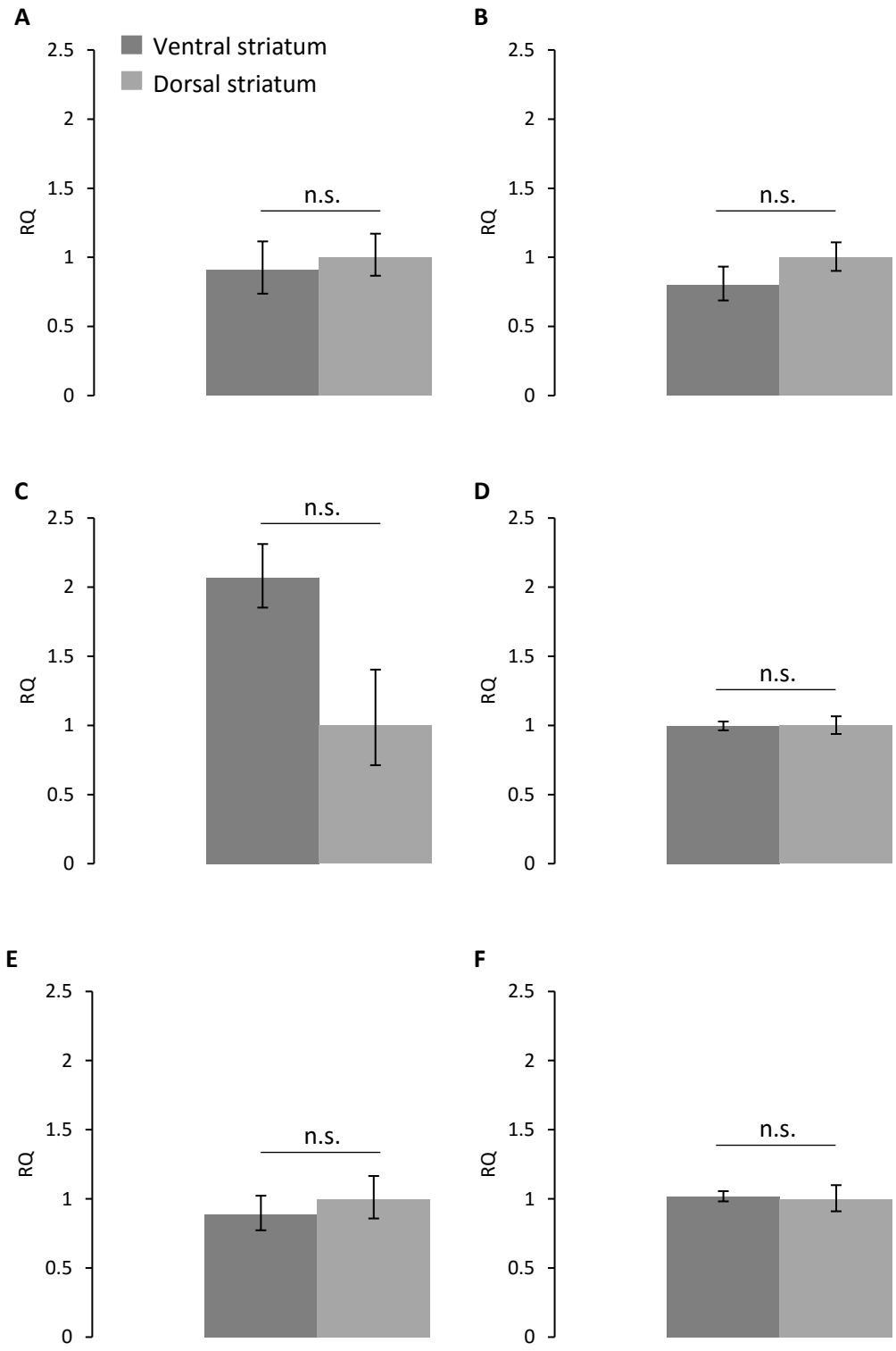
Group comparison	Gene of interest	Statistics
Hdh$^{+/+}$ untrained dorsal striatum vs *Hdh*$^{+/+}$ untrained ventral striatum	*Adora2A*	$t(8) = 1.062$, $p = 0.957$
	Arc	$t(8) = 3.032$, $p = 0.096$
	Bdnf	$t(8) = -0.795$, $p = 0.957$
	Creb1	$t(8) = -1.811$, $p = 0.432$
	Drd2	$t(8) = 1.972$, $p = 0.420$
	Homer1	$t(8) = -0.647$, $p = 0.957$
Hdh$^{+/+}$ SD trained dorsal striatum vs *Hdh*$^{+/+}$ SD trained ventral striatum	*Adora2A*	$t(8) = 0.329$, $p = 1.000$
	Arc	$t(8) = 1.140$, $p = 1.000$
	Bdnf	$t(8) = -2.112$, $p = 0.408$
	Creb1	$t(8) = 0.062$, $p = 1.000$
	Drd2	$t(8) = 0.511$, $p = 1.000$
	Homer1	$t(8) = -0.114$, $p = 1.000$
Hdh$^{Q150/+}$ untrained dorsal striatum vs *Hdh*$^{Q150/+}$ untrained ventral striatum	*Adora2A*	$t(8) = 0.892$, $p = 1.000$
	Arc	$t(8) = 1.246$, $p = 1.000$
	Bdnf	$t(8) = -0.381$, $p = 1.000$
	Creb1	$t(8) = -1.069$, $p = 1.000$
	Drd2	$t(8) = 0.830$, $p = 1.000$
	Homer1	$t(8) = 0.011$, $p = 1.000$
Hdh$^{Q150/+}$ SD trained dorsal striatum vs *Hdh*$^{Q150/+}$ SD trained ventral striatum	*Adora2A*	$t(8) = 2.315$, $p = 0.245$
	Arc	$t(8) = 0.659$, $p = 1.000$
	Bdnf	$t(8) = -1.323$, $p = 0.888$
	Creb1	$t(8) = -0.576$, $p = 1.000$
	Drd2	$t(8) = 2.799$, $p = 0.138$
	Homer1	$t(8) = 0.488$, $p = 1.000$

Appendix 1.11. RT-qPCR results for striatal region-dependent gene expression level changes. Specific two-group comparisons were analysed by independent samples t-tests, with Holm-Bonferroni sequential correction used for multiple hypothesis testing correction. Homogeneity of variances for data was assessed using Levene's test, and the Welch test used in the event of data violating this assumption.



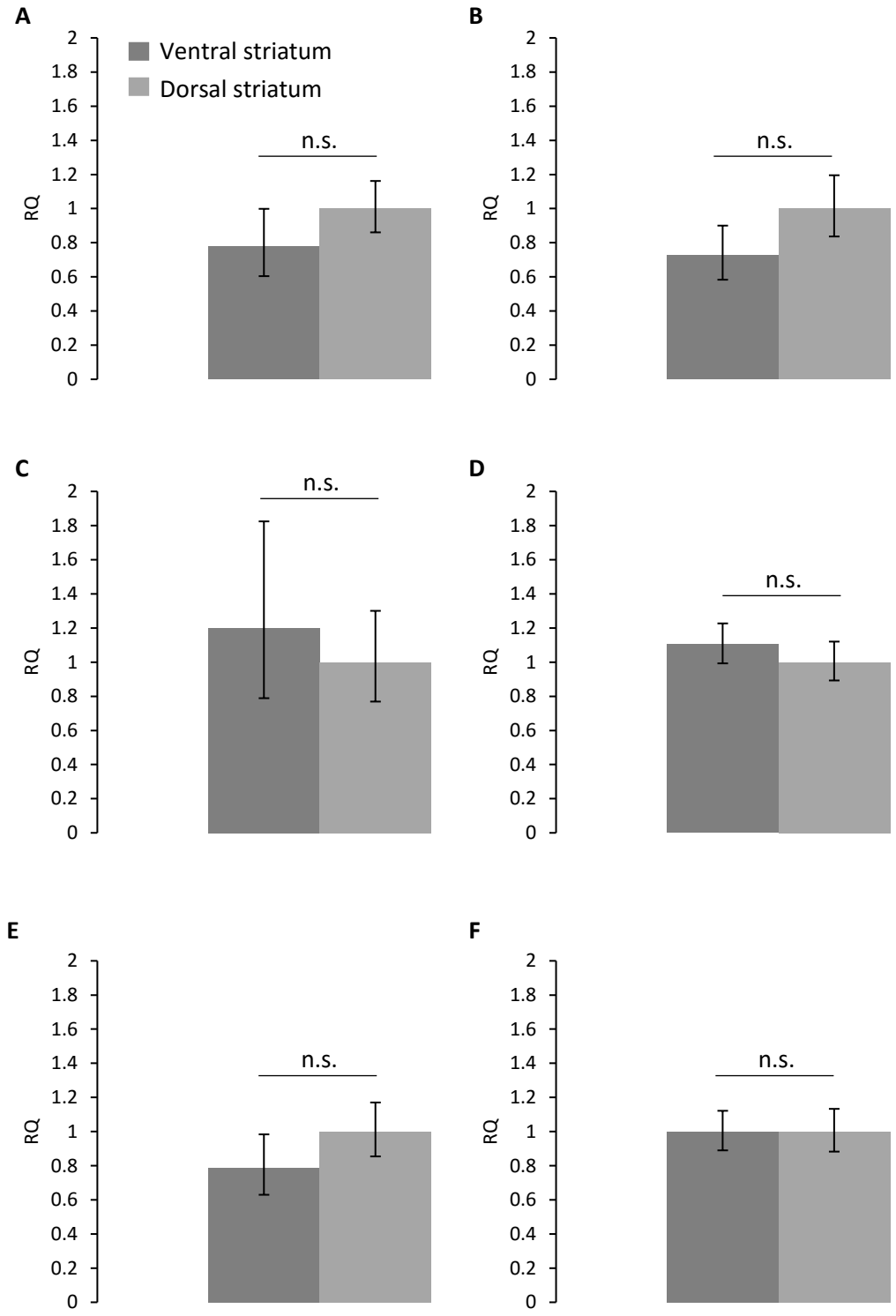
Appendix 1.12. RQ value comparison of dorsal and ventral striatal *Adora2A* (A), *Arc* (B), *Bdnf* (C), *Creb1* (D), *Drd2* (E), and *Homer1* (F) expression levels in untrained *Hdh*$^{+/+}$ animals following RT-qPCR. A trend in expression levels is not apparent between the dorsal and ventral striatum of untrained *Hdh*$^{+/+}$ mice. All gene expression level differences between groups were found to be non-significant (Appendix 1.11). Values shown are mean ± S.E.M.; n = 5/group.

n.s. = non-significant



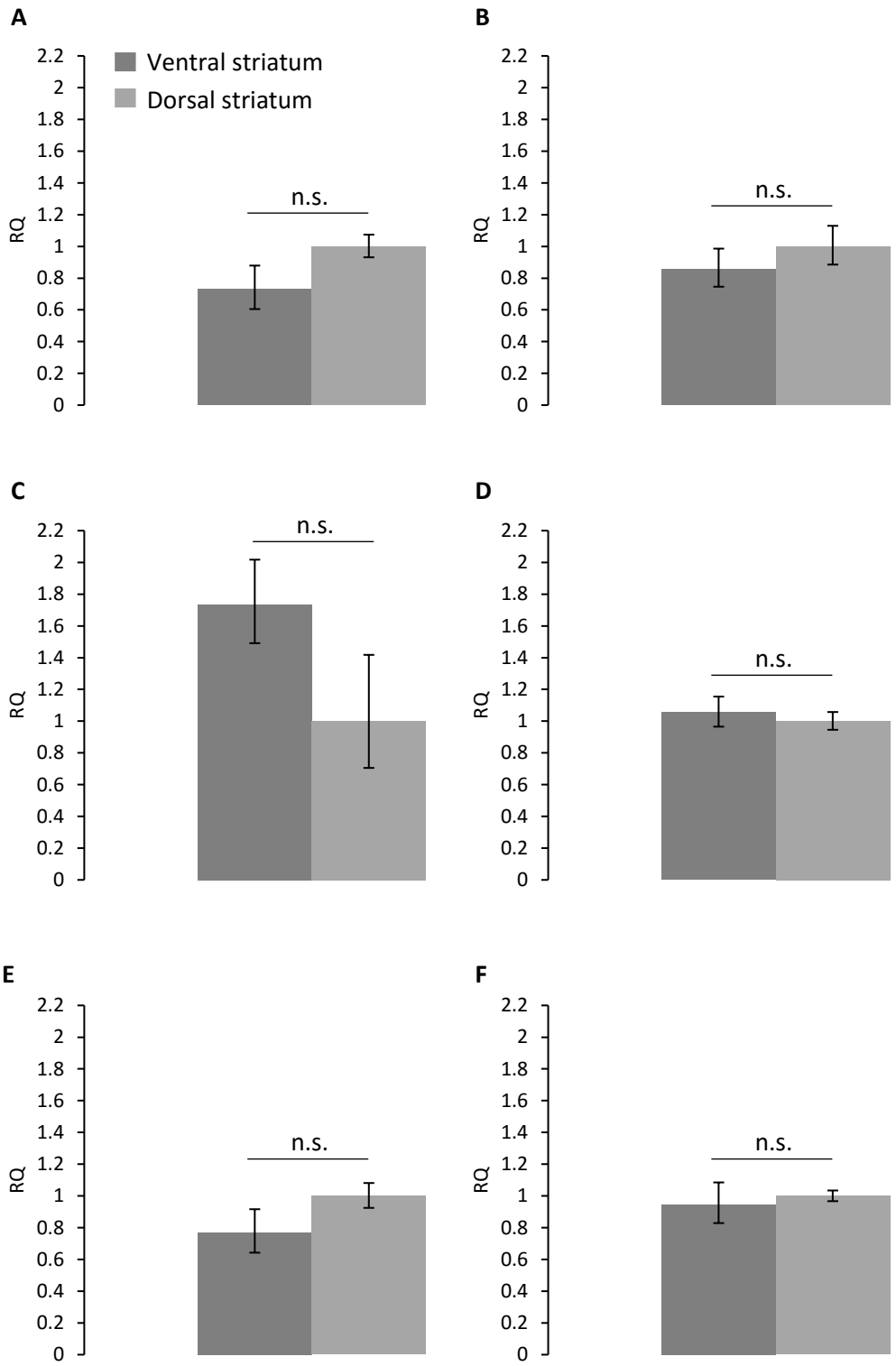
Appendix 1.13. RQ value comparison of dorsal and ventral striatal *Adora2A* (A), *Arc* (B), *Bdnf* (C), *Creb1* (D), *Drd2* (E), and *Homer1* (F) expression levels in SD trained *Hdh*$^{+/+}$ animals following RT-qPCR. A trend in expression levels is not apparent between the dorsal and ventral striatum of SD trained *Hdh*$^{+/+}$ mice. All gene expression level differences between groups were found to be non-significant (Appendix 1.11). Values shown are mean ± S.E.M.; n = 5/group.

n.s. = non-significant



Appendix 1.14. RQ value comparison of dorsal and ventral striatal *Adora2A* (A), *Arc* (B), *Bdnf* (C), *Creb1* (D), *Drd2* (E), and *Homer1* (F) expression levels in untrained *Hdh*$^{Q150/+}$ animals following RT-qPCR. A trend in expression levels is not apparent between the dorsal and ventral striatum of untrained *Hdh*$^{Q150/+}$ mice. All gene expression level differences between groups were found to be non-significant (Appendix 1.11). Values shown are mean ± S.E.M.; n = 5/group.

n.s. = non-significant



Appendix 1.14. RQ value comparison of dorsal and ventral striatal *Adora2A* (A), *Arc* (B), *Bdnf* (C), *Creb1* (D), *Drd2* (E), and *Homer1* (F) expression levels in SD trained $Hdh^{Q150/+}$ animals following RT-qPCR. A trend of decreased expression levels in the ventral striatum of SD trained $Hdh^{Q150/+}$ mice is identified in the genes investigated, with the exceptions of *Bdnf* (C) and *Creb1* (D). However, all gene expression level differences between groups were found to be non-significant (Appendix 1.11). Values shown are mean ± S.E.M.; n = 5/group.

n.s. = non-significant

Interaction	Gene of interest	Statistics
Genotype*Training	*Adora2A*	$F_{1, 32} = 0.590$, $p = 0.448$
	Arc	$F_{1, 32} = 0.947$, $p = 0.338$
	Bdnf	$F_{1, 32} = 0.089$, $p = 0.767$
	Creb1	$F_{1, 32} = 3.531$, $p = 0.069$
	Drd2	$F_{1, 32} = 3.788$, $p = 0.060$
	Homer1	$\mathbf{F_{1, 32} = 8.098, p = 0.008}$
Genotype*Striatal Region	*Adora2A*	$F_{1, 32} = 0.293$, $p = 0.592$
	Arc	$F_{1, 32} = 0.078$, $p = 0.782$
	Bdnf	$F_{1, 32} = 0.220$, $p = 0.643$
	Creb1	$F_{1, 32} = 0.045$, $p = 0.833$
	Drd2	$F_{1, 32} = 0.257$, $p = 0.616$
	Homer1	$F_{1, 32} = .0389$, $p = 0.537$
Training*Striatal Region	*Adora2A*	$F_{1, 32} = 0.014$, $p = 0.905$
	Arc	$F_{1, 32} = 0.578$, $p = 0.453$
	Bdnf	$F_{1, 32} = 0.553$, $p = 0.462$
	Creb1	$F_{1, 32} = 1.090$, $p = 0.304$
	Drd2	$F_{1, 32} = 0.987$, $p = 0.328$
	Homer1	$F_{1, 32} = 0.221$, $p = 0.642$
Genotype*Training*Striatal Region	*Adora2A*	$F_{1, 32} = 0.149$, $p = 0.702$
	Arc	$F_{1, 32} = 0.002$, $p = 0.963$
	Bdnf	$F_{1, 32} = 0.006$, $p = 0.940$
	Creb1	$F_{1, 32} = 0.253$, $p = 0.618$
	Drd2	$F_{1, 32} = 1.199$, $p = 0.282$
	Homer1	$F_{1, 32} = 0.002$, $p = 0.966$

Appendix 1.16. RT-qPCR results for striatal gene expression level change independent variable interactions. RT-qPCR data was analysed by a three-way ANOVA, with genotype (*Hdh*$^{+/+}$ or *Hdh*$^{Q150/+}$), training (untrained or SD) and striatal region (dorsal or ventral) as independent variables. The test in which a significant interaction was identified is highlighted in bold.