A Phenotypic Characterisation of the Hdh^{Q111} Mouse Model of Huntington's Disease.

This thesis is submitted for the degree of Doctor of Philosophy at Cardiff University

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September 2015

Dedicated to the memory of my grandparents; Alice, Roy, Pat and Ted.

Thesis Summary

The work presented in this thesis focuses on a behavioural and histological characterisation of the Hdh^{Q111} mouse model of Huntington's disease (HD). Numerous mouse models of HD are currently available; however, before their use, each must be fully understood, validated and characterised, which will allow the most appropriate model to be used in scientific research.

Chapter 1 provides a general introduction. **Chapter 2** states the materials and methods used in this thesis. The work presented in **Chapter 3** encompasses an extensive longitudinal immunohistological characterisation of the $Hdh^{Q111/+}$ mouse model, which includes immunohistochemical stains from animals at 3, 6, 9, 12, 15 and 18 months of age, to understand the development of the underlying neuropathology associated with this mouse model. **Chapter 4** builds on the knowledge gained from the previous chapter to explore the longitudinal progression of motor symptoms associated with the $Hdh^{Q111/+}$ mouse model.

In **Chapter 5** a longitudinal operant battery was conducted to explore cognitive dysfunction in the $Hdh^{Q111/+}$ mouse model. Operant tests of motivation, attention and implicit learning were conducted longitudinally at 6, 12 and 18 months of age. **Chapter 6** uses the knowledge gained from Chapter 5 to explore the effects of operant pre-training, at a young age, and the impact that this has on latter operant task performance and the development of the disease phenotype at an older age. To further understand the cognitive deficits observed in the $Hdh^{Q111/+}$ mouse model, **Chapter 7** presents the creation, optimisation and utilisation of an operant delayed matching and delayed non-matching to position (DMTP/DNMTP) task, which could be used to test working memory and reversal learning in mouse models of neurological diseases including HD. **Chapter 8** includes a general discussion of the work presented in this thesis.

The studies presented within this thesis demonstrate the development of a clear neuropathological profile as well as significant motor and cognitive deficits in the Hdh^{Q111} mouse model of Huntington's disease. We can therefore conclude that the Hdh^{Q111} mouse model is a valid mouse model for investigating the pathogenic mechanisms of the disease and for testing therapeutic interventions in HD.

Acknowledgements

I would firstly like to thank Steve and Anne for allowing me to become part of the Brain Repair Group (BRG), it has been an absolute pleasure to work in such a fantastic lab over the past 3 years. To all members of the BRG past and present, thank you all so much for helping me along the way. You have been great colleagues whom I can now call friends.

Particular thanks to my supervisors Steve and Simon. Steve, your vast knowledge is incredible, thank you so much for your support and wise words of encouragement over the last few years. Simon, thanks for your supervision and well done for managing to get me on a plane to America!

Thanks to Anne-Marie, Kate and Ngoc-Nga for your help, advice, enjoyable coffee time discussions and many many laughs. Particular thanks to Jane for your histology guidance, you are really missed. A big thanks to Mariah for your invaluable advice, knowledge and fantastic estate agent skills, you are a true superstar. Sophie, thank you for proof reading chapters, you have really helped me through the past couple of months, thank you. Zubeyde, thank you for teaching me lots of Turkish words, for your encouragement and advice and for all of your help with stereology.

To former PhD students and office mates; Andi, Lu, Amy, Vicky, Jess and Nan thank you for your support and believing that I would get there, in the end! Yat 'I have got no problems with that' Patel thank you for all of your help with mousey things, your advice and support, proof reading my chapters and another big thanks for being the lab taxi service! Claris thanks for arranging lots of fun lab activities, keep going and you will get there. Damla, thank you for believing in me and being a great friend, I can't wait to visit you in Canada soon.

To my current office mates, Harri, Susanne and David. Thanks for all of the laughs; jelly bean roulette, the song game and the fish tank have all been particular highlights. Keep ploughing on, you will all get there in the end and I will be the first to buy you a celebratory drink ☺. J-Sco (the abominable Scoman) keep at it, we are almost at the finish line, you can do it!

To the students that have come and gone in the BRG; Amber, Dean, Harry and Llinos thanks for the pub trips, meals out and for bringing lots of fun into the lab. You will all be brilliant in whatever you choose to do, best of luck for the future.

Susanne, Alison, Laura, Hannah and Lucy thank you for our many pub trips, dinners and BBQs. You guys are always able to take my mind of things and provide wine, whether I need it or not! You are all great friends, thank you for all being there for me whenever I needed it, you will all get there soon I have no doubts ☺.

To everyone in JBIOS, thank you for looking after my many mice. Special thanks to Sarah, Nicole and Alison for cleaning, feeding and watering and to Pat, Mike and Nicky for your help and advice.

Atilla, thanks for putting up with me over the past few months, for reminding me that I need to eat and sleep but more importantly to have fun. I wouldn't have been able to do this without you and I can't wait to support you on the way to becoming Dr. Randle!

Finally, thanks to my family, Mum, Dad, Claire and Kate, although I don't get to see you as much as I would like to, I know that you are always there at the end of a telephone. Claire and Kate, thanks for being there from the beginning and for being awesome sisters who rally round in times of need, I miss you both lots, come and visit soon!

Mum and Dad, thanks for always believing in me from the very beginning and supporting me every step of the way. I would not be the person I am today without you both, thanks for celebrating the good times and picking up the pieces in the not so good times. You have both done an incredible job bringing the three of us up and I will always be eternally grateful to you for your guidance, support and love.

Achievements during PhD

Publications

Yhnell, E., Dunnett, S.B., and Brooks, S.P. The utilisation of operant delayed matching and non-matching to position for probing cognitive flexibility and working memory in mouse models of Huntington's disease. *Journal of Neuroscience Methods* (2015) doi: 10.1016/j.jneumeth.2015.08.022

Yhnell, E., S. B. Dunnett, and S. P. Brooks. C04 A Longitudinal Behavioural Characterisation of the HdhQ111 Mouse Model of Huntington's Disease. *Journal of Neurology, Neurosurgery & Psychiatry* 85.Suppl 1 (2014): A26-A26.

Yhnell, E., Dunnett, S.B., and Brooks, S.P. The development of operant delayed matching to position for Huntington's disease mouse models. *NEUROREPORT*. Vol. 25. No. 3. 530 Walnut St, Philadelphia, PA 19106-3621 USA: Lippincott Williams & Wilkins, 2014.

Yhnell, E., Lelos, M.J., Brooks, S. P., and Dunnett, S.B.D. Cognitive training improves disease symptoms in the Hdh^{Q111} mouse model of Huntington's disease. *Manuscript in preparation.*

Travel Grants

Travel award (€200) to attend 24th Annual Meeting of the Network of European CNS Transplantation and Restoration (NECTAR), Galway, Ireland, 2014.

Travel Grant (£800) from Guarantors of Brain Travel to attend the Hereditary Disease Foundation (HDF) Conference in Boston, Massachusetts, 2014.

Prizes and Awards

The Biochemical Society Scientific Communication Writing Award, 2015 (Available: http://www.biochemistry.org/GetInvolved/ScienceCommunicationCompetition.aspx)

Best PhD talk at Careers in Neuroscience Satellite Event for NECTAR, Galway, 2014.

Peoples Award: Best Talk at Speaking of Science, Cardiff University, 2014.

Poster Prize for Neuroscience, Cardiff University, 2014.

Associate Fellow of the Higher Education Academy, 2013

Finalist for the Max Perutz Science Writing Award, MRC London, 2013
(Available at: http://www.mrc.ac.uk/documents/pdf/max-perutz-essays-2013/).

Judges Award: Best Talk at Speaking of Science, Cardiff University, 2013.

Biology Researchers in Schools Certificate, Cardiff University, 2013.

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Abbreviations

BAC	Bacterial artificial chromosome
CAG	Cytosine, Adenine and Guanine
CB1	Cannabinoid receptor
CNS	Central nervous system
D1	Dopamine 1 receptor
D2	Dopamine 2 receptor
DMTP	Delayed Matching to Position
DMNTP	Delayed Non-Matching to Position
FR	Fixed Ratio
5-CSRTT	Five - Choice Serial Reaction Time Task
GABA	gamma-aminobutryic acid
GPe	Globus pallidus external
GPi	globus pallidus internal
HD	Huntington's disease
HHT	Human huntingtin gene
HHT	Human huntingtin protein
Htt	Rodent huntingtin gene
Htt	Rodent huntingtin protein
IT15	Interesting transcript 15
JHD	Juvenile Huntington's disease
mHHT	Human mutant huntingtin gene
mHHT	Human mutant huntingtin protein
mHtt	Rodent mutant huntingtin gene
mHtt	Rodent mutant huntingtin protein
MoCA	Montreal Cognitive Assessment
MMSE	Mini Mental State Examination
MRI	Magnetic resonance imaging
MRS	Magnetic resonance spectroscopy
MSN	Medium spiny neurons
NII	Neuronal Intra-nuclear Inclusions
PR	Progressive Ratio
QA	Quinolinic acid

RPM	Revolutions per minute
S1	Stimulus light 1
S2	Stimulus light 2
SCA17	Spinocerebellar ataxia 17
SHIRPA	SmithKline Beecham, Harwell, Imperial College, Royal London Hospital, Phenotype Assessment
SILT	Serial Implicit Learning Task
SNc	substantia nigra compacta
SNr	substantia nigra reticulata
STN	subthalamic nucleus
TMS	Total motor score
TOI	Time out intervals
UHDRS	Unified Huntington's Disease Rating Scale
UPS	Ubiquitin proteosome system
WT	Wild Type
YAC	Yeast artificial chromosome

Chapter 1 : General Introduction

1.1. Background

The earliest recorded description of Huntington's disease (HD) was by Charles Oscar Waters and dates back to 1842, in a letter published in Robley Dunglison's *Practice of Medicine*. Waters accurately described a movement disorder, including its progressive chorea and hereditary nature. In addition to Waters, other early descriptions of the disease were produced by Charles Gorman in 1846, who described the occurrence of HD in localised geographical regions, and Johan Christian Lund in 1860, who described dementia associated with jerking movements causing chorea that had a hereditary nature.

However, the most widely acknowledged first description of HD was published in 1872 by the American physician George Huntington. Huntington clearly described patients that exhibited 'dance like movements' or chorea and accurately postulated cognitive deficits in patients as well as the hereditary nature of the disease (Huntington 1872). The worldwide prevalence of HD is now estimated to be approximately 3 per 100 000 (Harper 1992; Pringsheim *et al.* 2012). The disease is more common in European, American, and Australian populations where prevalence can be as high as 1 in 10 000 (McCusker *et al.* 2000; Orth 2011; Pringsheim *et al*. 2012; Evans *et al.* 2013). However, it should be considered that the estimated prevalence of the disease is heavily affected by the number of people presenting to the medical profession, the associated stigma of the disease and health care provisions available in particularly countries. Therefore, the estimated prevalence of the disease is likely to be an underestimation of those who are affected globally.

HD is an autosomal dominant inherited disorder which typically occurs in the third and fourth decades of life (Duyao *et al.* 1993). The progressive nature of the disease means symptoms will lead to death fifteen to twenty years after clinical diagnosis (Sørensen and Fenger 1992b; Vonsattel and DiFiglia 1998).

However, with increasing; awareness, understanding and therefore diagnosis of the disease, the time between diagnosis and mortality is often greater than has been previously predicted. Although some, limited, symptomatic treatments for HD are available (Ondo *et al.* 2002; Adam and Jankovic 2008; Mestre *et al.* 2009) reviewed in (Bonelli and Wenning 2006; Bonelli and Hofmann 2007), there is currently no cure.

1.2. The Genetic Cause of Huntington's Disease

The hereditary, autosomal dominant, nature of HD was postulated in the original descriptions of the disease (Waters 1842; Lund 1860; Huntington 1872). However, it was not until 1983 that the possible genetic cause of the disease was localised to chromosome 4p16.3 (Gusella *et al.* 1983). The precise genetic cause of HD is now known to be a trinucleotide polyglutamine repeat expansion of the nucleotides cytosine, adenine and guanine (CAG) within the first exon of the *huntingtin* gene (*HTT*) on the short arm of chromosome 4 (MacDonald *et al.* 1993). *HTT* is a large gene which spans ~210kb and contains 67 exons. *HTT* contains an open reading frame for a polypeptide of over 3140 residues, known as the huntingtin protein (HTT) (MacDonald *et al*. 1993).

The essential role of HTT in embryonic development and neurogenesis (White *et al.* 1997) has been demonstrated in knock-out mouse models (Nasir *et al.* 1995) and *HTT* gene inactivated models (Duyao *et al.* 1995; Zeitlin *et al.* 1995), which both exhibit embryonic lethality. Although attempts have been made to deduce the structure of HTT using alignment, modelling and crystallography studies (Poirier *et al.* 2005; Kelley *et al.* 2009; Kim *et al.* 2009), the exact structure, and therefore, function of the protein is still unknown. This is at least in part, due to the large size of the protein and the vast number of possible protein interactions (Wanker *et al.* 1997; Harjes and Wanker 2003; Li and Li 2004).

1.2.1. The Mutant Huntingtin Protein

Upon successful transcription and translation, the full length mutant huntingtin protein molecule (mHTT) is thought to undergo proteolysis, by either caspase or

calpain (Wellington *et al.* 2000; Wellington *et al.* 2002; Graham *et al.* 2006) due to the overlapping cleavage sites (Gafni *et al.* 2004). Proteolysis produces soluble monomeric fragments of mHTT (Kim *et al.* 2001; Landles *et al.* 2010) including N-terminal fragments which are thought to be more toxic than the full length mHTT (Hackam *et al.* 1998). Once formed, mHTT monomers increase in cytoplasmic concentration until a threshold concentration has been reached (Olshina *et al.* 2010). When the threshold value is reached monomers dimerise to form soluble intermediate oligomers which are in equilibrium with the monomer population (Ossato *et al.* 2010). Monomers and oligomers react together, via the formation of stabilising hydrogen bonds and covalent bonds between the glutamine residues of one mHTT and the amine residues of another, via the action of transglutaminase, to catalyse the formation of aggregate bodies both within the cytoplasm and the cellular nucleus (Zainelli *et al.* 2003; Landles *et al*. 2010; Marcellin *et al.* 2012), as shown in Figure 1.1. The aggregation of N-terminal fragments of mHTT within nuclear inclusions is thought to be proportional to the size of the CAG repeat length (Krobitsch and Lindquist 2000; Jana *et al.* 2001).

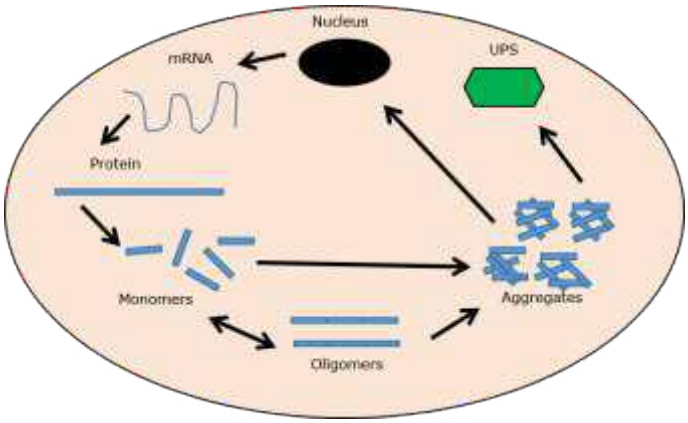

Figure 1.1. Schematic representation of aggregate formation in Huntington's disease. Once mutant huntingtin (*mHTT*) has been transcribed and translated, the protein is cleaved into soluble monomers, which are in equilibrium with the oligomeric pool which they form. Aggregate bodies are then formed which may be targeted to the ubiquitin proteosome system (UPS) for subsequent degradation via ubiquitination or transported to the nucleus.

The precise molecular role of mHTT, in neurodegeneration, and the associated effects of the protein, remains unclear. The expansion of CAG repeats, produces an abnormal three-dimensional protein structure which alters both the intra and

inter molecular interactions of the mutant protein (Huang *et al.* 1998; Nekooki-Machida *et al.* 2009). Despite a plethora of research investigating the structure, function and interaction of the mHTT, the precise sequence of events which cause the mutant protein to lead to the manifestation of the disease remains unclear.

1.2.2. CAG Repeat Length and Huntington's Disease

The precise number of CAG repeats within the mutant huntingtin gene (m*HTT*) has been shown to be a crucial factor in determining disease severity, penetrance and age of onset (Andrew *et al.* 1993; Duyao *et al*. 1993; Brandt *et al.* 1996; Brinkman *et al.* 1997a; Penney *et al.* 1997; Rosenblatt *et al.* 2006). The range of CAG repeats in unaffected individuals is typically 19-35, repeat lengths of 36-41 are considered to be within the range of low penetrance and often demonstrate variable penetrance (Langbehn *et al.* 2004), whereas repeat lengths of over 42 are considered to be pathogenic to the patient, often producing the full HD phenotype (Brinkman *et al.* 1997b; Menalled and Chesselet 2002). Estimates suggest that up to 90% of affected individuals are thought to have CAG repeat lengths in the rage of 40-50 repeats (Wexler 2004). The CAG trinucleotide repeat length observed in HD patients generally inversely correlates with the age of disease onset (Andrew *et al.* 1993). However, there is often a large degree of variation in age of onset and the range of symptoms exhibited, despite similarities in CAG repeat length (MacMillan *et al.* 1993).

Although, the number of CAG repeats is a critical factor in determining age of symptom onset, it has been suggested that CAG repeat length may account for only 40% of the variation in the age of disease onset (Wexler 2004). Therefore, other genetic, neurobiological and environmental factors have also been associated with disease progression and age of onset (Duyao *et al*. 1993; Kieburtz *et al.* 1994; Leeflang *et al.* 1995; Brinkman *et al*. 1997b; Wexler 2004; Andresen *et al.* 2007a; Andresen *et al.* 2007b; Gayán *et al.* 2008; Brocklebank *et al.* 2009). A particularly interesting example of this involves cases of monozygotic twins. Many studies have demonstrated that monozygotic twins

differ in the age of symptom onset, disease severity and disease progression despite both twins possessing an identical number of CAG repeats (Myrianthopoulos and Rowley 1960; Bird and Omenn 1975; Georgiou *et al.* 1999; Anca *et al.* 2004; Gomez-Esteban *et al.* 2006). While the reasons for this remain unclear, environmental, neurodevelopmental and epigenetic factors are all considered as possible reasons to explain the differences demonstrated in cases of monozygotic twins with HD (Petronis and Kennedy 1995; Crow 1997; Ketelaar *et al.* 2012) .

Genetic anticipation is demonstrated in HD and longer repeat lengths are often found in offspring in comparison to parents (Ranen *et al.* 1995). Molecularly, this has been attributed to the presence of a meitotically unstable intermediate allele (Goldberg *et al.* 1993), which can cause both sporadic and inherited cases of HD (Myers *et al.* 1993). Genetic anticipation is particularly prevalent in those cases which are passed through the male germ line (Erickson 1985; Ridley *et al.* 1988) and this is thought to be due to larger variations in CAG repeat length in sperm (Duyao *et al.* 1993; Telenius *et al.* 1995).

1.3. The Neuropathology of Huntington's Disease

1.3.1. The Structure of the Striatum

HD predominantly affects the striatum of the basal ganglia. In primates the basal ganglia comprises the striatum (including the caudate nucleus and putamen, which are divided by the internal capsule), the ventral striatum (nucleus accumbens and olfactory tubercle) and the internal and external segments of the globus pallidus (GPi and GPe, respectively). The pars reticulata and pars compacta of the substantia nigra (SNr, and SNc respectively) and the subthalamic nucleus (STN) are also functionally connected to the basal ganglia (Parent and Hazrati 1995). Although similar, the rodent basal ganglion lacks an internal capsule that divides the caudate nucleus from the putamen, thus the rodent striatum is a uniform structure, which has a similar functional organisation to the primate forebrain (Parent 1990).

1.3.2. Signal Transduction in the Striatum

Familiarity of the corticostriatal circuitry of the brain is essential in understanding the causes of HD. Transmission of neuronal signals through the basal ganglia, including the striatum, can occur via two pathways; the direct pathway and indirect pathways, as seen in Figure 1.2.

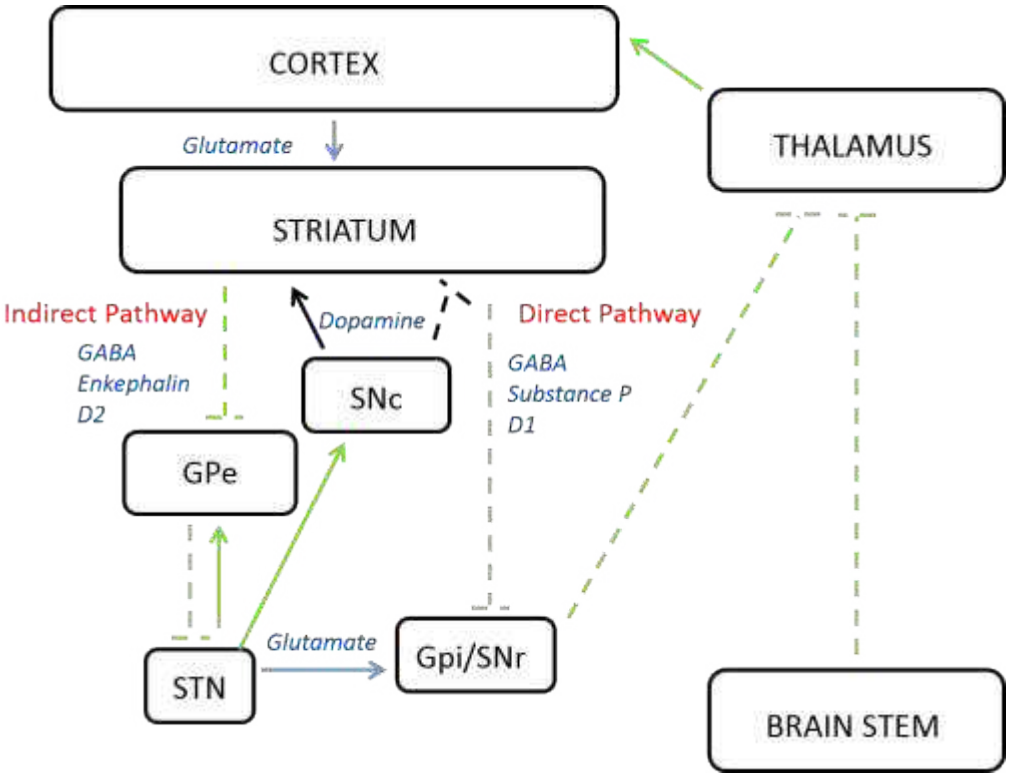

Figure 1.2. Connectivity map to show the direct and indirect pathways of the basal ganglia. Solid arrows represent excitatory connections and dashed arrows represent inhibitory connections. Green arrows indicate GABAergic signals, blue arrows represent glutamatergic signals and black arrows indicate dopaminergic signals. Excitatory efferent signals from cortical neurons stimulate the excitatory action of the direct pathway and dampen the inhibitory activity of the indirect pathway. (Abbreviations: D1: dopamine receptor 1; D2: dopamine receptor 2; GPe: globus pallidus extrenal; GPi: globus pallidus internal; STN: subthalamic nucleus; SNc: substantia nigra compacta; SNr: substantia nigra reticulata).

In the direct pathway glutamatergic input from the cortex to the striatum inhibits GABAergic neurons resulting in an excitatory cortical input into the motor cortex, the GPi and the thalamus. In the indirect pathway the glutamatergic input from the cortex to the striatum inhibits GABAergic release causing an inhibition of the action of the GPe. This causes excitatory activity from the STN to the

GPi/SNR, leading to the repression of excitatory signals from the thalamus and cortex and resulting in inhibition of the excitatory signals received by the motor cortex (Alexander *et al.* 1991). Dopamine 2 (D2) receptors are predominantly expressed in the indirect pathway and dopamine 1 (D1) receptors are expressed in the direct pathway (Gerfen *et al.* 1990; Gerfen *et al.* 1995).

In addition, the SNc projects a dopaminergic output into the basal ganglia, allowing the modulation of both the direct and indirect pathways. The direct and indirect pathways act via opposing mechanisms, with the direct pathway promoting movement and the indirect pathway preventing movement. Therefore, it is the balance of both pathways which controls the excitation and inhibition of motion (Albin *et al.* 1989; Surmeier *et al.* 2007). Both the direct and indirect pathways require careful regulation and modulation to allow striatally mediated functions, such as emotion, cognition and movement to occur in a controlled manner (Mink and Thach 1993). However, if the careful regulation and delicate balance between the direct and indirect pathways is lost, disease states such as Parkinson's disease and Huntington's disease can result (Cepeda *et al.* 2007), as shown in Figure 1.3.

1.3.3. Dysfunction of the Striatum in Huntington's Disease.

The medium spiny neuron (MSN) degradation, described in HD (Deng *et al.* 2004; Starr *et al.* 2008), results in a reduction in the action of the indirect pathway, which reduces overall inhibitory control, as the STN becomes more inhibited, this reduces excitatory stimulation to the GPi which in turn has a less inhibitory influence on the thalamus. Therefore, an increase in glutamatergic feedback to the cortex causes an over activation of the cortico-striato-thalamic feedback loop and this causes a lack of motor control as demonstrated in symptomatic HD patients (Hallett 1993; Graybiel *et al.* 1994), as shown in Figure 1.3.

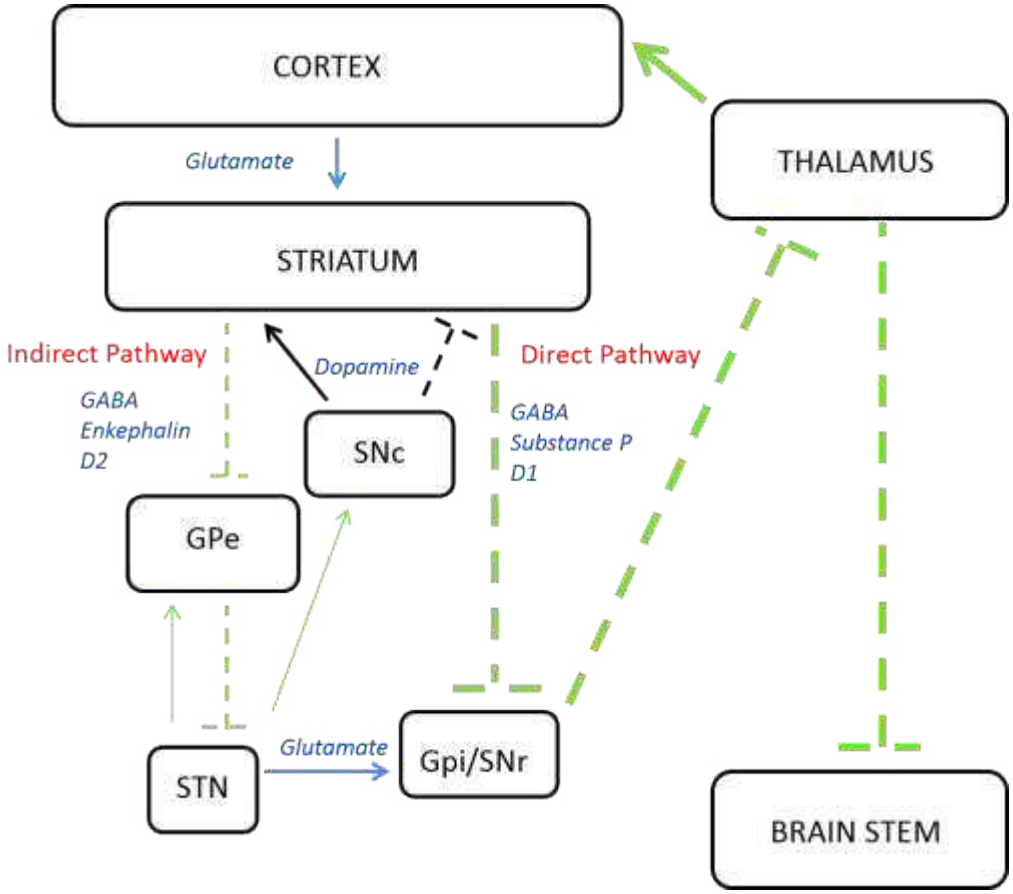

Figure 1.3. Connectivity map to show the alterations of the direct and indirect pathways in Huntington's disease. Solid arrows represent excitatory connections and dashed arrows represent inhibitory connections. Green arrows indicate GABAergic signals, blue arrows represent glutamatergic signals and black arrows indicate dopamine signals. Thicker arrows indicate a greater influence whereas thinner arrows indicate a decreased influence of the pathway. Excitatory efferent signals from cortical neurons stimulate the excitatory action of the direct pathway and dampen the inhibitory activity of the indirect pathway. (Abbreviations: D1: dopamine receptor 1; D2: dopamine receptor 2; GPe: globus pallidus extrenal; GPi: globus pallidus internal; STN: subthalamic nucleus; SNc: substantia nigra compacta; SNr: substantia nigra reticulata).

Although the indirect pathway (which is involved in preventing movement, as described above) is predominantly affected in the early symptomatic stages of HD, as the disease progresses choraic movements are often replaced by bradykinesia (Thompson *et al.* 1988; Berardelli *et al.* 1999b). The hypoactive phenotype reflects a more widespread neurodegeneration that includes loss of striatal MSNs and the D1 receptors of the direct pathway which causes a general loss of motor tone throughout the motor circuits (Thompson *et al*. 1988; Phillips *et al.* 1996; Berardelli *et al*. 1999b).

1.3.4. Gross Neuropathology of Huntington's Disease

Post mortem evidence, in human HD brains, generated an initial understanding of the gross neuropathology of HD in human patients, including cell loss and reduced size of the caudate and putamen (Lange *et al.* 1976; Vonsattel *et al.* 1985; Vonsattel and DiFiglia 1998). Although the caudate and putamen are the most obvious sites of neurodegeneration in HD, it is now being increasingly recognised, particularly through the use of MRI studies (Reading *et al.* 2004; Rosas *et al.* 2008; Tabrizi *et al.* 2009), that other brain regions including the cerebral cortex, cerebellum and hippocampus are also affected early in HD disease progression (Braak and Braak 1992; Heinsen *et al.* 1996; Rosas *et al*. 2008). The involvement of the cerebral cortex in HD was noted by Vonsattel who first introduced a grading system for scoring the severity of the neuropathology demonstrated in brain autopsies of HD patients (Vonsattel *et al.* 1985). Vonsattel graded the severity of neuropathology from 0-4; the higher the score the more severe the phenotype, patients with scores of 3 and 4 typically exhibited neurodegeneration of the cerebral cortex as well as the striatum (Vonsattel *et al*. 1985).

Recent advances in neuroimaging techniques have allowed *in vivo* brain atrophy measures to be correlated with disease stage and behavioural changes, to give a greater understanding of HD and the progression of the disease. Numerous imaging studies have concluded that structural changes in the brain, including; whole brain atrophy, caudate atrophy, white matter atrophy and grey matter atrophy, (Rosas *et al.* 2003; Reading *et al.* 2005; Henley *et al.* 2006; Rosas *et al.* 2008; Henley *et al.* 2009; Tabrizi *et al.* 2009; Hobbs *et al.* 2010; Tabrizi *et al.* 2011), can occur often decades prior to symptom onset. In addition, neuroimaging studies were included in the TRACK-HD study, which followed HD patients for three years in order to assess measurements which were suitable to use as 'outcome measures' in clinical trials. The TRACK-HD study allowed the correlation of functional change to symptom progression, disease burden and behavioural change (Tabrizi *et al.* 2009; Tabrizi *et al.* 2011; Tabrizi *et al.* 2013).

Therefore, there is now emerging evidence that HD is a disease of the whole brain rather than simply the striatum.

1.3.5. Cellular and Molecular Features of Huntington's Disease

HD is caused by a specific loss of MSNs within the striatum of the basal ganglia (Deng *et al*. 2004; Starr *et al.* 2008) which results in interruption of the corticostriatal circuitry (Figure 1.3). The MSNs of the striatum project GABAergic connections into the SNr and the internal and external segments of the globus pallidus and secrete the inhibitory neurotransmitter γ-aminobutryic acid (GABA), which dampens the activity of post-synaptic target neurons. In HD, degradation of the MSNs in the striatum, is indicated by a reduction in the expression of numerous receptor subtypes including; D1 and D2 receptors (Weeks *et al.* 1996a; Surmeier *et al.* 2007; Crook and Housman 2012), CB1 receptors (Denovan-Wright and Robertson 2000; Glass *et al.* 2000; Lastres-Becker *et al.* 2002; Glass *et al.* 2004; McCaw *et al.* 2004), NMDA receptors (Arzberger *et al.* 1997; Luthi-Carter *et al.* 2003) and TrkB receptors (Ginés *et al.* 2006). An alteration in the levels of the receptor subtypes produced by MSNs disrupts the delicate balance of the direct and indirect pathways and alters neuronal transmission. In addition, some studies have shown the loss of both D1 and D2 receptors in the striatum of asymptomatic HD patients (Seeman *et al.* 1989; Weeks *et al.* 1996b; Augood *et al.* 1997) thus indicating neuronal dysfunction prior to symptom onset.

Molecular aggregates that develop into neuronal intranuclear inclusions (NIIs) of mutant huntingtin protein, have been shown to form in neurons of HD affected individuals (Vonsattel *et al*. 1985; Sieradzan *et al.* 1999), often prior to the onset of symptoms (Gómez Tortosa *et al.* 2001). The precise cause of the formation of NIIs is still under debate. Many studies have indicated that inclusion formation is a toxic mechanism, which prevents the normal and proper functioning of the cells and neurons (Li *et al.* 2001; Lee *et al.* 2004; Miller *et al.* 2010). Others have shown that mHTT to be pro-apoptotic (Saudou *et al.* 1998) and mHTT aggregates have been shown to recruit wild type huntingtin, which is

anti-apoptotic (Ho *et al.* 2001), specifically through the N-terminus of mHTT (Huang *et al.* 1998). Therefore, NII formation could prevent the anti-apoptotic action of wild type huntingtin protein. However, others have concluded that NII formation may be a neuroprotective mechanism, at least in the first instance (Saudou et al. 1998; Guidetti *et al.* 2001; Zhou *et al.* 2001; Arrasate *et al.* 2004; Mitra *et al.* 2009). Interestingly, the short stop mouse model of HD demonstrates frequent and widespread NIIs, despite an absence of neurodegeneration or behavioural abnormalities (Slow *et al.* 2005), therefore suggesting, in this particular mouse model, that inclusion formation is not a pathogenic mechanism.

1.4. Clinical Symptoms of Huntington's Disease

Although HD is currently clinically diagnosed upon the onset of motor symptoms, cognitive and psychiatric deficits are now thought to precede later motor symptoms in the vast majority of cases (Diamond *et al.* 1992; Duff *et al.* 2007; Paulsen *et al.* 2008b; Tabrizi *et al.* 2009; Tabrizi *et al.* 2011; Tabrizi *et al.* 2013). The range of clinical symptoms demonstrated in HD led to the development of The Unified Huntington's Disease Rating Scale (UHDRS) (Kremer and Group 1996; Siesling *et al.* 1998), a clinical scale to assess and track disease severity across multiple domains including; motor function, cognitive function, behavioural abnormalities and functional capacity. The UHDRS is now commonly used clinically to characterise disease severity although variations of the original scale exist, for example for later stage patients (Youssov *et al.* 2013) or shortened versions of the original scale (Siesling *et al.* 1997).

1.4.1. Motor Symptoms

The more obvious motor symptoms of HD can include chorea, from the Greek meaning 'to dance', which results in uncontrollable involuntary jerking and writhing movements (Huntington 1872; Thompson *et al*. 1988; Kremer and Group 1996). Although, it should be noted that chorea is not present in all cases of HD, it is extremely common. Other obvious motor symptoms can, but do not

always include; hypokinesia including akinesia (impairment in starting a movement) (van Vugt *et al.* 1996) and bradykinesia (impairment and slowness in executing a movement) (Thompson *et al.* 1988), dystonia (abnormal muscle tone), rigidity, Tics and tremor (Huntington, 1872, (Hefter *et al.* 1987b; Phillips *et al*. 1996; Berardelli *et al*. 1999b; Louis *et al.* 1999). The abundance of motor symptoms significantly affect the gait of HD patients (Koller and Trimble 1985; Hausdorff *et al.* 1997; Reynolds *et al.* 1999; Grimbergen *et al.* 2008; Rao *et al.* 2008), commonly leading to falls and stumbles (Grimbergen *et al.* 2008; Busse *et al.* 2009) and an eventual inability to walk. In addition to the more obvious motor symptoms of HD, ideomotor apraxia, difficulty in the planning and completing motor tasks, is common (Knopman and Nissen 1991; Shelton and Knopman 1991). Furthermore, impairments in the learning of motor skills (Heindel *et al.* 1988; Willingham and Koroshetz 1993) and dysphagia are common symptoms during the progression of the disease (Leopold and Kagel 1985; Hunt and Walker 1989; Kagel and Leopold 1992; Hamakawa *et al.* 2004; Heemskerk and Roos 2011).

Increasingly, subtle motor abnormalities, which often proceed the more obvious motor symptoms observed upon clinical diagnosis are being described in HD patients (de Boo *et al.* 1997). Subtle motor abnormalities, in self-paced finger tapping, gait and oculomotor measures were found to occur often decades prior to clinical diagnosis in HD patients (Paulsen *et al.* 2008b; Biglan *et al.* 2009; Tabrizi *et al.* 2009; Tabrizi *et al.* 2011; Tabrizi *et al.* 2013). In addition, an abundance of literature describes ocular motor abnormalities in both asymptomatic patients and patients in the early stages of the disease (Leigh *et al.* 1983; Rothlind *et al.* 1993; Blekher *et al.* 2004; Golding *et al.* 2006).

Despite the clear motor symptoms associated with HD, research is increasingly focused upon the psychiatric and cognitive symptoms that are often apparent earlier in the disease progression (Duff *et al.* 2007; Paulsen *et al.* 2008b; Tabrizi *et al.* 2009; Paulsen 2010; Tabrizi *et al*. 2011; Tabrizi *et al.* 2013), as these symptoms offer an earlier stage for potential therapeutic intervention in HD.

1.4.2. Psychiatric Symptoms

Psychiatric symptoms are common in HD patients; it has been estimated that up to 98% of HD patients exhibit some sort of neuropsychiatric symptoms (Paulsen *et al.* 2001b). Psychiatric symptoms typically begin prior to motor dysfunction and are thought to worsen as motor symptoms become more prevalent and disease burden increases (Duff *et al.* 2007; van Duijn *et al.* 2013). It has been estimated that up to 80% of HD patients develop a non-cognitive psychiatric disorder within fifteen years of clinical diagnosis (Leroi *et al.* 2002), and some studies have estimated that up to a fifth of HD patients suffer with severe psychiatric problems (Orth 2011). Depression (Slaughter *et al.* 2001b), apathy, irritability, aggression and anxiety (Julien *et al.* 2007) are all common behavioural symptoms of HD (Burns *et al.* 1990; Craufurd *et al.* 2001; Paulsen *et al.* 2001b; Slaughter *et al.* 2001a; Duff *et al.* 2007; Reedeker *et al.* 2012; van Duijn *et al.* 2013).

The PREDICT-HD study examined a large cohort of affected individuals and found that the range of psychiatric problems (apathy, anxiety, obsessive compulsive disorder and depression) exhibited in HD pre-symptomatic carriers was far greater than non-affected individuals and that the psychiatric problems had strong correlation with difficulties in undertaking everyday tasks (Duff *et al*. 2007; Paulsen *et al.* 2008a). The suicide risk in HD patients and families affected by HD is high (Schoenfeld *et al.* 1984; Lipe *et al.* 1993; Almqvist *et al.* 1999) with suicide rates estimated to be up to four times greater in HD families compared to the general population (Di Maio *et al.* 1993). Some studies suggest that suicide attempts can be as high as ~25% in symptomatic patients with ~5% committing suicide (Farrer *et al.* 1986). Suicidal ideation is demonstrated in both pre-symptomatic and symptomatic patients (Paulsen *et al.* 2005; Hubers *et al.* 2012) with the presence of pre-existing neuropsychiatric problems such as; depression, anxiety and aggression often increasing suicidal ideation (Hubers *et al.* 2013).

Sleep disturbances are often common in HD patients and alterations in circadian rhythm have been shown to effect the sleep-wake cycle, often causing nocturnal sleeping patterns (Hansotia *et al.* 1985; Wiegand *et al.* 1991; Morton *et al.* 2005; Arnulf *et al.* 2008; Aziz *et al.* 2008; Videnovic *et al.* 2009). The sleep disturbances observed may also contribute to the more general cognitive and psychiatric symptoms shown in HD (Drummond and Brown 2001; Aziz *et al.* 2008).

1.4.3. Cognitive Symptoms

Cognitive deficits that precede profound motor dysfunction have been demonstrated in HD patients (Diamond *et al*. 1992; Lawrence *et al.* 1998b; Lawrence *et al.* 1998c; Kirkwood *et al.* 2000; Lemiere *et al.* 2004; Duff *et al*. 2007; Robins Wahlin *et al.* 2007; Paulsen *et al*. 2008b; Tabrizi *et al*. 2009; Tabrizi *et al*. 2011; Tabrizi *et al*. 2013). The Unified Huntington's Disease Rating Scale (UHDRS), is the most commonly used clinical scale to assess the clinical symptoms of HD. Although, this scale has been designed to classify the overall severity of HD, across a range of clinical symptoms. Therefore, although the UHDRS contains a cognitive component, other assessments have been specifically designed to assess cognitive dysfunction. In addition to the UHDRS, a range of assessments are used to classify cognitive dysfunction in HD including the Mini-Mental State Examination (MMSE) (Folstein *et al.* 1975) and the Montreal Cognitive Assessment (MoCA) (Videnovic *et al.* 2010). However, cognitive assessments such as these were not specifically designed to measure cognitive dysfunction in terms of HD. Therefore the usefulness, validity and sensitivity of cognitive assessments such as the MMSE and MoCA for specifically classifying cognitive dysfunction in HD patients are disputed (Feher *et al.* 1992; Videnovic *et al*. 2010; Gluhm *et al.* 2013).

However, cognitive assessments are used in disease diagnosis and are crucial in the categorisation of disease progression, but the vast and broad array of cognitive symptoms demonstrated in HD, can make the overall classification of cognitive dysfunction difficult. Cognitive dysfunction has been demonstrated

relatively early in HD disease across a broad arrange of cognitive tasks (Stout *et al.* 2011) demonstrating specific deficits in: perseverance (Craufurd *et al*. 2001; Thompson *et al.* 2002a; Aron *et al.* 2003), reversal learning (Lawrence *et al.* 1996; Lawrence *et al.* 1999), alternation (Kirkwood *et al.* 1999), set shifting (Lawrence et al. 1998b), olfactory recognition and discrimination (Moberg *et al.* 1987; Nordin *et al.* 1995; Lazic *et al.* 2007), procedural memory (Caine *et al.* 1977; Butters *et al.* 1985; Snowden *et al.* 2002), judgement and risk tasking (Craufurd *et al.* 2001; Stout *et al.* 2001), facial recognition and emotional recognition problems (Jacobs *et al.* 1995; Sprengelmeyer *et al.* 1996; Gray *et al.* 1997; Sprengelmeyer *et al.* 1997; Milders *et al.* 2003; Johnson *et al.* 2007; Kipps *et al.* 2007; Snowden *et al.* 2008).

Cognitive and psychiatric symptoms typically occur relatively early in disease progression (Diamond *et al*. 1992; Kirkwood *et al*. 2000; Lemiere *et al*. 2004; Robins Wahlin *et al*. 2007) and thus allow an opportunity for early potential therapeutic intervention, targeting HD as early as possible. Furthermore, there is considerable interplay between underlying cognitive and psychiatric symptoms and motor and peripheral symptoms which are also demonstrated in the disease.

1.4.4. Peripheral Symptoms

Further to a range of symptoms associated with the central nervous system (CNS), HD also causes many peripheral symptoms which can significantly affect the quality of life of patients. Weight loss is often demonstrated from the early stages of HD (Djousse *et al.* 2002) and progresses as disease burden increases (Robbinsa *et al.* 2006). The degree of weight loss observed has been correlated with an increase in CAG repeat length (Seong *et al.* 2005; Aziz et al. 2008), although the precise reasons for the observed weight loss remain unclear. An abundance of literature has examined metabolic problems in HD patients (Koroshetz *et al.* 1997; Lodi *et al.* 2000; Mochel *et al.* 2007a; Goodman *et al.* 2008; Mochel and Haller 2011), including mitochondrial dysfunction (Panov *et al.* 2002; Quintanilla and Johnson 2009; Kim *et al.* 2010; Oliveira 2010), which may relate to the weight loss observed.

In addition, to the underlying metabolic dysfunction, motor symptoms such as dysphagia and tremor (Huntington 1872; Hefter *et al*. 1987b; Phillips *et al*. 1996; Berardelli *et al*. 1999b; Louis *et al*. 1999), which are centrally controlled, not only increase sedentary energy expenditure (Pratley *et al.* 2000) but also physically affect the ability of patients in the late stages of HD to consume food independently (Leopold and Kagel 1985). Furthermore, underlying psychological symptoms such as apathy, lack of motivation and depression may also affect the willingness of the patient to consume food independently. Many other peripheral symptoms are shown in HD patients including; cardiac dysfunction and failure (Chiu and Alexander 1982; Lanska *et al.* 1988; Sørensen and Fenger 1992a), skeletal muscle abnormalities (Lodi *et al*. 2000; Turner *et al.* 2007; Busse *et al.* 2008a), endocrine dysfunction including diabetes (Farrer 1985) and gastrointestinal problems (Andrich *et al.* 2009; van der Burg *et al.* 2011).

HD is characterised by a range of symptoms, which can interact, and often vary in their severity and nature. Therefore, although there are commonalities in the symptoms observed in HD patients, the exact profile and severity of symptoms will vary depending on the individual patient.

1.5. Juvenile Huntington's Disease

The CAG trinucleotide repeat length observed in HD patients generally inversely correlates with the age of disease onset (Andrew *et al.* 1993). But patients with particularly long CAG repeat lengths, typically in the range of 60 -120 repeats will develop juvenile Huntington's disease (JHD) (Douglas *et al.* 2013), although cases of JHD have been described in patients with as few as 48 CAG repeats (Ribaï *et al.* 2007). Juvenile cases of HD account for approximately 10% of all cases and are typically diagnosed before 20 years of age (Van Dijk *et al.* 1986b; Douglas *et al.* 2013). Some patients with expansions of up to 250 CAG repeats (Nance *et al.* 1999) have been shown to develop clinical symptoms as

young as 18 months of age (Nicolas *et al.* 2011), although such large expansions are exceedingly rare.

In the majority of cases, JHD is inherited from the paternal germ line and genetic anticipation is observed (Van Dijk *et al.* 1986a). However unlike HD, JHD is thought to have a more rapid rate of disease severity and progression (Van Dijk *et al.* 1986a; Bhidayasiri and Tarsy 2012). The cognitive decline and psychiatric problems associated with JHD are similar to adult onset HD, including; depression, poor school performance, memory problems, irritability, depression and social withdrawal (Gómez-Tortosa *et al.* 1998; Srivastava *et al.* 1999; Ribaï *et al.* 2007; Bhidayasiri and Tarsy 2012). However, unlike adult onset HD which demonstrates choreic movements, JHD typically demonstrates motor symptoms of rigidity, bradykinesia and dystonia (Van Dijk *et al.* 1986a; O'Shea and Falvey 1991; Srivastava *et al.* 1999; Geevasinga *et al.* 2006). Furthermore, seizures and epilepsy are common in JHD patients. In addition to the characteristic motor disturbances observed in JHD the neuropathology of the disease is thought to be more widespread, with nuclear aggregates forming in the cortex (Gutekunst *et al.* 1999) as well as the striatum rapidly during the disease progression (Vonsattel and DiFiglia 1998; Nance and Myers 2001).

1.6. Animal Models of Huntington's Disease

Since the discovery of the mutant gene which causes HD (MacDonald *et al.* 1993), a vast range of animal models of HD have now been developed which include non-mammalian organisms such as; *Caenorhabditis elegans* (Faber *et al.* 1999; Parker *et al.* 2001)*, Drosophila melanogaster* (Jackson *et al.* 1998; Warrick *et al.* 1998; Lee *et al.* 2004) and *Danio rerio* (Karlovich *et al.* 1998; Schiffer *et al.* 2007). In addition numerous mammalian models of HD have been developed including; mice (Mangiarini *et al.* 1996; White *et al.* 1997; Reddy *et al.* 1998; Schilling *et al.* 1999; Wheeler *et al.* 1999; Yamamoto *et al.* 2000; Laforet *et al.* 2001; Lin *et al.* 2001; Menalled *et al.* 2003; Slow *et al.* 2003; Slow et al. 2005; Gray *et al.* 2008; Menalled *et al.* 2012; Cheng *et al.* 2013), rats (de

Almeida *et al.* 2002; von Hörsten *et al.* 2003; Yu-Taeger *et al.* 2012), pigs (Yang *et al.* 2010), sheep (Jacobsen *et al.* 2010) and monkeys (Palfi *et al.* 2007; Yang *et al.* 2008). Each of these animal models of HD has its own particular merits for modelling different aspects of the disease, however the focus of this thesis is on murine models of HD, of which there are great number.

1.6.1. Lesion Rodent Models of Huntington's Disease

The first mouse models of HD were lesions models, where mice were given excitotoxic lesions of the striatum (Coyle and Schwarcz 1976; Sanberg *et al.* 1989; Borlongan *et al.* 1995) to investigate the functional role of the striatum in HD. Lesion models were generated by administration of either kainic (Coyle and Schwarcz 1976; Sanberg *et al.* 1978), ibotenic (Schwarcz *et al.* 1979) or quinolinic (Schwarcz *et al.* 1983) acids into the striatum, thus causing the apoptotic death of cells within the striatum (DiFiglia 1990).

Arguably the most valid rodent lesion models were lesioned with quinolinic acid (QA) which caused the relatively specific apoptotic cell death of medium spiny neurons (MSNs) within the striatum with comparatively little effect on the giant cholinergic interneurons of the striatum (Schwarcz *et al.* 1983; Beal *et al.* 1988). Furthermore, the motor abnormalities demonstrated in late stage HD patients were replicated in these lesion models (Schwarcz *et al*. 1983; Beal *et al*. 1988; Ma *et al.* 2012). Classical lesion models of HD have substantially increased our understanding into the role of the corticostriatal pathway and the striatum in HD; they produce a rapid behavioural phenotype which allows the efficient validation of both novel behavioural tests and potential therapeutics. Although elegant in their nature, lesion models do not produce consistent results that accurately reflect the genetic basis and thus the progressive and gradual nature of HD, as seen in human patients.

1.6.2. Genetic Mouse Models of Huntington's Disease

Once the m*HTT* gene that causes HD had been identified (MacDonald *et al.* 1993) attempts to create genetic models of HD that mimicked the human

condition in terms of expressing m*HTT* in the appropriate proteomic and genomic context were undertaken. The first mouse models created to investigate the effect of *HTT* in HD were knock-out models which demonstrated embryonic lethality (Duyao *et al.* 1995; Nasir *et al.* 1995). These results indicated the importance of the wild type huntingtin protein (Htt) in early embryonic development (Reiner *et al.* 2003) although they did not increase our understanding of how mutant huntingtin protein (mHtt) causes HD. Therefore, in order to assess the effect of *HTT* in HD, it was necessary to further manipulate the huntingtin gene to produce more genetically modified animals which are more reflective of the human condition. Although a large number of transgenic mouse models of HD have been created, comparatively few transgenic rat models of HD have been produced (Table 1.1.).

1.6.2.1 Rat Models of Huntington's Disease

The first rat model of HD was produced using lenitviral vectors that coded for the partial amino acid sequences of either HTT or mHTT (including a range of CAG repeat sizes) which were driven by endogenous rat promoters. The lentiviral vectors were injected bilaterally into the striatum of the adult rat (de Almeida *et al.* 2002). Ubiquitinated mHHT aggregates were first seen 1 week after the lentiviral vector injection; these progressively worsened over a 12 week period. In addition, decreased dopamine expression and cell death were shown within the rat striatum over the course of the 12 week study. The molecular and histological analysis of the lentiviral vector model of HD demonstrated that many hallmarks of HD were present. However, the rapid nature and appearance of the neuropathology including aggregate formation shown in this viral model is not fully reflective of the progressive nature of the human disease. Therefore, genetically modified rat models which express the transgene for HD were produced to perhaps more accurately replicate the progressive nature of the disease.

A transgenic rat model of HD (tgHD) was successfully created that contained the human mutation with 51 CAG repeats (von Hörsten *et al.* 2003). This model

demonstrated cognitive impairments of spatial memory on the radial arm maze at 10 months of age, decreased anxiety in the elevated plus maze from 2 months of age and motor dysfunction on the rotarod at 10 months of age (von Hörsten *et al.* 2003). In addition, neuropathology was indicative of HD including; striatal shrinkage and enlarged lateral ventricles at 8 months of age (von Hörsten *et al.* 2003). Others have also shown early cognitive dysfunction in a range of tasks including operant choice reaction time tasks, spatial operant reversal and object recognition and recall tasks (Cao *et al.* 2006; Fink *et al.* 2012; Zeef *et al.* 2012a; Zeef *et al.* 2012b) and motor deficits including hyperkinetic behavior (Zeef *et al.* 2012b) and abnormal locomotor activity in open field (Cao *et al.* 2006). However, variable results have been obtained in this model, with only subtle but progressive cognitive deficits demonstrated in female rats on response accuracy and response time in the operant serial implicit learning task (SILT) (Brooks *et al.* 2009). In addition, another study using both male and female rats showed only modest motor deficits on the rotarod and inconsistent cognitive impairments on the water T-maze test of reversal learning (Fielding *et al.* 2012). However, these apparent differences demonstrated in the behaviour of TgHD rat may be attributable to sex differences which have been highlighted previously (Bode *et al.* 2008).

Another transgenic rat model, the BACHD rat, was originally developed by Yu-Taeger *et al*, as a model of HD. The model was generated using a human bacterial artificial chromosome (BAC) that contained the full length m*HTT* with a 97 CAG/CAA repeat length, under the control of the associated human regulatory elements. The first description of the BACHD rat described a HD like phenotype with early and progressive motor deficits on the rotarod from 1 month of age, alterations in gait at 14 months of age and a clasping phenotype which was evident from 3 weeks of age (Yu-Taeger *et al.* 2012). In addition, altered anxiety like behaviour was observed in the elevated plus maze from 4 months of age and progressive mHTT aggregate formation was seen in both the cortex and striatum (Yu-Taeger *et al.* 2012). Hyperactivity was demonstrated in open field

at 1 month of age followed by hypoactivity from 4 months of age. Rotarod deficits were present at 2 months of age and additional gait abnormalities were observed at 12 months of age (Yu-Taeger *et al.* 2012). However, weight, novel object recognition and acoustic startle response were unchanged at all ages in comparison to wild type animals (Yah-se *et al.* 2013b). Additional cognitive testing on the BACHD rat indicated a lower freezing response upon re-exposure to foot shock, which may be indicative of problems with associative learning (Yah-se *et al.* 2013a). Reversal learning deficits were shown in a cross maze task at 6 months of age and deficits in contextualised fear conditioning were evident from 4 months of age (Yah-se *et al*. 2013a). Therefore, in comparison to the transgenic rat models of HD described, the vast range of transgenic mouse models of HD that are available appear to produce more reproducible results.

Table 1.1. Rat Models of Huntington's Disease - There is a range of HD rat models available for the study of HD. Initially virus vec
models and then genetically modified rat models were created. *CMV= cytomegalovirus, #PGK = phosphoglycerate kinase.

Rat Model	CAG repeat length	Background	Construct	Promoter	Life span	Motor deficits
Lenti-viral (de Almeida *et al.* 2002)	44, 66 and 82	Wistar	Lentiviral- mediated delivery of mHTT injected into the striatum, under control of endogenous mouse promoter.	Mouse PGK# and CMV*	Unknown – study conducted up until 12 weeks of age.	Not reported
tgHD (von Hörsten *et al*. 2003)	51	Sprague Dawley	Rat cDNA fragment under control of the rat endogenous promoter.	Rat huntingtin	24 months	Rotarod (von Hörsten *et al*. 2003 Open field (Cao *et al*. 2006). Hyperkinetic locomotor activity (Zeef *et al.* 2012b). SILT (Brooks *et al.* 2009)
BACHD (Yu-Taeger *et al.* 2012)	97 CAG/CAA	Sprague Dawley	Replacement of exon 1 with human BAC full length m*HTT* sequence including 97 CAG/CAA repeats under control of associated human regulatory elements	Human huntingtin	Unknown – characterised up until 72 weeks of age	Rotarod, gait abnormalities, hypoactivity and clasping (Yu-Taeger *et al.* 2012; Yah-se *et a* 2013b).

1.6.2.2. Transgenic Mouse Models of Huntington's Disease

The first genetically modified mouse models of HD produced were transgenic models, which contained either the full length or a fragment of exon 1 of human *HTT* randomly integrated into the murine genome (Menalled and Chesselet 2002). A range of transgenic mouse models of HD have now been created (Table 1.2.), which demonstrate different genetic constructs and manipulations, thus producing different behavioural and neuropathological phenotypes.

The first successfully generated transgenic mouse models of HD were termed the R6 lines, which included R6/0, R6/T, R6/1, R6/2, and R6/5 (Mangiarini *et al.* 1996). These lines carried a fragment of m*HTT* exon 1 randomly integrated into the murine genome, thus endogenous and mutant proteins were both expressed. Of the five R6 lines originally generated (Mangiarini *et al.* 1996) the R6/1 and R6/2 lines have been most widely studied and utilised in HD research (Mangiarini *et al*. 1996; Carter *et al.* 1999; Hurlbert *et al.* 1999; Lione *et al.* 1999; Bolivar *et al.* 2004; Mazarakis *et al.* 2005; Stack *et al.* 2005; Lazic et al. 2007; Hodges *et al.* 2008; Brooks *et al.* 2012f).

The R6/1 transgenic mouse model (Mangiarini *et al*. 1996) produces a subtle phenotype, relative to the R6/2 model, due to the comparatively shorter CAG repeat length (~115 rather the ~145 of the R6/2). Although symptoms still develop relatively early including; motor deficits on rotarod by 8 weeks of age (Brooks *et al.* 2012g), hypoactive locomotor activity (Hodges *et al.* 2008), clasping (Naver *et al.* 2003), alterations in gait and deficits in coordination as demonstrated on balance beam (Brooks *et al*. 2012f). Acoustic startle response pre-pulse reactivity deficits have been reported at 12 weeks of age, (Brooks *et al*. 2012g) as has, weight loss by 12 weeks of age (Brooks *et al*. 2012f). Cognitive deficits have previously been demonstrated in; reference memory in the Morris water maze (Brooks *et al*. 2012f), tests of exploratory behaviour (Bolivar *et al.* 2004; Hodges *et al.* 2008) and tests of sensory discrimination (Mazarakis *et al.* 2005).

The R6/2 mouse model has been extensively studied and demonstrates a severe and aggressive phenotype with motor deficits detectable as early as 5 weeks of age (Mangiarini *et al.* 1996; Carter *et al.* 1999; Stack *et al.* 2005). Cognitive deficits are also demonstrated in reversal learning in the water T-maze and fear conditioning tasks (Lione *et al.* 1999). Other developmental abnormalities quickly develop including; weight loss, diabetes (Hurlbert *et al.* 1999), increased metabolism (van der Burg *et al.* 2008) and seizures, resulting in an early death which typically occurs by 12 weeks of age (Mangiarini *et al.* 1996). A detailed study used MRI to correlate motor symptoms in the R6/2 mouse, with regional brain volume loss. MRI measurements indicated that rather than striatal atrophy, lack of striatal growth occurred from 8 weeks of age (Rattray *et al.* 2013).

Table 1.2. Transgenic Mouse Models of Huntington's Disease # The YAC128 is the most commonly used of this mouse line although others exist with shorter repeat lengths (Hodgeson *et al*)., NII = neuronal nuclear inclusions, *CMV= cytomegalovirus.

Mouse Model	CAG repeat length	Background	Construct	Promoter	Life span	Motor deficits	Cognitive deficits	Neuropathology
R6/1 (Mangiarini *et al.* 1996)	116	CBA x C57BL/6J	m*HTT* fragment including exon 1 and part of the first intron integrated into mouse genome.	Human huntingtin	30-40 weeks	Rotarod (Brooks *et al*. 2012f), hyperactivity then hypoactivity, clasping, rearing and gait. (Naver *et al.* 2003).	Exploratory behaviour (Bolivar *et al*. 2004), olfactory abnormalities (Lazic *et al.* 2007) elevated plus-maze (Naver *et al.* 2003), sensory discrimination (Mazarakis *et al. 2005*)	NII's from 8 weeks of age (Hansson *et al.* 2001)
R6/2 (Mangiarini *et al.* 1996)	144	CBA x C57BL/6J	m*HTT* fragment including exon 1 and part of the first intron integrated into mouse genome.	Human huntingtin	10-12 weeks	Rotarod, grip strength, clasping, weight loss, tremor, seizures and open field deficits. (Carter *et al.* 1999; Hurlbert *et al.* 1999; Stack *et al.* 2005; Cowin *et al.* 2012; Rattray *et al.* 2013)	Water T-maze, fear conditioning, visual cliff avoidance (Lione *et al.* 1999) Fear conditioning (Cowin *et al.* 2012)	Reduced brain weight (Davies *et al.* 1997) Reduced striatal volume (Hansson *et al.* 1999) NII's at from 4 weeks of age (Morton *et al.* 2000; Meade *et al.* 2002) Age related loss of regional brain volumes (Rattray *et al.* 2013)

Mouse Model	CAG repeat length	Background	Construct	Promoter	Life span	Motor deficits	Cognitive deficits	Neuropathology
N171-82Q (Schilling *et al.* 1999)	82	C3H/HEJX C57Bl/6J	m*HTT* fragment (longer than R6 lines) including exon 1 and part of the first intron integrated into mouse genome.	Mouse prion protein	20-40 weeks	Rotarod deficits (Klivenyi *et al.* 2006).	Morris water maze deficits in latency to find platform (Potter *et al.* 2010)	NII's at 11 months (Schilling *et al.* 1999)
YAC128[#] (Slow *et al.* 2003).	128	FVB/N	Complete m*HTT* gene integrated into mouse genome.	Human huntingtin	Up to 27 months	Motor abnormalities on rotarod and open field (Menalled *et al.* 2003; Van Raamsdonk *et al.* 2005).	Water T-maze set shifting procedure (Brooks *et al.* 2012e). Depressive like behaviour (Pouladi *et al.* 2009) operant deficits in SILT (Brooks *et al.* 2012h).	Decreased brain weight and striatal volume from 9 months, decrease in cortical volume from 12 months. Diffuse mHTT staining at 12 months but no inclusions (Slow *et al.* 2003).

Mouse Model	CAG repeat length	Background	Construct	Promoter	Life span	Motor deficits	Cognitive deficits	Neuropathology
Hu97/18 (Southwell *et al.* 2013)	97	FVB/N	Fully humanised transgene, one transgene is expanded and the other is wild type therefore the model is heterozygous.	Human huntingtin gene	Unknown – study conducted until 12 months of age.	Motor deficits on Rotarod from 2 months of age (although this is not demonstrated longitudinally). Decrease in repetitive movements and jumping from 2 months of age.(Southwell *et al.* 2013).	Motor learning deficit in Rotarod. Spatial learning deficits in novel object recognition. Anxiety in open field and elevated plus maze and depressive like behaviour in forced swim task. (Southwell *et al.* 2013)	Reduced cortical and striatal volume at 12 months of age. (Southwell *et al.* 2013). Progressive synaptic dysfunction and altered plasticity from 9 months of age (Kolodziejczyk *et al.* 2014)
Ubi-G-HTT84Q (Cheng *et al.,* 2013)	84	FVB/N	N-terminal fragment of *mHTT* fused to EGFP	Human ubiquitin promoter	Study conducted until 13 months – terminated due to ill health	Clasping from 8.5 months, gait and rotarod deficits at 13 months (Cheng *et al.*, 2013).	None reported	NII's at 12 months of age (Cheng *et al,* 2013)

Another transgenic mouse model of HD, the YAC (yeast artificial chromosome) mouse model expresses the full length *mHTT* gene, therefore the *mHTT* is under the control of human regulatory elements. A variety of YAC models have been created with different CAG repeat lengths (Hodgson *et al.* 1999), although the most widely utilised model is the YAC128, containing 128 CAG trinucleotide repeats (Slow *et al*. 2003). The YAC128 model has been shown to have cognitive deficits in set shifting strategy and changing between rules (Van Raamsdonk *et al*. 2005; Brooks *et al*. 2012e) as well as reversal learning deficits in the water T-maze (Van Raamsdonk *et al.* 2005) and a depressive like behaviour in the forced swim test (Pouladi *et al.* 2009). Abnormalities of motor ability including hypoactivity (Menalled *et al.* 2003; Van Raamsdonk *et al.* 2005) and a decreased latency to fall from the rotarod (Menalled *et al.* 2003; Van Raamsdonk *et al.* 2005; Pouladi *et al.* 2012) have also been demonstrated at 8 weeks of age. Less pre-pulse inhibition in response to acoustic startle at 52 weeks of age has also been observed (Van Raamsdonk *et al.* 2005). Although, others have reported no significant differences in pre-pulse inhibition testing (Menalled *et al.* 2003), although these observed differences may well be due to differences in background strain.

Although a range of deficits including; motor, cognitive and psychiatric have been described in the YAC128 mouse model, some longitudinal studies have suggested that behavioural deficits do not worsen over time (Menalled *et al.* 2003; Van Raamsdonk *et al.* 2005) The YAC128 model has a relatively long life span of 108 weeks (Slow *et al.* 2003). The behavioural differences observed may be attributable to differences in background strain. A behavioural characterisation of the YAC128 mouse model across different background strains has shown that although YAC128 mice on all background strains (FVB/N, 129 and C57BL/6) develop motor and cognitive deficits significant differences in the degree of deficit are observed in YAC128 mice of different background strains on rotarod and open field tests as well as differences in weight and total striatal volume (Van Raamsdonk *et al.* 2007).

The N171-82Q transgenic mouse model (Schilling *et al.* 1999) contains a truncated *mHTT* gene which encodes the first 171 amino acids of the mutant protein; this model has a similar life span as the R6/1 model and develops similar motor deficits on the rotarod at 12 weeks of age (Schilling *et al.* 1999; Klivenyi *et al.* 2006). Although motor impairments have been demonstrated, cognitive deficits within this mouse model have not been widely reported. But, decreased latency to locate the platform in a Morris water maze has been shown (Potter *et al.* 2010), which is reflective of episodic memory deficits. However, the N171-82Q mouse model has been used in a range of studies investigating possible therapeutic treatments for HD (Andreassen *et al.* 2001; Schilling *et al.* 2001; Gardian *et al.* 2005; Duan *et al.* 2008).

The BACHD (bacterial artificial chromosome) mouse model (Gray *et al*. 2008) is a conditional model of HD which, similar to the BACHD rat model described earlier (Yu-Taeger *et al.* 2012), contains 97 trinucleotide repeats of both CAG and CAA. Motor deficits have been demonstrated on the accelerating rotarod as early as 8 weeks of age (Pouladi *et al*. 2012; Yah-se *et al.* 2013c) as have deficits in motor coordination (Abada *et al.* 2013). In addition, cognitive deficits in set shifting, fear conditioning (Abada *et al.* 2013) and startle response (Pouladi *et al.* 2012) have been described. Furthermore, a depressive like behaviour has been shown in the forced swim test (Pouladi *et al.* 2012) as has anxiety like behaviour in a zero maze test. The BACHD mouse model has a life span of up to 72 weeks (Gray *et al.* 2008). In addition, this mouse model demonstrates a clear obese phenotype from as early on as 2 months of age (Gray *et al.* 2008), which may contribute to the observed phenotypic differences. An obese phenotype is not evident in HD patients. Therefore, this unusual obese phenotype may have an inadvertent effect on task performance. Kudwa *et al*, elegantly studied the obese phenotype of BACHD mice and the effect that this had on behavioural tasks using food restricted and non-restricted BACHD mice. They found that increased body weight accounts for some, but not all, of the observed HD like behaviour in BACHD mice, specifically the hypoactive

phenotype observed in open field testing (Kudwa *et al.* 2013). Therefore, the obese phenotype observed in the BACHD mouse may inadvertently inflate the degree of difference observed between BACHD mice and controls in behavioural tests.

In addition to the transgenic mouse lines described above, which have been extensively characterised, a number of additional HD mouse lines have been described in the literature, although the volume of research conducted on these mouse lines is comparatively limited.

The short stop model of HD was serendipitously discovered while generating YAC128 mice (Slow *et al.* 2005). This specific mouse line was discovered to have a truncation 3' of the CAG repeat tract and therefore it expressed a shortened fragment of the *HTT* gene, resulting from a premature stop codon. Progressive formation of NII's was found from 3 months of age in the striatum, cortex and hippocampus of these animals. Behaviorally, no rotarod deficits were demonstrated at 12 months of age. Furthermore, short stop mice did not exhibit neuronal degeneration, as there were no differences in overall brain weight, striatal volume or overall number of neurons, at 12 or 18 months of age. In addition, short stop mice were shown to be resistant to NMDA-induced excitotoxicity, when they were given bilateral striatal injections of the NMDA receptor agonist QA (Slow *et al.* 2005). Therefore, despite the formation of NII's from as early as 3 months of age, this mouse line does not present with the behavioural problems or neurodegeneration which is demonstrated in HD.

The HD48 and HD89 mouse lines (Reddy *et al.* 1998) contain the full length human *mHTT* under the control of the endogenous cytomegalovirus (CMV) promoter. Motor deficits including alterations in clasping and hyperkinetic followed by hypokinetic behaviour have been described (Reddy *et al.* 1999). In addition, NII's have also been demonstrated in the striatum (Reddy *et al.* 1999). Both the HD46 and HD100 mouse lines originally described by Laforet *et al*. 2001, expressed the N-terminal fragment of *mHTT* under the control of

endogenous neuronal specific endolase. These animals demonstrated progressive motor deficits on rotarod, deficits in clasping behavior, alterations in gait and locomotor activity from 3 months of age (Laforet *et al.* 2001). NII's were found predominantly in the striatum and also in cortical layers 2/3, 5 and 6, diffuse staining was also significantly greater in the striatum in comparison to the cortex. Alterations in the electrophysiology of striatal neurons were evident with NMDA current densities significantly larger in transgenic animals in comparison to wild type animals (Laforet *et al.* 2001).

The HD94-Tet regulated mouse model of HD expresses 94 CAG repeats under the control of a CamkIIa tetracycline regulated promoter (Yamamoto *et al.* 2000). Motor problems including hind limb clasping at 8 weeks of age and a full body clasp at 28 weeks of age have been described and in addition, a hypoactive phenotype in open field was noted at 36 weeks of age (Yamamoto *et al.* 2000). NII formation in the striatum has been demonstrated from 8 weeks of age, as have a decrease in striatal volume and an increase in ventricle size (Yamamoto *et al.* 2000).

The Hu97/18 mouse line, generated by Southwell and colleagues (2013), is the first transgenic mouse model of HD that possesses both *HTT* genes that are fully humanised. The mutation is heterozygous and therefore the model carries one *mHTT* gene containing 97 CAG repeats and another *HTT* gene with 18 CAG repeats (Southwell *et al.* 2013). Hu97/18 mice demonstrated increased body weight in comparison to Hu18/18 animals from 4 months, a similar finding to that observed in BACHD mice (Gray *et al.* 2008). No significant difference was demonstrated in latency to fall from the rotarod in Hu97/18 mice in comparison to Hu18/18 mice, over a 12 month time period, although the ability to initially learn the task at 2 months of age was significantly impaired (Southwell *et al.* 2013) . Locomotor activity was the same in both genotypes over a 12 month period, although Hu97/18 mice were observed performing increased repetitive movements and decreased jumping at both 2 and 4 months of age. Increased anxiety was demonstrated by fewer entries into the centre of the field in

comparison to Hu18/18 animals at 3 months of age, and by less time spent in the arms of the elevated plus maze and 3 and 6 months of age (Southwell *et al.* 2013). A depressive behavior was also observed at 12 months of age in the forced swim test. Finally, cognitive deficits in spatial learning were demonstrated in a novel object recognition task at 6 and 9 months of age and object recognition deficits at 9 months of age. Reduced striatal and cortical volumes were both demonstrated, although there was a trend in reduction of DARPP-32 staining in the corpus callosum, it did not reach significance (Southwell *et al.* 2013).

The Ubi-G-HTT84Q mouse model of HD is a newly generated mouse model which contains GFP fused with exon 1 of human *mHTT* with 84 CAG repeats, under the control of the human ubiquitin promoter (Cheng *et al.* 2013). Clasping behavior was observed from 8.5 months of age, as was decreased latency to fall from the rotarod and gait abnormalities were evident at 13 months of age (Cheng *et al.* 2013). In addition, NII's were observed in the striatum and cortex at 12 months of age (Cheng *et al.* 2013).

Transgenic HD mouse models have been used in the development and validation of a range of behavioral tasks and therapeutic studies due to their rapidly progressing and relatively severe phenotype. However the rapid disease progression demonstrated in transgenic mouse lines does not accurately mimic the human condition. In addition, the random integration of the *mHTT* gene into the murine genome means that both endogenous and mutant huntingtin genes are expressed, which is not the case in the human condition. In order to overcome these issues with transgenic mouse lines, numerous genetically modified knock-in mouse models have been created in which the mutant gene is specifically targeted into the murine genome.

1.6.2.3. Knock-in Mouse Models of Huntington's Disease

After the successful results gained from transgenic mouse models of HD, knock-in mouse models were developed (Table 1.3.) in an attempt to more accurately represent the human disease. Knock-in models contain the mutant gene targeted

into the endogenous mouse *Htt*, therefore the mutant protein is expressed in its appropriate proteomic and genomic context. The generation of knock-in rodent models of HD has largely been limited to mice due to the extensive mouse genetic toolbox which is available; whereas the development of a similar genetic toolbox for rats has been slower, with only recent advances in germline competent rat ES cell lines (Buehr *et al.* 2008; Li *et al.* 2008) and zinc finger nucleases (Geurts *et al.* 2010; Cui *et al.* 2011; Huang *et al.* 2011) which are required for complex genetic manipulations.

A large range of knock-in mouse models have now been created (Menalled 2005). The behavioural results observed in knock-in mouse models initially proved disappointing as they did not show the aggressive and overt motor deficits first detected in the R6/2 transgenic model (Mangiarini *et al.* 1996). However, additional testing revealed knock-in models typically develop subtle early behavioural phenotypes which gradually worsen (White *et al.* 1997; Wheeler *et al.* 1999; Menalled *et al.* 2003; Heng *et al.* 2010). Early behavioural abnormalities have now been demonstrated across a range of knock-in models (Table 1.3) (Van Raamsdonk *et al*. 2005; Trueman *et al.* 2007; Trueman *et al.* 2012e) and are thought to resemble the pre-symptomatic stages of HD. The severity of both motor and cognitive symptoms generally correlates with the number of CAG repeats contained within the mouse model (Lin *et al.* 2001).

The Hdh^{Q50} mouse model originally described by White *et al*., 1997 contained a chimeric insertion of 48 CAG repeats into the endogenous mouse *HTT* gene, which are under the control of the endogenous mouse promoter. However, no overt behavioural abnormalities have been described in motor, cognitive or psychiatric tasks in comparison to wild type animals.

The Hdh^{71} and Hdh^{94} mouse lines were generated by Menalled *et al*., (2000). Both mouse lines contains a targeted chimeric insertion of either 71 or 94 CAG repeats into exon 1, of the mouse *HTT* gene. No abnormal phenotype was observed in the either of the mouse lines in clasping or weight loss at 3 months

of age (Menalled *et al.* 2000). No cognitive deficits have yet been reported. Immunohistochemistry for substance P and enkephalin was performed but reductions were only found in Hdh^{94} animals at 8 months of age (Menalled *et al*. 2000).

The Hdh^{Q92} mouse model originally created by Wheeler and colleagues (Wheeler *et al.* 1999) has been extensively studied by Trueman *et al* in homozygous ($Hdh^{Q92/Q92}$) animals. This mouse model has a range of cognitive deficits on 9-hole box operant tests, including deficits in the serial implicit learning task (SILT), that probes learning and attention, as young as 16 weeks of age (Trueman *et al.* 2007), although the observed deficit is not progressive with age (Trueman *et al.* 2008b). Deficits were observed in the five choice serial reaction time task (5-CSRTT), in measures of attention and reaction time, at 16 weeks of age (Trueman *et al.* 2012b). Further deficits were observed in the delayed alternation operant task, a test of working memory, at 20 weeks of age (Trueman *et al*. 2012e) and the progressive ratio operant task of motivation (Trueman *et al.* 2009b) at 108 weeks of age. In addition, cognitive impairments of spatial working memory have also been demonstrated in non-operant tasks including the Morris water maze (Brooks *et al.* 2012c) from 16 weeks of age. Motor impairments have been demonstrated in the Hdh^{Q92} mouse line in a range of tests including; increased locomotor activity in open field, decreased latency to fall from the rotarod, reduced grip strength and prepulse inhibition in acoustic startle from 16 weeks of age (Trueman *et al*. 2009b; Brooks *et al*. 2012c). Immunohistological analysis revealed very specific neuropathology with dense NII formation in the striatum, the piriform cortex and the olfactory tubercle at 4 months of age which progressively worsened over time (Bayram-Weston *et al.* 2012b).

The Hdh^{Q111} mouse model is the subject of this thesis. The original Hdh^{Q111} mouse model of HD was generated on a CD1 background and contained a chimeric genetic alteration which targeted the human polyglutamine expansion into the endogenous murine *Htt* gene. This resulted in the majority of exon 1 and

part of intron 1 being replaced with human DNA containing ~111 CAG repeats, under the control of the endogenous murine promoter (Wheeler *et al.* 1999) as shown in Figure 1.4.

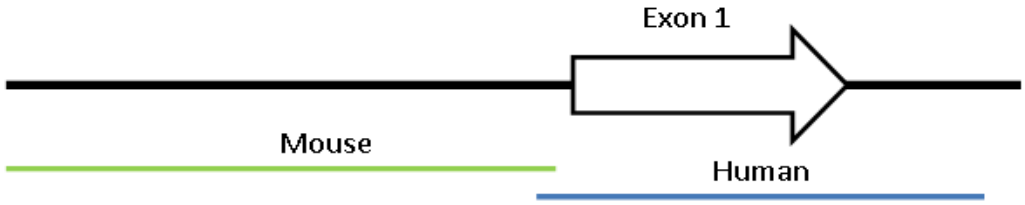

Figure 1.4. Schematic representation of the genetic construct contained within the Hdh^{Q111} mouse model. Mouse endogenous gene is indicated in green and the inserted human construct is indicated in blue, it contains the whole of exon 1 and part of the first intron. Adapted from Wheeler *et al.,* 1999.

Early behavioural data from Hdh^{Q111} mice on a CD1 background revealed only subtle differences in gait at 24 months of age (Wheeler *et al.* 2002b), although these results were demonstrated in both homozygous (Hdh$^{Q111/Q111}$) and heterozygous animals (Hdh$^{Q111/+}$). Furthermore, decreased latency to fall from the rotarod at 100 weeks of age and weight loss beginning at 28 weeks of age have also been demonstrated (Menalled *et al.* 2009). However, no significant differences were observed in comparison to wild type animals in terms of; locomotor activity, grip strength, rear climbing, gait, light-dark choice test or prepulse inhibition (Menalled *et al.* 2009). Anxiety like behaviour has been demonstrated in only male Hdh^{Q111} mice on a CD1 background in a range of tests including; splash test, forced swim test, open field and novelty suppressed feeding at 13 weeks of age (Orvoen *et al.* 2012). NII's have been demonstrated in the striatum of Hdh^{Q111} animals at 10 months of age (Wheeler *et al.,* 2000). Some limited studies have been performed in Hdh^{Q111} mice on a C57BL/6J background. Long terms memory deficits were observed in novel object recognition and in spatial memory in the Morris water maze task, however this study only used male animals (Giralt *et al.* 2012).

The first description of the Hdh^{Q140} mouse line demonstrated initial hyperactivity in locomotor activity analysis and increased rearing, followed by hypoactivity by 16 weeks of age and gait abnormalities at 52 weeks of age (Menalled *et al.*

2003). Since the original description of the Hdh^{Q140} mouse line Hickey *et al.,* 2008 extended the characterisation of the mouse line demonstrating a range of motor abnormalities including; reduced climbing, decreased latency to fall from the rotarod and reduced running wheel performance from 6 weeks of age. In addition, increased anxiety was shown in a light dark box, as was an increase in fear conditioning response from 16 weeks of age (Hickey *et al.* 2008). Furthermore, Rising *et al*, (2001) confirmed that motor deficits were seen in this mouse model in increased rearing frequency and decreased latency to fall from the rotarod, although these deficits were not progressive over time. NII's were demonstrated in both the striatum and cortex from 6 months of age which progressively worsened over time (Rising *et al.* 2011).

The original characterisation of the Hdh^{Q150} mouse line showed motor and gait abnormalities from 25 weeks of age as well as reactive gliosis and intranuclear inclusion formation in the striatum, indicative of HD pathology in human patients (Lin *et al.* 2001). Further studies on this mouse line have confirmed a HD phenotype. Woodman *et al.,* 2007 used a modified SHIRPA (Smith Kline Beecham Harwell Imperial College Royal London Hospital phenotype assessment) test to assess the behavioural phenotype of Hdh^{Q150} mice up to 72 weeks of age. Animals appeared normal until 60 weeks of age when poor grooming, increased anxiety, aggression and irritability were observed. At 88 weeks of age Hdh^{Q150} animals exhibited no fear response and were unkempt, in comparison to wild type littermates, the authors compare the phenotype of these animals to transgenic R6/1 mice at 36 weeks of age.

Table 1.3. Knock-in Mouse Models of Huntington's disease. *This mouse line will be the subject of this thesis, NII's = neuronal nuclear inclusions.

Mouse Model	CAG repeat length	Background	Construct	Promoter	Life span	Motor Deficits	Cognitive Deficits	Neuropathology
Hdh^{Q50} (White *et al.* 1997)	48	129/CD1	Chimeric human/mouse exon 1 inserted into the murine Htt gene.	Endogenous mouse *mHTT*	Normal	No observed deficits (White *et al.* 1997)	None reported	No abnormalities reported
Hdh71 (Menalled *et al*. 2000)	71	129SvXC57BL/6J	Chimeric human/mouse exon 1 inserted into the murine Htt gene.	Endogenous mouse *mHTT*	Normal	None observed (Menalled *et al.* 2000)	None reported	No significant differences reported (Menalled *et al*. 2000)
Hdh^{Q92} (Wheeler *et al.* 1999)	90	129/CD1	Chimeric human/mouse exon 1 inserted into the murine Htt gene.	Endogenous mouse *mHTT*	Normal	Rotarod and open field (Trueman *et al.* 2009b).	Operant: Delayed alternation (Trueman *et al*. 2012e), and SILT (Trueman *et al*. 2007, 2008b).	Progressive NII formation from 4 months of age in the striatum and olfactory tubercle (Bayram-Weston *et al*. 2012). NII formation from 12 months of age (Wheeler *et al.* 2000)
Hdh94 (Menalled *et al*. 2000)	94	129SvXC57BL/6J	Chimeric human/mouse exon 1 inserted into the murine Htt gene	Endogenous mouse *mHTT*	Normal	None found (Menalled *et al.* 2000)	None reported	Reduced enkephalin mRNA at 8 monthsof age (Menalled *et al.* 2000).

Mouse Model	CAG repeat length	Background	Construct	Promoter	Life span	Motor Deficits	Cognitive Deficits	Neuropathology
Hdh^{Q111} (Wheeler *et al.* 1999)*	109	129/CD1	Chimeric human/mouse exon 1 inserted into the murine Htt gene.	Endogenous mouse *mHTT*	Normal	Mild reduction in startle (Menalled *et al.* 2009)	Increase in anxiety reported on a CD1 background (Orvoen *et al.* 2012). Long term memory deficits (Giralt *et al.* 2012)	NII's from 10 months of age (Wheeler *et al.* 2000)
Hdh^{Q140} (Menalled *et al.* 2003)	140	C57/BL6	Chimeric human/mouse exon 1 inserted into the murine Htt gene.	Endogenous mouse *mHTT*	Normal	Rotarod, open field and wheeling running (Hickey *et al.* 2008) Decrease in rearing, decreased latency to fall on rotarod. (Rising *et al.* 2011)	Fear conditioning and anxiety (Hickey *et al.* 2008).	NII's in striatum from 6 months of age (Rising *et al.* 2011)
Hdh^{Q150} (Lin *et al.* 2001)	150	C57/BL6	Replacement of endogenous mouse CAG repeats with human CAG repeats.	Endogenous mouse *mHTT*	Normal	Grip strength (Woodman *et al.* 2007) Rotarod and grip strength (Brooks *et al.* 2012d)	Pre pulse inhibition Water maze task (Brooks *et al.* 2012d) Set shifting deficits (Brooks *et al.* 2006)	NIIs from 25 weeks of age (Lin *et al.* 2001)
Hdh^{Q175} (Menalled *et al.* 2012)	188	C57/BL6	Chimeric human/mouse exon 1 inserted into the murine Htt gene	Endogenous mouse *mHTT*	90 weeks	Pre-seizure activity, body tremor, abnormal gait, limb splay (Heikkinen *et al.* 2012), piloerection (Menalled *et al.* 2012), Rotarod, weight and gait deficits (Smith *et al.* 2014)	Two- choice swim test (Heikkinen *et al.* 2012). Circadian rhythm deficits (Loh *et al.* 2013). Decreased spontaneous alternation (Smith *et al.* 2014)	Hyperexcitability in MSNs and attenuated glutamate transmission (Heikkinen *et al.* 2012). Axonal and synaptic protein alterations (Smith *et al.* 2014)

Mouse Model	CAG repeat length	Background	Construct	Promoter	Life span	Motor Deficits	Cognitive Deficits	Neuropathology
Hdh^{Q200} (Heng *et al.* 2010)	200	C57/BL6	Replacement of endogenous mouse CAG repeats with human CAG repeats.	Endogenous mouse *mHTT*	Unknown – study conducted until 80 weeks.	Weight loss from 40 weeks, imbalance and co-ordination deficits at 50 weeks, gait deficits at 60 weeks, hypoactivity, thin, grip deficits and poorly groomed at 80 weeks of age (Heng, 2010).	None reported	mHTT aggregate formation at 9 weeks of age, NII's by 20 weeks (Heng *et al.* 2010)
$Hdh^{6/72}$ (Shelbourne *et al.* 1999)	72	C57/BL6/129	CAG repeat insertion into exon 1 of endogenous mouse gene	Endogenous mouse *mHTT*	Normal	None reported	Increased aggression in the resident intruder task at 9-12 months of age (Shelbourne *et al.* 1999).	Smaller brain weights at 4-6 months of age. No other immunochemical changes observed (Shelbourne *et al.* 1999).
$Hdh^{4/80}$ (Shelbourne *et al.* 1999)	80	C57/BL6/129	CAG repeat insertion into exon 1 of endogenous mouse gene	Endogenous mouse *mHTT*	Normal	None reported	Increased aggression in the resident intruder task at 9-12 months of age (Shelbourne *et al.* 1999).	Smaller brain weights at 4-6 months of age. No other immunochemical changes observed (Shelbourne *et al.* 1999).

In addition, Woodman *et al.* (2007) demonstrated motor abnormalities including; rotarod deficits at 72 weeks and grip strength deficits at 24 weeks of age in Hdh^{Q150} animals. Furthermore, homozygous Hdh^{Q150} animals showed cognitive impairments in spatial learning and navigation in the Morris water maze task at 16 weeks of age, abnormalities in acoustic startle and prepulse inhibition tests at 24 weeks of age, weight loss from 61 weeks of age and rotarod impairments from 84 weeks of age (Brooks *et al*. 2012d; Brooks *et al*. 2012g). Molecular investigations regarding the neurobiology of the Hdh^{Q150} mouse line demonstrated an inverse correlation between soluble and aggregate mHtt fragments in the striatum from 3 to 8 months of age, after this time period the pool of soluble mHtt fragments remained constant (Marcellin *et al*. 2012). Furthermore, Young *et al*, demonstrated that Hdh^{Q150} mice exhibited wide spread NII formation in the striatum, olfactory bulb, hippocampus and cerebellum, extra-nuclear aggregate formation was increased in the dentate gyrus and decreased levels of DARPP-32 were also shown (Young *et al.* 2013).

The Hdh^{Q175} (zQ175) mouse line has recently been developed and characterised (Menalled *et al.* 2012), this line demonstrates behavioural deficits in heterozygous animals from 4 weeks of age, including; hypoactivity in open field testing as young as 8 weeks of age, increased rearing at 8 weeks of age, decreased latency to fall from the rotarod at 30 weeks of age, and weight loss at 52 weeks of age. Behavioural deficits were found to be significantly greater in homozygous animals than in heterozygous animals when compared to wild type, in; reduced grip strength, decreased climbing ability, reduced cognitive ability in a discrimination reference memory two-choice swim task and overall decreased life span (Menalled *et al.* 2012). Additional testing on the zQ175 mouse line performed by Heikkinen *et al*. (2012), confirmed the behavioural deficits previously demonstrated by Menalled *et al*. 2012 and extended the characterisation to include electrophysiological, morphological, volumetric, magnetic resonance imaging (MRI) and magnetic resonance spectroscopy (MRS) data. Similar deficits were demonstrated in both studies in open field

demonstrating reduced activity at 16 weeks of age, increased rearing frequency at 8 weeks of age (Heikkinen *et al.* 2012). In addition, electrophysiology data demonstrated hyperexcitability in the MSNs of zQ175 animals, attenuated glutamate transmission was also shown and MRI analysis revealed decreased whole brain, cortical and striatal volumes in comparison to wild type animals (Heikkinen *et al*. 2012).

More, recently work on the zQ175 mouse line (Smith *et al.* 2014) has demonstrated altered gait at 1 month of age, decreased grooming and rotarod deficits at 6 months of age, decreases in spontaneous alternation and nest building capacity at 16 months of age and a failure to gain weight beginning at 12 months of age (Smith *et al.* 2014). Molecular analyses revealed NIIs in the striatum which progressed from 6 months of age. In addition, selective neuronal vulnerability was demonstrated in the striatum and cortex, with decreased cortical and striatal volumes shown at 12 months of age. Finally, levels of the synaptic protein dynein were decreased in the striatum of zQ175 animals at 6 months of age, cytoskeletal and axonal transport changes were seen at 12 months of age (Smith *et al.* 2014).

Hdh^{Q200} animals demonstrated perhaps the most severe neuropathology of all the knock-in mouse lines, with aggregate formation as young as 9 weeks of age, NII formation by 20 weeks of age and motor deficits at 50 weeks of age. (Heng *et al.* 2005). The severity of the phenotype is due at least in part to the length of the CAG repeat, which is under the control of the endogenous mouse promoter.

Both $Hdh^{4/80}$ and $Hdh^{6/72}$ mouse lines were generated by Shelbourne *et al*. 1999. The knock in mouse lines generated contained 80 and 72 CAG repeats respectively, specifically targeted into exon 1 of the mouse endogenous *HTT* gene, under the control of the mouse endogenous promoter. Both groups of knock-in animals demonstrated significantly smaller brain weights at 4-6 months of age. Although no other abnormal immunohistochemical changes were found in terms of neuropathology. Increased aggression was demonstrated in male

knock-in animals in comparison to wild type animals, at 9 -12 months of age in the resident intruder task (Shelbourne *et al.* 1999). No motor abnormalities have yet been reported in these mouse lines.

A large number of knock-in mouse models which recapitulate many of the symptoms of HD are available for use in scientific research (Table 1.3). However, these have been generated using different genetic constructs and on different background strains, which produce altered HD phenotypes. Therefore, in order to evaluate which mouse models to use in future research studies, the longitudinal phenotype of each mouse model must be characterised, considered and evaluated against other mouse models to determine which mouse model is the most appropriate for use.

Previous work in the Hdh^{Q92} mouse model demonstrated relatively selective neuropathology and robust but subtle behavioural changes in homozygous animals (Wheeler *et al.* 2000a; Trueman *et al*. 2007, 2008a; Trueman *et al.* 2009a; Brooks *et al.* 2012b; Trueman *et al.* 2012a; Trueman *et al.* 2012d). The Hdh^{Q111} mouse model contains a similar genetic construct to the Hdh^{Q92} mouse model, although the CAG repeat length is expanded. Therefore, the Hdh^{Q111} mouse model was chosen as the subject of this thesis based on previous work in the Hdh^{Q92} model and because a longitudinal behavioural analysis is yet to be performed in Hdh^{Q111} animals. Hdh^{Q111} animals were bred on to a C57BL/6J background as this background strain in more responsive to operant testing (unpublished data). In addition, the use of a C57BL/6 background strain allows comparison with other HD mouse models bred onto the same background.

1.7. Thesis Aims

This thesis has the following aims;

1) To understand the development of neuropathological and behavioural change in the Hdh^{Q111} mouse model of HD.

2) To determine the range and severity of phenotypic changes in the Hdh^{Q111} mouse model and how these relate to other mouse models of HD and the human condition.

3) To develop and test novel behavioural tasks for use in mouse models of neurological diseases, including HD.

Chapter 2 : Materials and Methods

2.1. Animal Husbandry and Legislation

For all experiments animals were group housed, although in some instances male animals had to be separated and singly housed to prevent fighting. All experiments were conducted in adult mice, the exact age of the mice used in testing is stated in the Materials and Methods section of each chapter. All animals were maintained on a 12 hour light/dark circadian schedule (06.00 hours lights on, 18.00 hours lights off), in a temperature controlled environment (21ºC ± 2ºC). Animals were given *ad-libitum* access to food and water unless otherwise stated. Animals were housed with one chew stick and cardboard tube per cage. Animals were weighed and health checked weekly, except when under food or water restriction, where daily health checks and weighing was required. All experiments were conducted in accordance with the United Kingdom Animals Scientific Procedures Act (ASPA) 1986. From 1st January 2013, the European Union (E.U.) Directive 2010/63/EU was implemented into UK law by an update of ASPA 1986.

In the instances that C57BL/6J animals were used (for the experiments outlined in Chapter 7), these were obtained commercially, from Charles River (Margate, Kent, U.K.), at 6-8 weeks of age and tail marked with permanent marker to allow identification. Hdh^{Q111} knock-in mice were originally obtained from Jax® (Bar Harbour, Maine, U.S.A.) and bred inhouse on a C57BL/6J background, typically using $Hdh^{Q111/+}$ and wild type pairings. After a minimum of 10 generations C57BL/6J animals were used to re-establish the colony and reduce the level of genetic variance. Animals were genotyped using standard PCR methods (see 2.1.1). The number of CAG repeats present in the breeding colony had expanded since the original description (Wheeler *et al*. 2000a). $Hdh^{Q111/+}$ animals trinucleotide repeat length ranged from 125-145 CAG repeats (typically containing an average of 138 CAG repeats) on the affected allele. The CAG

repeat length in wild type animals and in the unaffected allele of $Hdh^{Q111/+}$ animals, was typically 7 CAG repeats. Litters were weaned, ear notched for identification purposes and tail tipped for genotyping.

2.1.1. Genotyping

Upon weaning, all Hdh^{Q111} animals were tail tipped for genotyping purposes. Ethyl chloride anaesthetic spray (Vidant Pharma Ltd, Surrey, U.K.) was applied to the tip of the tail before removing a 1mm section. The tail was then cauterised with a silver nitrate pen and samples were collected in Eppendorf tubes. All samples were then shipped on dry ice to Laragen Inc. (Culver City, California, U.S.A). All genotyping was performed by Laragen Inc. using probe based qPCR to generate the length of the CAG repeat on both chromosomes and therefore the genotype of each individual animal.

2.2. Behavioural Techniques

2.2.1. Rotarod

Rotarod testing was used to provide an overall assessment of motor co-ordination and balance. Animals received 5 days of training on a rotarod apparatus (Ugo Basile, Model Number 47600, Varese, Italy), as shown in Figure 2.1. Day 1 consisted of training with the rod accelerating from 5 revolutions per minute (rpm) to 24rpm. Training days 2 to 5 consisted of training with the rod accelerating from 5rpm to 44rpm. During the training period animals were put back onto the accelerating rod if they fell from the apparatus. Training sessions lasted a maximum of 300 seconds (5 minutes) each and were completed on consecutive days. Animals were then tested on the apparatus which consisted of 2 trials (accelerating from 5rpm to 44rpm in a single session). The latency to fall from the rod was recorded for each trial following the activation of a button situated below the rotarod. The values from both trials were averaged to provide the rotarod latency statistic. If the mouse remained on the accelerating beam for whole 5 minute testing period, they were removed at the end of the testing session and given a score of 300 seconds.

Figure 2.1. Photograph of the rotarod apparatus used in behavioural testing.
The rotarod was manufactured by Ugo Basile S.R.I. 47600, VA, Italy.

2.2.2. Grip Strength

Grip strength testing was used to determine motor function and the strength of the fore limbs and hind limbs. Animals were placed on a mesh grid (30cm x 30cm) which had a wooden border. For testing, towels were placed under the apparatus to provide a comfortable landing surface. The grid was then inverted 30cm above the bench by placing 4 wooden posts under the border of the grid, such that the mouse was suspended upside down on the under surface (Figure 2.2). Latency to fall from the grid was recorded; the maximum time allowed per trial was 60 seconds. Each animal was tested twice on the same day, the times were averaged to provide the grip strength statistic.

Figure 2.2. Photograph of the inverted grid used in grip strength behavioural testing.
Photograph of the grip strength grid apparatus (30cm x 30cm) suspended on the wooden border of the grid using 4 posts such that the grid is raised 30cm above the bench. For testing, towels were placed underneath the apparatus.

2.2.3. Balance Beam

Animals were tested on a balance beam apparatus (1m in length, 17º angle of ascent, with a 1.5cm to 0.5cm taper across the width) to determine motor co-ordination and balance, as shown in Figure 2.3A. Animals were trained on the apparatus to encourage traversing of the beam to the 'house' box located at the high end. Training day 1 consisted of placing the mouse on the beam initially close to the 'house' box and then increasing the distances away from the 'house box' until the animal was at the start area of the beam. The mouse was then placed facing away from the 'house' box at the end of the beam (to allow turn time to be measured as a measure of motor coordination) and encouraged to turn around and fully traverse the beam, as shown in Figure 2.3B. The second day of training consisted of 2 trials where the animal was placed facing away from the 'house' box and needed to turn around and traverse the beam to enter the 'house' box at the top. Testing was conducted by 2 consecutive trials which were videotaped to enable analysis. The animal was placed at the far end of the balance beam facing away from the 'house' box; the time taken to turn around and the time taken to traverse the beam were recorded. Between each trial mice were given 1 hour to recover before the next trial. While traversing the beam foot slips for the front and hind legs on each side were recorded, one side was live scored and the other video recorded to allow bilateral analysis.

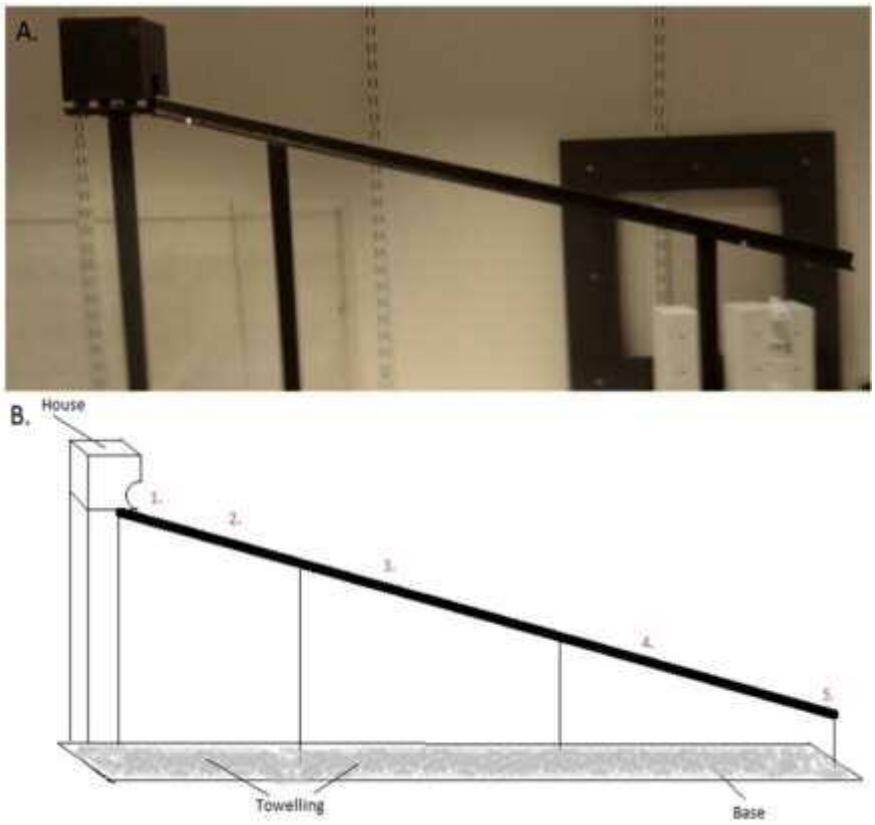

Figure 2.3. Schematic representation of the balance beam apparatus used for testing and the associated training schedule. A. The balance beam apparatus was constructed from wood painted black, with a goal 'house' located at the top of the beam. 1m of the beam was used for testing, the beam was 17º from horizontal and towels were used to provide cushioning at the base on the apparatus. **B.** During training animals were initially placed at position 1 and encouraged to traverse the beam and enter the house located at the top. Mice were subsequently placed further down the beam (positions 2, 3, 4 and 5) and similarly encouraged to run along the beam to enter the house at the top.

2.2.4. Spontaneous Automated Locomotor Activity

Animals were placed in a clear Perspex cage (dimensions 40cm x 24cm x 18cm) on a metal rack through which 3 infrared beams were able to pass, crossing the base of each cage, as shown in Figure 2.4. Locomotor activity was recorded, via non-perseverative infrared beam breaks, for a total of 32 hours on MED-PC® (version 4) software (Vermont, USA) to determine changes circadian cycle mediated activity levels. Animals were allowed *ad-libitum* access to both food and water which were placed at opposite ends of the clear Perspex cage. Lamps were placed into the testing room and set on a timer to ensure that animals were

maintained on their standard 12 hour light/dark cycle, lights on at 06.00 hours and lights off at 18.00 hours.

Figure 2.4. Photograph of automated locomotor activity cage.
Each clear Perspex cage (dimensions 40cm x 24cm x 18cm) was placed on a metal rack through which 3 infrared beams were able to pass. *Ad-libitum* food and water was provided at opposite ends of the cage. Locomotor activity was recorded, via infrared beam breaks, for a total of 32 hours on MED-PC® software (Vermont, USA).

2.2.5. Sucrose Consumption

Sucrose consumption was measured as an indicator of motivation and physical ability to consume a palatable reward. Sucrose consumption testing was conducted for 8 hours during the light phase from 09.00 hours to 17.00 hours. Animals were singly housed and placed in a standard cage (dimensions 45cm x 20cm x 15cm) with *ad-libitum* access to water and access to 4 grams of sucrose pellets (TestDiet®, Minnesota, U.S.A.) placed in a plastic dish in the centre of the cage. At 17.00 hours the quantity of sucrose consumed was recorded, as was the body weight of the animal. Animals were then returned to their home cages. Sucrose consumption testing occurred for a total of 4 consecutive days.

2.2.6. Food Consumption

Animals were singly housed in a standard cage (dimensions 45cm x 20cm x 15cm) and allowed *ad-libitum* access to both food and water for a 16 hour overnight period. The quantity of food consumed overnight was recorded the following morning as a measure of motivation and physical ability to consume food. Food consumption testing occurred for a total of 4 consecutive days.

2.2.6. Gait Analysis

Measurement of gait analysis was conducted to determine any development in gait abnormalities, using a clear Perspex corridor apparatus (65cm x 5cm x 15 cm) which was lined with a pre-cut piece of white paper, as shown in Figure 2.5A. Animals were trained to run to the enclosed darkened box at the end of the corridor by placing the mouse at the far end of the corridor and encouraging them to move towards the goal box at the end. Training was conducted twice for each mouse until the animal readily ran to the end box without encouragement.

For testing, the paws of the animal were painted with non-toxic paint (TTS paint, Nottinghamshire, UK); red was used for the front paws and blue for the hind paws. The animal was then placed at the near end of the apparatus and ran to the enclosed goal box at the far end of the apparatus, leaving a print of associated foot prints on the paper at the base of the apparatus. The mouse was then placed into a water bath to wash and groom before being returned to the home cage. The paper print (Figure 2.5B) was then allowed to air dry to enable analysis (Figure 2.5C) of; stride length, width and overlap for both the front and hind paws of each animal. For each animal, this was calculated using 4 paw prints (Figure 2.5C), this allowed 3 values to be calculated for each measurement (stride length, stride width and overlap) which were then averaged to provide gait measurements.

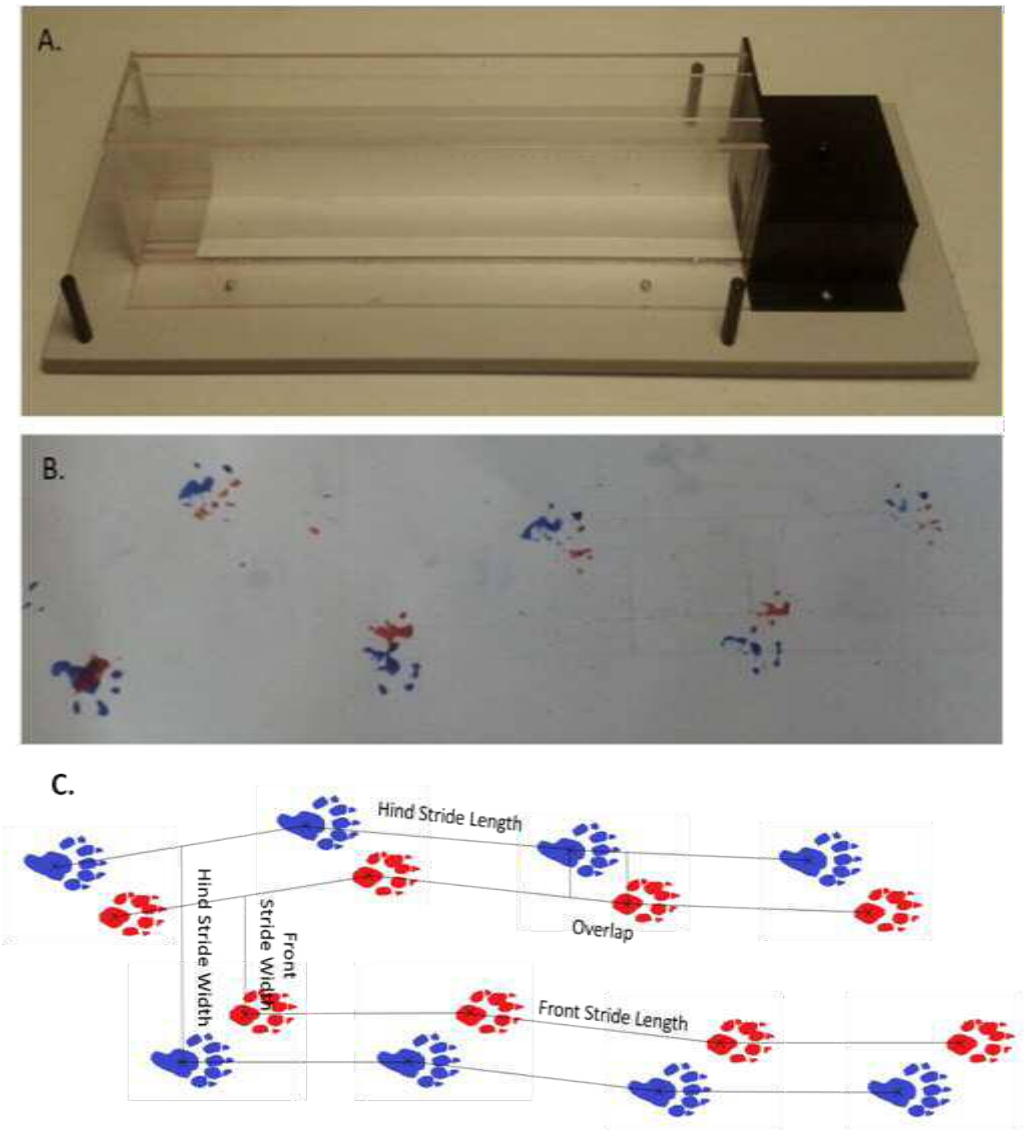

Figure 2.5. Gait analysis apparatus and representative images of the results obtained. **A.** A photograph of the gait corridor apparatus used in gait analysis. **B.** Representative image of the results obtained from gait analysis. **C.** Schematic illustration of gait analysis measurements of stride length, stride width and stride overlap.

2.3. Operant Techniques

2.3.1. 9-hole Operant Box Apparatus

Operant testing was conducted in a total of 16 9-hole operant boxes (Campden Instruments, Loughborough, UK), measuring 14cm x 13.5cm x 13.5cm, controlled by a BehaviourNet Controller BNC MKII operating system (Campden

Instruments, Loughborough, UK). Each operant box (Figure 2.6A) constituted a sound attenuating chamber which enclosed the 9-hole box made of aluminium on all sides with a clear Perspex lid. Each box contained a litter tray beneath the metal grid floor. The rear wall of each chamber was curved and contained a horizontal array of nine holes (11mm in diameter, placed 2mm apart and 15mm above floor level) numbered 1-9 from left to right.

Each hole contained photocell infrared beams which were localised at the front of each hole to detect nose pokes. At the rear of each hole a white light emitting device (LED) acted as the target visual stimulus. Only holes 1, 3, 5, 7 and 9 were used in any given test presented in this Thesis; consequently black plastic film was placed in the light array to block unused holes (2, 4, 6 and 8) to prevent their use. A peristaltic pump delivered liquid reinforcement in the form of strawberry milk (Yazoo®, Campina Ltd, Horsham, UK) to a reward magazine at the front of the box, located opposite the 9-hole array (Figure 2.6). The reward size delivered into the magazine differed depending on the operant task, as stated in the results chapter. However, typically a 5µl reward was delivered, which required a 100 millisecond pump duration.

Reward delivery to the magazine was signalled by an LED located above the magazine and nose entry into the magazine was detected by an infrared beam located across the opening of the magazine. 'House lights' were also located on the side walls of the operant chamber, which illuminated to signal the end of a trial or time out intervals (TOI) within trials. Background noises were provided by an extractor fan and computer operating system. Each of the 9-hole operant boxes was modified to include a Skinner-like responding set up located opposite the 9-hole array (Figure 2.6B). As stated, for operant tasks either the 9-hole or the Skinner-like configuration was used.

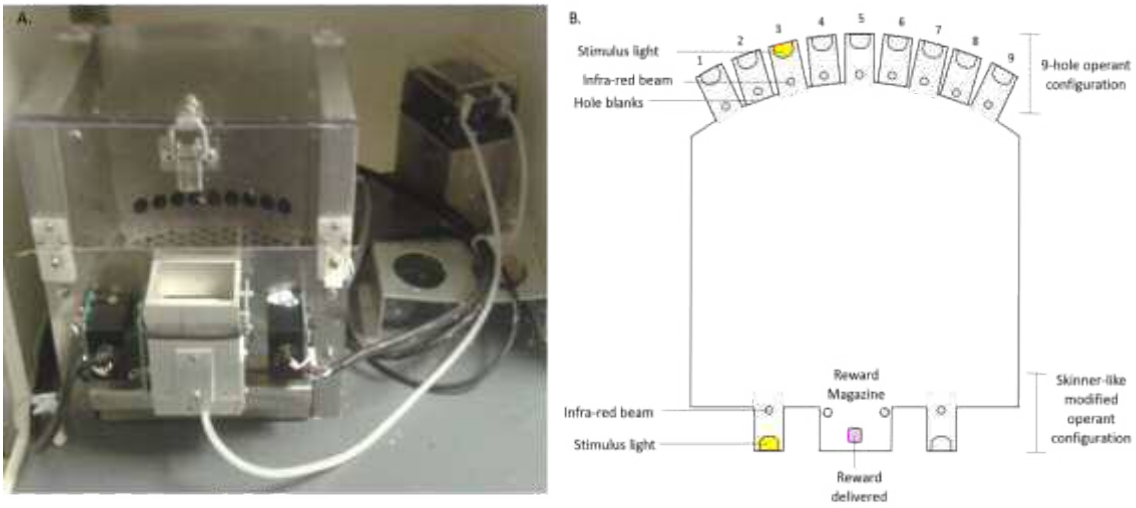

Figure 2.6. A. Overall picture of the mouse operant boxes used including the 9-hole and 'Skinner-like' set ups. **B.** Schematic of the operant box apparatus including both the 9-hole and 'Skinner-like' set ups. In the 9-hole set up the back response wall contained 9 stimulus lights, 5 of which were used in operant testing. Holes 1, 3, 5, 7 and 9 contained stimulus lights and photocell detectors to detect nose pokes via breaking of an infrared beam. Holes 2, 4, 6 and 8 were covered with well blanks. In the 'Skinner-like' set up 2 response holes were located either side of the reward magazine. Each nose poke contained a stimulus light and an infrared beam to detect nose poke responses.

2.3.2. Skinner-like Modified Operant Box Apparatus

The 16 9-hole operant boxes described above in 2.3.1. were modified to include additional Skinner-like nose poke response holes located either side of the reward magazine (Figure 2.6B). Each response hole measured 11mm in diameter and contained photocell infrared beams localised at the front to detect nose pokes and a white LED at the rear to act as a visual target. When the Skinner-like operant configuration was not in use the response holes were covered using black plastic inserts to prevent unwanted responding.

2.3.3. Water Restriction Regime

1 week prior to the beginning of operant testing gradual water restriction was introduced and animals were habituated to strawberry milk reward (Yazoo®, Campina Ltd, Horsham, UK) within their home cages, to prevent any neophobic response. Water restriction was introduced such that the time animals went without water was gradually increased daily for 1 week. Once fully water restricted animals were maintained on a restriction schedule of 3 hours access to water, which was given daily in their home cages for 5 days a week for the duration of operant testing. Animals were allowed *ad-libitum* access to water,

from Friday afternoon until Sunday afternoon to meet with licencing requirements provided by the Home Office.

2.3.4. 9-hole Box Operant Tests

To summarise the range of operant tests used in this Thesis, the primary purpose of each of operant test is shown below in Table 2.1. Although it should be noted that although the operant tests have a primary purpose there are also secondary measures which are discussed in more detail in the relevant chapters.

Table 2.1. Summary of operant tests used.

Operant Test	Primary cognitive function designed to probe
Fixed ratio (FR)	Acquisition and amenability in responding
Progressive ratio (PR)	Motivation
5-choice serial reaction time ask (5-CSRTT)	Attention
Serial implicit learning task (SILT)	Implicit learning
Delayed matching and non- matching to position (DMTP/DNMTP)	Working memory and reversal learning

2.3.4.1. 9-hole Box Training

At approximately 10 weeks of age mice were introduced to the 9-hole operant box apparatus. Initial training in responding into the magazine began with the non-contingent delivery of 150µl of strawberry milk into the magazine and illumination of the magazine light, for 1 day. Reward retrieval was detected via infrared beam breaks, which were triggered following nose entry and withdrawal from the magazine. After successful reward retrieval the magazine light was extinguished. The process was repeated until the 20 minute session time had elapsed.

After 1 day of magazine training, mice were taught to nose poke into the stimulus LED lights on a simple fixed ratio (FR1) schedule of reinforcement, for each trial, the central hole in the array of the operant box was illuminated. To obtain reward, mice were required to respond to the stimulus light in the central hole of the array via a single nose poke response. A correct nose poke in response to the stimulus light triggered the centre light to extinguish and the simultaneous illumination of the magazine light and delivery of a 5µl strawberry milkshake reward into the magazine. Upon withdrawal of the animal's nose from the magazine, the next trial was initiated with the illumination of the centre light. In order to promote learning, the centre light was painted with strawberry milk to encourage nose poking into the illuminated hole. Mice were trained on this programme until they had achieved over 100 pokes into the centre hole within the 20 min time period without additional painting of the centre hole with strawberry milk.

2.3.4.2. Fixed Ratio (FR) Testing

Fixed ratio (FR) operant testing was conducted after the 9-hole training described above in 2.4.1. The purpose of this test was to investigate the acquisition and amenability to respond when varying sizes of reward were used. Mice were required to poke into the central hole of the array, in response to illumination, to obtain reward. The operant programme utilised varying reward sizes of 2.5µl, 5.0µl and 7.5µl of strawberry milk, which were delivered into the reward magazine. Animals were reinforced with 5.0µl of reward for the first 3 days of testing, then 2.5µl of reward for the next 3 days, before finally rewarding animals with 7.5µl of reward for the final 3 days. The number of pokes into both the central hole and magazine was recorded in response to reward size, over a 45 minute session time.

2.3.4.3. Progressive Ratio (PR) Testing

The progressive ratio (PR) operant task tested the motivation of mice in terms of how many nose poke presses they were prepared to make to obtain a given level

of reward before ceasing to respond. The PR schedule of reinforcement required progressively more nose poke responses, to be made on each successive trial, into the illuminated central hole of the array for successive rewards. The number of responses required to obtain a reward increased by 3 additional responses every 3 trials. The first trial required 1 response for 1 reward, the next 3 trials required 3 responses for 1 reward, and the next 3 trials required 6 responses for 1 reward etc. There was no upper limit to the number of trials that could be initiated. The motivation of the animals to a obtain reward was recorded by measuring how quickly they stopped responding for a pre-defined period of time (30 seconds), termed the "break point". The overall ratio attained within the session was also recorded as a measure of motivation. Reward sizes of 2.5µl, 5µl and 7.5µl were used on separate days in PR testing. A 2.5µl reward size was used for the first 3 days of testing, the 7.5µl reward size was used for the next 3 days of testing and the 5.0µl reward size was used in the final 3 days of testing. Each session lasted for 60 minutes.

2.3.4.4. 5-Choice Serial Reaction Time Task (5-CSRTT) Testing

In the 5-Choice Serial Reaction Time Task (5-CSRTT) animals were trained to respond, via nose poking, to a stimulus light which was presented randomly across the 5 hole light array (Figure 2.6.), in order to receive a 5µl reward. The purpose of this task was to investigate attentional performance and spatial awareness. Three different stimulus lengths were used (10, 2 and 0.5 seconds), which increased the difficulty and attentional load of the task. For the first 10 days of testing a 10 second stimulus length was presented. A stimulus length of 2 seconds was then used for the next 5 days and a stimulus length of 0.5 seconds was used for the final 5 days of testing. Therefore, the attentional load of the task increased with decreasing stimulus length. If a response was not made within the stimulus length time, or the subsequent 10 seconds after the presentation of the stimulus (termed a 'limited hold') the light was extinguished and a 'time out' period of 10 seconds was initiated by illumination of the house light. The process was repeated until the 30 minute session time had elapsed.

2.3.4.5. Serial Implicit Learning Task (SILT) Testing

For the Serial Implicit Learning Task (SILT) animals were trained to respond to a 2-step sequence of stimulus lights in order to receive a reward. A continuous stimulus light was randomly presented in 1 of the 5 holes across the array (as in the 5-CSRTT). Then, a correct response to the first stimulus light (S1) resulted in the simultaneous extinguishing of this continuous light stimulus and illumination of a second light (S2). A correct response to S2 resulted in delivery of a 5µl reward into the magazine. A predictable stimulus sequence (hole 7 was always illuminated after hole 3) was embedded among other unpredictable sequences in order to probe implicit learning. The programme was repeated until the 30 minute session time had elapsed. The latency to respond accurately to S1 and S2 on each trial was recorded, subdivided according to the S1 holes and S2 step sizes, and whether the S2 choice was predictable.

2.3.4.6. Delayed Matching and Non-Matching to Position (DNMTP and DMTP) Tasks

The delayed matching to position and delayed non-matching to position (DMTP and DNMTP) operant tasks, were designed to test working memory and reversal learning in mice. The development of the DMTP and DNMTP operant tasks is the focus of Chapter 7 and thus a more in depth description of the task is given there.

The DMTP and DNMTP tasks were conducted in both the 9-hole operant apparatus (as described in Chapter 2, section 2.3.1.) and a modified 'Skinner-like' operant apparatus (as described in Chapter 2, section 2.3.2). However, the responses required were the same in each manipulation of the task. The animal was required to nose poke into a continuously illuminated stimulus; the animal was then re-centralised, via nose poking, before being presented with 2 stimulus lights that were illuminated simultaneously. The animal was then required to match the response with either the stimulus light that it previously responded into (the DMTP task) or in the reversal of the task, the stimulus light which it had not

previously responded to (the DNMTP task), to obtain a 5µl strawberry milk reward. The testing procedure was repeated until the 30 min session time had elapsed.

2.4. Histological Techniques

2.4.1. Tissue Preparation

Animals were culled via cervical dislocation and the whole brain dissected from the skull. The whole brain was divided into sagittal hemispheres using a razor blade. The right hemisphere was micro-dissected into constituent brain regions, snap frozen in liquid nitrogen and stored at -80°C, for subsequent gene expression analysis (undertaken by colleagues and not reported in this thesis). The left hemisphere was post fixed in 4% paraformaldehyde (PFA) at room temperature for 24 hours on a shaker and then transferred into 25% sucrose in phosphate buffered saline (PBS) solution for subsequent immunohistochemical analysis. For a full list of solutions and recipes see the Appendix.

2.4.2. Immunohistochemistry of Free Floating Sections

The left hemisphere of each brain (see tissue preparation 2.4.1. above) was frozen on a sliding sledge freezing microtome (Leitz, Wetzlar), cut coronally into a 1 in 12 series of 40µm sections and collected into 48 well plates (Thermo, Fisher Scientific) which contained an anti-freeze solution of di-sodium hydrogen orthophosphate. Sections were stored at -20°C until required. A 1 in 12 series of sections was selected from the 48 well plate and placed into a single well of a 25 well immunohistochemistry basket. The sections were washed in tris-buffered saline (TBS) to remove residual antifreeze, then endogenous peroxidase activity was quenched for 5 minutes using 10% H_2O_2 (VWR, West Sussex, UK) and methanol (Sigma-Aldrich, Dorset, UK) before further TBS washes were applied. Sections were blocked in 3% serum in Triton-X tris-buffered saline (TXTBS) for 1 hour to block non-specific binding sites. Sections were incubated in a solution of TXTBS, 1% serum and primary antibody, overnight at room temperature. For the primary antibody concentrations used for each antibody see Table 2.2.

Table 2.2. Primary antibody information summary.

Primary Antibody	Used to stain	Concentration Used	Species Raised in	Blocking serum	Supplier and Reference
DARPP-32	Medium spiny neurons	1: 30000	Mouse	Horse	Cornell University (AB_2314285) (Ouimet *et al.* 1984)
Neu-N	Neurons	1: 2000	Mouse	Horse	Millipore (MAB 377) (Mullen *et al.* 1992)
S830	Mutant huntingtin	1: 25000	Sheep	Horse	Gift from Gillian Bates (King's College London). (Sathasivam *et al.* 2001)

Following overnight incubation in primary antibody, sections were washed in TBS before incubation in biotinylated secondary antibody at a concentration of 1:200 for all stains for 2 hours at room temperature. Sections were then washed in TBS and incubated in an ABC kit (Vector Laboratories Ltd, Peterborough, Cambridgeshire), using a 1 in 200 dilution of both solutions A and B for 2 hours. The specific secondary antibodies used are specified in the methods section of each experimental chapter, although a summary of the secondary antibodies used in this Thesis is shown below in Table 2.3.

Table 2.3. Secondary antibody information summary

Secondary Antibody	Supplier
Biotinylated Horse Anti-Mouse IgG Antibody, rat adsorbed*	Vector (BA2001)
Biotinylated Horse Anti-Goat IgG Antibody*	Vector (BA9500)

* All secondary antibodies were used at a 1: 200 concentration.

Sections were washed in TRIS non-saline (TNS) before visualisation of the tissue bound antibody using the chromogen 3-3'-diaminobenzadine (DAB) supplied from DAKO. A 1:5 concentration of DAB in TNS was initiated by the addition of a 0.25% of H_2O_2. The reaction was allowed to develop until the stain could be clearly seen under the microscope. To stop the reaction sections were washed in TBS before being mounted on to double subbed 1% gelatinised slides

(Thermo Scientific, Menzel Gläser). Slides were allowed to air dry before dehydration in an industrial methylated spirit (IMS) alcohol ladder of increasing concentrations (70%, 95% and 100%) and were then cleared in 100% xylene. All slides were then cover slipped using distyrene plasticizer and xylene (DPX) (Thermo Scientific, Raymond Lamb, Leicestershire, UK) mountant and allowed to dry before subsequent analysis.

2.4.3. Cresyl Violet Staining.

Cresyl violet (CV) staining was used to gain an overall visualisation of cellular morphology by staining Nissl substance in the cytoplasm of neurons and glia. A 1 in 12 series of sections was selected from the 48 well plate and mounted onto glass slides double subbed with 1% gelatine and allowed to air dry. Once dry, slides were placed for 5 minutes in each of the following solutions: 70% IMS, 95% IMS, 100% IMS, 50/50 chloroform alcohol, 95% IMS, 70% IMS, distilled water, cresyl violet solution and distilled water. Sections were then dehydrated in 70% and 95% IMS for 5 minutes each, then destained by gentle agitation in acid alcohol solution for up to 5 minutes until an appropriate level of staining required was obtained. Additional dehydration in 100% IMS was performed, before clearing in xylene and cover slipping using DPX mountant.

2.5. Stereological Analysis and Image Acquisition

Stereological quantification was conducted using Visiopharm Integrator System (VIS, version 4.4.6.9) software on an Olympus Canada Inc. Q-Imaging Microscope. For each slide striatal sections were outlined under a 1.25X objective lens. Defined striatal sections were then sampled in a systematically automated fashion and cells were counted using oil under the 100X objective lens. The counting frame size used was 285µm^2. A representative image of the counting frame used is shown below in Figure 2.7. Cells that are within the counting frame and touching either of the green lines were included in the count, whereas cells that touched either of the red lines were excluded.

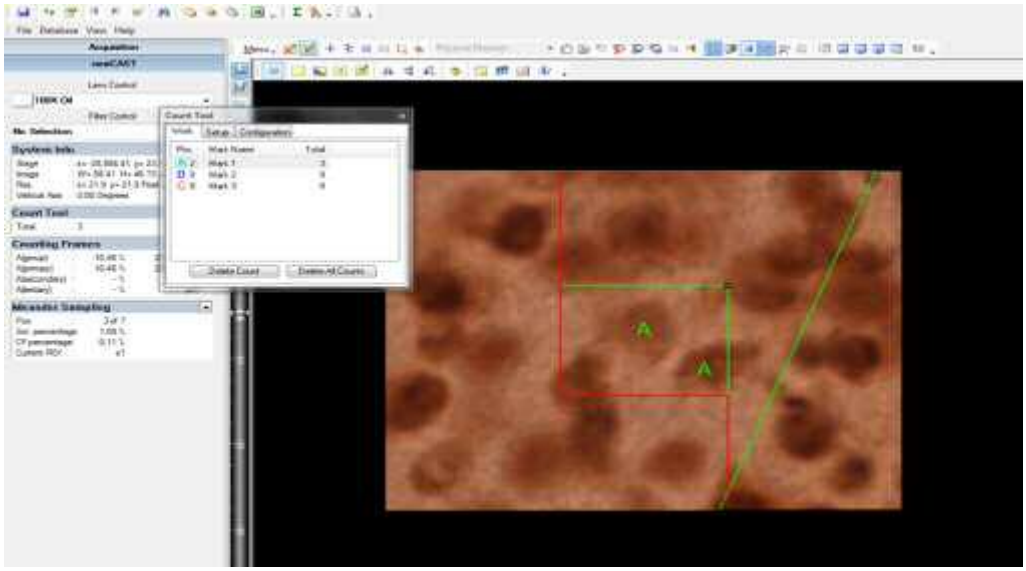

Figure 2.7. Representative image of stereological analysis conducted in Visiopharm Integrator System (VIS) software. The 285µm^2 counting frame can be seen in the centre of the image, as a square with a red and green border. Cells that were clearly within the counting frame and not touching the red lines were counted (2 cells in this image labelled with the letter 'A'). If a cell touched the red line within the counting frame it was excluded. The striatal border of this section can be seen to the right of the image as the green dashed line.

The number of animals used in each stereological analysis differed depending on the experiment, therefore the precise number of animals used is provided in each experimental chapter. The total number of cells (C) in each striatal section was calculated using the following formula:

$$C = \Sigma c \times (\Sigma A \times (\Sigma n \times a)) \times f$$

Where:
C = estimated total number of cells
Σc = total number of cells counted
ΣA = sum of all striatal areas
Σn = total number of frames allocated to the included striatal area
a = area of sampling user grid (285μm^2)
f = frequency of sectioning

An Abercrombie correction factor was then applied to the total number of calculated cells using the following formula (Abercrombie 1946):

aC = C x (ST /(ST+D)

Where:
aC = Abercrombie corrected cell count
C = estimated total number of cells
ST = section thickness
D = cell diameter.

Representative images of the stained sections were acquired using a Leica Microsystems Ltd microscope (Heerbrugg, Switzerland, Model CH-9435) and Leica Application Suite (LAS, version 3.8) software.

2.6. Statistical Analysis

All statistical analyses were performed in IBM SPSS Statistic 20 software for windows. Repeated measures analysis of variance (ANOVA) tests followed by simple effects analysis were conducted for the majority of behavioural tests. Although, the exact statistical analysis performed in each case is stated in the relevant methods section for each experimental chapter. Where significance was found *post-hoc* tests with Bonferroni corrections were applied to identify the locus of effects and their interaction(s). The critical significance level used throughout was $\alpha = 0.05$.

Chapter 3 : A longitudinal histological characterisation of the Hdh^{Q111} mouse model of Huntington's disease.

3.1. Introduction

An early neuropathological hallmark of HD is the selective loss of GABAergic medium spiny neurons (MSNs) within the striatum (Deng *et al*. 2004; Starr *et al*. 2008), with the relative sparing of other neuronal populations (Ferrante *et al.* 1985; Ferrante *et al.* 1987a; Ferrante *et al.* 1987b; Reiner *et al.* 1988). As MSNs are the major striatal afferents from the neocortex and the thalamus, the loss of these projections causes cortical atrophy which has also been shown to be a neuropathological feature of HD (Suzanne *et al.* 1988; Halliday *et al.* 1998; Rosas *et al.* 2002).

Another characteristic neuropathological hallmark of HD is the presence of protein aggregates and the progressive formation of inclusions of mutant huntingtin (mHTT), which can be both intranuclear and extranuclear (Vonsattel *et al*. 1985; DiFiglia *et al.* 1997; Gutekunst *et al*. 1999; Sieradzan *et al*. 1999). The precise cause of the formation of neuronal intranuclear inclusions (NIIs) is still under debate. While many studies have indicated that inclusion formation is a toxic mechanism (Li *et al*., 2001; Lee *et al*, 2004; Miller *et al*.,2010); others have shown mHTT to be pro-apoptotic, leading to the degradation of inclusions (Saudou *et al*., 1998; Ho *et al*., 2001; Huang *et al*., 1998). Therefore, the formation of NIIs may be a neuroprotective mechanism (Saudou *et al.* 1998; Guidetti *et al.* 2001; Zhou *et al.* 2001; Arrasate *et al.*, 2004, Mitra *et al*., 2009), at least in the first instance. Nevertheless, at the very least the presence of NIIs is a neuronal abnormality which is a characteristic neuropathological hallmark of HD, and has previously been shown in both transgenic (Davies *et al*. 1997; Schilling *et al*. 1999; Morton *et al*. 2000; Hansson *et al*. 2001; Meade *et al*. 2002;

Bayram-Weston *et al.* 2012c, d; Cheng *et al*. 2013) and knock-in (Wheeler *et al.* 2000b; Lin *et al*. 2001; Menalled *et al*. 2003; Heng *et al*. 2010; Rising *et al*. 2011; Bayram-Weston *et al.* 2012a; Bayram-Weston *et al.* 2012e) HD mouse models.

HD was previously thought to be a relatively focused disease of the striatum, causing both a gross reduction in striatal volume and enlargement of the lateral ventricles (Vonsattel *et al.* 1985; Aylward *et al.* 2004). Although, recent advances in *in vivo* imaging technology have demonstrated more widespread atrophy, which can affect: the cerebrum, total white matter, cerebral cortex, caudate, putamen, globus pallidus, amygdala, hippocampus, brainstem, and cerebellum (Thieben *et al.* 2002; Rosas *et al*. 2003; Reading *et al*. 2005; Henley *et al*. 2006; Rosas *et al*. 2008; Henley *et al*. 2009; Tabrizi *et al*. 2009; Hobbs *et al*. 2010; Tabrizi *et al*. 2011), often in the mid to early stages of the disease.

To determine the validity of the Hdh^{Q111} mouse model in recapitulating the neuropathological features of the human disease, immunohistochemical stains were conducted longitudinally to determine any neuropathological changes which occurred during the disease progression.

3.2. Aims

The aim was to characterise the development of neuropathology in the Hdh^{Q111} mouse model of HD, longitudinally over 18 months using histological and stereological techniques, and to determine its face and predictive validity as a model of HD.

3.3. Materials and Methods

3.3.1. Animals

Animals were group housed and allowed *ad-libitum* access to both food and water. Animals were housed with a single cardboard tube and wooden chew stick per cage to provide modest enrichment. All animals were maintained on a 12 hour light:dark circadian schedule (06.00 hours lights on, 18.00 hours lights off),

in a temperature controlled environment (21ºC ± 2ºC). Animals were weaned, genotyped and ear notched for identification purposes at 4 weeks of age (as described in Chapter 2) and allowed to age to the required time point before being humanely euthanised.

A total of 48 mice (24 $Hdh^{Q111/+}$) were used in this histological characterisation, a total of n = 4 for each genotype and age. $Hdh^{Q111/+}$ animals contained an average CAG repeat length of 138, which ranged from 132 to 142 CAG repeats.

3.3.2. Tissue Preparation

Animals were allowed to age to the required time point and were then culled via cervical dislocation and the whole brain dissected from the skull. The tissue was prepared as previously described in section 2.4.1. of Chapter 2.

3.3.3. Immunohistochemical Staining of Sections

Immunohistochemical staining was conducted utilising DARPP-32, Neu-N and S830 primary antibodies, as previously described in section 2.4.2 of Chapter 2.

3.3.4. Cresyl Violet Staining

Cresyl violet staining was conducted as previously described in section 2.4.3. of Chapter 2.

3.3.5. Stereological Quantification and Image Acquisition

Stereological quantification and image acquisition was conducted as previously described in section 2.5 of Chapter 2.

3.3.6. Statistical Analysis

All statistical analyses were performed in IBM SPSS Statistic 20 software. Repeated measures analysis of variance (ANOVA) tests followed by simple effects analysis were conducted for the majority of analyses. For s830 staining One-way ANOVAs were performed. Where significance was found *post-hoc*

tests with Bonferroni corrections were applied. The critical significance level used throughout was $\alpha = 0.05$.

3.4. Results

3.4.1. Cresyl Violet Results

The total number of CV stained cells within the striatum did not differ between $Hdh^{Q111/+}$ and wild type animals (Genotype; $F_{1,6} = 0.006$, p = n.s.), nor did the number of CV stained cells change as animals aged (Age; $F_{5,30} = 2.13$, p = n.s). There was also no significant interaction effect (Age x Genotype; $F_{5,30} = 0.508$, p = n.s.) as can be seen in Figure 3.1.

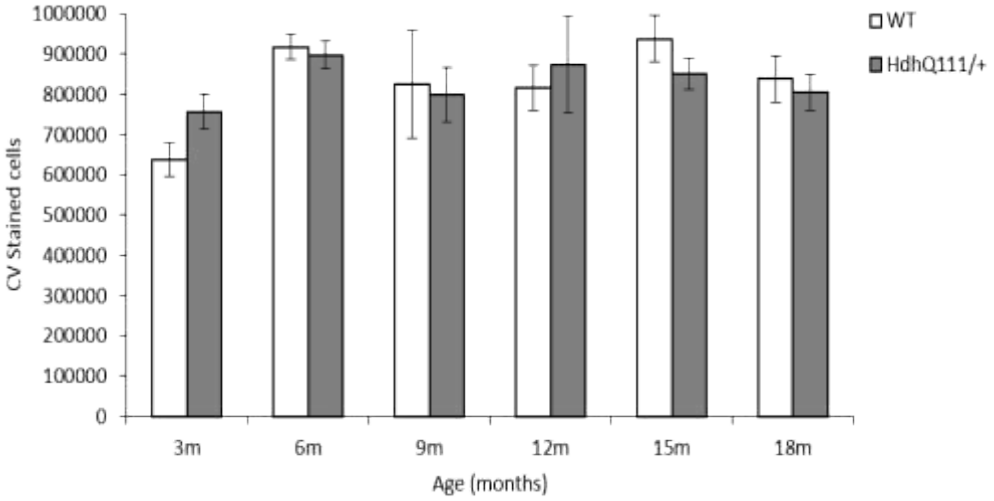

Figure 3.1. Cresyl violet (CV) longitudinal stereological results for $Hdh^{Q111/+}$ animals. $Hdh^{Q111/+}$ animals showed no statistically significant difference in the number of CV stained cells in comparison to wild type animals. Error bars represent ± standard error of the mean. N=4 in all cases.

The striatal volume, as measured by CV staining, showed that $Hdh^{Q111/+}$ animals demonstrated significant striatal atrophy in comparison to wild type animals (Genotype; $F_{1,6} = 11.98$, $p<0.01$). Although this difference was not progressive as animals aged (Age x Genotype; $F_{5, 30} = 7.14$, p = n.s.), a significant decrease in striatal volume was observed (Age; $F_{5,30} = 7.14$, $p <0.001$) as shown in Figure 3.2.

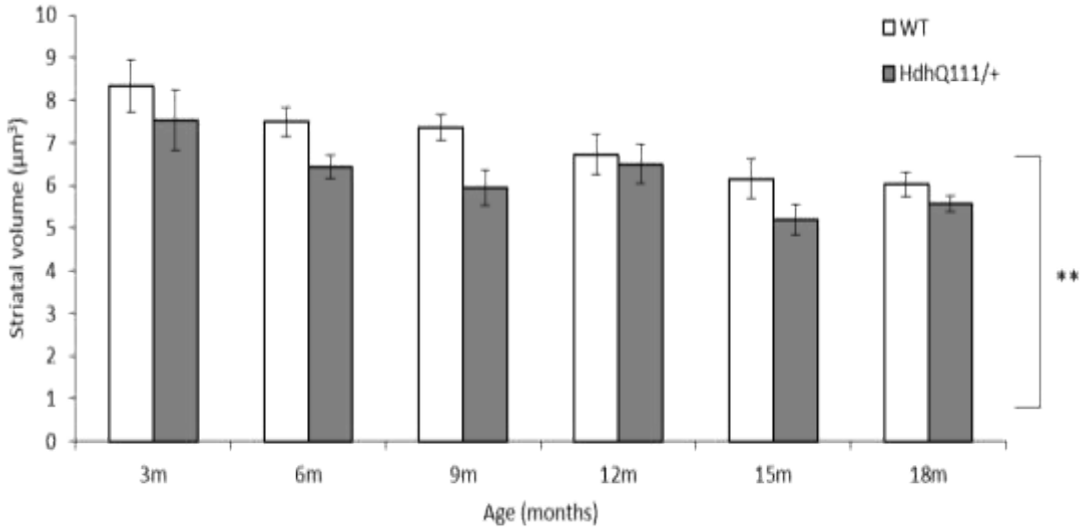

Figure 3.2. Striatal volume results for $Hdh^{Q111/+}$ animals. $Hdh^{Q111/+}$ animals showed significantly decreased striatal volumes in comparison to wild type animals, although this was not shown to be progressive as animals aged. Error bars represent ± standard error of the mean. N=4 in all cases. Statistical significance considers all ages ** $p <0.01$.

3.4.2. Neu-N Results

The number of Neu-N stained cells within striatum did not significantly differ between $Hdh^{Q111/+}$ and wild type animals (Genotype; $F_{1,6} = 1.25$, p = n.s.), although the number of Neu-N stained cells did significantly decrease as animals aged (Age; $F_{5,30} = 7.24$, $p <0.001$). Despite this, there was no significant interaction effect (Age x Genotype; $F_{5,30} = 0.93$, p = n.s.), as shown in Figure 3.3.

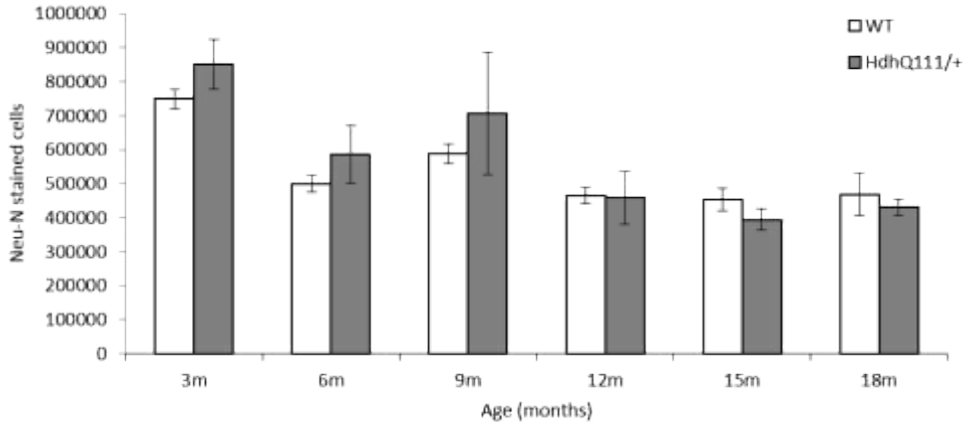

Figure 3.3. Neu-N longitudinal results of stereological analysis for Hdh$^{Q111/+}$ animals. Hdh$^{Q111/+}$ animals showed no statistically significant difference in the number of Neu-N stained cells in comparison to wild type animals. Although the number of cells significantly decreased as animals aged. Error bars represent ± standard error of the mean. N=4 in all cases.

3.4.3. DARPP-32 Results

A photographic representation of the differences in DARPP-32 staining in the striatum of wild type and Hdh$^{Q111/+}$ animals is shown below in Figure 3.4. The number of DARPP-32 stained cells within the striatum decreased as animals aged (Age; $F_{5,30}$ = 23.7, p <0.001). Hdh$^{Q111/+}$ animals had significantly fewer DARPP-32 stained cells within the striatum than wild type animals (Genotype; $F_{1,6}$ = 1.93, p <0.001), although this was not progressive as animals aged (Age x Genotype; $F_{5, 30}$ = 1.93, p = n.s.), as shown in Figure 3.5.

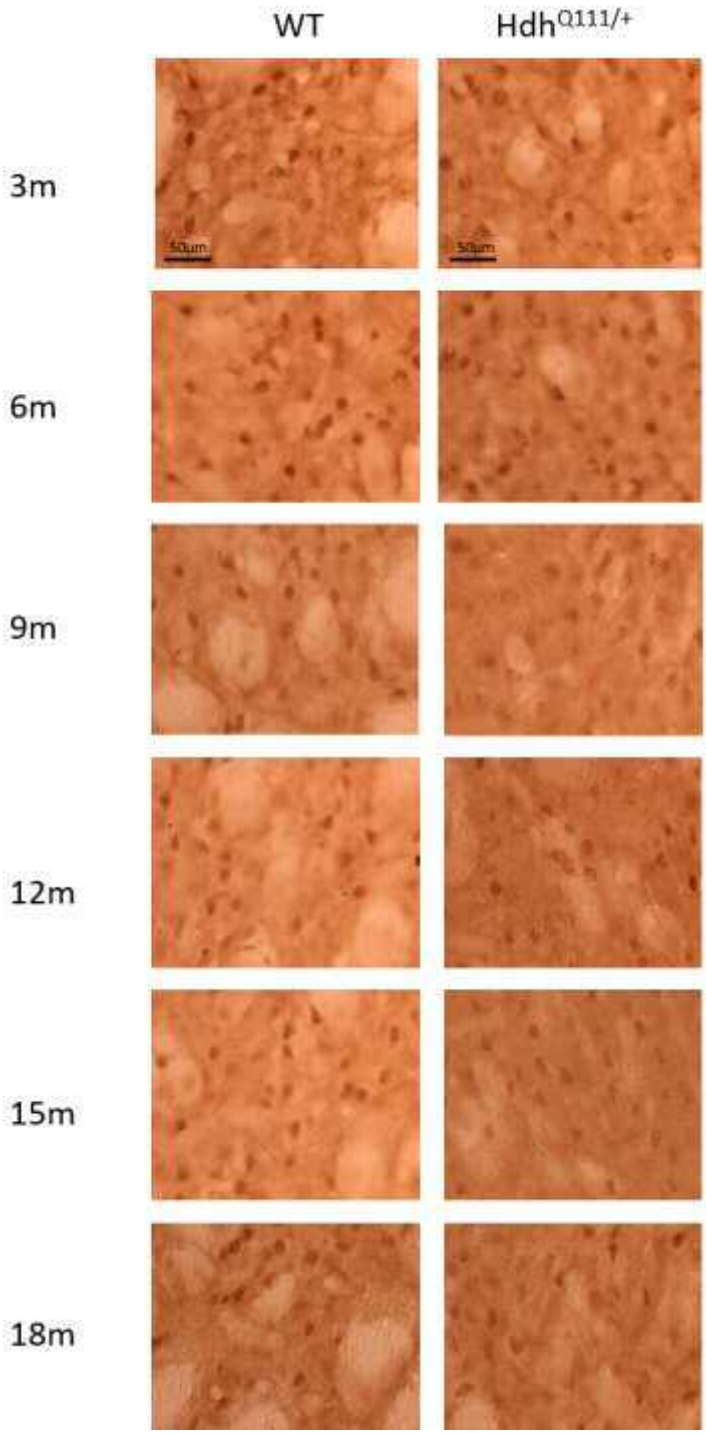

Figure 3.4. Representative light microscope images of DARPP-32 staining in the striatum of both wild type and Hdh$^{Q111/+}$ animals over time. Scale bars represent 50μm throughout and all photos were obtained using X40 magnification objective lens.

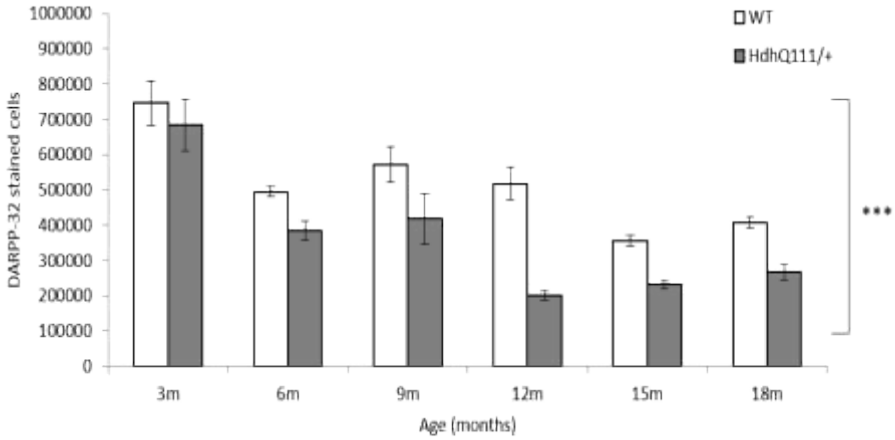

Figure 3.5. DARPP-32 longitudinal results of stereological analysis for Hdh$^{Q111/+}$ animals. The number of DARPP-32 positive cells significantly decreased as animals aged. Hdh$^{Q111/+}$ animals had significantly fewer DARPP-32 positive cells than wild type animals, although this was not shown to be progressive over time. Error bars represent ± standard error of the mean. N=4 in all cases. Statistical significance is presented considering all ages of testing *** $p<0.001$.

3.4.4. S830 Results

Representative light microscope images of the progression of S830 immunoreactivity within the Hdh$^{Q111/+}$ mouse striatum are shown below in Figure 3.6. All staining was negative in wild type animals. NIIs can clearly be seen from 6 months of age and form progressively until they are prevalent by 18 months of age.

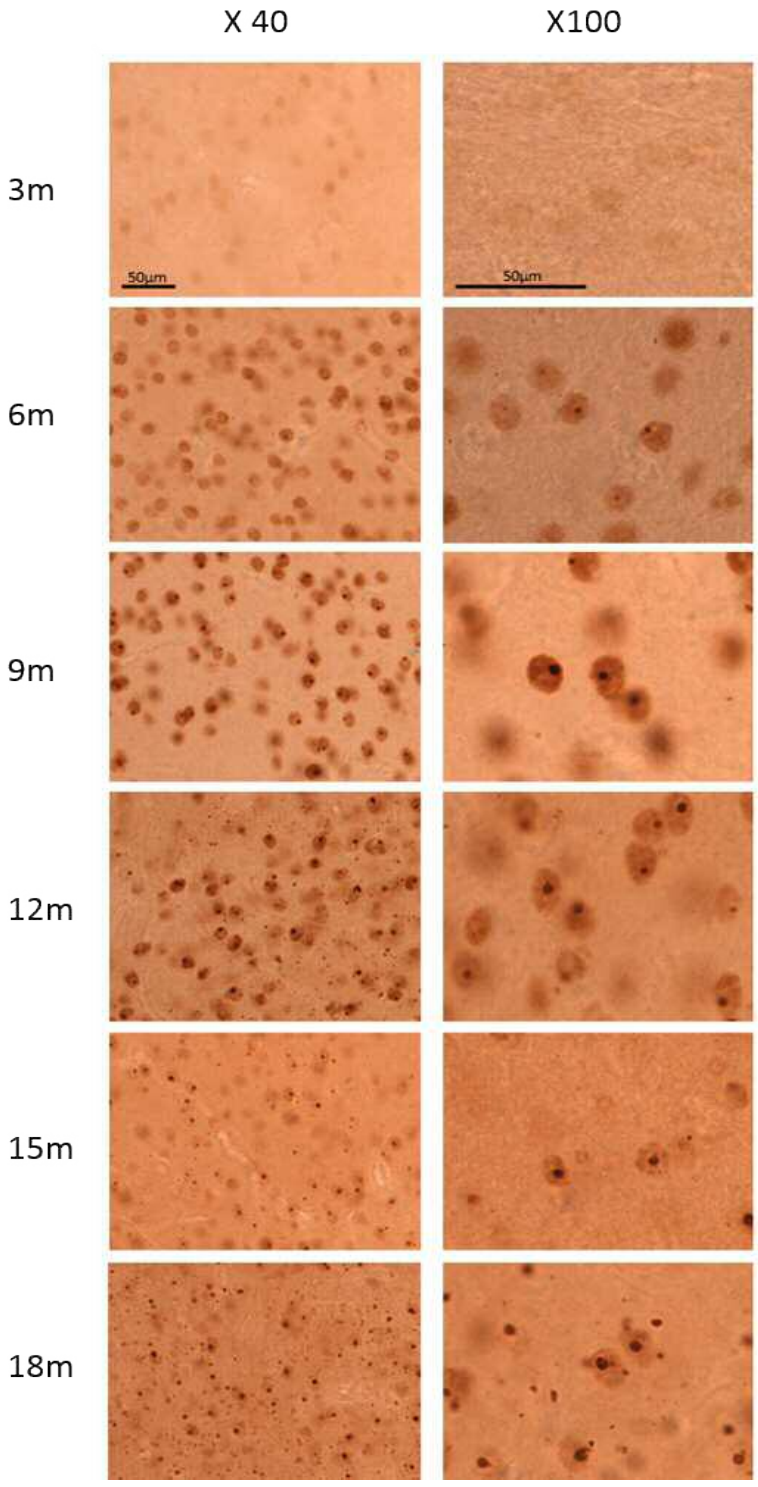

Figure 3.6. Representative light microscope images of S830 staining in the striatum of Hdh$^{Q111/+}$ animals over time. Images on the left were taken at X40 and images on the right were taken at X100. Scale bars represent 50µm throughout.

The total number of cells affected by mHtt, as determined by stereological quantification of S830 staining, demonstrated that significantly more striatal cells were affected by mHtt as animals aged (Age; $F_{5,15} = 5.05$, $p < 0.01$), as shown in Figure 3.7.

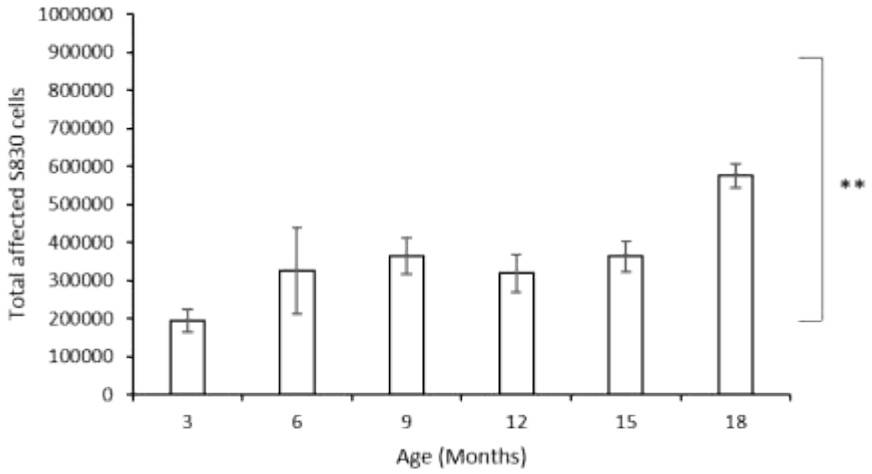

Figure 3.7. Total cells affected by S830 staining in $Hdh^{Q111/+}$ animals longitudinally. The number of cells affected by S830 staining significantly increased as animals aged. Error bars represent ± standard error of the mean. N=4 in all cases. Statistical significance is presented considering all ages of testing ** $p<0.01$.

Diffuse mHtt staining and overt mHtt inclusion staining were measured relative to each other. Diffuse mHtt staining was demonstrated in the striatum of $Hdh^{Q111/+}$ animals at 3 months of age and decreased as animals aged (Age; $F_{5,30} = 6.70$, $p < 0.001$). No diffuse mHtt staining was present in $Hdh^{Q111/+}$ animals at 18 months of age (Figure 3.8). Overt mHtt inclusions began to appear in the striatum at 6 months of age and were prevalent by 18 months of age (Figure 3.8). As animals aged, the number of cells affected by mHtt diffuse staining significantly decreased and the number of cells affected by overt mHtt inclusions significantly increased (Staining x Age; $F_{5,30} = 30.85$, $p<0.001$), as shown in Figure 3.8.

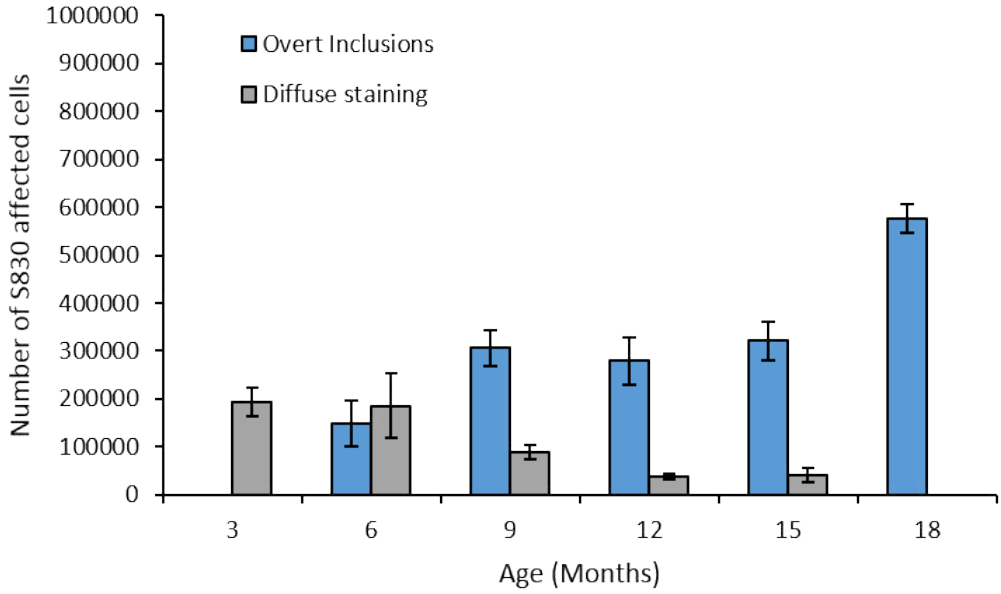

Figure 3.8. Cells affected by diffuse mHtt staining and mHtt inclusions longitudinally in Hdh$^{Q111/+}$ animals. The number of cells affected by diffuse mHtt staining significantly decreased as animals aged and the number of cells affected by overt mHtt inclusions significantly increased as animals aged. Error bars represent ± standard error of the mean. N=4 in all cases.

Furthermore, measurement of mHtt inclusion width in the striatum of $Hdh^{Q111/+}$ animals demonstrated that mHtt inclusions became significantly wider as animals aged (Figure 3.9).

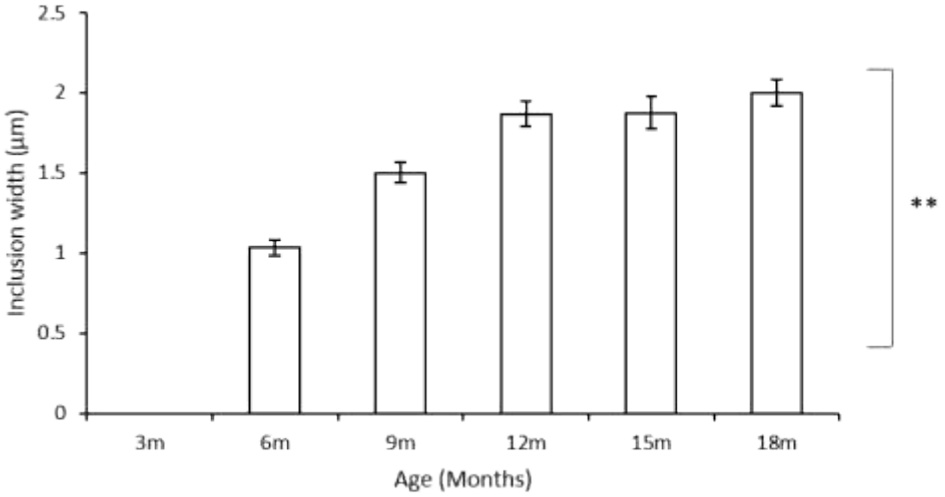

Figure 3.9. mHtt inclusion width measured longitudinally in $Hdh^{Q111/+}$ animals. The width of mHtt inclusions significantly increased as animals aged. Error bars represent ± standard error of the mean. N=4 in all cases. Statistical significance is presented considering all ages of testing *** $p<0.01$.

Stereological quantification of s830 mHtt inclusion staining was restricted to the striatum. Although, overt mHtt inclusions were also seen in other brain regions, including; the olfactory tubercle, piriform cortex, motor cortex, hippocampus and cerebellum. Although these observations were not quantified, overt mHtt inclusions were widespread throughout the brain of $Hdh^{Q111/+}$ animals by 18 months of age.

3.5. Discussion

The $Hdh^{Q111/+}$ mouse model of HD demonstrated striatal atrophy, as measured by cresyl violet staining and loss of DARPP-32 staining, which may be indicative of a loss of MSNs. Diffuse mHtt staining was seen from 3 month of age and was present in small amounts as animals aged until 18 months of age, where it was no longer evident. NIIs were shown to progressively develop from 6 months of age and were prevalent throughout the striatum at 18 months of age. This observation is in accordance with literature in other mouse models of HD

which shows mHtt aggregates progressively reduce as overt mHtt inclusions are formed (Davies *et al*. 1997; Wheeler *et al*. 2000b; Bayram-Weston *et al*. 2012b, c).

A significant reduction in the number of DARPP-32 positive cells in the striatum was shown in $Hdh^{Q111/+}$ animals, which may be indicative of a loss of MSNs, which is a hallmark of HD pathology (Deng *et al*. 2004; Starr *et al*. 2008). Studies of MSN degradation in HD mice are limited, in comparison to the number of studies regarding mHtt aggregate and inclusion formation; although our findings are in accordance with other studies in transgenic (Bibb *et al.* 2000; van Dellen *et al.* 2000) and knock-in mouse models (Hickey *et al*. 2008) which demonstrated a loss of DARPP-32 positive MSNs in the striatum of HD mice. No significant interaction effect between Age x Genotype was demonstrated for MSN loss, thus it cannot be considered to be progressive over time, as it was in previous studies. However, the lack of significant interaction is likely to be due to the small sample size considered in the statistical analysis. An in depth discussion regarding the effects of sample size on the statistical analysis is given in the General Discussion in Chapter 8.

Despite a reduction in the number of DARPP-32 stained cells in $Hdh^{Q111/+}$ animals, there was no significant difference in the number of cresyl violet stained cells in the striatum. In order to further explore the neuropathology in the $Hdh^{Q111/+}$ mouse model, in future studies, 'glial-like' nuclei could be quantified in the cresyl violet stain to determine if any gliosis occurs alongside striatal shrinkage.

In comparison to previous studies of the neuropathology in the Hdh^{Q111} mouse model (Wheeler *et al*. 2000b; Wheeler *et al.* 2002a), which demonstrated inclusion formation from 10 months of age, our findings demonstrate the appearance of NIIs 4 months earlier, at 6 months of age. However, previous characterisations of NIIs in Hdh^{Q111} animals were conducted using a CD1 background strain and a C57BL6/J background strain was used here. The mouse

background strain is known to be an important factor in the disease progression of HD and has previously been shown to modify the phenotype observed in the YAC128 model (Van Raamsdonk *et al*. 2007) and the Hdh^{Q111} model (Lloret *et al.* 2006). Lloret *et al*., concluded that NIIs develop most rapidly on the C57BL/6 background, which is supported by the results obtained here. Furthermore, it is important to consider that previous characterisations of the NIIs in the Hdh^{Q111} mouse model have used the EM48 antibody (Wheeler *et al*. 2000b; Wheeler *et al*. 2002a), rather than the S830 antibody that was used here.

The Hdh^{Q92} mouse model, which is most genetically similar to the Hdh^{Q111} mouse model, also demonstrates a different neuropathological phenotype when different genetic background strains are used. When Hdh^{Q92} animals were bred on a CD1 background strain, they demonstrated NII formation from ~12 months of age (Wheeler *et al*. 2000b), whereas when bred on a C57BL/6J background, NIIs were present from 6 months of age (Bayram-Weston *et al*. 2012b). Therefore, when our findings are compared to Hdh^{Q92} animals on the same background strain (Bayram-Weston *et al*. 2012b) they demonstrate a similar neuropathological profile with NIIs present in the striatum from 6 months of age. Although NII formation in other brain regions was not quantified, it seems that Hdh^{Q111} animals demonstrate a similar pattern of NII formation in the olfactory tubercle and piriform cortex as that previously described in Hdh^{Q92} animals. Although, it should be noted that the neuropathological analysis of Hdh^{Q92} animals on a C57BL6/J background was conducted in homozygous animals, rather than the heterozygous animals used here, therefore there does not seem to be a dosage effect in terms of comparative neuropathology. $Hdh^{Q111/+}$ mice demonstrated striatal atrophy, which has not previously been shown in the Hdh^{Q92} mouse model, therefore it could be the case that the large polyglutamine repeat length evokes a more rapid striatal volume reduction in HD mice.

Comparison of our findings to the results obtained in Hdh^{Q140} and Hdh^{Q150} animals (Lin *et al*. 2001; Rising *et al*. 2011) demonstrates a similar appearance of NIIs at 6 months of age. Due to the genetic expansion of the polyglutamine

repeat length, the Hdh^{Q111} animals used here had an average of 138 CAG repeats. Therefore, it is perhaps unsurprising that our Hdh^{Q111} animals demonstrated a similar neuropathological phenotype as that previously observed in Hdh^{Q140} and Hdh^{Q150} animals, due to the similar CAG repeat length. However, when our findings are compared to those observed in the zQ175 mouse model (Carty *et al.* 2015), NIIs were visible 2 months later in zQ175 mice in comparison to $Hdh^{Q111/+}$ mice. Therefore, it could be concluded that the appearance of NIIs inversely correlates with polyglutamine repeat length.

In comparison transgenic mouse models of HD such as the R6/1 (Hansson *et al*. 1999; Bayram-Weston *et al*. 2012c) and R6/2 (Morton *et al*. 2000; Meade *et al*. 2002) models, which first develop NIIs at 8 and 4 weeks of age respectively; $Hdh^{Q111/+}$ animals show a more slowly progressive and less severe neuropathology in terms of striatal volume reduction and NII formation. However, in comparison to other transgenic models such as the N171-82Q (Schilling *et al*. 1999) and the *Ubi*-G-HTT84Q (Cheng *et al*. 2013) models, $Hdh^{Q111/+}$ animals demonstrate a comparatively earlier appearance of NIIs which are more progressive and less severe throughout the life of the animal.

The aim of this neuropathological characterisation was to initially determine if the $Hdh^{Q111/+}$ mouse model replicated the neuropathology of the human condition. Therefore, due to time restrictions, in the first instance, stereological quantifications were restricted to the striatum. However, in future analyses it would be interesting to consider the neuropathology demonstrated in other brain areas. Particularly as HD is increasingly being recognised as a disease that affects the whole brain, including cortical atrophy (Rosas *et al.* 2002; Rosas *et al.* 2003; Tabrizi *et al.* 2009; Tabrizi *et al.* 2011) and because mHTT inclusions were seen in other brain regions such as the olfactory tubercle and the piriform cortex. Furthermore, additional characterisations of the neuropathology in Hdh^{Q111} mice may wish to consider performing immunohistochemical analysis for measures of factors which are required for normal neuronal functions such

as; tyrosine hydroxylase (TH), brain derived neurotrophic factor (BDNF), dopamine 1 (D1) and dopamine 2 (D2) receptors.

In conclusion, the $Hdh^{Q111/+}$ mouse model displays a progressive neuropathological phenotype, with a decrease in striatal volume and evidence of MSN cell loss. Diffuse mHtt staining was demonstrated from 3 months of age and was apparent until 15 months of age. Overt NIIs were observed from 6 months of age and were prevalent by 18 months of age. NIIs were also distributed more widely throughout the brain by 18 months of age, although this was not quantified. The neuropathological phenotype observed in $Hdh^{Q111/+}$ mice is therefore less severe than that observed in other transgenic mouse models and resembles the neuropathology seen in adult onset human HD patients.

Chapter 4 : A longitudinal motor characterisation of the Hdh^{Q111} mouse model of Huntington's disease.

4.1. Introduction

The presentation of overt motor symptoms, has been shown to occur during the disease progression of HD in both the human condition (Hefter *et al.* 1987a; Kremer and Group 1996) and in knock-in mouse models (Hickey *et al*. 2008; Trueman *et al*. 2009b; Heng *et al*. 2010; Brooks *et al*. 2012d; Smith *et al*. 2014). A clinical diagnosis of manifest HD is currently based on the total motor score (TMS) of the Unified Huntington's Disease Rating Scale (UHDRS), because there is a lack of sensitive cognitive assessments available to diagnose manifest HD. Therefore, in order to determine whether the Hdh^{Q111} mouse model accurately reflects the human condition of HD, it is important to determine if and when motor symptoms develop.

A behavioural test battery of motor function was applied longitudinally to determine the time course of symptom development in the Hdh^{Q111} mouse model of HD. The accelerating rotarod was used to assess motor co-ordination, as it has previously been used extensively to classify motor dysfunction in a range of HD mouse lines (Laforet *et al*. 2001; Hickey *et al*. 2008; Trueman *et al*. 2009b; Heng *et al*. 2010; Rising *et al*. 2011; Brooks *et al*. 2012d, c; Brooks *et al*. 2012f; Cheng *et al*. 2013; Southwell *et al*. 2013; Smith *et al*. 2014). A balance beam apparatus was used to determine abnormalities in co-ordinated movement and balance, which have been demonstrated in HD patients (Johnson *et al.* 2000) and mouse models (Heng *et al.* 2007; Brooks *et al*. 2012d, c; Brooks *et al*. 2012f). In addition, a further test of motor function utilising an inverted lid to test grip strength was used, as this has been used previously to determine motor function and strength in HD mice (Menalled *et al*. 2009; Brooks *et al*. 2012c, d).

Spontaneous locomotor activity testing was included in the motor test battery to investigate changes in both general locomotor activity levels and disruption to circadian rhythms. Circadian rhythm disruptions predominantly manifest as sleep disturbances in HD patients (Wiegand *et al*. 1991; Videnovic *et al*. 2009; Aziz *et al.* 2010) and have also been described in HD mouse models (Hefter *et al*. 1987a; Carter *et al*. 1999; Morton *et al*. 2005; Menalled *et al*. 2009; Wulff *et al.* 2010). Gait abnormalities have been shown in HD patients (Koller and Trimble 1985; Hausdorff *et al.* 1998; Reynolds Jr *et al.* 1999; Delval *et al.* 2006; Grimbergen *et al*. 2008; Rao *et al*. 2008) and HD mouse models (Lin *et al*. 2001), including the Hdh^{Q111} mouse model (Menalled *et al.* 2009). Thus, gait analysis was included in the motor test battery to determine any gait abnormalities. Finally, consumption tests were included to determine if animals were able to consume food adequately. Difficulties in swallowing, are evident in the later stages of the human disease (Leopold and Kagel 1985; Hunt and Walker 1989; Kagel and Leopold 1992), thus reducing the ability to consume food.

In addition to overt motor symptoms, weight loss is common in HD patients (Kremer and Roos 1992; Stoy and McKay 2000; Van Raamsdonk *et al.* 2006), even in the early stages of the disease (Djousse *et al*. 2002). Although the precise reasons for weight loss in HD remains unclear it is likely that both the underlying metabolic dysfunction (Koroshetz *et al*. 1997; Mochel *et al.* 2007b) and the chorea and movement abnormalities associated with the disease both contribute to the weight loss observed. Decreases in body weight as HD progresses have also been described in a range of both transgenic and knock-in mouse models (Carter *et al*. 1999; Brooks *et al*. 2012d, c; Brooks *et al*. 2012f), including the Hdh^{Q111} mouse line (Oroven *et al.,* 2012). However, the BACHD transgenic mouse line demonstrates an increase in body weight during HD disease progression (Gray *et al*. 2008). Therefore, it was necessary to determine the body weight progression of the Hdh^{Q111} animals to compare and contrast any weight changes seen to further determine how accurately the Hdh^{Q111} mouse model accurately reflects the human condition. Furthermore, weight loss was used to

determine the health and wellbeing of animals, a 20% weight loss of free feeding body weight was used to define a humane endpoint in experimental procedures.

4.2. Aims

To characterise the developmental time course, severity and nature of the motor phenotype in the Hdh^{Q111} mouse model of HD, using a longitudinal battery of motor tests.

4.3. Materials and Methods.

4.3.1. Animals.

A total of 56 age matched mice were used (36 $Hdh^{Q111/+}$ animals, with a CAG repeat length range 131-143 and an average of 138 CAG repeats). Of the 36 $Hdh^{Q111/+}$ mice, 16 were male and 21 were female. Of the 20 wild type mice 10 were female and 10 were male. Animals were housed in pairs or threes, although some animals had to be separated and singly housed to prevent fighting. Testing occurred during the light phase from 12.00 hours to 18.00 hours. A female $Hdh^{Q111/+}$ animal was culled at 12 months of age, and 2 male animals (1 WT and 1 $Hdh^{Q111/+}$) were culled at 16 months of age due to health issues unrelated to HD.

4.3.2. Behavioural Methods

The behavioural methods used in this experimental chapter have been described fully in the Materials and Methods sections of Chapter 2, therefore only a brief summary of each method is given below.

Animals were tested at 3 monthly intervals in a behavioural test battery that included: locomotor activity, rotarod, grip strength and balance beam. In addition, at six monthly intervals additional tests of food and sucrose consumption and gait analysis, were added into the test battery, as shown in Figure 4.1.

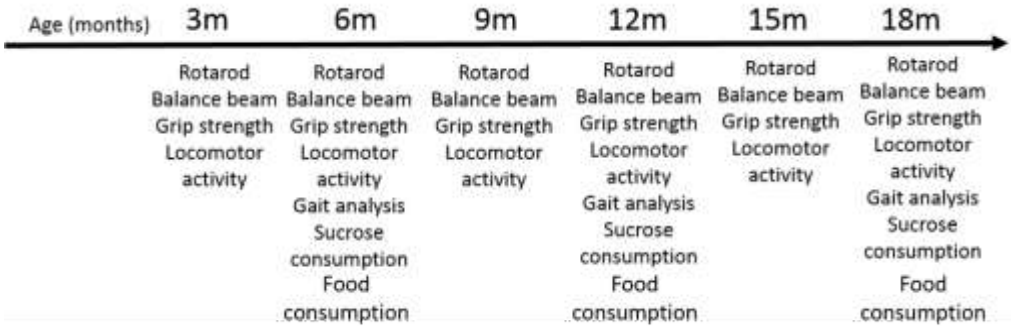

Figure 4.1. Longitudinal hand testing time scale.
Animals were tested in the longitudinal testing battery every 3 months. Rotarod, balance beam, grip strength and locomotor activity testing occurred every 3 months, from 3 months of age until 18 months of age. Additional tests of gait analysis, food consumption and sucrose consumption were completed at the 6 month, 12 month and 18 month time points.

4.3.2.1. Weight Progression

Animals were weighed monthly between 15.00 hours and 16.00 hours.

4.3.2.2. Spontaneous Locomotor Activity

Spontaneous locomotor activity was recorded for a total of 32 hours on MED-PC® software (Med Associates, Vermont, USA). Animals were maintained on a 12 hour light/dark cycle from 06.00 hours to 18.00 hours. Animals were allowed *ad-libitum* access to both powdered food and water, which were placed at opposite ends of the activity box.

4.3.2.3. Rotarod

Animals received 5 days of training on a rotarod apparatus, training sessions lasted a maximum of 300 seconds each and were completed on consecutive days. Testing consisted of 2 trials (accelerating from 5rpm to 44rpm), the latency to fall from the beam was recorded for each trial. If the mouse remained on the accelerating beam for the 5 minute testing period, they were removed and given a score of 300 seconds.

4.3.2.4. Grip Strength

Animals were placed on a mesh grid which was inverted such that the mouse was upside down on the under surface of the grid. Latency to fall was recorded,

the maximum time allowed per trial was 60 seconds. Each animal was tested twice.

4.3.2.5. Balance Beam

Animals were trained on the balance beam apparatus to encourage traversing of the beam to the goal box located at the elevated high end of the beam. Testing consisted of 2 separate trials which were videotaped to enable analysis. The animal was placed at the far end of the balance beam; the time taken to turn around and traverse the beam to reach the goal box located at the top were recorded. While traversing the beam foot slips of the front and hind legs on each side were recorded, one side was live scored and the other video recorded to enable future analysis.

4.3.2.6. Sucrose Consumption

Animals were singly housed with *ad-libitum* access to water and 4 grams of sucrose pellets placed in a dish at the centre of the cage. Sucrose consumption was measured for 8 hours during the light phase from 09.00 to 17.00 hours. At 17.00 hours the quantity of remaining sucrose and the animals were weighed. Sucrose consumption testing occurred for a total of 4 consecutive days.

4.3.2.7. Food Consumption

Animals were singly housed in a standard cage and allowed *ad-libitum* access to both food and water for a 16 hour overnight period (17.00 hours to 09.00 hours). The quantity of food consumed overnight and the weight of the animal was recorded the following morning. Food consumption testing occurred for a total of 4 consecutive days.

4.3.2.8. Gait Analysis

Animals were tested using a clear Perspex corridor apparatus lined with a pre-cut piece of white paper. Animals were trained to run to the enclosed darkened box at the end of the corridor. For testing, the paws of the animal were painted with non-toxic paint. The animal was placed at the far end of the apparatus and

ran to the enclosed goal box at the far end, leaving a print at the base of the apparatus. Stride length, stride width and overlap were then analysed over 3 consecutive strides. Each animal was tested once.

4.3.3. Statistical Analysis

Statistical analyses were conducted in IBM SPSS 20 Statistics Software. Typically, three way split plot analyses with ANOVA were used with repeated measures of age and between subject measures of genotype and sex, followed by simple effects analysis. Where significance was found *post-hoc* tests with Bonferroni corrections were performed. In the cases where missing values were present, due to animals needing to be culled for health reasons, missing data were estimated by an unbiased iterative interpolation procedure within the IBM SPSS statistical analysis. The critical significance level used throughout was $\alpha = 0.05$.

4.4. Motor Characterisation Results

4.4.1. Weight Progression Results

Male animals were significantly heavier than their female counterparts throughout the 18 months of testing (Sex; $F_{1,52} = 248.05$, $p<0.001$). Wild type and $Hdh^{Q111/+}$ animals demonstrated similar body weights at the earlier testing time points, however $Hdh^{Q111/+}$ animals subsequently lost body weight in comparison to their wild type littermates, as demonstrated by the divergence of the wild type and $Hdh^{Q111/+}$ weight curves for both sexes similarly (Age x Genotype; $F_{16,832} = 6.98$, $p<0.001$). *Post-hoc* tests confirmed that the significant difference in body weight began at 11 months of age ($p<0.05$) (as shown in Figure 4.2.), and was subsequently shown at 12 months ($p<0.01$), 13 months ($p<0.01$), 14 months ($p<0.05$), 15 months ($p<0.01$), 16 months ($p<0.001$), 17 months ($p<0.001$) and 18 months ($p<0.001$) of age.

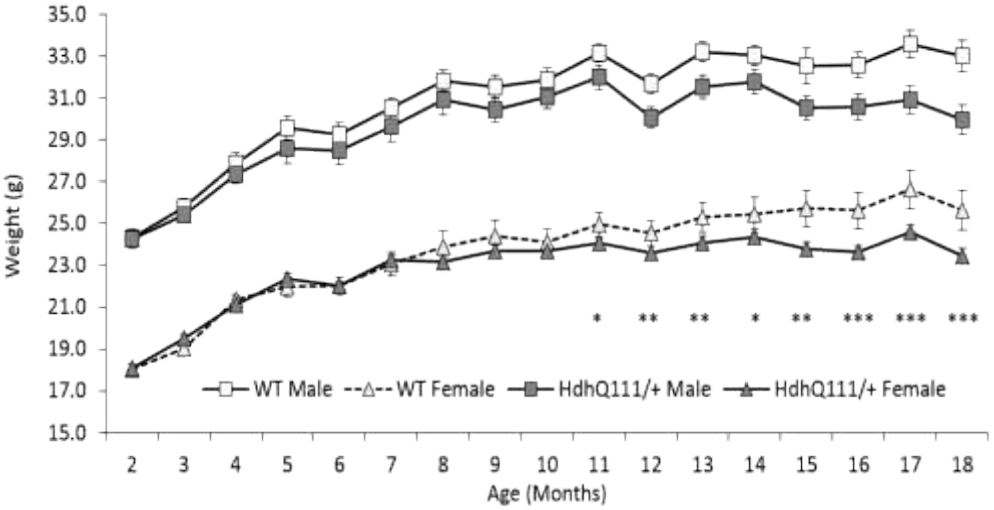

Figure 4.2. Weight progression of $Hdh^{Q111/+}$ animals.
Mice were weighed at 2 months of age and subsequently every month. The data represents the mean weight of $Hdh^{Q111/+}$ and wild type animals averaged to provide the data point. The data represents a total 56 animals, 36 $Hdh^{Q111/+}$ animals (20 female and 16 male) and 20 wild type animals (10 female and 10 male). Error bars represent ± standard error of the mean. Significant differences are stated for males and females combined * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

4.4.2. Spontaneous Locomotor Activity Results

Spontaneous locomotor activity was tested longitudinally in $Hdh^{Q111/+}$ animals (Figure 4.3.). Animals displayed decreased levels of locomotor activity as they aged (Age; $F_{5,255} = 24.42$, $p<0.001$). Furthermore, a significant effect of time of day was observed (Time; $F_{24,1224} = 116.65$, $p<0.001$), with animals generally becoming more active in the dark phase (18.00 hours to 06.00 hours) than in the light phase (Figure 4.3.). However no significant differences were observed in locomotor activity between $Hdh^{Q111/+}$ and wild type animals (Genotype; $F_{1,52} = 0.002$, p = n.s.).

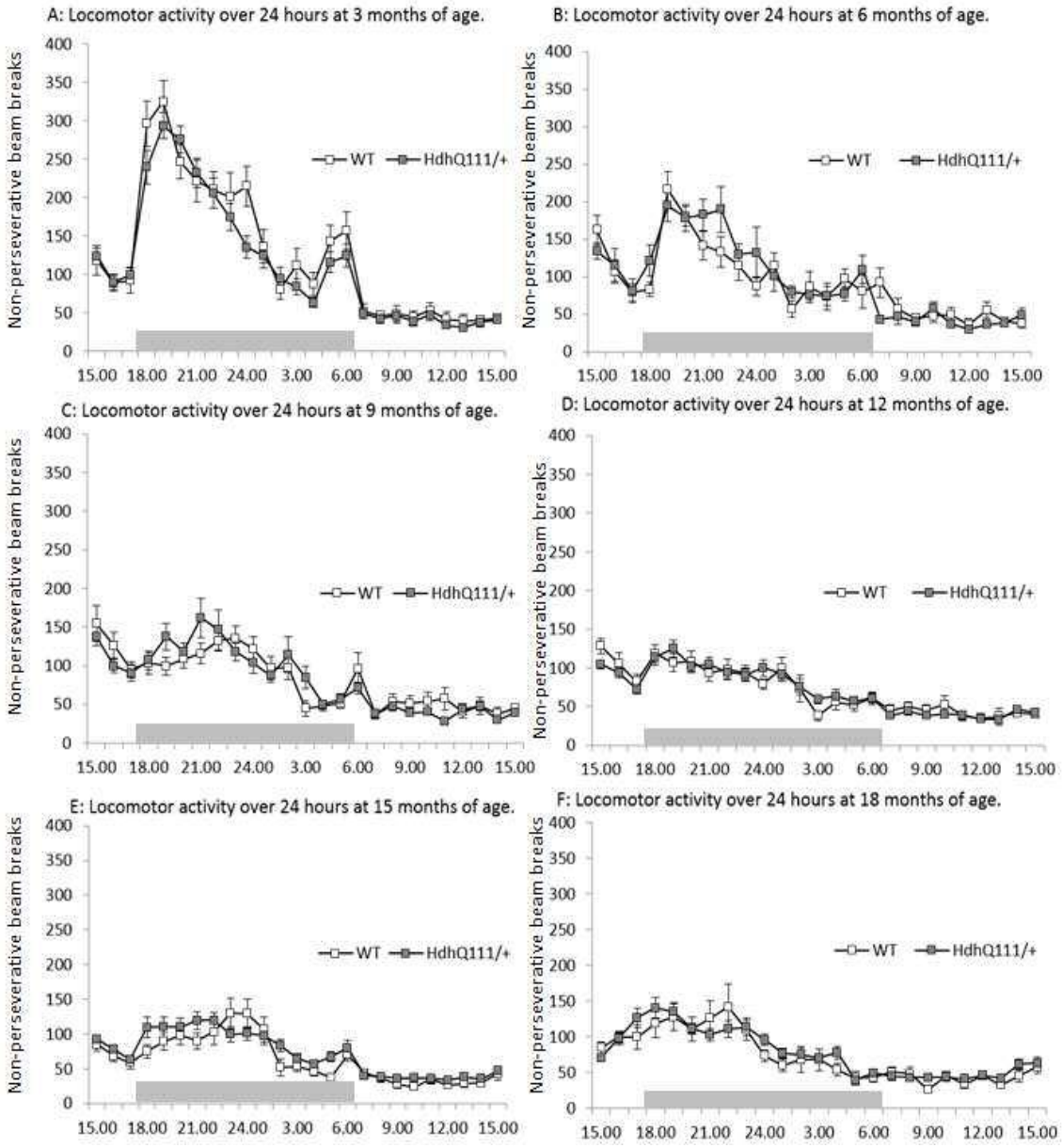

Figure 4.3. Spontaneous locomotor activity longitudinal results for Hdh$^{Q111/+}$ animals.
Results are shown as an average over 24 hours (from 15.00 hours to 15.00 hours) at each time point. The data represents a total 56 animals, 36 Hdh$^{Q111/+}$ animals (20 female and 16 male) and 20 wild type animals (10 female and 10 male). A main effect of time was demonstrated at each age, with animals generally more active in the dark phase (18.00 hours to 06.00 hours). No significant differences were demonstrated between genotypes at any age. Error bars represent ± standard error of the mean.

4.4.3. Grip Strength Results

$Hdh^{Q111/+}$ animals displayed no impairments in grip strength performance, in comparison to wild type animals (Genotype; $F_{1,52} = 1.39$, p = n.s.) (Figure 4.4.). As animals aged their latency to fall from the grid increased (Age; $F_{5,260} = 3.83$, p=0.002). Interestingly, male animals performed significantly worse on the grip strength test than female animals (Sex; $F_{1,52} = 6.68$, p=0.013, data not shown). Although this sex difference was shown for both $Hdh^{Q111/+}$ and wild type animals (Genotype x Sex: $F_{1,52} = 0.58$, p = n.s.).

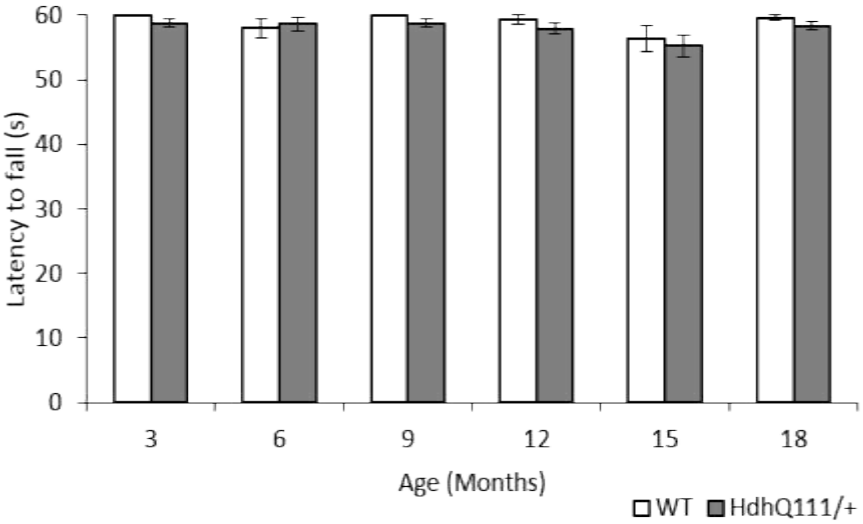

Figure 4.4. Grip strength for $Hdh^{Q111/+}$ animals over 18 months.
Mice were tested at 3 months of age and subsequently every 3 months. The average latency to fall represents an average of 2 trials for $Hdh^{Q111/+}$ and wild type animals at each time point. The data represents a total 56 animals, 36 $Hdh^{Q111/+}$ animals (20 female and 16 male) and 20 wild type animals (10 female and 10 male). Grip strength results demonstrated no significant differences between $Hdh^{Q111/+}$ or wild type animals at any time points. Error bars represent ± standard error of the mean.

4.4.4. Rotarod Results

Rotarod performance over the 18 months of testing demonstrated that $Hdh^{Q111/+}$ animals displayed a decreased latency to fall from the rotarod in comparison to wild type animals (Genotype; $F_{1,52} = 5.83$, $p<0.05$) (Figure 4.5.). A highly significant effect of age was seen, with animals displaying a decreased latency to fall from the rotarod as they aged (Age; $F_{5,260} = 15.98$, $p<0.001$). There was a trend for $Hdh^{Q111/+}$ animals to have a decreased latency to fall from the rotarod as they aged, at 12 months, 15 months and 18 months of age in comparison to

wild type animals, but this effect failed to meet significance (Age x Genotype; $F_{5,260} = 1.35$, p = n.s.). Interestingly male animals performed significantly worse than female animals throughout rotarod testing (Sex; $F_{1,52} = 10.46$, $p<0.01$, data not shown).

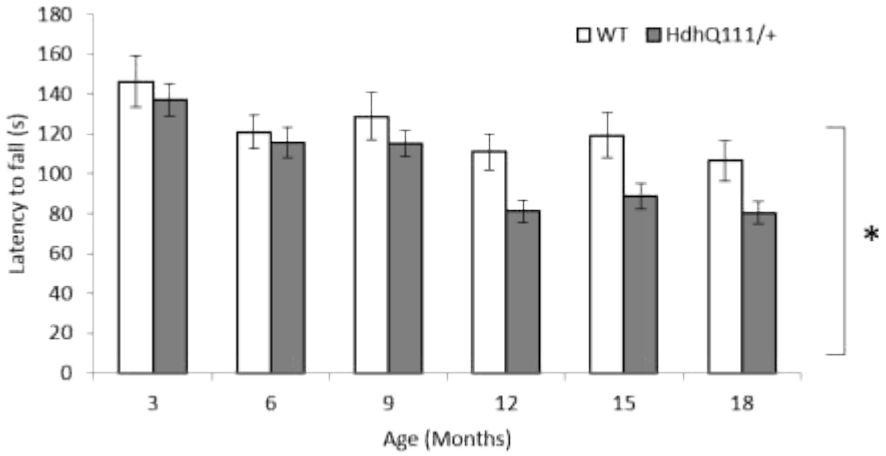

Figure 4.5. Rotarod performance in $Hdh^{Q111/+}$ animals over 18 months.
The data represents a total 56 animals, 36 $Hdh^{Q111/+}$ animals (20 female and 16 male) and 20 wild type animals (10 female and 10 male). Data are shown as an average of 2 trials. Error bars represent ± standard error of the mean. * $p<0.05$.

4.4.5. Balance Beam Results

$Hdh^{Q111/+}$ animals were significantly impaired in numerous aspects of balance beam performance in comparison to wild type animals (Figure 4.6.). As shown in Figure 4.6A, latency to turn on the balance beam was progressively slower as animals aged (Age; $F_{5,260} = 13.82$, $p<0.001$). Although $Hdh^{Q111/+}$ animals did not differ from wild type animals at younger ages, they were progressively slower to turn than wild type controls (Figure 4.6A, Age x Genotype; $F_{5,260} = 3.376$, $p<0.01$), from 9 months of age ($p<0.05$) and subsequently at 12 months ($p<0.05$), 15 months ($p<0.01$) and 18 months of age ($p<0.01$). Interestingly, female animals were shown to turn on the beam significantly faster than male animals (Sex; $F_{1,52} = 5.40$, $p<0.05$, data not shown).

Male animals were slower to traverse the balance beam than female animals (Sex; $F_{1,52} = 13.78$, $p<0.001$). Although $Hdh^{Q111/+}$ animals did not differ in traverse time from wild type animals at the earliest ages, they were progressively slower to traverse the balance beam than wild type animals as they aged (Age x

Genotype; $F_{1,52} = 15.29$, $p<0.001$), at 9 months ($p<0.01$), 15 months ($p<0.001$) and 18 months of age ($p<0.001$), as shown in Figure 4.6B.

Measurement of foot slips made while traversing the balance beam (Figure 4.6C) demonstrated that the number of foot slips made by $Hdh^{Q111/+}$ animals did not differ from wild type animals at younger ages. However, $Hdh^{Q111/+}$ animals made progressively more foot slips than wild type animals as they aged (Age x Genotype; $F_{5,260} = 40.56$, $p<0.001$) from 12 months of age ($p<0.001$) and subsequently at 15 months ($p<0.001$) and 18 months of age ($p<0.001$).

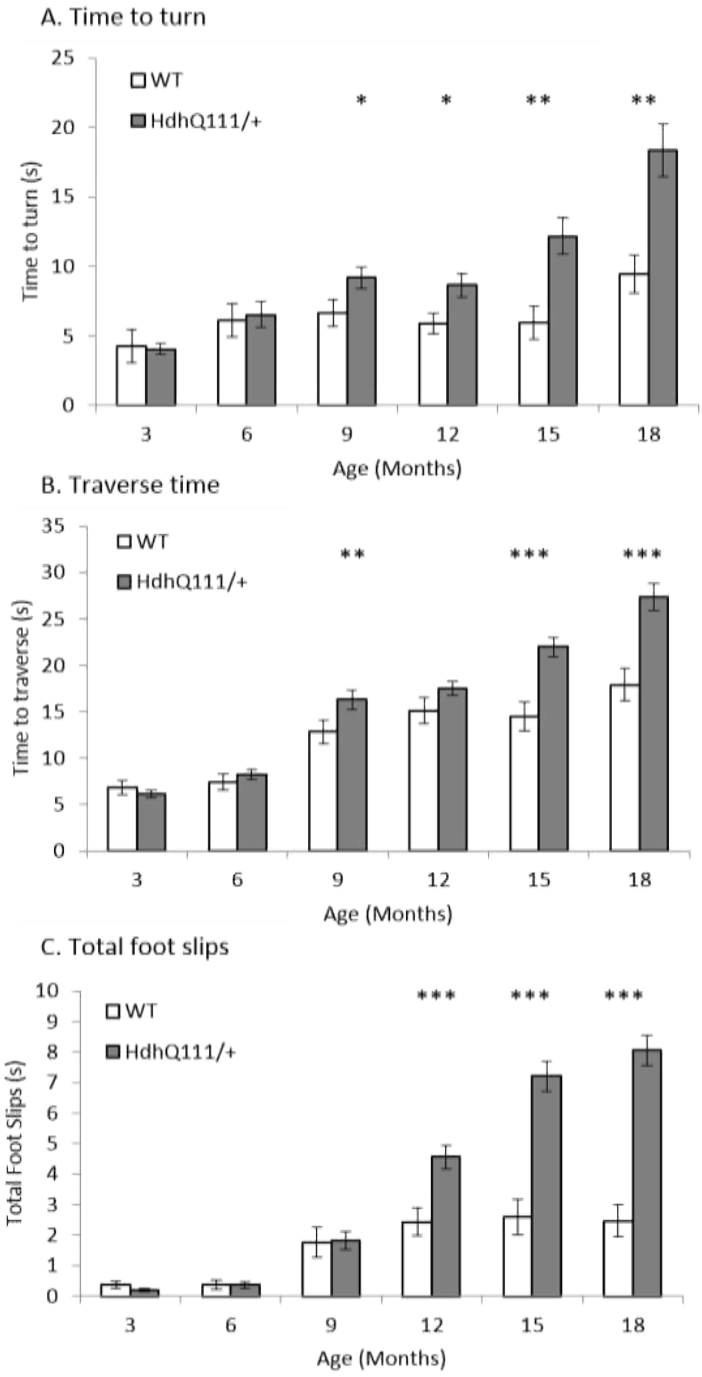

Figure 4.6. Balance beam performance in Hdh$^{Q111/+}$ animals over 18 months.
A. Time to turn on the balance beam demonstrated Hdh$^{Q111/+}$ animals were significantly slower to turn on the beam than wild type animals, from 9 months of age. B. Traverse time on the balance beam showed Hdh$^{Q111/+}$ animals were significantly slower to traverse the beam than wild type animals from 9 months of age. C. Total number of foot slips made while crossing the beam demonstrated Hdh$^{Q111/+}$ animals made significantly more foot slips from 12 months of age. The data represents a total 56 animals, 36 Hdh$^{Q111/+}$ animals (20 female and 16 male) and 20 wild type animals (10 female and 10 male). Error bars represent ± standard error of the mean. * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

4.4.6. Sucrose Consumption Results

Sucrose consumption did not differ between $Hdh^{Q111/+}$ and wild type animals (Figure 4.7., Genotype; $F_{1,52} = 2.50$, p = n.s.). However, it was shown that the quantity of sucrose consumed decreased as animals aged (Age; $F_{2,104} = 228.78$, $p<0.001$). Furthermore, no sex differences in sucrose consumption were seen (Sex; $F_{1,52} = 5.82$, p = n.s.).

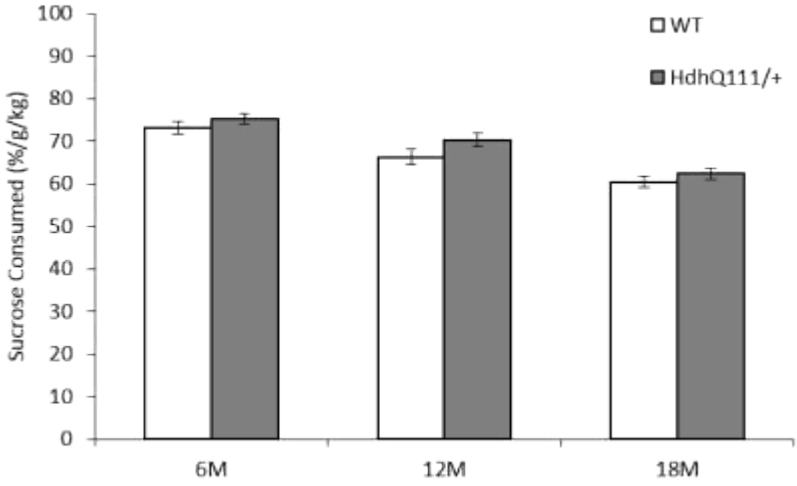

Figure 4.7. Sucrose consumption in $Hdh^{Q111/+}$ animals over 18 months.
The data represents a total 56 animals, 36 $Hdh^{Q111/+}$ animals (20 female and 16 male) and 20 wild type animals (10 female and 10 male). The data shown is an average of the 4 days of testing. Error bars represent ± standard error of the mean.

4.4.7. Food Consumption Results

The quantity of food consumed did not differ between $Hdh^{Q111/+}$ and wild type animals (Figure 4.8, Genotype; $F_{1,52} = 2.70$, p = n.s.). However, animals consumed significantly more food as they aged (Age; $F_{2,104} = 40.14$, $p<0.001$), despite this no significant interaction effect was demonstrated (Age x Genotype; $F_{2,104} = 0.198$, p = n.s.). Furthermore, male animals consumed significantly more food than female animals (Sex; $F_{1,52} = 6.55$, $p<0.05$). Interestingly female $Hdh^{Q111/+}$ animals consumed significantly less food than female wild type animals (Genotype x Sex; $F_{1,52} = 7.79$, $p<0.05$, data not shown).

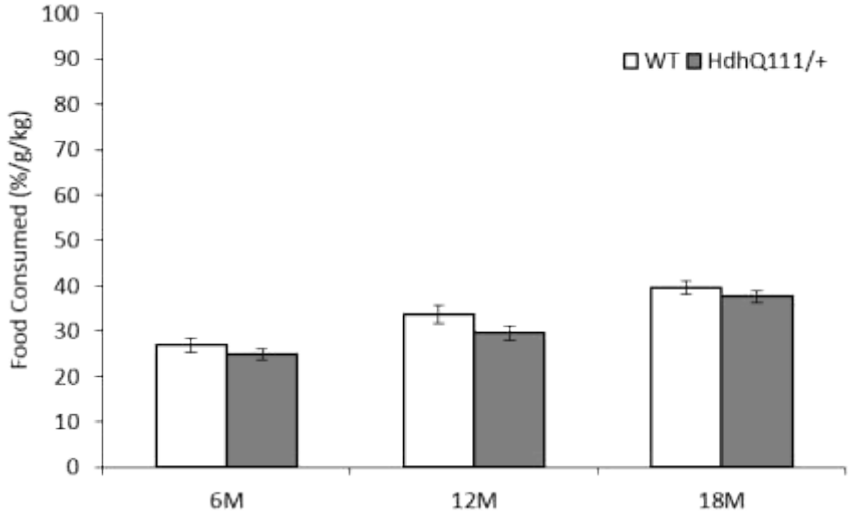

Figure 4.8. Food consumption of standard laboratory chow in $Hdh^{Q111/+}$ animals over 18 months. The data represents a total 56 animals, 36 $Hdh^{Q111/+}$ animals (20 female and 16 male) and 20 wild type animals (10 female and 10 male). The data shown is an average from 4 days of testing. Error bars represent standard ± error of the mean.

4.4.8. Gait Analysis Results

$Hdh^{Q111/+}$ animals did not show any impairments in gait performance in comparison to wild type animals in stride length (Genotype; $F_{1,52} = 0.04$, p = n.s.), stride width (Genotype; $F_{1,52} = 0.02$, p = n.s.) or stride overlap (Genotype; $F_{1,52} = 0.02$, p = n.s.), as shown in Figure 4.9. However, as animals aged, their stride length decreased (Age; $F_{2,104} = 15.37$, $p<0.001$) (Figure 4.9A), as did their stride overlap (Age; $F_{2,104} = 4.36$, $p<0.05$) (Figure 4.9C). However, no significant effect of age was seen in stride width (Age; $F_{1,52} = 3.61$, p = n.s.) (Figure 4.9B).

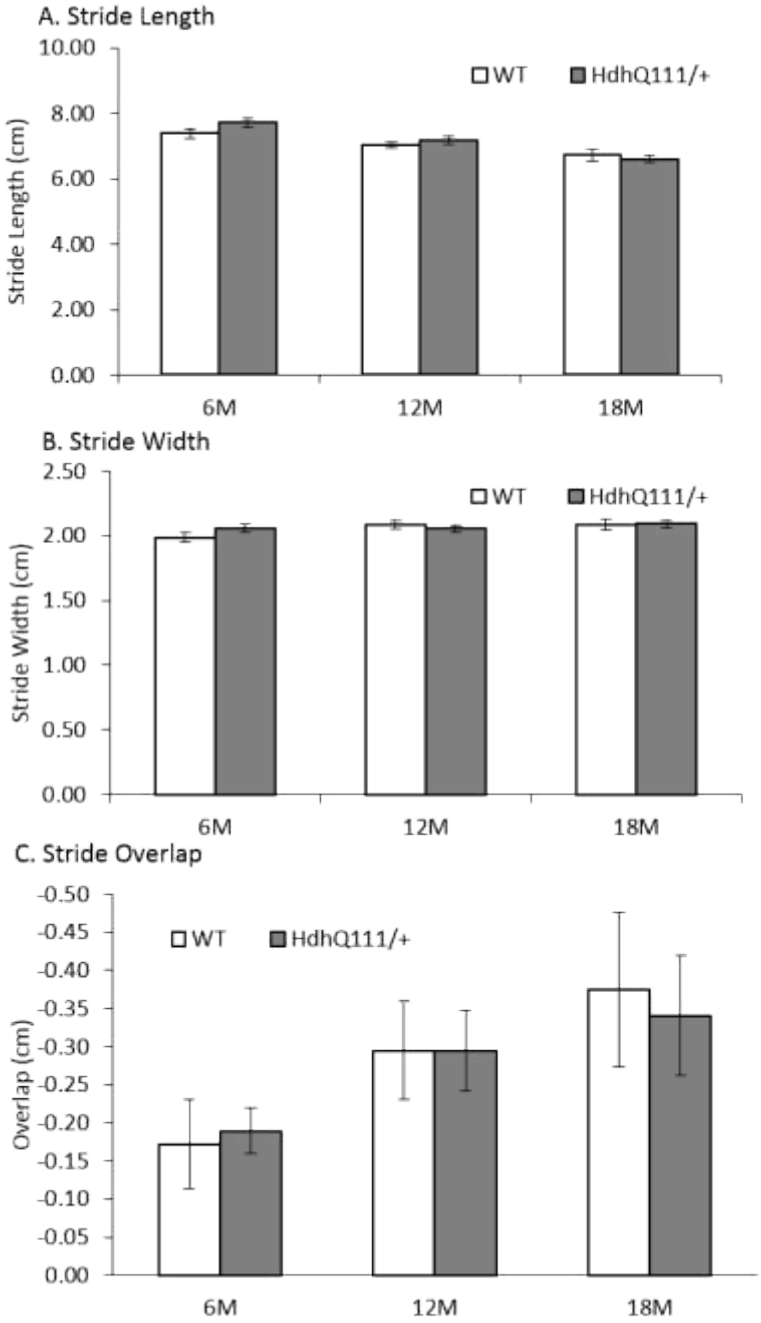

Figure 4.9. Gait analysis results for Hdh$^{Q111/+}$ animals over 18 months.
No significant differences were observed between Hdh$^{Q111/+}$ and wild type animals in any measure of gait analysis. The data represents a total 56 animals, 36 Hdh$^{Q111/+}$ animals (20 female and 16 male) and 20 wild type animals (10 female and 10 male). Error bars represent ± standard error of the mean.

4.5. Discussion

The results of the longitudinal motor characterisation of $Hdh^{Q111/+}$ animals presented in this chapter demonstrate that significant motor deficits occur in $Hdh^{Q111/+}$ animals, as measured in balance beam performance from 9 months of age and a decreased latency to fall from the rotarod from 12 months of age. $Hdh^{Q111/+}$ animals also had a significantly lighter body weight than wild type animals from 11 months of age.

The observed weight differences replicate previous findings in other mouse models of HD, which shows progressive weight loss as disease burden increases (Carter *et al*. 1999; Brooks *et al*. 2012d, c; Brooks *et al*. 2012f). Although, the observed onset of weight loss differs for each specific mouse strain. Furthermore, the progressive weight loss demonstrated in $Hdh^{Q111/+}$ animals is reflective of the weight loss shown in the human condition (Kremer and Roos 1992; Stoy and McKay 2000; Djousse *et al*. 2002; Aziz *et al*. 2008). Although the underlying reasons for the associated weight loss are still unknown, the lack of observable chorea in $Hdh^{Q111/+}$ mice, despite weight loss, may be indicative of an underlying metabolic problem which causes the observed weight loss. This suggestion has been explored in human HD patients (Mazziotta *et al.* 1987; Browne *et al.* 1997; Koroshetz *et al.* 1997) and in other mouse strains (Duan *et al.* 2003a; Tsang *et al.* 2006; Weydt *et al.* 2006; van der Burg *et al*. 2008), although, it is yet to be confirmed in the Hdh^{Q111} mouse model of HD. Furthermore, the results obtained on several of the motor tests can be sensitive to the confounding effects of body weight. The effect, if any that the weight changes had on the motor read outs presented here is uncertain. Although, if weight were to influence the motor results obtained, it may be that heavier animals, which are in this case wild type animals, are likely to be disadvantaged in certain tasks such as the rotarod, in comparison to $Hdh^{Q111/+}$ animals, which is not the case in the observed results. However, it could also be argued that a decreased body weight may be advantageous in other motor tests such as the balance beam. If this was the case, $Hdh^{Q111/+}$ animals would not show a deficit

in comparison to wild type animals, which is not the case in the observed results, as balance beam deficits are shown in $Hdh^{Q111/+}$ animals despite significant weight loss in comparison to wild type animals.

Significant motor deficits were seen in $Hdh^{Q111/+}$ animals in turn time and traverse time on the balance beam apparatus from 9 months of age, thus suggesting an inability to initiate movement and subsequently move along the beam. Furthermore, the increased number of foot slips that $Hdh^{Q111/+}$ animals made while crossing the balance beam compared to wild type animals, at 12, 15 and 18 months of age, are indicative of progressive impairments in motor co-ordination and balance. Sex differences were demonstrated, with female animals generally displaying a more rapid time to turn and traverse the beam, although these differences between sexes were not consistently produced over time and their level of significance was not as high as that of the genotype differences. Problems with balance are important to consider in people who are affected by HD, as this can lead to an increased risk of falls (Busse *et al*. 2009) and thus, a reduced quality of life (Helder *et al.* 2001; Ready *et al.* 2008; Ho *et al.* 2009).

Further motor deficits were demonstrated in $Hdh^{Q111/+}$ animals in rotarod performance, as $Hdh^{Q111/+}$ animals displayed a decreased latency to fall from the rotarod in comparison to wild type animals. These deficits in rotarod performance are again indicative of problems with co-ordinated motor movement in $Hdh^{Q111/+}$ animals. Significant gender differences were observed in rotarod performance, with female animals performing significantly better on the rotarod than male animals. These gender differences may be attributable to differences in weight between male and female animals which were shown at all of the testing time points. This suggestion has previously been described in other outbred mouse strains (McFadyen *et al.* 2003).

Although $Hdh^{Q111/+}$ animals displayed significant deficits in balance beam traverse time from 9 months of age, no significant deficits were seen in spontaneous locomotor activity. Therefore, it may be the case that when these

animals are free to move in the locomotor activity cages no significant differences in activity are observed. Whereas, when $Hdh^{Q111/+}$ animals are challenged to initiate movement and traverse a beam or accelerate on a rotarod, they find the task significantly harder than wild type animals. Therefore, the lack of observable deficits in spontaneous locomotor activity suggests that the deficits seen in both rotarod and balance beam are indeed indicative of a specific involvement in motor co-ordination and balance.

Spontaneous locomotor activity was studied in the $Hdh^{Q111/+}$ mouse model of HD because significant alternations in circadian rhythm have been previously reported in HD patients (Wiegand *et al.* 1991; Videnovic *et al.* 2009; Aziz *et al.* 2010; Wulff *et al.* 2010); however, such differences were not observed in this case in the $Hdh^{Q111/+}$ mouse model. Furthermore, overall, animals displayed decreased activity as they aged, although this may be the result of the natural aging process it may also be attributable to habituation effects. As animals were tested in the motor test battery every 3 months, they were required to be tested a total of 6 times within the testing battery. Therefore, it could be the case that animals became increasingly habituated to the locomotor activity boxes and thus moved less during testing. This is suggested in particular by the large decline in locomotor activity between the first and second tests, at 3 months of age and 6 months of age respectively, before other motor deficits were apparent. Furthermore, this suggestion may also be valid for the other motor tasks. Because the same animals were used repeatedly in the longitudinal testing paradigm, this meant that animals received repeated testing on the motor tasks at a young age; therefore this exposure and testing on the motor tests at a young age may affect future motor test read outs. To prevent this occurring in future longitudinal motor characterisations, a new group of animals could be tested at each time point, although this would have considerable ethical, financial and logistical implications. A full discussion of the experimental design used in longitudinal testing is given in the General Discussion in Chapter 8.

The lack of genotype differences in grip strength is perhaps unsurprising due to the relatively insensitive nature of the behavioural test, with the majority of animals performing close to the 60 second ceiling in the protocol. This grip strength test is highly likely to be influenced by differences in body weight, with heavier animals falling from the grid more rapidly than lighter animals. However, if this were to be the case it is likely to disadvantage wild type animals rather than $Hdh^{Q111/+}$ animals. Furthermore, the time that each grip strength trial was conducted for (60 seconds) is in accordance with the relevant licencing requirements to minimise pain, suffering, distress and lasting harm to animals. However, this may not be long enough to draw out any genotype differences. It is therefore plausible that extending the test duration to 2 or 3 minutes may increase the sensitivity of the task, but this is yet to be investigated. Therefore, the grip strength behavioural test, which in this case utilised an inverted grid, may not be a sensitive test measure. Attempts to use mechanical grip strength metres (Van Riezen and Boersma 1969) have been used successfully in observing deficits in grip strength in other genetically modified mouse models (Barnéoud *et al.* 1997; Glynn *et al.* 2005) and in the R6/2 mouse model of HD (Hickey *et al.* 2005). Although, typically mechanical grip strength meters only test forelimb grip strength, and it can be difficult to motivate mice to sustain clinging onto the bar of the grip strength meter.

Sucrose and food consumption measures demonstrated no significant differences between $Hdh^{Q111/+}$ and wild type animals. These measures were included in the testing battery to probe motivation and physical ability to consume palatable and less palatable food stuffs. Although problems with food consumption including dysphagia have been shown in HD patients (Leopold and Kagel 1985; Hunt and Walker 1989; Kagel and Leopold 1992), these symptoms often occur in the later stages of the disease and therefore the behavioural phenotype of the $Hdh^{Q111/+}$ mouse model may be too subtle to demonstrate significant behavioural differences in these measures.

Gait abnormalities have previously been observed in both $Hdh^{Q111/+}$ and $Hdh^{Q111/Q111}$ mice at 24 months of age (Wheeler *et al*. 2002a), although these animals were bred on a CD1 background strain, which is different to the background strain of the animals used here. No gait abnormalities were observed in the $Hdh^{Q111/+}$ animals in this case, although the characterisation was only conducted until 18 months of age, therefore it may be the case that such abnormalities would have developed if the characterisation were to be extended to 24 months of age. However, significant motor abnormalities were demonstrated in other motor tests and thus for practical, financial and welfare reasons it was pre-planned to end the motor characterisation at 18 months of age.

Gender differences were demonstrated in some of the motor tasks, with female animals often performing better than male animals. Typically, this was an independent influence on the behavioural results and there were no interactions demonstrated between sex and genotype. The gender differences between male and female animals do not appear to be stable within the behavioural results of the motor tests. Therefore, gender differences could be attributable to the oestrous cycle of female animals. Although it is plausible to use a single sex of animals in behavioural testing, HD is a disease which affects both genders equally (Wexler 2004) and thus both genders were used in behavioural testing to reflect this. Gender differences have previously been observed in the Hdh^{Q111} mouse line, with male animals demonstrating increased anxiety (Oroven *et al.,* 2012). However Oroven *et al*., used $Hdh^{Q111/Q111}$ animals and therefore an anxious phenotype may not be observed in $Hdh^{Q111/+}$ mice. Anxiety measurements were not conducted in this particular study and may have been demonstrated if anxiety was to be investigated.

The longitudinal motor characterisation presented here, demonstrated a gradual decrease in body weight in $Hdh^{Q111/+}$ animals, with respect to their wild type littermates. The emergence of motor deficits in balance beam performance from 9 months of age, and in rotarod performance in $Hdh^{Q111/+}$ animals, demonstrates subtle and progressive motor deficits which resemble the early stages of motor

dysfunction in HD, as the deficits are specific in nature and lack the gross dyskinesia observed in late stage HD patients.

Chapter 5 : Longitudinal operant testing to probe cognitive dysfunction in the Hdh^{Q111} mouse model of Huntington's disease.

5.1. Introduction

In the previous chapter, motor dysfunction was demonstrated in $Hdh^{Q111/+}$ mice in both balance beam and in rotarod performance measures. However, behavioural deficits that are present prior to the onset of motor dysfunction have been shown in HD patients. Specific deficits can include; lack of motivation, depression, apathy, anxiety and reduced ability to switch tasks, have been demonstrated in human HD patients prior to the presentation of profound motor dysfunction (Diamond *et al*. 1992; Kirkwood *et al*. 2000; Aron *et al*. 2003; Lemiere *et al*. 2004; Duff *et al*. 2007; Robins Wahlin *et al*. 2007; Paulsen *et al*. 2008b). Behavioural deficits have further been demonstrated in a range of knock-in HD mouse models, these include specific deficits in; motivation, attention, extra dimensional set-shifting and working memory (Lione *et al*. 1999; Van Raamsdonk *et al.* 2005; Brooks *et al.* 2006; Trueman *et al*. 2007, 2008b; Brooks *et al.* 2012d, c; Brooks *et al.* 2012e; Brooks *et al.* 2012f; Giralt *et al.* 2012; Trueman *et al.* 2012b; Trueman *et al.* 2012e). Cognitive and behavioural dysfunctions such as these, have been shown to significantly affect the daily activities of people with HD, often reducing independence, causing isolation and reducing overall quality of life (Helder *et al*. 2001; Ready *et al*. 2008; Ho *et al*. 2009). Therefore, cognitive dysfunctions are a core early symptom of HD and in order to assess whether a mouse model of HD is valid and replicative of the human condition a longitudinal characterisation of the development of any associated cognitive deficits is necessary.

Cognitive deficits have previously been investigated in mouse models of HD using swimming tests including the Morris water maze (Lione *et al*. 1999; Van

Raamsdonk *et al.* 2005; Brooks *et al.* 2012a; Brooks *et al.* 2012c, d; Brooks *et al.* 2012f) and the water T-maze apparatus (Van Raamsdonk *et al.* 2005; Brooks *et al.* 2012a; Brooks *et al.* 2012e). However, in comparison to these swimming tests, operant testing allows more a sensitive, unbiased, rapid and automated way of testing for specific cognitive deficits. Operant testing has previously been utilised in numerous HD mouse models to determine cognitive dysfunction in a range of operant tasks, often in the absence of motor symptoms (Balci *et al.* 2009; Brooks *et al*. 2012e; Brooks *et al*. 2012h; Oakeshott *et al.* 2012; Trueman *et al*. 2012b; Trueman *et al*. 2012e). Therefore, we sought to determine the cognitive phenotype of the Hdh^{Q111} mouse model of HD utilising a longitudinal operant test battery which was designed to probe specific and discrete cognitive functions.

There is evidence for the use of the frontal striatal system, which is known to be affected in HD, in cognitive learning (Poldrack *et al.* 1999) which may affect the ability of people with HD to learn new cognitive tasks. HD patients have previously been shown to be unable to acquire certain tasks (Heindel *et al*. 1988) due to deficits particularly in procedural learning (Knopman and Nissen 1991). Therefore, the ability of $Hdh^{Q111/+}$ animals to initially learn and then subsequently reacquire the task of 'nose poking' was explored using a standard fixed ratio (FR1) schedule.

Once animals had successfully acquired the 'nose poke' response, they were tested on an FR1 schedule which utilised different reward sizes. Thus, perception of the value of the different reward sizes was tested. Motivation and apathetic behaviours in responding were explored utilising a progressive ratio (PR) operant task which required animals to respond to a light stimulus an increasing number of times to obtain a reward. This measured the motivation and willingness of animals to maintain responding for a reward when the level of responding required to achieve this reward successively increased. Exploration of motivational problems in $Hdh^{Q111/+}$ animals was probed as alterations in motivation have previously been described in the PR task in other mouse models

of HD (Oakeshott *et al.* 2012) and in HD patients (Burns *et al*. 1990; Paulsen *et al*. 2001b; Thompson *et al.* 2002b). In addition, different reward sizes were utilised to explore perception of value in responding to reward and flexibility in responding to the different reward sizes.

The five choice serial reaction time task (5-CSRTT) was designed to probe attentional function and spatial awareness, as deficits in executive functions, including spatial perception and attentional sharing have previously been demonstrated in HD patients (Sprengelmeyer *et al.* 1995; Claus and Mohr 1996; Lawrence *et al*. 1998b; Murphy *et al.* 2000; Lemiere *et al*. 2004; Finke *et al.* 2006; Delval *et al.* 2008; Thompson *et al.* 2010). These specific cognitive deficits have been shown to significantly affect everyday activities, increasing the risk of falls and reducing quality of life for people with HD (Helder *et al*. 2001; Ready *et al.* 2008). The stimulus length was decreased in the 5-CSRTT to increase the attentional load of the task. Animals were therefore required to pay attention to the stimulus lights, utilising spatial awareness and attention in order to respond accurately, particularly when the shorter stimulus lengths were applied.

The serial implicit learning task (SILT) was designed as an extension of the 5-CSRTT, it therefore required an additional response (a total of two responses) to obtain a reward. This task was included in the test battery to probe attention and spatial awareness, as the 5CSRTT does but, in addition, to test implicit learning. Previous attempts to explore implicit learning deficits in HD patients have demonstrated mixed results; while some have shown clear implicit learning impairments, prior to the onset of motor symptoms (Knopman and Nissen 1991; Willingham and Koroshetz 1993; Ghilardi *et al.* 2008), others have concluded that implicit learning is unaffected in the HD patient population (Bylsma *et al.* 1991; Brown *et al.* 2001). Therefore, a SILT operant task was included in the operant test battery to investigate if any deficits in implicit learning were observed in the $Hdh^{Q111/+}$ mouse model of HD.

The operant test battery used in this experiment therefore comprised; initial stimulus response training, acquisition and maintenance of a simple fixed ratio 1 schedule (FR1), progressive ratio (PR) to determine intrinsic levels of motivation, the five choice serial reaction time task (5CSRTT) to probe attentional dysfunction and the serial implicit learning task (SILT) to determine attentional function and implicit learning. The inclusion of these operant tasks within the test battery was necessary to determine cognitive deficits associated with the Hdh^{Q111} mouse model of HD.

5.2. Aims

To determine the face and predictive validity of the Hdh^{Q111} mouse model of HD by characterising the nature, severity and time course of cognitive deficits using an operant test battery.

5.3. Materials and Methods

5.3.1. Animals

A total of 16 animals, 7 $Hdh^{Q111/+}$ (4 female and 7 male) and 9 wild type (5 female and 4 male) were used. $Hdh^{Q111/+}$ animals contained an average of 137 CAG repeats, which ranged from 122 repeats to 142 repeats. A female $Hdh^{Q111/+}$ animal became ill at approximately 13 months of age, this animal was humanely euthanised. For further details regarding animal husbandry see section 2.1 of Chapter 2.

Operant testing occurred between 08.00 and 12.00 hours, 5 days per week. 1 week prior to operant testing animals were gradually water restricted and habituated to strawberry milkshake (Yazoo® Campina Ltd, Horsham, UK) in their home cages. For the duration of operant testing animals were restricted to 3 hours of access to water per day, given after behavioural testing between the hours of 14.00 hours and 17.00 hours. Throughout the experiments animals were allowed *ad-libitum* access to standard laboratory chow food.

The operant testing battery took approximately 3 months to complete at each age of testing (Figure 5.1). Therefore, the time point stated (6, 12 or 18 months of age) represents the mid-point in each phase of testing, with mice reaching the stated age of testing typically during the 5-CSRTT test.

5.3.2. 9-hole Operant Box Apparatus

A full description of the 9-hole operant boxes used in behavioural testing is given in section 2.3.1. of Chapter 2. It should be noted that only holes; 1, 3, 5, 7 and 9 were used in any given test presented in this experimental chapter. Consequently black plastic film was placed in the light array to block unused holes (2, 4, 6 and 8) to prevent their use.

5.3.3. 9-hole Operant Box Testing

An operant test battery was conducted longitudinally, as shown in Figure 5.1, at 6 months, 12 months and 18 months of age. A full description of the operant tests used is given in section 2.3.4. of Chapter 2, however a brief description is also given below.

At 10 weeks of age mice were introduced to the 9-hole operant chambers. Magazine training began with the non-contingent delivery of 150µl of strawberry milk into the magazine and illumination of the magazine light, for 1 day. Reward retrieval was detected by nose entry then withdrawal from the magazine, which broke the infrared beam across the magazine. After successful reward retrieval the magazine light was extinguished. The process was repeated until the 20 minute session time had elapsed.

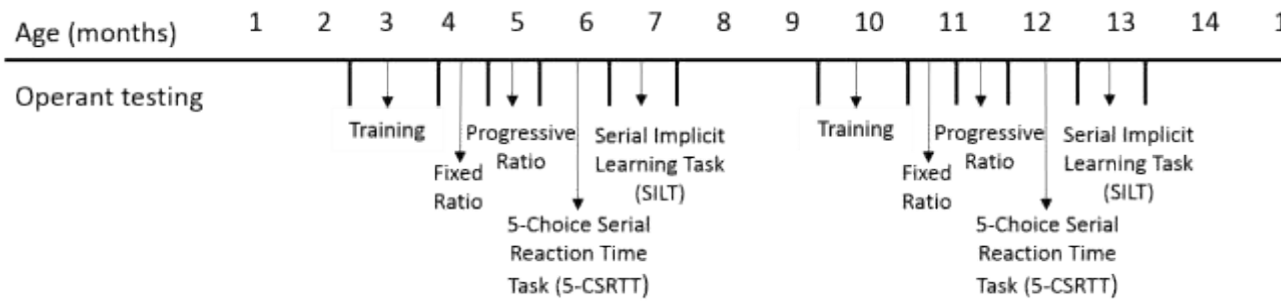

Figure 5.1. Schematic representation of operant testing timeline. Animals were tested in the operant battery at 6 monthly intervals a age. Due to the time it took to complete the operant battery animals began testing one and a half months prior to testing for that time poin months after the required time point.

After magazine training, mice were taught to nose poke on a simple FR1 schedule of reinforcement which required illumination of the central hole in the array of the 9-hole operant box. To obtain reward, mice were required to respond to the stimulus light in hole 5 via a single nose poke. A correct nose poke in response to the stimulus light triggered the extinguishing of the centre light and simultaneous illumination of the magazine light and delivery of 5µl of reward into the magazine. Upon withdrawal of the animal's nose from the magazine, the next trial was initiated with the illumination of the central light. In order to promote learning the central light was painted with strawberry milk to encourage nose poking into the illuminated hole. Mice were trained on this program until they had achieved over 100 pokes into the central hole within the 20 minute session without encouragement from painting of the holes.

5.3.3.1. Fixed Ratio (FR) Testing

FR testing was conducted after FR1 training (described above). The purpose of this test was to test investigate the amenability of responding to the delivery of varying reward sizes. Mice were required to poke into the central hole of the array, in response to illumination, to obtain reward. The program utilised varying reward sizes of 2.5µl, 5.0µl and 7.5µl, which were delivered into the magazine. Animals were reinforced with 5.0µl of reward for the first 3 days of testing, 2.5µl of reward for the next 3 days, before finally rewarding animals with 7.5µl of reward for the final 3 days of testing. The number of pokes into the central hole was recorded in response to reward size, over the 45 minute session time.

5.3.3.2. Progressive Ratio (PR) Testing

PR task tested the motivation of the mice to obtain reward. The PR schedule of reinforcement required an increasing number of nose poke responses, into the illuminated central hole of the array to obtain successive rewards. The number of responses required to obtain reward increased by 3 after the repetition of each ratio (responses required per reward; 1, 1, 1, 3, 3, 3, 6, 6, 6, 9, 9, 9 etc). The motivation of the animals to obtain reward was recorded as the ratio attained after a 60 minute session and by measuring the response ratio attained before a

defined period of non-responding (30 seconds), this time period was termed a break point. The ratio attained by each animal at each break point was recorded. Each session lasted for 1 hour.

5.3.3.3. 5-Choice Serial Reaction Time Task (5-CSRTT) Testing

In the 5-CSRTT animals were trained to respond, via nose poking, to a stimulus light which was presented randomly across the 5 hole array, in order to receive a reward. The task was modified from those previously described in rats (Cole and Robbins 1989) to be conducted in mice. The purpose of this task was to investigate spatial awareness and attentional performance. The use of 3 different stimulus lengths (10 seconds, 2 seconds and 0.5 seconds) increased the difficulty and attentional load of the task. The stimulus length used became increasing shorter over the testing period. For the first 10 days of testing a 10 second stimulus length was presented, a stimulus length of 2 seconds was used for the next 5 days and a stimulus length of 0.5 seconds was used for the final 5 five days. If a response was not made within 10 seconds after the presentation of the stimulus (termed a 'limited hold'), the light was extinguished (termed a 'limited hold') and a time-out period of 10 seconds was initiated by illumination of the house light. The process was repeated until the 30 minute session time had elapsed.

5.3.3.4. Serial Implicit Learning Task (SILT) Testing

For SILT testing animals were trained to respond to a 2-step sequence of lights in order to receive a reward. A continuous stimulus light was randomly presented in one of the 5 holes across the array. A correct response to the first stimulus light (S1) resulted in the simultaneous extinguishing of this light and illumination of a second light (S2). A correct response to S2 resulted in delivery of a 5µl reward into the magazine. A predictable stimulus sequence (hole 7 was always illuminated after hole 3) was embedded among other unpredictable sequences in order to probe implicit learning. The test was repeated until the 30 minute session time had elapsed.

5.3.4. Statistical Analysis

Statistical analyses were conducted in IBM SPSS Statistic 20 software. Repeated measures ANOVAs, were conducted, followed by simple effects analysis. Where significance was found *post-hoc* analyses with Bonferroni corrections were performed. In the cases where missing values were present, data were estimated by an unbiased iterative interpolation procedure within the statistical analysis. The critical significance level used throughout was $\alpha = 0.05$.

5.4. Results

5.4.1. Training Results

All animals were trained on a simple FR1 schedule. Initial, training was conducted for 13 days to allow animals to learn and acquire the nose poke response. At subsequent testing ages, animals were required to complete FR1 training again for 8 days at each testing age to reacquire nose poke responding after a rest period in testing.

During initial training in the nose poke response, animals were trained for a total of 13 days until the levels of responding had plateaued. No significant differences in performance were demonstrated between wild type and $Hdh^{Q111/+}$ animals (Genotype; $F_{1,12} = 0.51$, p = n.s.) or between sexes (Sex; $F_{1,12} = 1.95$, p = n.s.) (Figure 5.2). However, all animals made an increasing number of nose poke responses over subsequent days (Time; $F_{12,144} = 24.15$, $p<0.001$), as they successfully acquired the nose poke response.

Animals were re-trained on the FR1 schedule for 8 days at 12 months of age for the second testing age. $Hdh^{Q111/+}$ animals were able to reacquire the nose poke response to the same degree as wild type animals (Genotype; $F_{1,12} = 3.01$, p = n.s.). No significant sex differences were observed (Sex; $F_{1,12} = 1.26$, p = n.s.). But, as was seen at the earlier time point, a significant overall effect of time was demonstrated (Time; $F_{7,84} = 44.67$, $p<0.001$), with the overall number of responses increasing on subsequent days as shown in Figure 5.2., as animals reacquired the nose poke response.

At the final 18 month age of testing, animals were placed on the training schedule for 8 days. $Hdh^{Q111/+}$ animals were unable to make as many nose poke responses as wild type animals over the 8 days of training (Genotype; $F_{1,12} = 5.85$, $p<0.05$). Thus indicating that $Hdh^{Q111/+}$ animals were now unable to reacquire the nose poke response in the same way that wild type animals were able to do so. There was a trend for female animals to make more responses than male animals at 18 months of age, although this trend failed to meet significance (Sex; $F_{1,12} = 4.650$, $p = 0.052$). A significant overall effect of time was also seen, as animals learnt to reacquire the nose poke response on subsequent days of training (Time; $F_{7,48} = 4.217$, $p<0.05$).

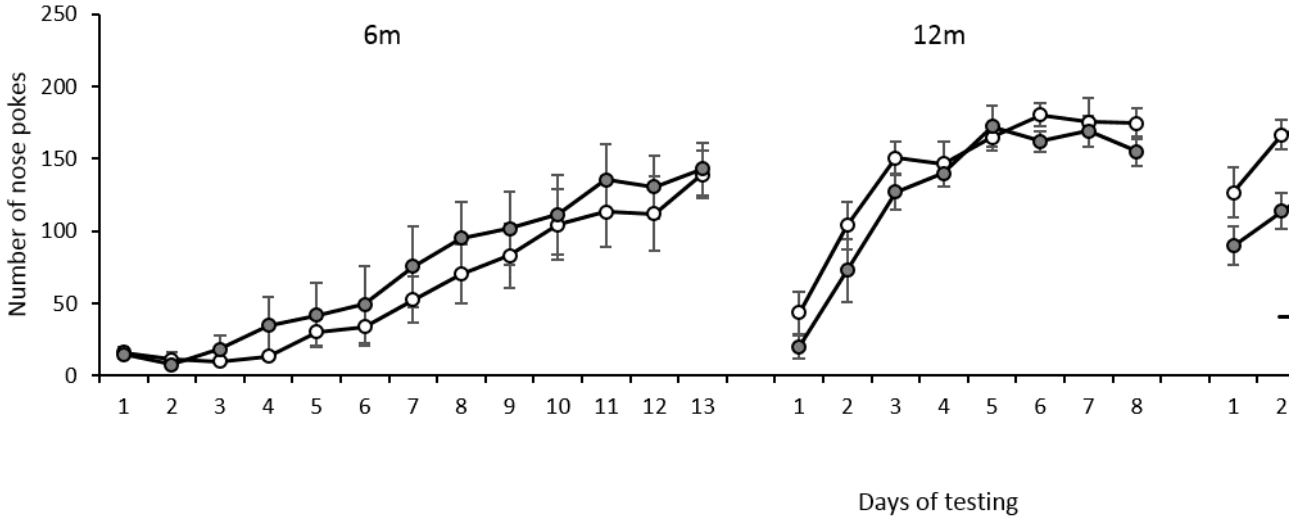

Figure 5.2. Longitudinal Operant Training Results for Hdh$^{Q111/+}$ animals. Nose poke training raining at 6 months of age revealed no ge main effect of time was seen. Training at 12 months of age demonstrated no significant effect of sex or genotype although a significant ma months of age revealed Hdh$^{Q111/+}$ animals made significantly fewer nose poke responses than wild type animals. An overall significant eff a total of 16 mice, 7 Hdh^{Q111+} (4 female and 3 male) and 9 wild type (5 female and 4 male). Data are presented as the average numbe Hdh$^{Q111/+}$ or wild type mice across each day of testing at each age. Error bars represent ± standard error of the mean, * $p<0.05$.

animals (Genotype; $F_{1,12} = 11.355$, $p<0.01$). Despite a trend for $Hdh^{Q111/+}$ animals to make fewer responses than wild type animals as they aged, this trend failed to meet significance (Age x Genotype; $F_{2,24} = 3.078$, $p=0.065$). $Hdh^{Q111/+}$ animals made significantly fewer responses to the smallest 2.5µl reward in comparison to wild type animals when all ages were considered (Genotype x Reward size; $F_{2,24} = 11.88$, $p<0.001$), this effect was largely driven by the results seen at the 18 month time point, as shown in Figure 5.3.

$Hdh^{Q111/+}$ animals made a similar number of responses as wild type animals at each reward size at 6 months of age and 12 months of age, as shown in Figure 5.3. However, at 18 months of age, $Hdh^{Q111/+}$ animals made fewer responses than wild type animals at all reward sizes. Therefore, restricted analysis of the 18 month time point only, demonstrated that $Hdh^{Q111/+}$ animals made significantly fewer responses at all reward sizes in comparison to wild type controls (Genotype; $F_{1,12} = 12.65$, $p<0.01$), which was not seen at either of the earlier ages (Figure 5.3.). Interestingly, $Hdh^{Q111/+}$ female animals made significantly fewer responses than female wild type animals when all testing conditions were considered (Sex; $F_{1,12} = 13.57$, $p<0.01$).

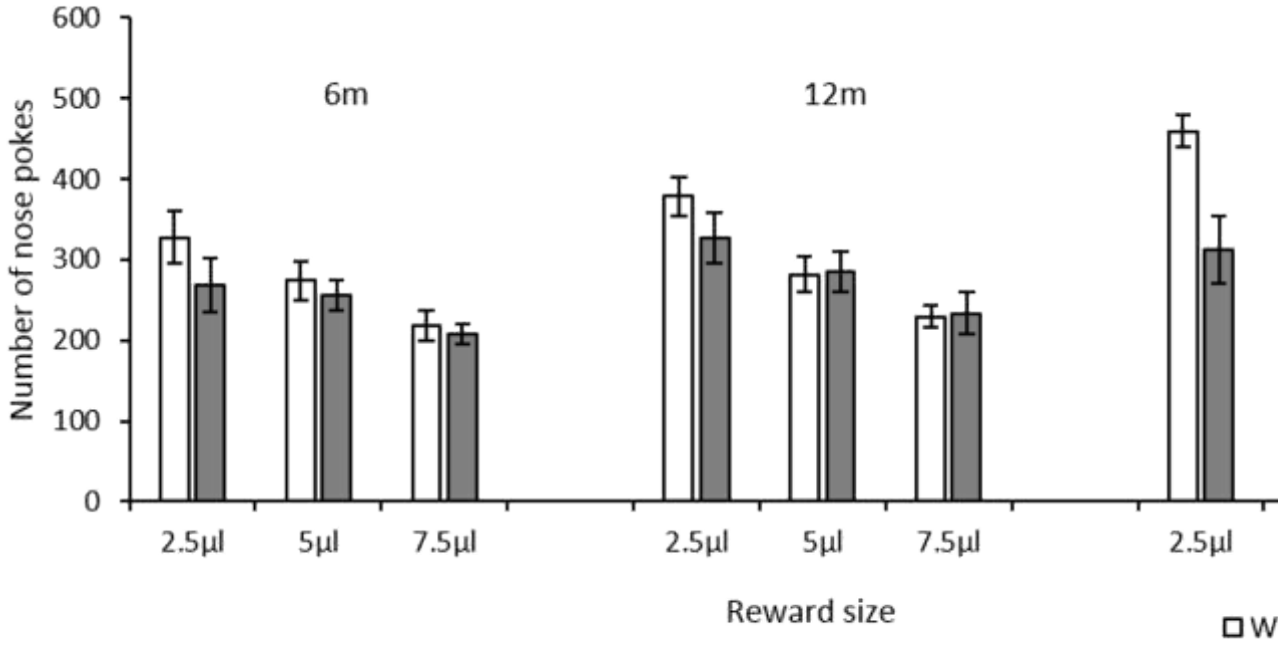

Figure 5.3. Longitudinal Fixed Ratio (FR) Results for Hdh$^{Q111/+}$ animals. FR results demonstrated a significant overall effect of rewar smaller reward sizes. Hdh$^{Q111/+}$ animals made significantly fewer responses than wild type animals when all testing conditions were conside fewer responses than wild type animals, which was driven by the result at the 18 month time point. Data are shown for a total of 16 mice wild type (5 female and 4 male). Data are shown as the average number of responses made over 3 days of testing for Hdh$^{Q111/+}$ or wild type error of the mean. Significance considers genotype differences over all ages of testing, ** $p<0.01$.

wild type animals (Genotype; $F_{1,12} = 4.26$; $p < 0.05$), although this deficit was not shown to be progressive over time (Age x Genotype; $F_{2,24} = 0.542$, p = n.s.). Furthermore, no significant sex differences were demonstrated (Sex; $F_{1,12} = 4.26$, p = n.s.).

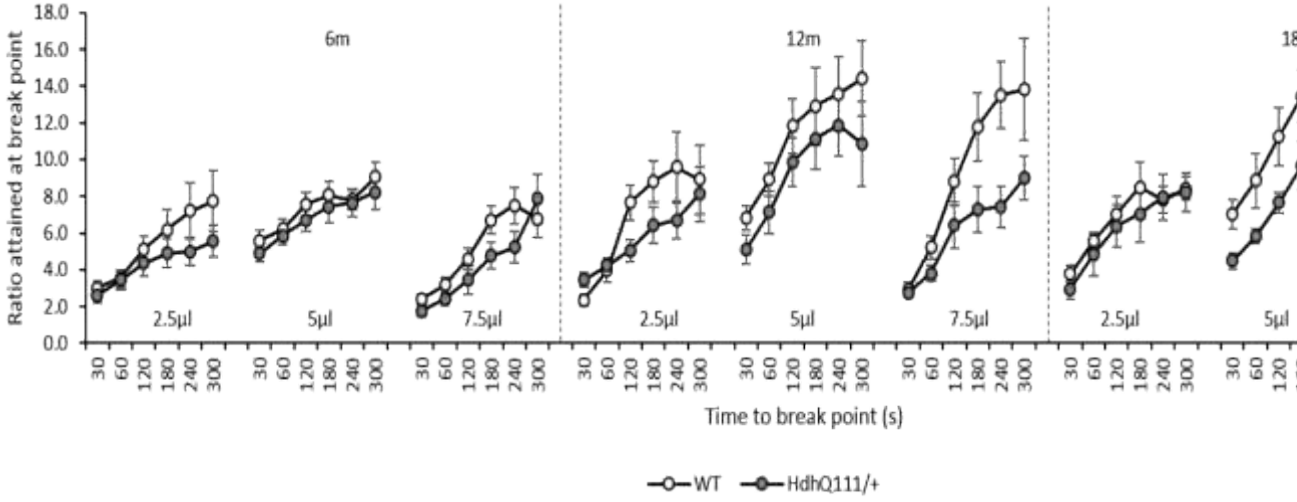

Figure 5.4. Longitudinal Progressive Ratio (PR) Results for Hdh$^{Q111/+}$ animals. Animals were able to reach a higher ratio of respondin were used in testing and animals were able to reach higher ratios of responding at higher break points. Hdh$^{Q111/+}$ animals demonstrated a r to wild type animals, although this deficit was not shown to be progressive over time. Data are shown for a total of 16 mice, 7 Hdh^{Q111+} (female and 4 male). Data are shown as the average number of responses made over 3 days of testing, for each reward size, for Hdh$^{Q111/+}$ c ± standard error of the mean. Significance considers genotype differences over all ages of testing,* p<0.05.

5.4.4. Five Choice Serial Reaction Time Task (5-CSRTT) Results

Animals initiated significantly fewer trials in the 5-CSRTT when the stimulus length was decreased and the attentional load of the task was therefore increased, at all testing ages (Figure 5.5: Stimulus length; $F_{2,24} = 118.49$, $p<0.001$).

$Hdh^{Q111/+}$ animals initiated significantly fewer trials than wild type animals when all ages and stimulus lengths were considered (Genotype; $F_{1,12} = 10.14$, $p<0.01$), although no significant interaction effect was demonstrated (Age x Genotype; $F_{2,24} = 0.43$, p = n.s.). However, restricted analysis of the 18 month age demonstrated a highly significant difference between genotypes, with $Hdh^{Q111/+}$ animals initiating significantly fewer trials than wild type animals at all stimulus lengths (Genotype; $F_{1,12} = 8.04$, $p<0.05$).

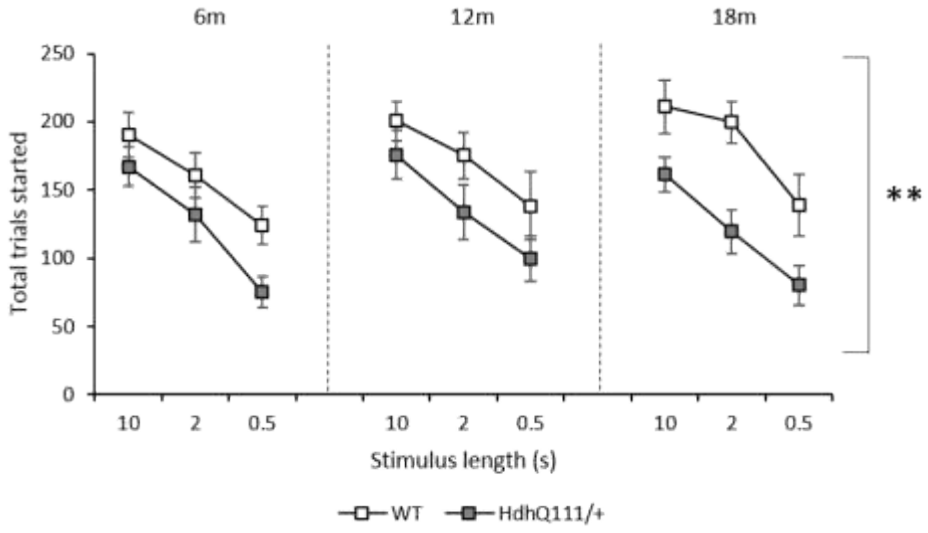

Figure 5.5. Total trials started in the 5-Choice Serial Reaction Time Task (5-CSRTT) longitudinally in $Hdh^{Q111/+}$ animals. Animals initiated significantly fewer trials as the stimulus length was decreased, at all ages. $Hdh^{Q111/+}$ animals initiated significantly fewer trials than wild type animals, largely due to the magnitude of difference in the number of initiated trials at 18 months of age. Data are shown for a total of 16 mice, 7 Hdh^{Q111+} (4 female and 3 male) and 9 wild type (5 female and 4 male) and is the average number of responses made over 5 days of testing at each stimulus length. Error bars represent ± standard error of the mean. Significance considers genotype differences over all ages of testing, ** $p<0.01$.

Decreasing the stimulus length led animals to make significantly less accurate responses (Stimulus length; $F_{2,24} = 291.86$, $p<0.001$) when all testing ages were considered (Figure 5.6.). Furthermore, animals were significantly more accurate in responding into the central hole in comparison to the peripheral holes in all testing conditions (Hole; $F_{4,48} = 25.12$, $p<0.001$).

When all testing ages were included in the analysis, $Hdh^{Q111/+}$ animals were significantly less accurate in responding in the 5-CSRTT in comparison to wild type animals (Genotype; $F_{1,12} = 5.00$, $p<0.05$), although this deficit was stable over time (Age x Genotype; $F_{2,24} = 0.33$, p = n.s.). The overall genotype difference was largely driven by deficits observed at the shortest 0.5 second stimulus length, rather than at the longer stimulus lengths of 10 seconds and 2 seconds, as shown in Figure 5.6.

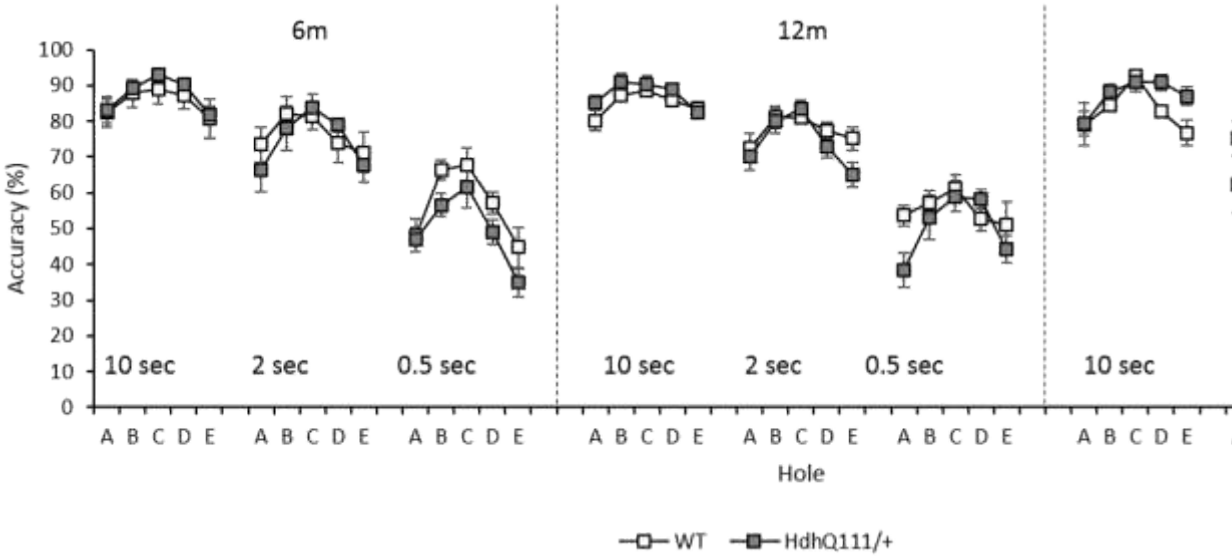

Figure 5.6. Accuracy in the 5-Choice Serial Reaction Time Task (5-CSRTT) longitudinally in Hdh$^{Q111/+}$ animals. Animals were sig shorter stimulus lengths and were significantly more accurate is responding to the central hole in comparison to the peripheral holes. I accurate than wild type animals, although no Genotype x Age interaction was demonstrated. Data are shown for a total of 16 mice, 7 Hd type (5 female and 4 male) and is the average number of responses made over 5 days of testing at each stimulus length. Error bars represent ± considers genotype differences over all ages of testing, * $p<0.05$.

The $Hdh^{Q111/+}$ animals were significantly slower to respond to stimulus in the 5-CSRTT than wild type animals, when all testing ages were considered, (Genotype; $F_{1,12} = 13.19$, $p<0.01$), however no statistically significant interaction effect was observed (Age x Genotype; $F_{2,24} = 0.23$, p = n.s.). Restricted analysis of each age revealed a progressive phenotype; no significant differences in response time were observed between $Hdh^{Q111/+}$ and wild type animals 6 months of age; (Genotype; $F_{1,12} = 7.56$, $p = 0.123$), but $Hdh^{Q111/+}$ animals were significantly slower to respond than wild type animals at 12 months of age (Genotype; $F_{1,12} = 8.29$, $p<0.05$) and 18 months of age (Genotype; $F_{1,12} = 12.72$, $p<0.01$).

Interestingly, in female $Hdh^{Q111/+}$ animals the response time was significantly slower than in female wild type animals in all experimental conditions (Genotype x Sex; $F_{1,12} = 10.80$, $p<0.01$), although no significant differences were observed between the response time performance of male animals.

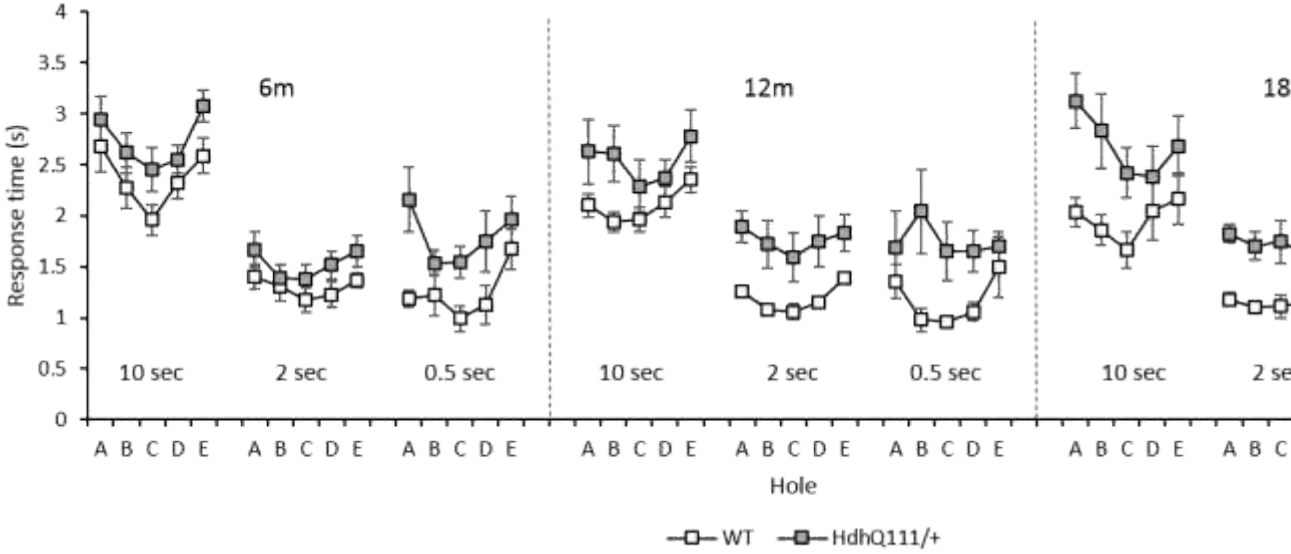

Figure 5.7. Response time in the 5-Choice Serial Reaction Time Task (5-CSRTT) longitudinally in Hdh$^{Q111/+}$ animals. Animals respo and into the central hole in comparison to peripheral holes. Hdh$^{Q111/+}$ animals were significantly slower to respond to the stimulus than wild were significantly slower to respond than female wild type animals. Data are shown for a total of 16 mice, 7 Hdh^{Q111+} (4 female and 3 mal and is the average number of responses made over 5 days of testing at each stimulus length. Error bars represent ± standard error of the differences over all ages of testing, ** $p<0.01$.

for $Hdh^{Q111/+}$ animals to make more time-out responses than wild type animals when restricted analysis was performed at 18 months of age, this failed to meet significance (Genotype; $F_{1,12}$ =1.85, p = n.s.).

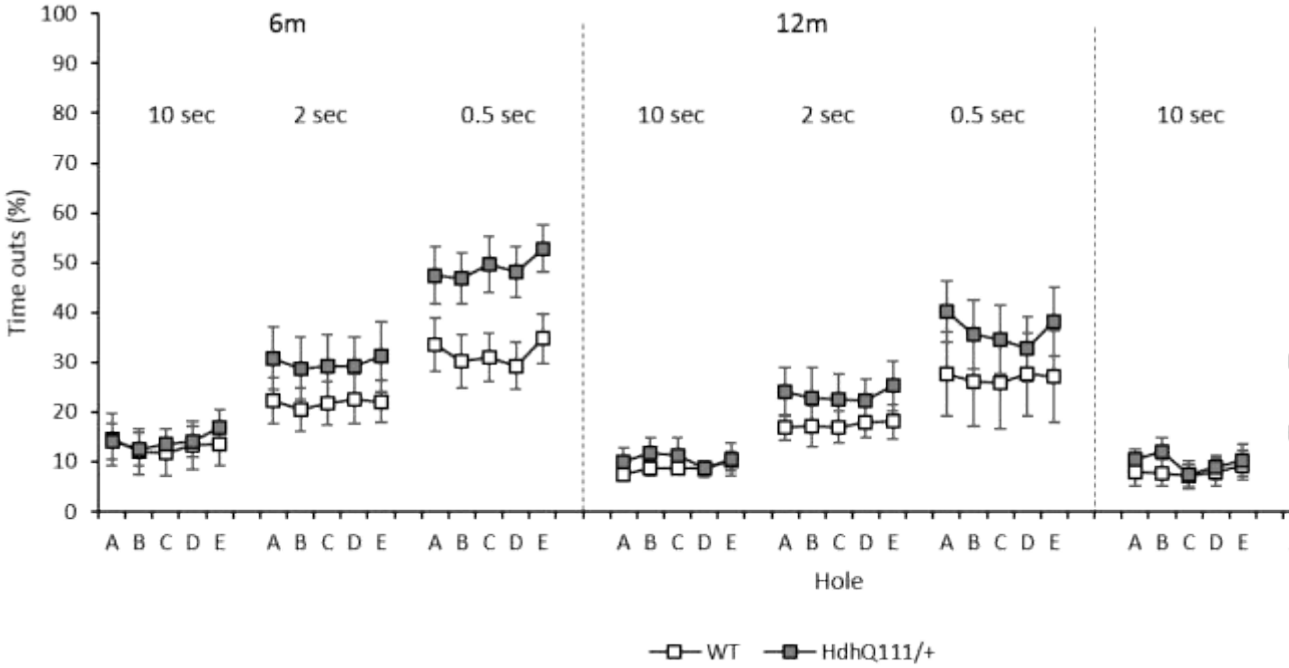

Figure 5.8. The number of time outs made in the 5-Choice Serial Reaction Time Task (5-CSRTT) longitudinally in Hdh$^{Q111/+}$ an time outs as stimulus length decreased. Animals also made significantly more time outs at the peripheral holes in comparison to the centr mice, 7 Hdh^{Q111+} (4 female and 3 male) and 9 wild type (5 female and 4 male) and is the average number of responses made over 5 days bars represent ± standard error of the mean.

5.4.5. Serial Implicit Learning Task (SILT) Results

SILT analysis comprised of S1 and S2 accuracy and response time measures to probe attention and motor function, as well as accuracy and response time measures for the predictable sequence (hole 3 - hole 7) as a measure of implicit learning.

Animals were significantly less accurate in S1 responding accuracy in the SILT when the stimulus length was decreased from 2 seconds to 0.5 seconds (Stimulus length; $F_{1,12} = 6.53$, $p<0.05$), as shown in Figure 5.9. Furthermore, animals were more accurate in responding into the central hole rather than the peripheral holes (Hole; $F_{4,48} = 19.27$, $p<0.001$). $Hdh^{Q111/+}$ animals made significantly slower responses than wild type animals (Genotype; $F_{1,12} = 8.40$, $p <0.05$) and this deficit was stable over time (Age x Genotype; $F_{2,24} = 2.66$, p = n.s.).

However, restricted analysis at each testing age confirmed a deficit in S1 response accuracy in $Hdh^{Q111/+}$ animals in comparison to wild type animals, which was not seen at 6 months of age (Genotype; $F_{1,12} = 0.101$, $p = 0.756$) or 12 months of age (Genotype; $F_{1,12} = 1.109$, $p = 0.313$) but was demonstrated at 18 months of age; (Genotype; $F_{1,12} = 5.97$, $p<0.05$), with $Hdh^{Q111/+}$ animals making significantly less accurate responses in comparison to wild type animals.

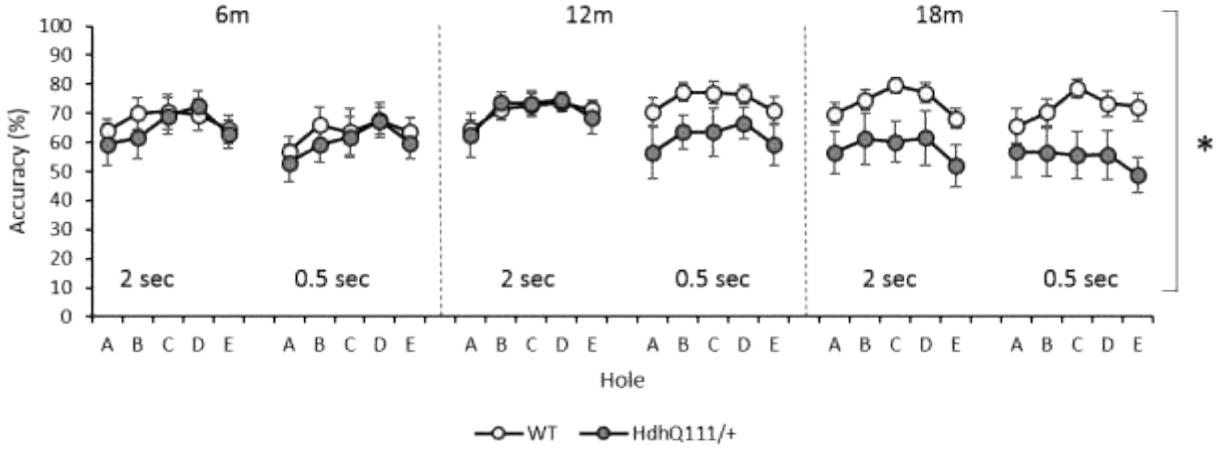

Figure 5.9. S1 Accuracy Serial Implicit Learning Task (SILT) results for $Hdh^{Q111/+}$ animals longitudinally. Animals were more accurate in responding into the central hole at shortest (2 second) stimulus length. Data are shown for a total of 16 mice, 7 Hdh^{Q111+} ((4 female and 3 male) and 9 wild type (5 female and 4 male) and is the average number of responses made over 5 days of testing at each stimulus length. Error bars represent ± standard error of the mean. Significance considers genotype differences over all ages of testing, * $p<0.05$.

Animals were significantly faster to respond to the S1 stimulus when the stimulus length was reduced (Stimulus length; $F_{1,12} = 11.70$, $p<0.01$) when all testing ages were considered, as shown in Figure 5.10. Furthermore, animals were significantly faster to respond into the central hole rather than the peripheral holes (Hole; $F_{4,48} = 11.60$, $p<0.001$), as shown in Figure 5.10. When all testing ages were considered, $Hdh^{Q111/+}$ showed an increased S1 response time in comparison to wild type animals (Genotype; $F_{1,12} = 8.41$, $p<0.05$), but this deficit was not shown to be progressive over time (Genotype x Age; $F_{2,24} = 0.160$, p = n.s.). Restricted analyses to each time point demonstrated that, $Hdh^{Q111/+}$ animals displayed similar levels of S1 response time at 6 months of age (Genotype; $F_{1,12} = 3.42$, $p = 0.089$) but were significantly impaired in responding at 12 months (Genotype; $F_{1,12} = 12.36$, $p<0.01$) and 18 months of age (Genotype; $F_{1,12} = 6.55$, $p<0.05$).

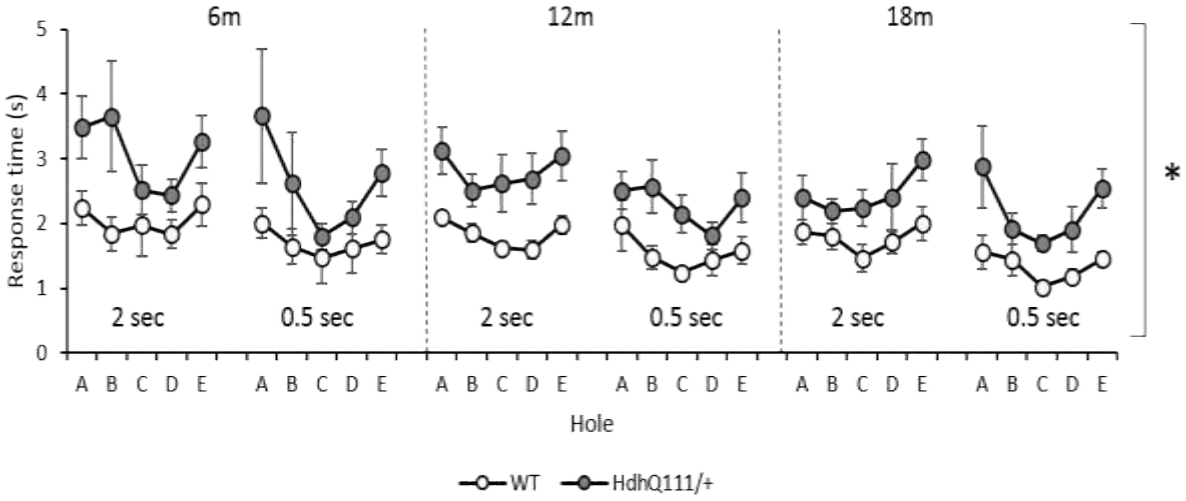

Figure 5.10. S1 Response Time Serial Implicit Learning Task (SILT) results for Hdh$^{Q111/+}$ animals longitudinally. Animals were faster to respond into the central hole at the shorter 0.5 second stimulus length. Hdh$^{Q111/+}$ animals were significantly slower to respond across all task manipulations in comparison to wild type animals. Data are shown for a total of 16 mice, 7 Hdh^{Q111+} (4 female and 3 male) and 9 wild type (5 female and 4 male) and is the average number of responses made over the final 5 days of testing at each stimulus length. Error bars represent ± standard error of the mean. Significance considers genotype differences over all ages of testing, *p<0.05.

In the second response required in the SILT task (S2), mice were significantly less accurate to respond when the step size of the response was increased (Step; $F_{3,36} = 228.22$, $p<0.001$) and when the stimulus length was decreased (Stimulus length; $F_{1,12} = 169.19$, $p<0.001$), as shown in Figure 5.11. Hdh$^{Q111/+}$ animals were significantly less accurate in S2 responding in comparison to wild type animals when all testing ages were considered (Genotype; $F_{1,12} = 14.76$, $p<0.01$), although this deficit was not shown to be progressive over time (Age x Genotype; $F_{2,24} = 4.31$, p = n.s.). Nevertheless, restricted analysis of each testing age demonstrated no significant difference between Hdh$^{Q111/+}$ animals and wild type animals in S2 response accuracy at 6 months of age (Genotype; $F_{1,12} = 2.09$, $p = 0.174$). Although Hdh$^{Q111/+}$ animals demonstrated a significant S2 accuracy deficit in comparison to wild type animals at 12 months of age (Genotype; $F_{1,12} = 11.55$, $p<0.01$) and 18 months of age (Genotype; $F_{1,12} = 18.83$, $p<0.01$). Interestingly, female Hdh$^{Q111/+}$ animals were shown to be significantly less accurate in comparison to female wild type animals (Genotype x Sex; $F_{1,12} = 5.61$, $p<0.05$), although this was not shown for male animals.

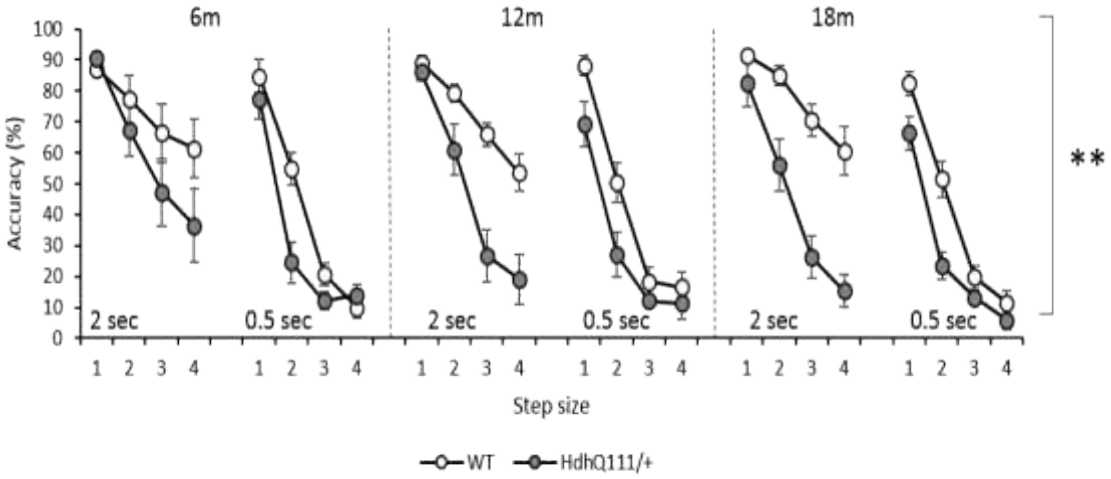

Figure 5.11. S2 Accuracy in Responding in the Serial Implicit Learning Task (SILT) for Hdh$^{Q111/+}$ animals longitudinally. Data are shown for a total of 16 mice, 7 Hdh^{Q111+} (4 female and 3 male) and 9 wild type (5 female and 4 male) and is the average number of responses made over the final 5 days of testing at each stimulus length. Error bars represent ± standard error of the mean. Significance considers genotype differences over all ages of testing, ** p<0.01.

Animals were significantly slower to respond to the S2 stimulus when the step size was increased (Step; $F_{3,36} = 42.35$, $p<0.001$) and when the stimulus length was decreased (Stimulus length; $F_{1,12} = 33.44$, $p<0.001$), as shown in Figure 5.12. Hdh$^{Q111/+}$ animals were shown to be significantly slower to respond to the S2 stimulus when all ages were considered (Genotype; $F_{1,12} = 6.45$, $p<0.05$), this finding was driven by the results obtained using the 2 second stimulus length and was consistent over time (Age x Genotype; $F_{2,24} = 0.43$, p = n.s.).

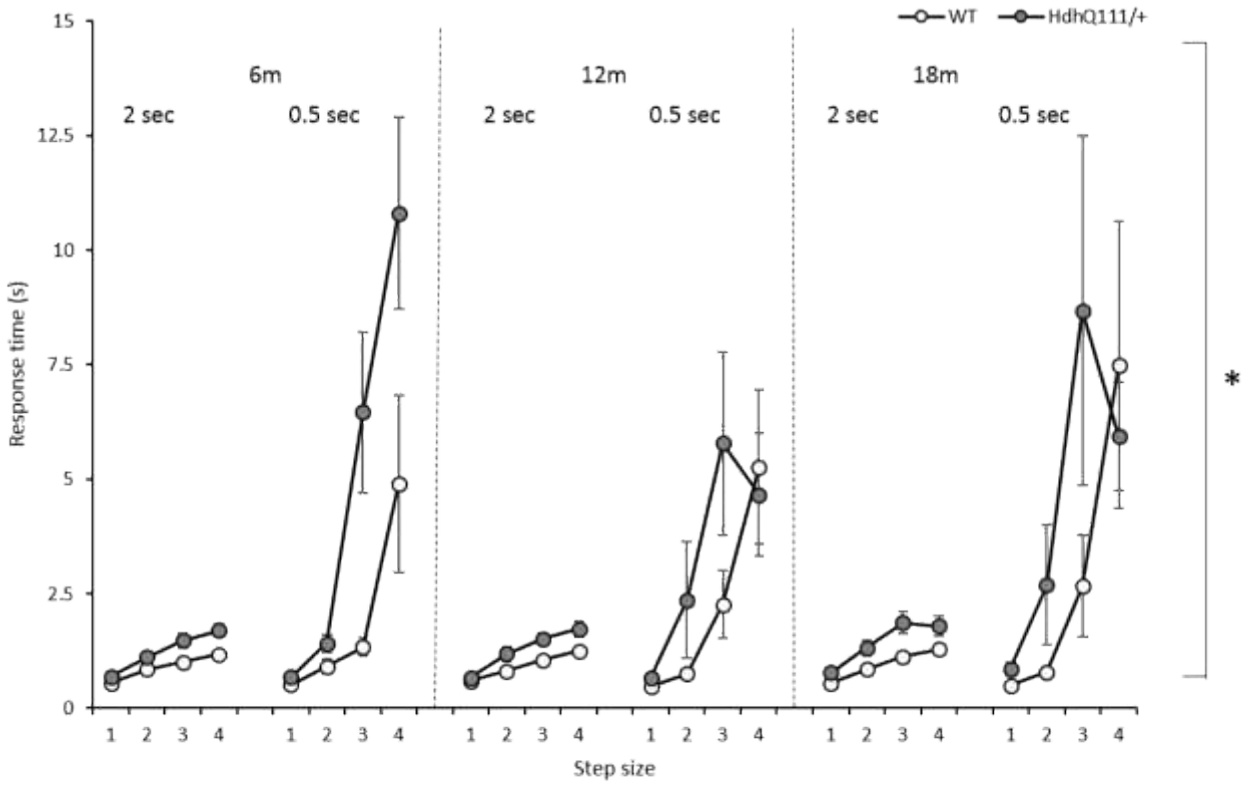

Figure 5.12. S2 Response Time in the Serial Implicit Learning Task (SILT) in Hdh$^{Q111/+}$ animals longitudinally. Animals took significantly longer to respond at the shorter 0.5 second stimulus length and as the step size increased. Hdh$^{Q111/+}$ animals were significantly slower to respond than wild type animals when all testing conditions were considered. Data are shown for a total of 16 mice, 7 Hdh^{Q111+} (4 female and 3 male) and 9 wild type (5 female and 4 male) and is the average number of responses made over the final 5 days of testing at each stimulus length. Error bars represent ± standard error of the mean. Significance considers genotype differences over all ages of testing, * $p<0.05$.

S2 accuracy in responding to the stimulus was considered in light of the predictability of the stimulus, which was designed to probe implicit learning (Figure 5.13). Mice were significantly less accurate when the stimulus length was decreased (Stimulus length; $F_{1,12} = 247.91$, $p<0.001$). Furthermore, animals were significantly more accurate when the stimulus was predictable rather than unpredictable (Predictability; $F_{1,12} = 17.04$, $p<0.01$). Hdh$^{Q111/+}$ animals were significantly less accurate overall in responding in comparison to wild type animals regardless of the predictability of the stimulus (Genotype; $F_{1,12} = 12.61$, $p<0.01$). However, the predictability of the stimulus did not confer any

significant benefit to either wild type animals or $Hdh^{Q111/+}$ animals (Genotype x Predictability; $F_{1,12} = 0.45$, p = n.s.), as shown in Figure 5.13.

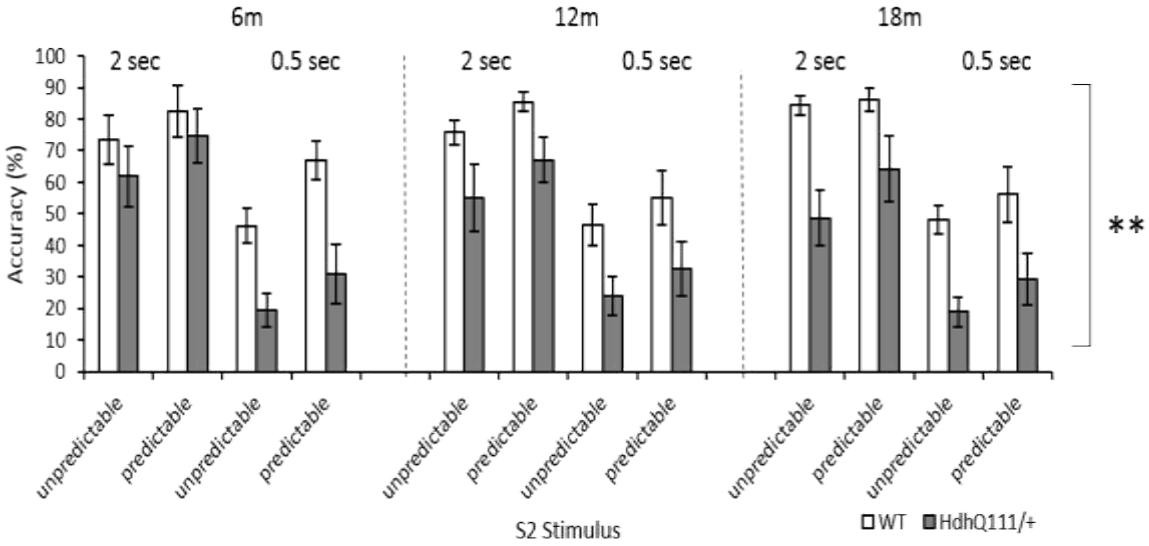

Figure 5.13. S2 Response Accuracy in the Serial Reaction Time Task (SILT) for predictability of stimulus in $Hdh^{Q111/+}$ animals longitudinally. Data are shown for a total of 16 mice, 7 Hdh^{Q111+} (4 female and 3 male) and 9 wild type (5 female and 4 male) and is the average number of responses made over the final 5 days of testing at each stimulus length. Error bars represent ± standard error of the mean. Significance considers genotype differences over all ages of testing, * p<0.05.

The predictability of the S2 stimulus in the SILT did not confer any significant benefit to how rapidly animals were able to respond to the stimulus (Predictability; $F_{1,12} = 0.27$, p = n.s.) (Figure 5.14.). There was considerable variation among response times, in some instances, therefore no significant effect of stimulus length was seen in this case (Stimulus length; $F_{1,12} = 0.15$, p = n.s.). Although, $Hdh^{Q111/+}$ animals showed a trend to be significantly slower than wild type animals when all testing conditions were considered, this trend failed to meet the threshold for statistical significance (Genotype; $F_{1,12} = 4.328$, p = 0.062).

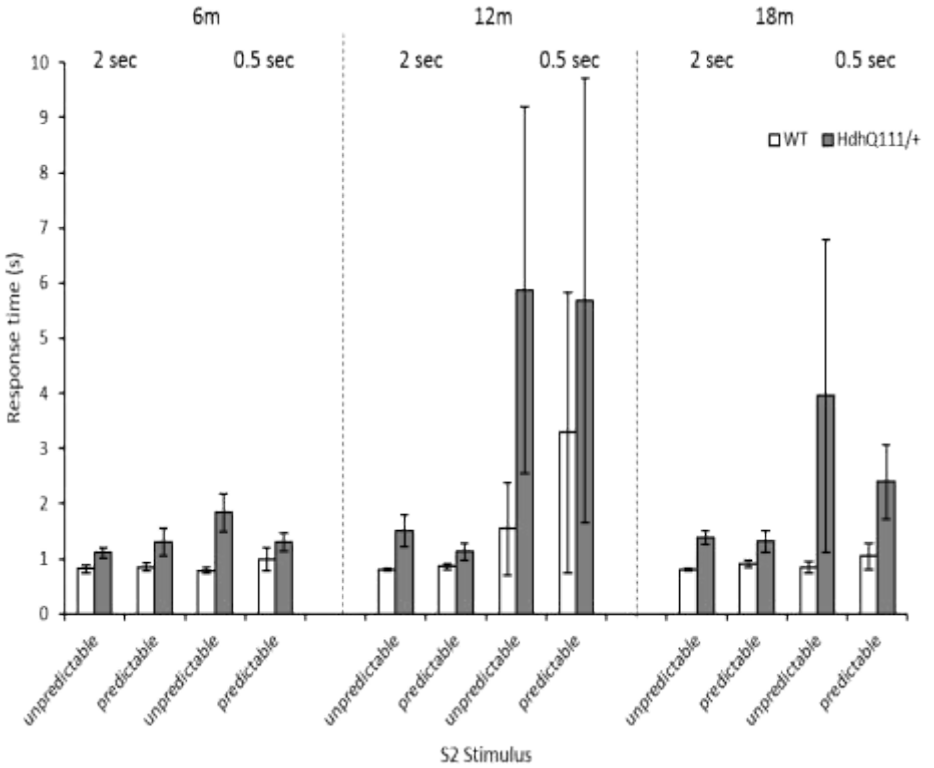

Figure 5.14. S2 Response Time in the Serial Implicit Learning Task (SILT) for predictability of stimulus in Hdh$^{Q111/+}$ animals longitudinally. Data are shown for a total of 16 mice, 7 Hdh^{Q111+} (4 female and 3 male) and 9 wild type (5 female and 4 male) and is the average number of responses made over the final 5 days of testing at each stimulus length. Error bars represent ± standard error of the mean.

5.5. Discussion

Longitudinal operant testing revealed specific cognitive deficits in Hdh$^{Q111/+}$ animals in comparison to wild type animals in FR, PR, 5-CSRTT and SILT in the operant test battery. The data demonstrated that Hdh$^{Q111/+}$ animals were most significantly impaired in comparison to wild type animals at 18 months of age, which was demonstrated with restricted statistical analyses. Our results therefore suggest that the Hdh$^{Q111/+}$ mouse demonstrates an insidious and subtle disease progression that was not detectable in this operant test battery throughout most of the life of the animal.

Initial training in the nose poke response and subsequent reacquisition of the nose poke response demonstrated an inability for Hdh$^{Q111/+}$ animals to initiate as many trials as wild type animals at the oldest 18 month time point. This observation is also reflected in the results of the 5-CSRTT, with Hdh$^{Q111/+}$

animals initiating significantly fewer trials than wild type animals at 18 months of age. Due to the motor deficits demonstrated in the previous chapter, which occur from 9 months of age and are prevalent by 12 months of age, the reduction in responding may be due to significant motor impairments which are also reflected in the response time measures. Therefore, $Hdh^{Q111/+}$ animals may not be physically able to initiate a response as rapidly as wild type animals at 18 months of age and therefore initiate fewer trials within the testing session. This suggestion is further confirmed by the response time deficits shown in the 5-CSRTT task and the SILT at 18 months of age. The operant apparatus is extremely sensitive to detecting response time deficits, which are indicative of motor dysfunction, therefore it may be the case that operant testing is able to detect motor dysfunction more sensitively than the other motor tests used in Chapter 4. It may also be the case that the response time deficits observed in $Hdh^{Q111/+}$ animals are reflective of an inability to initiate movement. Difficulties in the initiation of movement were described in $Hdh^{Q111/+}$ animals progressively from 9 months of age on the balance beam in Chapter 4. Although the initiation of movement has not yet been thoroughly explored in HD mouse models, people with HD have showed a decrease in the initiation of movement from early in the disease progression (Bradshaw *et al.* 1992; Smith *et al.* 2000).

Upon the introduction of different reward sizes into the FR1 testing schedule a general effect of reward size was seen, with all animals making significantly more responses for smaller reward sizes. This effect may be due to animals taking a greater time to consume larger rewards, or there may be an effect of satiation, with animals becoming satiated when they consume larger rewards. However, at the 2.5µl reward size $Hdh^{Q111/+}$ animals responded less than wild type animals, this effect was driven by the large difference observed at 18 months of age, although it was not seen at any other reward size. This may be due to an underlying apathetic phenotype in $Hdh^{Q111/+}$ animals, which means that they respond less than wild type animals for smaller rewards. Apathy has been shown in HD patients (Burns *et al*. 1990; van Duijn *et al*. 2013), although

typically this occurs early in the disease progression, often prior to the onset of motor dysfunction. Therefore, if apathy were causing the results observed in $Hdh^{Q111/+}$ mice, apathy is expressed relatively late in the disease progression in comparison to the human condition.

However, significant differences were observed between $Hdh^{Q111/+}$ animals and wild type animals in progressive ratio performance, thus indicating that $Hdh^{Q111/+}$ mice are impaired in motivation in comparison to wild type animals. These results are comparable to those previously observed in the Hdh^{Q92} (Trueman *et al*. 2009b), zQ175 and BAC mouse models (Oakeshott *et al.* 2012) which demonstrated reduced motivation in HD mice in comparison to wild type controls at 15 months and 73 weeks respectively. However, previous studies utilised food restriction in their PR tasks, and it may be the case that mice are comparatively more motivated when placed on food restriction rather than the water restriction that was imposed in this experiment. Furthermore, the studies cannot be directly compared due to the fact that Oaksehott *et al*., utilised a different version of the PR task which required higher ratios of responding to obtain reward and a longer session time. Although, Trueman *et al.*, utilised the same version of the PR testing regime, this study used homozygous $Hdh^{Q92/Q92}$ animals, whereas to replicate the human condition heterozygous $Hdh^{Q111/+}$ animals were used here. Furthermore, previous studies conducted the PR task in animals at a single time point and not longitudinally and thus, it may be the case that if $Hdh^{Q111/+}$ animals were tested solely at 18 months of age they would show a motivational deficit in the PR task in comparison to wild type animals. The motivational deficits observed in the PR task in the $Hdh^{Q111/+}$ mouse model are in accordance with previous studies in the human patient population (Burns *et al*. 1990; Paulsen *et al*. 2001b; Thompson *et al*. 2002b), which demonstrate motivational problems early in the disease progression of HD, prior to the onset of motor symptoms. Thus, although motivational deficits were shown in the $Hdh^{Q111/+}$ mouse model they were not progressive over time and were present alongside the motor dysfunction shown in Chapter 4.

Highly significant effects of stimulus length and response hole location were demonstrated in the 5-CSRTT, across all testing conditions. This was unsurprising given the attentional load of the task was increased when the stimulus length was decreased. The inability for $Hdh^{Q111/+}$ animals to initiate as many trials as wild type animals in the 5-CSRTT at 18 months of age at all stimulus lengths is indicative of a general inability to complete the task. The precise reason for this inability to complete the task could be due to multiple underlying cognitive factors, such as depression, apathy or lack of motivation. But, given the considerable motor dysfunction that was demonstrated in $Hdh^{Q111/+}$ animals at 18 months of age, in the previous chapter, it is perhaps the case that animals are simply unable to physically get to the stimulus to make a response within the required time.

$Hdh^{Q111/+}$ animals were able to maintain their response accuracy in the 5-CSRTT over consecutive testing ages, despite considerable response time deficits which developed as they aged. Therefore, it may be the case that $Hdh^{Q111/+}$ animals are able to maintain their accuracy in responding in the 5-CSRTT, at the detriment of responding slower to the stimulus. This suggestion is also supported by the fact that $Hdh^{Q111/+}$ animals did not demonstrate a significant difference in the number of time outs made in comparison to wild type animals. Therefore, $Hdh^{Q111/+}$ animals are able to respond to the stimulus and to the same degree of accuracy as wild type animals, although it may take them longer to do so.

A previous study in the Hdh^{Q92} mouse model of HD utilised the 5-CSRTT to demonstrate that $Hdh^{Q92/Q92}$ animals had a significant deficit in accuracy in responding in the 5-CSRTT when the stimulus was pseudo-randomly generated with stimuli of different durations (Trueman *et al.* 2012c). The experimental design employed by Trueman *et al*., was different from the version of the 5-CSRTT employed in this study, where the stimulus length was decreased over consecutive days. Therefore, the way in which the 5-CSRTT was implemented in this experiment, through gradually decreasing the stimulus length during testing, may have allowed animals to respond with increasing accuracy.

Whereas, if the 5-CSRTT was implemented in $Hdh^{Q111/+}$ mice, as it was by Trueman *et al*., with pseudo-randomly generated stimuli of different durations which further increases the attentional load of the task, deficits in attentional function may have been observed.

In the SILT $Hdh^{Q111/+}$ animals were significantly slower in responding to both the S1 and S2 stimulus, than wild type animals, thus further reflecting the motor deficits previously observed in $Hdh^{Q111/+}$ animals. The S1 accuracy deficits demonstrated in $Hdh^{Q111/+}$ animals at 18 months of age were surprising, given that these deficits did not exist in the 5-CSRTT. Therefore, it may be the case that when the complexity of the operant task is increased and requires two responses, animals are unable to maintain the accuracy of responding as was shown in the 5-CSRTT. It could also be the case that motor dysfunction prevents animals from responding to the S1 SILT stimulus accurately. Although, the deficits observed in the SILT task in S2 accuracy among $Hdh^{Q111/+}$ animals may also be attributed to motor problems, these deficits may further be explained by the eccentricity of response required in this task, which has been previously demonstrated in rats with striatal lesions (Lelos *et al.* 2012).

In the SILT animals were able to respond with increasing accuracy to the S2 predictable stimulus, although this benefit was not reflected in S2 response time. This demonstrates that both wild type and $Hdh^{Q111/+}$ animals are able to learn the implicit learning component of the task. Therefore, it can be concluded that $Hdh^{Q111/+}$ animals have no deficit in implicit learning in comparison to wild type animals. Generally $Hdh^{Q111/+}$ animals were significantly slower in responding in the SILT task, than wild type animals, thus further reflecting the motor deficits observed in $Hdh^{Q111/+}$ animals in the previous chapter. Previous studies regarding implicit learning in the HD patient population have produced mixed results, with some demonstrating deficits in implicit learning in people with HD (Knopman and Nissen 1991; Willingham and Koroshetz 1993; Ghilardi *et al*. 2008), whereas others have shown no significant difference in implicit learning between people with HD and controls (Bylsma *et al.* 1991; Brown *et al.* 2001). Our results

support the hypothesis that there are no significant deficits in implicit learning performance in the $Hdh^{Q111/+}$ mice in comparison to wild type animals.

Due to the experimental aim of characterising the $Hdh^{Q111/+}$ mouse model, the operant tasks utilised in the operant testing battery were designed to run for a pre-determined session time. This experimental design ensured that exposure to the operant box was the same among animals. Although, when $Hdh^{Q111/+}$ animals began significantly fewer trials than wild type animals, it meant that wild type animals were exposed to comparatively more reward and thus practice effects may have been demonstrated within the data. Therefore, at the later ages, practice effects may be observed in the data due to wild type animals performing comparatively more trials than $Hdh^{Q111/+}$ animals on subsequent days. However, because the aim of this study was to characterise the Hdh^{Q111} mouse model, this finding could not have been foreseen. Thus future manipulations of these operant tasks would be designed such that the animal is required to complete a set number of trials during the task to eliminate any possible practice effects among testing groups.

In addition, the longitudinal operant battery was performed such that the same animals underwent repeated testing in the operant test battery. Therefore, animals were extensively exposed to the operant boxes, and the associated responding, obtaining of reward and the associated water restriction for an extended period of time. Thus, it may be the case that the extensive training and testing from a young age may mask some of the cognitive deficits associated with $Hdh^{Q111/+}$ animals. Although the experiment was performed with the aim of characterising the cognitive phenotype of the Hdh^{Q111} mouse model of HD, it may be that repeated exposure and testing on operant tasks of cognition inadvertently modified subsequent operant behaviours. Therefore, in future behavioural characterisations, it may be necessary to use a separate testing group for each of the experimental time points, to eliminate any such effects, although this will have considerable ethical and financial implications. An extensive

discussion regarding the possible effects of repeatedly testing the same animals in a longitudinal study is presented in the General Discussion in Chapter 8.

The suggestion of practice effects caused by cognitive training is also clinically relevant, as studies in other neurodegenerative disease including Alzheimer's disease (Davis *et al.* 2001; Farina *et al.* 2002; Clare *et al.* 2003) and Parkinson's disease (Sinforiani *et al.* 2004; Sammer *et al.* 2006; Milman *et al.* 2014; Atias *et al.* 2015) have considered cognitive training as a non-pharmacological therapeutic intervention. These studies used cognitive training, via repeatedly conducting specific cognitive tasks on computer programmes, to improve both motor and cognitive disease symptoms. Therefore, these patient studies demonstrate that the way that operant testing has been performed in this experimental design may mask or even perhaps improve some of the behavioural deficits seen in the $Hdh^{Q111/+}$ mouse model of HD. To explore this possibility a thorough investigation into the effects of repeatedly testing $Hdh^{Q111/+}$ mice on the 5-CSRTT is presented in the following chapter.

Chapter 6 : Can pre-training in an operant task modify subsequent behaviour and neuropathology in the Hdh^{Q111} mouse model of Huntington's disease?

6.1. Introduction

In the previous chapter, operant tests were utilised to demonstrate significant cognitive deficits in the $Hdh^{Q111/+}$ mouse model of HD, although the observed deficits were perhaps not as progressive or severe as was to be expected based on previous results in other mouse models of HD (Trueman *et al*. 2007; Trueman *et al*. 2009a; Brooks *et al*. 2012h; Trueman *et al*. 2012c). This led to the hypothesis that the experimental design employed in the previous chapter (repeatedly conducting the same operant test battery in the same animals) may lead to an altered behavioural phenotype, which may not have been observed if separate groups of animals had been used for each age of operant behavioural testing.

Attentional deficits were observed in the 5-choice serial reaction time task (5-CSRTT) in $Hdh^{Q111/+}$ animals at 18 months of age (as seen in Chapter 5). However, previous literature in HD patients (Lawrence *et al*. 1996; Lawrence *et al.* 1998a; Lemiere *et al.* 2004) and other HD mouse lines (Trueman *et al.* 2007; Trueman *et al.* 2012c) suggests that problems with attention are a specific early deficit within the progression of HD. Therefore, it may be the case that previous testing on the 5-CSRTT, at a young age, may improve attentional performance in $Hdh^{Q111/+}$ animals when they are subsequently tested in the same task at a later age.

This hypothesis is further supported by human studies which suggest cognitive training can improve executive function in healthy individuals as they age (Ball *et al.* 2002; Willis *et al.* 2006). Furthermore, in the patient population cognitive

training studies have been conducted in other neurodegenerative diseases including Parkinson's disease (PD) (Sinforiani *et al*. 2004; Sammer *et al*. 2006; París *et al.* 2011; Milman *et al*. 2014) and Alzheimer's disease (AD) (Hofmann *et al.* 1996; Davis *et al*. 2001; Farina *et al*. 2002; Clare *et al*. 2003; Clare and Woods 2004). These studies have demonstrated that cognitive training, specifically focused on tasks of executive function, can improve both cognitive and motor outcomes in PD and AD patients.

6.2. Aims

To determine if pre-training in an operant task (the 5-CSRTT), at a young age, modifies the subsequent behavioural and neuropathological phenotype of $Hdh^{Q111/+}$ animals at an older age.

6.3. Materials and Methods

6.3.1. Animals

A total of 43 animals were used in this experiment, which included 21 $Hdh^{Q111/+}$ animals (11 female and 10 male) and 22 wild type animals (13 female and 9 male). Animals were split equally into three testing groups based on sex and genotype (see Experimental Design section 6.3.2., Table 6.1). $Hdh^{Q111/+}$ animals were genotyped by Laragen Inc (see Chapter 2 section 2.1.1.) and contained an average CAG repeat length of 141 repeats, which ranged from 134-149 repeats.

Animals were housed in mixed genotype and single sex groups and were kept in a temperature controlled environment (21°C±1°C) on a 12 hour light dark cycle (lights on 06.00 hours, lights off 18.00 hours). Animals were given *ad-libitum* access to standard laboratory chow food, and a water restriction regime was imposed for the duration of operant testing.

6.3.2. Experimental Design

The animal testing groups used in this experiment are shown below in Table 6.1. Operant pre-training commenced in the operant boxes at 4 months of age. The operant pre-training given to animals differed depending on the testing group. The first group received 15 days of nose poke training and then 20 days of

training in an attentional task (the 5-choice serial reaction time task). The second group received a comparable total number of days (35 days) of nose poke training; however, for the final 20 days an extended nose poke training regime was used, as detailed below in 6.3.4.2. The third testing group were cage controls and therefore received no exposure to, or training in, the operant boxes; however they were handled and water restricted in the same way as the other testing groups.

Table 6.1. Animal testing groups.
*An $Hdh^{Q111/+}$ animal in testing group 3 became ill at approximately 8 months of age and was therefore culled reducing the number of $Hdh^{Q111/+}$ animals in the testing group to 7. 5-CSRTT refers to the 5-Choice Serial Reaction Time Task.

	Pre-training (4 months of age)	**Testing (12 months of age)**
Group 1 Attentional pre-training (n=14, 7 $Hdh^{Q111/+}$)	Nose poke training (15 days) 5-CSRTT training (20 days)	Nose poke training (15 days) 5-CSRTT testing (20 days)
Group 2 Non-attentional pre-training (n=14, 6 $Hdh^{Q111/+}$)	Nose poke training (15 days) Extended nose poke training (20 days)	Nose poke training (15 days) 5-CSRTT testing (20 days)
Group 3 Control (n=15, 8 $Hdh^{Q111/+}$*)	-	Nose poke training (15 days) 5-CSRTT testing (20 days)

6.3.3. Operant Apparatus

Operant testing was conducted in 16 9-hole operant boxes as previous described in Chapter 2 (section 2.3.1.). For the 5-choice serial reaction time task (5-CSRTT) utilised in this chapter only the 9-hole operant set up was required and thus the response holes required for the 'Skinner like' operant set up were covered with black plastic for operant testing, as were holes 2,4 ,6 and 8 of the 9-hole array.

6.3.4. Operant Pre-training

One week prior to the beginning of operant pre-training all animals were gradually water restricted, to a final restriction of 3 hours of access to water per

day. Animals were introduced to Yazoo© strawberry milk reward, by placing dishes of strawberry milk into their home cages. Animals then received operant pre-training as described below and indicated in Table 6.1. Animals were tested in operant boxes 5 days per week and were given *ad-libitum* access to water from Friday evenings until Sunday evenings.

6.3.4.1. Nose Poke Pre-training

At 4 months of age the animals in Groups 1 and 2 received 15 days of nose poke training (as previously described in section 2.3.4.1. of Chapter 2). Briefly, animals were required to learn to poke into the central stimulus light of the 9-hole array. Once an animal had correctly poked into the illuminated light stimulus it was extinguished and 5µl of strawberry milk reward was simultaneously delivered into the reward magazine. The programme was 20 minutes in duration.

6.3.4.2. Extended Nose Poke Pre-training

In addition to the nose poke pre-training described above, the animals in testing Group 2 received 20 days of extended nose poke training (Table 6.1). The extended nose poke training programme was designed as a comparable task to the 5-CSRTT (as detailed below), although without the attentional training component. Animals were still required to make nose pokes to obtain reward, but the location of the response hole differed on subsequent days of pre-training, to prevent perseverative responding. The operant programme was designed to randomly select a light stimulus which the animal was required to poke into for the duration of the 30 minute training session to obtain reward a 5µl strawberry milk reward.

6.3.4.3. 5-Choice Serial Reaction Time Task (5-CSRTT) Pre-training

After 15 days of nose poke pre-training, the animals in testing Group 1 (see 6.3.4.1. above and Table 6.1) animals were tested in the 5-choice serial reaction time task (5-CSRTT). The details of the 5-CSRTT programme utilised have previously been described (see section 2.3.4.4. in Chapter 2).

6.3.5. Operant Testing

Operant testing was conducted in all groups of animals at 12 months of age. Animals were gradually water restricted one week prior to operant testing and introduced to strawberry milkshake within their home cages.

6.3.5.1. Nose Poke Testing

All animals were placed on the nose poke training programme for 15 days, as described above in 6.4.3.1.

6.3.5.2. 5-Choice Serial Reaction Time Task (5-CSRTT) Testing

After the completion of nose poke training, animals were tested on the 5-CSRTT as previously described (see section 2.3.4.4. in Chapter 2). Testing lasted for a total of 20 days, for the first 10 days of testing a 10 second stimulus length was used. For the next 5 days of testing a 2 second stimulus length was used, and for the final 5 days of testing a 0.5 second stimulus length was used.

6.3.6. Tissue Preparation

At the end of operant testing animals were culled via cervical dislocation and the tissue was prepared as previously described in Chapter 2, section 2.4.1.

6.3.7. Immunohistochemistry of Free Floating Sections

Immunohistochemistry was conducted on the left hemisphere of each brain as described in Chapter 2, section 2.4.2. The primary antibodies used for immunohistochemical staining included; S830, Neu-N and DARPP-32. Cresyl violet staining was also conducted as previously described in Chapter 2, section 2.4.3.

6.3.8. Stereological Analysis and Image Acquisition

Stereological analysis and image acquisition was conducted as previously described in Chapter 2, section 2.5.

6.3.9. Statistical Analysis

Statistical analyses were performed in IBM SPSS Statistic 20 software for windows. Repeated measures ANOVA tests followed by simple effects analysis

were conducted for behavioural tests. For histological analysis univariate ANOVA tests followed by simple effects analysis were conducted. Where significance was found *post-hoc* tests with Bonferroni corrections were applied to identify the locus of effects and their interaction(s). The critical significance level used throughout was $\alpha = 0.05$.

6.4. Results

6.4.1. Pre-training Results

The pre-training given to testing Groups 1 and 2 (see Table 6.1) demonstrated that both $Hdh^{Q111/+}$ animals and wild type animals were able to acquire and learn the nose poke response equally well, initiating approximately 160 nose pokes per session, after 15 days of pre-training (Figure 6.1A, Genotype; $F_{1,10} = 8.56$, p = n.s.). Furthermore, comparison of testing Groups 1 and 2 demonstrated similar levels of acquisition in learning the nose poke response (Figure 6.1B, Group; $F_{1,10} = 32.56$, p = n.s.), with both groups initiating approximately 160 trials per session by the end of the 15 days of pre-training.

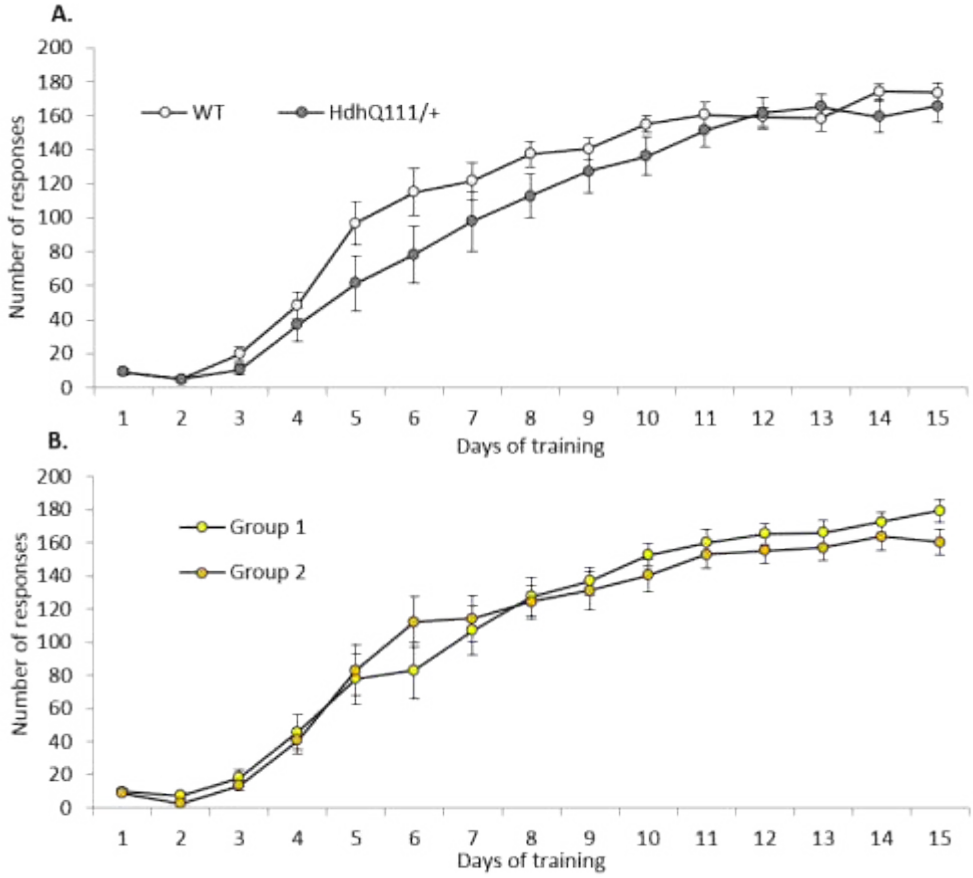

Figure 6.1. Nose poke pre-training results. A. Both $Hdh^{Q111/+}$ and wild type animals demonstrated similar acquisition and learning of the nose poke response, performing approximately 160 nose pokes per session after 15 days of pre-training **B.** Both testing groups 1 and 2, which comprised both $Hdh^{Q111/+}$ and wild type animals, demonstrated similar acquisition and learning of the nose poke response, performing approximately 160 nose pokes per session after 15 days of pre-training. The data in panel A, are shown for a total of 28 animals, 13 $Hdh^{Q111/+}$ and 15 wild type. Group 1 contained a total of 14 days animals, 7 of which were $Hdh^{Q111/+}$ and group 2 contained 14 animals, 6 of which were $Hdh^{Q111/+}$. Data are shown as an average for each day, error bars represent ± standard error of the mean.

Testing Group 2 were given extended 20 days of nose poke training after initial nose poke training. Although there was a trend for $Hdh^{Q111/+}$ animals to make fewer responses than wild type animals, throughout the 20 days of extended nose poke pre-training (Figure 6.2), this trend failed to meet the conventional threshold of significance (Genotype; $F_{1,12} = 4.10$, $p=0.066$).

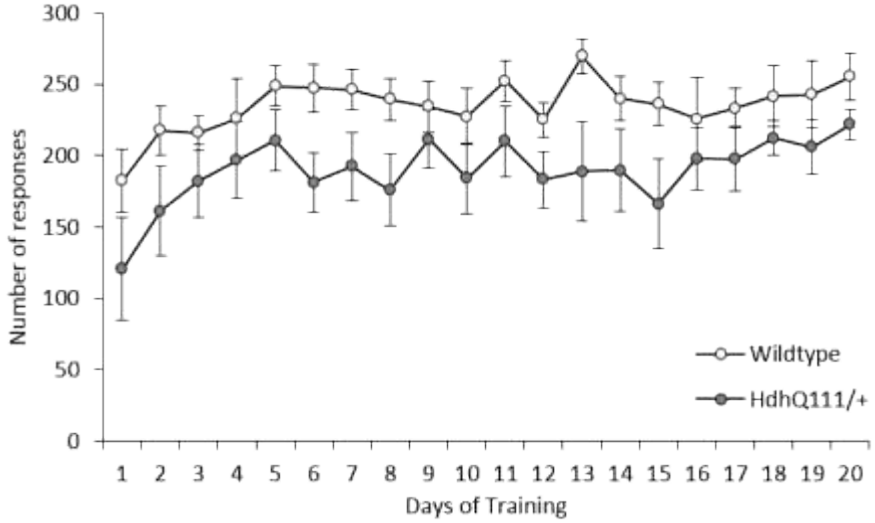

Figure 6.2. Extended nose poke pre-training results. There was a trend for $Hdh^{Q111/+}$ animals to make fewer nose poke responses than wild type animals during extended nose poke training, although this trend failed to meet conventional levels of significance. Data are shown for a total of 14 animals, 6 of which were $Hdh^{Q111/+}$. Error bars represent ± standard error of the mean.

Testing Group 1 were given pre-training in the 5-CSRTT after initial nose poke training. $Hdh^{Q111/+}$ animals demonstrated similar overall performance in the 5-CSRTT as wild type animals in all measures of task performance (Figure 6.3). $Hdh^{Q111/+}$ animals began a similar number of trials in the 5-CSRTT as wild type animals (Genotype: ($F_{1,10}$ = 0.08, p = n.s.), and there was a clear effect of stimulus length, with fewer trials initiated at the shorter stimulus lengths (Figure 6.3A, Stimulus length: $F_{2,20}$ = 85.54, p<0.001). $Hdh^{Q111/+}$ animals were as accurate in the 5-CSRTT as wild type animals, at all stimulus lengths (Figure 6.3B, Genotype: $F_{1,10}$ = 0.02, p = n.s.). Animals became less accurate as stimulus length was decreased (Stimulus length: $F_{2,20}$ = 419.47, p<0.001) and were more accurate in responding into the central hole in comparison to the holes further away from the centre (Hole: $F_{4,40}$ = 14.88, p<0.001). $Hdh^{Q111/+}$ animals displayed similar response times as wild type animals in the 5-CSRTT (Figure 6.3C, Genotype: $F_{1,10}$ = 0.13, p = n.s.), and reducing the stimulus length decreased the response time (Stimulus length; $F_{2,20}$ = 67.70, p<0.001). Faster responses were made towards the central hole of the array in comparison to the holes further from the centre (Hole: $F_{4,40}$ = 6.88, p<0.001).

The number of time outs made in the 5-CSRTT was the same in $Hdh^{Q111/+}$ and wild type animals, although animals made more time outs when shorter stimulus lengths were used, see Figure 6.3D (Stimulus length: $F_{2,20} = 31.34$, $p<0.001$).

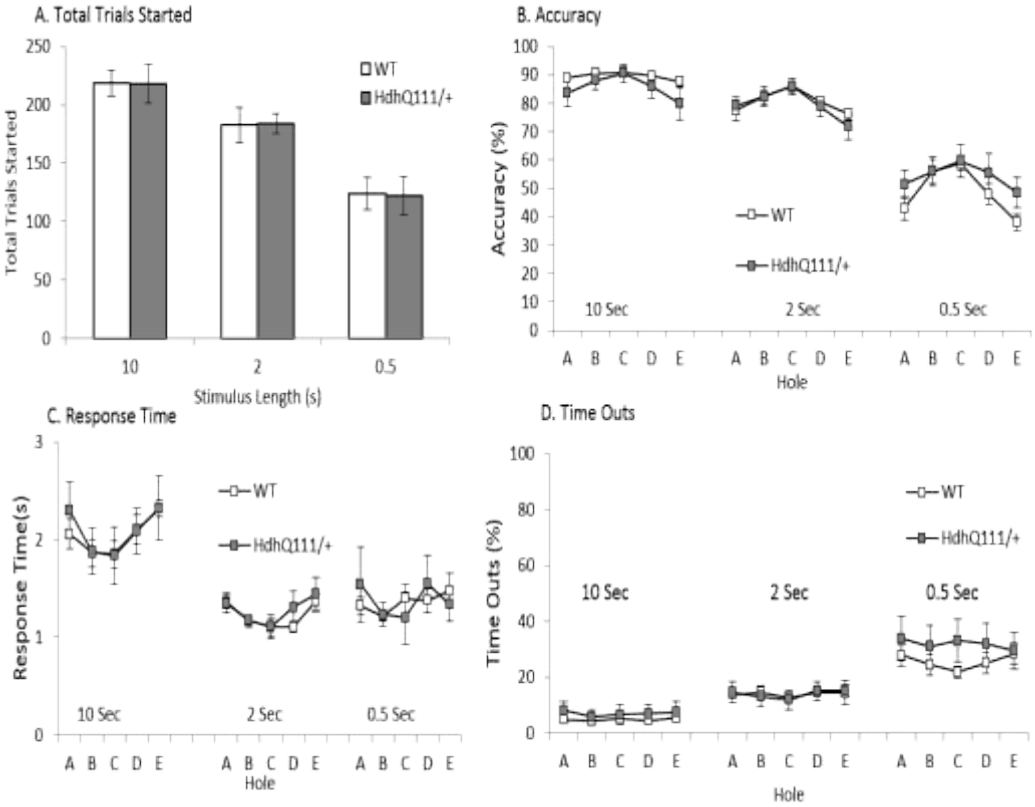

Figure 6.3. 5-Choice serial reaction time task (5-CSRTT) pre-training results. A. Total trials started did not differ among genotypes although fewer trials were initiated at the shorter stimulus lengths. **B.** Accuracy in responding did not differ between $Hdh^{Q111/+}$ or wild type animals although animals were more accurate toward the central hole and at the longest stimulus lengths. **C.** Response time did not differ between $Hdh^{Q111/+}$ and wild type animals although animals responded significantly faster into the central hole and at the shortest stimulus lengths. **D.** The number of time outs made did not differ between $Hdh^{Q111/+}$ and wild type animals although more time outs were made at the shorter stimulus lengths. The data represents 14 animals, 7 of which were $Hdh^{Q111/+}$ animals, which began operant testing at 4 months of age. Data are shown as an average of the last 5 days of testing at each stimulus length. Error bars represent ± standard error of the mean.

6.4.2. Testing Results

6.4.2.1. Operant pre-training alters the ability to acquire the nose poke response.

Animals who had received pre-training in either an operant attentional task (Group 1) or an operant non-attentional task (Group 2) were able to re-acquire the nose poke response rapidly, performing approximately 150 responses after 15 days of testing (Figure 6.4.). However, the animals who were naive to the operant boxes (Group 3) took significantly longer to acquire the nose poke response than those who had previously been trained in the operant boxes (Groups 1 and 2) (Group: $F_{2,36} = 14.17$, $p<0.01$). After 15 days of testing the wild type animals who were naive to the operant boxes were able to perform comparable levels of responses as $Hdh^{Q111/+}$ animals who had previously received pre-training, although they were not able to perform as many trials as wild type animals who had received pre-training. $Hdh^{Q111/+}$ animals who were naive to the operant boxes were noticeably unable to initiate as many trials as the animals in the other testing groups. An overall genotype effect was seen (Genotype: $F_{1,36} = 17.57$, $p<0.001$), although the interaction between genotype and group failed to meet convetional levels of significance (Age x Genotype: $F_{1,36} = 2.83$, p = n.s.).

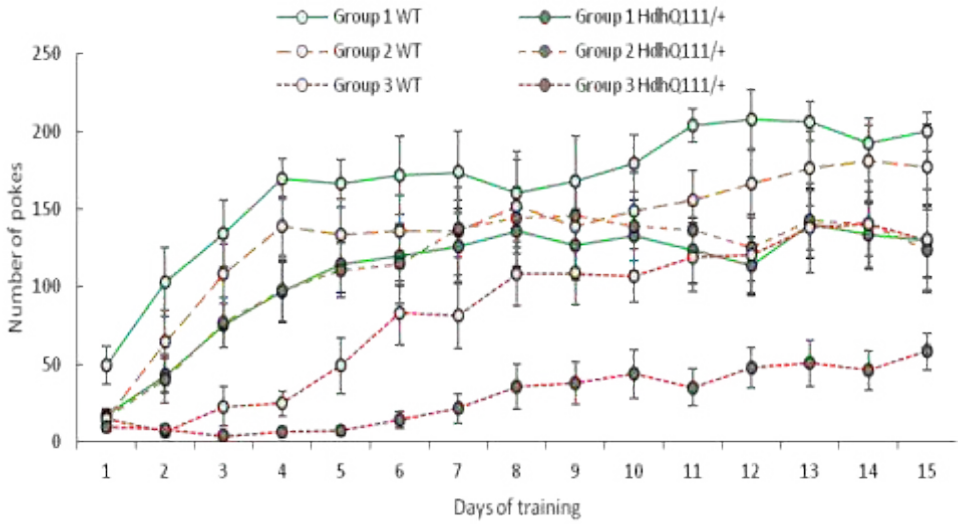

Figure 6.4. Acquisition of a nose poke response at 12 months of age. All animals began testing at 12 months of age. The data represents a total of 43 animals, which were split into three testing groups, as detailed above in Table 6.1. Error bars represent ± standard error of the mean.

6.4.2.1. Does operant pre-training in an attentional task modify subsequent operant behaviour in the 5-CSRTT?

Animals who had received pre-training on the 5-CSRTT task at a young age initiated significantly more trials in the 5-CSRTT in comparison to animals who had not received pre-training in the task (Group: $F_{1,23} = 21.83$, $p<0.001$), as seen in Figure 6.5A. $Hdh^{Q111/+}$ animals initiated fewer trials than wild type animals (Genotype: $F_{1,23} = 46.58$, $p<0.001$). A highly significant effect of stimulus length was seen, with fewer trials were initiated at the shorter 0.5 second stimulus length, in comparison to the longer 2 second stimulus length was used in testing (Stimulus length: $F_{1,23} = 31.40$, $p<0.001$).

Despite a trend for $Hdh^{Q111/+}$ animals to demonstrate a greater attentional deficit, in response accuracy, than wild type animals when they had not received pre-training, this trend failed to meet the threshold for conventional levels of significance (Genotype: $F_{1,23} = 4.21$, $p = 0.052$). Nevertheless, both wild type and $Hdh^{Q111/+}$ animals who had received pre-training in the 5-CSRTT were significantly more accurate in responding in the 5-CSRTT than those who had not received pre-training (Group: $F_{1,23} = 30.86$, $p<0.001$, Figure 6.5B).

The group of animals that were pre-trained in the 5-CSRTT demonstrated improved response times in comparison to animals who were trained in the task at an older age (Group; $F_{1,23} = 37.36$, $p<0.001$). Interestingly, $Hdh^{Q111/+}$ animals were significantly improved in response time in the 5-CSRTT, in comparison to $Hdh^{Q111/+}$ animals that were not pre-trained on the task (Group x Genotype: $F_{1,23} = 6.41$, $p<0.05$), as shown in Figure 6.5C. This pattern of results was also reflected in the number of time-outs made in the 5-CSRTT (Figure 6.5D). Animals who were pre-trained in the 5-CSRTT made significantly fewer time outs in comparison to animals who were not pre-trained on the task (Group: $F_{1,23} = 17.76$, $p<0.001$). $Hdh^{Q111/+}$ animals made significantly fewer time outs in the task when they had received pre-training in the 5-CSRTT at a young age, in comparison to $Hdh^{Q111/+}$ animals who received training at an older age (Group x Genotype: $F_{1,23} = 11.26$, $p<0.01$).

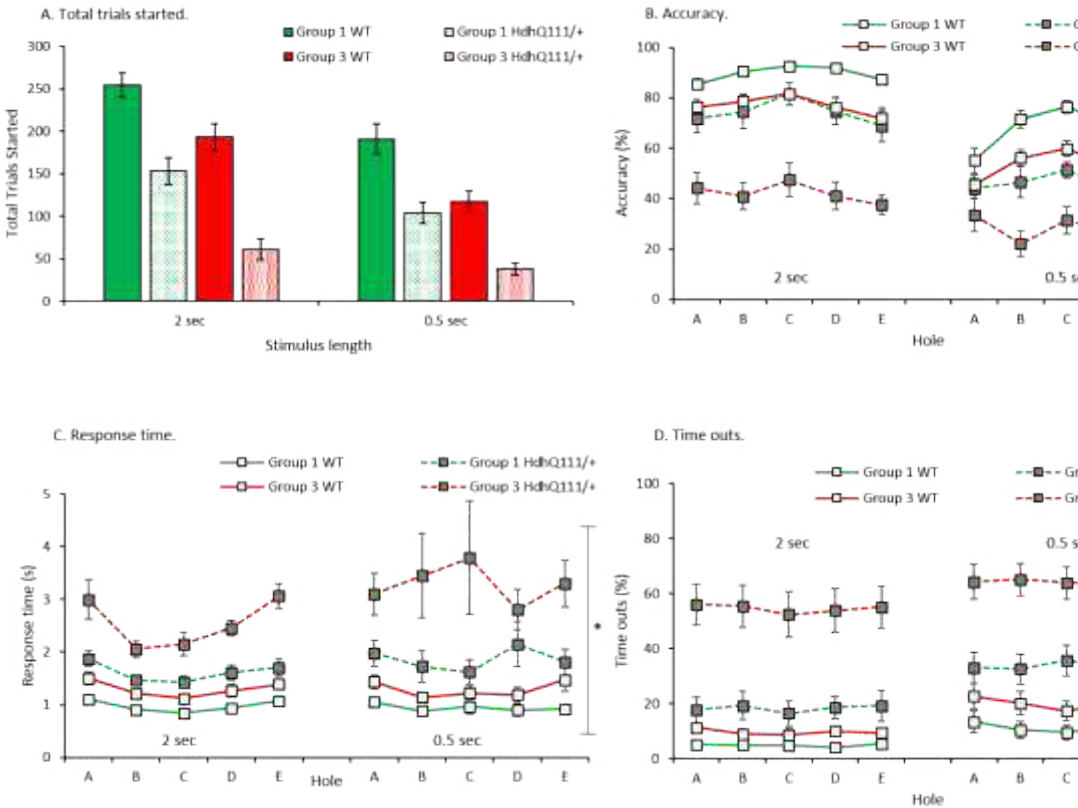

Figure 6.5. Attentional pre-training improves subsequent performance in the 5-CSRTT. A. Total trials started, demonstrated that pre-tr
B. Accuracy in responding was higher in the pre-training groups. **C.** Response time was faster in the pre-trained groups and significantly i
training. **D.** Time outs made as a percentage of total responses were decreased in the group that received pre-training and significantly decr
pre-training. Data represents a total of 27 animals (Group 1 n=14 (7 WT and 7 $Hdh^{Q111/+}$) and Group 3, n=14 (7 WT and 6 $Hdh^{Q111/+}$)) Data a
2 second and 0.5 second stimulus lengths. Only significant interaction effects are shown. Error bars represent ± standard error of the mean. * p

$F_{1,24}$ = 21.98, p<0.001) and fewer trials were initiated at the shorter 0.5 second stimulus length in comparison to the 2 second stimulus length (Stimulus length: $F_{1,24}$ = 40.94, p<0.001), as shown in Figure 6.6A. Animals who had received previous attentional pre-training were significantly more accurate in the 5-CSRTT in comparison to animals who had received comparable pre-training in an non-attentional task (Group: $F_{1,24}$ = 8.84, p<0.01). Although $Hdh^{Q111/+}$ animals were less accurate than wild type animals (Genotype: $F_{1,24}$ = 18.16, p<0.001), no significant interaction was demonstrated (Group x Genotype: $F_{1,24}$ = 1.29, p = n.s., Figure 6.6B).

Despite a trend for animals who had received pre-training on an attentional task to demonstrate faster response times in the 5-CSRTT than animals who had received equivalent pre-training in a non-attentional task (Figure 6.6C), this trend failed to meet the threshold for statistical significance (Group: $F_{1,24}$ = 3.61, p=0.07). Furthermore, animals that received pre-training in an attentional task made significantly fewer time outs in the 5-CSRTT in comparison to animals that had received pre-training in a non-attentional task (Group: $F_{1,24}$ = 13.57, p<0.05). $Hdh^{Q111/+}$ animals made more time outs than wild type animals (Genotype: $F_{1,24}$ = 19.84, p<0.001), despite a trend for $Hdh^{Q111/+}$ animals who had received pre-training in an attentional task to make fewer time outs than $Hdh^{Q111/+}$ animals who had received non-attentional pre-training, this interaction effect failed to meet significance (Group x Genotype: $F_{1,24}$ = 3.73, p=0.065).

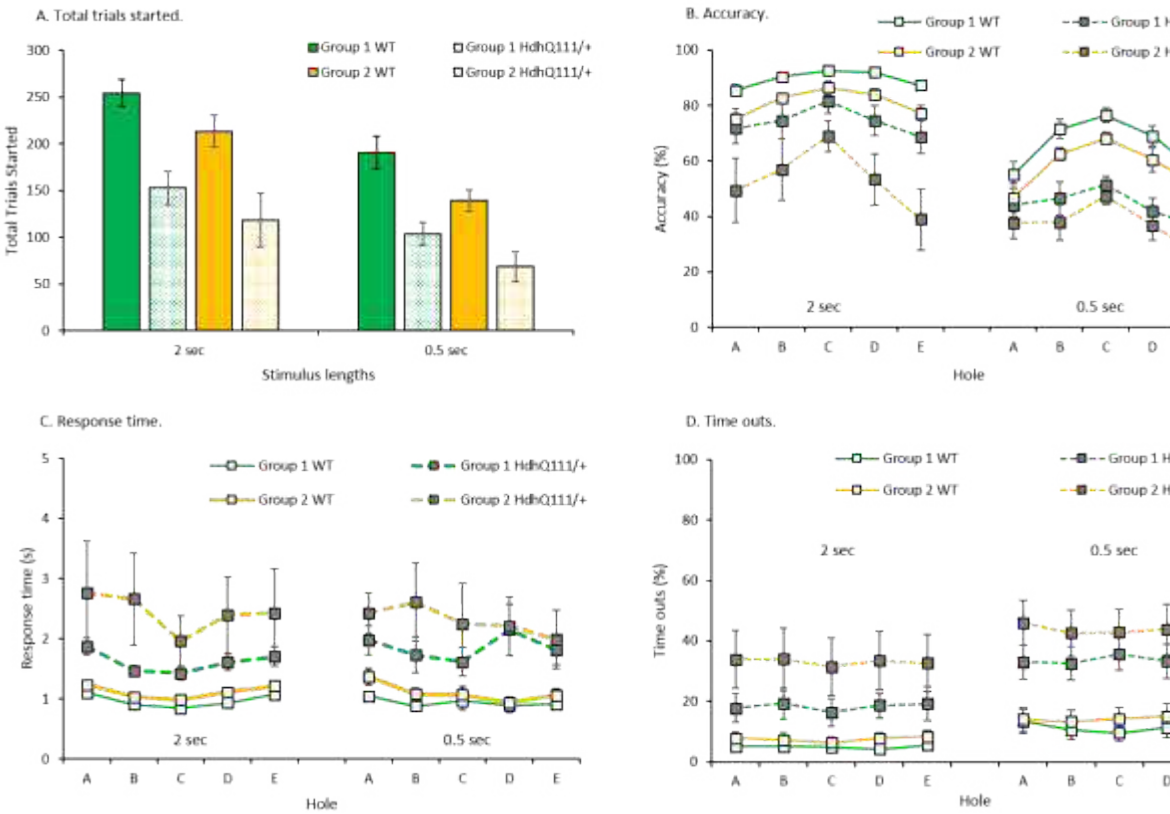

Figure 6.6. Comparison of attentional and non-attentional pre-training on subsequent 5-CSRTT performance. A. Total trials started der stimulus length and by $Hdh^{Q111/+}$ animals in comparison to wild type animals. **B.** Accuracy in responding was improved in animals who had comparison to animals who had received pre-training in a non-attentional task. **C.** Response time was decreased in $Hdh^{Q111/+}$ animals but no effi outs made were significantly. The data represents a total of 28 animals (group 1 contained 14 animals, 7 of which were $Hdh^{Q111/+}$ and grou $Hdh^{Q111/+}$). Error bars represent ± standard error of the mean.

or $Hdh^{Q111/+}$ animals (Group; $F_{2,35} = 1.58$, p = n.s.) (Figure 6.7B). The number of cells that stained positive for Neu-N did not differ in wild type or $Hdh^{Q111/+}$ animals when pre-training was given to any of the testing groups (Group; $F_{2,33} = 0.13$, p = n.s.), as shown in Figure 6.7C. Furthermore, pre-training had no significant effect on the number of DARPP-32 positive cells in either wild type or $Hdh^{Q111/+}$ animals (Group; $F_{2,36} = 1.9o$, p = n.s.), as shown in Figure 6.7D.

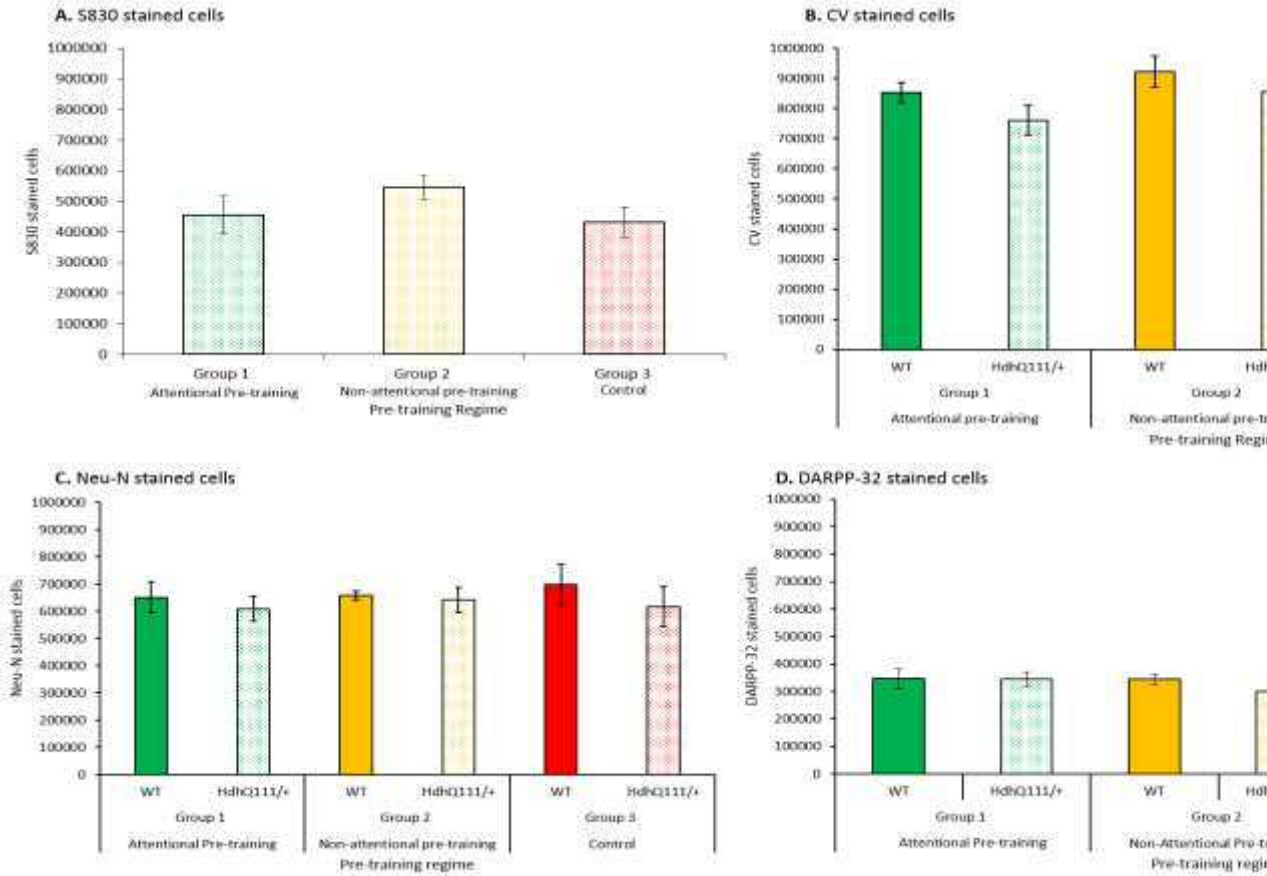

Figure 6.7. Histological results from operant pre-training. A. S830 cells counts demonstrated the number of affected cells was similar in al similar across all testing groups **C.** Neu-N staining was similar across all testing groups **D.** DARPP-32 staining was similar across all testing g striatum. Error bars represent ± standard error of the mean.

6.5. Discussion

When animals were pre-trained on the 5-CSRTT of attention at a young age, they demonstrated significant improvements in the all behavioural measures of the 5-CSRTT when they were tested at an older age, in comparison to naïve animals. These improvements included; initiating a larger number of trials, making more accurate responses, making faster responses and making significantly fewer time outs. Cognitive training has been previously demonstrated to prevent cognitive decline that would otherwise appear as part of the normal aging process in both mice (Gower and Lamberty 1993; Forster *et al.* 1996) and humans (Levy 1994; Hanninen *et al.* 1996; Ball *et al*. 2002; Persson *et al.* 2006; Willis *et al*. 2006; Schönknecht *et al.* 2014).

Environmental enrichment has previously been used to improve cognitive function in HD mice (Nithianantharajah *et al.* 2008; Wood *et al.* 2010; Wood *et al.* 2011) although, specifically using operant cognitive training to improve cognitive function is yet to be explored in HD. Operant pre-training in an attentional task was shown to significantly improve operant performance in $Hdh^{Q111/+}$ animals in the 5-CSRTT, as animals were significantly faster to respond and made fewer time outs, than comparable $Hdh^{Q111/+}$ animals who had not received attentional pre-training and were naïve to the operant boxes. This is the first time that operant cognitive training has been used to show improved cognitive function in HD mice. These findings are supported by patient studies in other neurodegenerative diseases including PD (Sinforiani *et al*. 2004; Sammer *et al*. 2006; París *et al*. 2011; Milman *et al*. 2014) and AD (Hofmann *et al*. 1996; Davis *et al*. 2001; Farina *et al*. 2002; Clare *et al*. 2003; Clare and Woods 2004), which demonstrated that cognitive training improved both motor and cognitive disease symptoms in AD and PD patients. $Hdh^{Q111/+}$ animals were shown to have improved response time when they had received attentional pre-training, which may therefore be reflective of an improvement in motor function. Both $Hdh^{Q111/+}$ and wild type animals that had received attentional pre-training

demonstrated improved attentional ability, therefore these results are important to consider with respect to the HD population, as well as in other neurodegenerative diseases and the aging population in general.

The specific type of pre-training implemented in the cognitive training intervention is also an important factor that was considered here. While pre-training in an attentional task at a young age significantly improved attention at an older age, comparable pre-training in a non-attentional task did not improve attention in the 5-CSRTT at an older age. Therefore, it can be concluded that the attentional pre-training given to animals demonstrated task specific improvements in cognitive function. In future iterations of this study it would be interesting to give attentional pre-training in the 5-CSRTT and then later test animals on a different operant task of attention to probe whether the training provides task specific benefits or more general benefits of executive function which may be translatable to other tasks. In the present study, equivalent pre-training in a non-attentional task demonstrated that animals were able to initiate as many trials and respond as quickly in the 5-CSRTT as animals who has received pre-training in an attentional task. Therefore, although it can be concluded that the attentional pre-training specifically improved attentional performance at a later age, equivalent pre-training also improved other operant behaviours.

Therefore, it can be concluded that pre-training animals at a young age in an operant task will modify subsequent operant behaviours in both wild type and $Hdh^{Q111/+}$ animals. This is a particularly important issue in the behavioural characterisation of mouse lines, such as the characterisation conducted in Chapter 5. Although the type of pre-training given in this experiment was short when compared to the operant testing battery implemented in Chapter 5, clear behavioural differences were noted even after this comparatively short pre-training period. In addition, the pre-training was given at 4 months of age and animals were tested in the operant task at 12 months of age, therefore despite the 8 month gap animals were still able to gain a significant benefit from the

previous cognitive training. Therefore, pre-training or previously testing animals in operant tasks may mask or perhaps improve some of the behavioural deficits observed in the HD.

Furthermore, implementing cognitive training at a young age has the potential to improve HD symptoms, as was demonstrated in the response time performance of $Hdh^{Q111/+}$ animals in the 5-CSRTT. This suggestion is of huge clinical interest as it demonstrates that the HD brain may demonstrate a degree of plasticity, particularly if cognitive training can be implemented at a young age. Moreover, other environmental enrichment activities such as exercise have previously shown benefit in animals models of HD (Hockly *et al.* 2002; Pang *et al.* 2006; Harrison *et al.* 2013) and HD patients (Busse and Rosser 2007; Busse *et al.* 2008b; Khalil *et al.* 2012; Busse *et al.* 2013; Khalil *et al.* 2013). Although such exercise based therapies can be difficult to implement in this patient population and can lead to an increased risk of falls (Busse *et al*. 2009). Therefore, it may be the case that cognitive based intervention therapies which may utilise computer programmes, have a greater degree of acceptance and uptake as a therapeutic intervention this specific patient population in comparison to exercise interventions.

The 5-CSRTT was used in this case to measure attentional function in the $Hdh^{Q111/+}$ mouse model of HD. Although attentional deficits have previously been shown in HD patients (Lawrence *et al*. 1998a; Lawrence *et al*. 1998c), more recently difficulty in sharing attention, often using dual tasks, has been shown to be a more robust measure of determining attentional dysfunction in HD (Sprengelmeyer *et al*. 1995; Claus and Mohr 1996; Lawrence *et al*. 1998b; Murphy *et al*. 2000; Lemiere *et al*. 2004; Finke *et al*. 2006; Delval *et al*. 2008; Thompson *et al*. 2010; Vaportzis *et al.* 2015). Therefore, in future manipulations of this study it may be the case that using a dual task as a measure of attentional dysfunction may produce even clearer and more definitive results.

Finally, immunohistochemical and stereological analyses were conducted to determine any gross neuropathological changes caused as a result of the pre-training regimes. As the pre-training intervention was relatively short, the lack of any significant differences between the numbers of S830 affected cells, cresyl violet, Neu-N or DARPP-32 stained cells between testing groups is unsurprising. However, it may be the case that the neuronal connections underlying the observed behaviour have been strengthened.

The study conducted in this experimental chapter demonstrated that operant pre-training, implemented at a young age, can modify the subsequent operant behaviour of $Hdh^{Q111/+}$ animals. These results are important to consider in the future experimental design of longitudinal behavioural characterisations which may utilise operant behavioural testing. Perhaps of most clinical significance is the finding that operant pre-training can modify and improve future behavioural symptoms in the $Hdh^{Q111/+}$ mouse model of HD. This leads to the exciting possibility cognitive training could be used as a therapeutic intervention for people with HD.

Chapter 7 : The development and utilisation of an operant delayed matching and non-matching to position operant task for mouse models of neurodegenerative diseases.

Disclaimer: the results of this Experimental Chapter have recently been published. Yhnell E, et al. The utilisation of operant delayed matching and non-matching to position for probing cognitive flexibility and working memory in mouse models of Huntington's disease. J Neurosci Methods (2015), http://dx.doi.org/10.1016/j.jneumeth.2015.08.022

7.1. Introduction

In HD, reversal learning deficits and perseveration are particular features of both the human disease (Lawrence *et al*. 1998b; Lawrence *et al*. 1999) and the HD mouse (Lione *et al*. 1999; Trueman *et al*. 2007; Brooks *et al*. 2012e). Furthermore, short term memory problems develop in HD patients comparatively early in the disease progression (Kirkwood *et al.* 2001). Therefore, reversal learning and short term memory deficits in the Hdh^{Q111} mouse model of HD require more investigation to understand if they accurately replicate the symptoms demonstrated in the human disease.

Delayed matching to position (DMTP) and delayed non-matching to position (DNMTP) tasks allow an investigation of both working memory and reversal learning. DMTP and DNMTP tasks performed in rodents, typically utilise a water T-maze apparatus (Beracochea and Jaffard 1995; Gibbs 2002; Johnson *et al.* 2002). However, water T-maze tasks are often time consuming to run, are strongly affected by motor deficits and are open to subjectivity. Furthermore, the swimming component of the task subjects the animal to additional stress.

However, operant versions of the DMTP and DNMTP tasks allow a rapid, automated and highly sensitive way of measuring deficits in working memory, perseverative responding and reversal learning.

The DMTP operant protocol was initially developed in rats utilising Skinner boxes (Dunnett *et al.* 1989). Since the original description, operant DMTP testing has been used in numerous other rat studies (Dunnett *et al.* 1988; Bushnell 1990; Cole *et al.* 1993; Stephens and Cole 1996; Yamada *et al.* 2005). The DMTP and DNMTP tasks were originally developed to explore the effects of lesions and associated drug treatments in rats (Dunnett 1985; Dunnett *et al.* 1989). However, the use of DMTP and DNMTP testing protocols in mouse studies has been comparatively limited (Beracochea and Jaffard 1995; Estapé and Steckler 2001) and DMTP and DNMTP operant protocols are yet to be investigated in HD mice.

The large number of mouse models of HD that now exist means that there is a need to continually develop novel behavioural tasks to better understand, validate and explore the behavioural symptoms that are demonstrated in these mouse models. Therefore, a DMTP and DNMTP operant protocol was designed to test working memory and reversal learning in $Hdh^{Q111/+}$ animals. Initially, the DMTP and DNMTP protocol was performed in standard 9-hole operant boxes. However, the operant boxes were subsequently modified to include an additional 'Skinner-like' operant set up with nose poke responses placed either side of the magazine. Therefore, a comparison between the DMTP and DNMTP protocols conducted in the standard 9-hole and modified 'Skinner like' operant boxes was able to be conducted. Based on the results of this comparison study, the DMTP and DNMTP tasks were conducted in the more efficient 'Skinner-like' operant experimental set up to test working memory and reversal learning in the $Hdh^{Q111/+}$ mouse model of HD.

7.2. Aims.

To create, establish and test an operant delayed matching to position (DMTP) and delayed non-matching to position (DNMTP) task for use in mouse models of neurodegenerative diseases, including HD.

7.3. Materials and Methods.

7.3.1. Animals

The C57BL/6J animals used in the comparison of the DMTP and DNMTP tasks in differing operant apparatus were obtained from Harlan (Oxfordshire, UK) at 8 weeks of age. 14 C57BL/6J male animals were used in testing of the DMTP and DNMTP task in the conventional 9-hole operant apparatus set up and 15 C57BL/6J male animals were used in the testing of the DMTP and DNMTP task in the 'Skinner-like' operant apparatus set up. Mice were, housed in pairs, although some had to be separated and housed singly to prevent fighting.

For the testing of the DMTP and DNMTP tasks in the $Hdh^{Q111/+}$ mouse model, a total of 21 littermate animals were used, 12 $Hdh^{Q111/+}$ (6 female and 6 male) and 9 wild type (5 female and 4 male). Animals were weaned at 3-4 weeks of age and tail tipped for genotyping (Laragen, Inc.). CAG repeat length in $Hdh^{Q111/+}$ animals ranged from 134-145 repeats with an average of 140 repeats. $Hdh^{Q111/+}$ animals began operant testing at 8 months of age.

Operant testing occurred between 08.00 hours and 14.00 hours five days per week. One week prior to the beginning of operant testing animals were habituated to Yazoo© strawberry milkshake in their home cages and gradually water restricted. For the duration of behavioural testing animals were restricted to 3 hours of access to water per day which was given in their home cages between the hours of 14.00 hours and 17.00 hours. Animals were allowed *ad-libitum* access to food throughout behavioural testing and *ad-libitum* access to water from Friday evening to Sunday evenings.

7.3.2. Apparatus

Testing apparatus consisted on 16 operant boxes, as described in section 2.3.1. of Chapter 2. Of particular importance to note, was the modification of the existing 9-hole operant boxes to include a 'Skinner-like' set up in addition to the existing 9-hole set up. The 'Skinner-like' operant set up was located opposite the 9-hole array, two nose poke holes were positioned either side of the reward magazine, as shown in Figure 7.1.

7.3.3. DMTP Training.

The operant training protocols were the same for both the 9-hole set up and the 'Skinner-like' set up. In all cases, initial training in the operant boxes began with the non-contingent delivery of 150µl of Yazoo® strawberry milk into the magazine and illumination of the magazine light. Reward retrieval from the magazine was detected via infrared beam breaks. After successful reward retrieval the magazine light was extinguished. The process was repeated until the 20 minute session time had elapsed. Magazine training was conducted for 1 20 minute session.

After initial magazine training animals were required to learn the nose poke response. The responding locations differed based on the operant apparatus set up, as shown in Figure 7.1. In the 9-hole operant apparatus configuration, animals were required to learn to poke into the central hole of the 9-hole array and receive reward from the magazine located behind the 9-hole array, on the opposite wall, as shown in Figure 7.1A. Whereas, in the Skinner-like operant apparatus configuration, animals were required to learn to poke into a response hole which was located either side of the reward magazine, and then into the reward magazine, located adjacent to the response location, as shown in, Figure 7.1B.

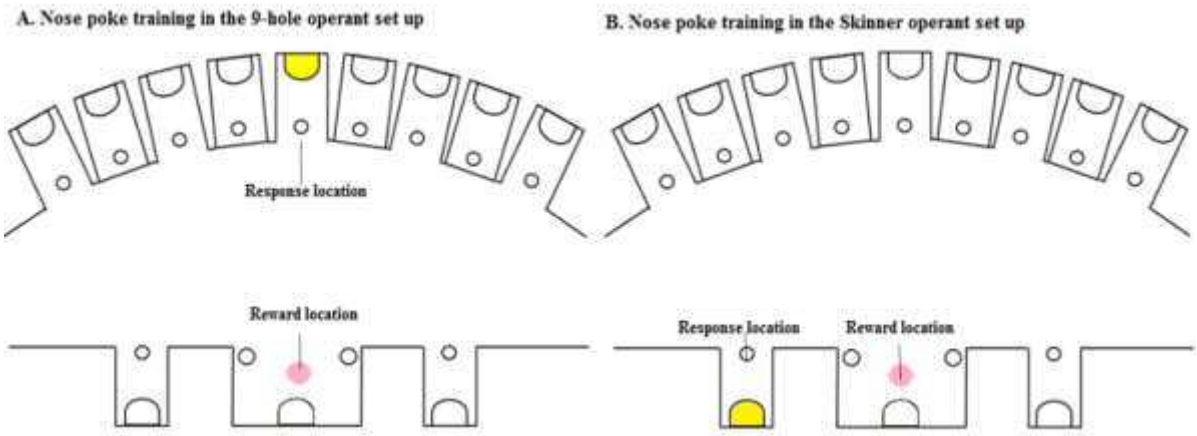

Figure 7.1. Training response locations in different operant set ups.
A. The 9-hole operant apparatus configuration demonstrates a 9-hole array with a reward magazine located behind the 9-hole array **B.** In the 'Skinner-like' operant configuration the response locations are located either side of the reward magazine.

Once animals had successfully learned to the nose poke response, by making more than 100 responses on 3 consecutive days, they were moved on to the next phase of the training schedule. The response locations for each training schedule differed based on the operant configuration used (as shown in Figure 7.2). Training was conducted in phases, one additional poke response was required in each phase of training, as shown in Figure 7.2. In the 9-hole configuration animals were trained on each phase until responding had plateaued, which took a total of 7 days. In the 'Skinner-like' configuration animals were trained on each phase until their responding had plateaued, this took a total of 5 days. Each training phase programme lasted for 30 minutes. At the end of training, animals were moved on to the full DMTP testing schedule, initially without delays, which required a total of three correct responses to obtain a reward.

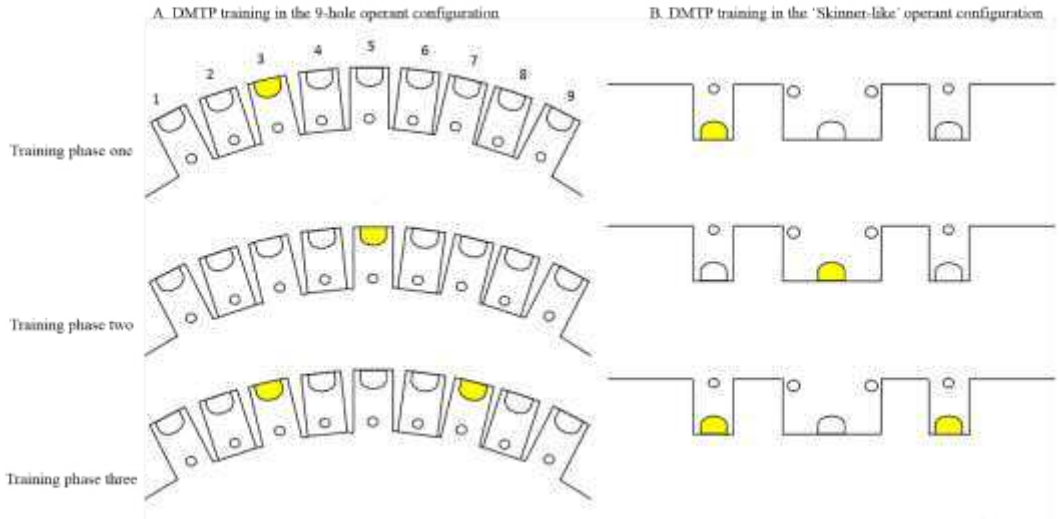

Figure 7.2. Training responses required in different operant set ups for DMTP training.
A. Training in the 9-hole operant configuration required additional nose poke responses to be made in different stages of the operant training regime. The animal was required to poke into either hole 3 or 7 (hole 3 is demonstrated here), then into the central hole, finally the animal was presented with both holes 3 and 7 simultaneously to respond into **B.** Training in the 'Skinner-like' operant set up was the same as in the 9-hole set up but the response locations differed. The animal was required to respond into the hole either to the left or right of the magazine (right is shown here) before poking back into the magazine and being presented with both left and right hole simultaneously.

7.3.4. DMTP and DNMTP Testing.

Upon completion of the training schedule described above, animals were tested on the full DMTP program (which required the same responses as the third training phase shown in Figure 7.2.), initially without delays. Animals were required to make a total of three correct responses before being rewarded. The accuracy in responding to 'matching to the position' was recorded daily for a total of 18 days, until animals had averaged approximately a 90% accuracy. After this, the delay schedule was introduced. In the optimisation experiment of different operant set-ups two different delays lengths of 2 and 10 seconds were utilised. When $Hdh^{Q111/+}$ animals were tested three different delays of 2, 5 and 10 seconds were used. The delays were presented immediately prior to the illumination of both holes. The length of the delay was pseudo-randomly generated by the computer programme for each individual trial. Testing on the DMTP operant programme with delays was conducted for 10 days.

After animals had learned the DMTP task and responded successfully, the delay schedule was introduced for 10 days, they were then tested in the reversal of delayed matching to position (DMTP) task, delayed non-matching to position (DNMTP). As animals had prior training in responding, they were initially introduced to the full DNMTP testing schedule without delays. The testing schedule was the same as the DMTP task, however, in this task, animals were required to make the opposite response to the one that they had previously made to successfully obtain a reward. The total program length was 30 minutes. Animals were tested on the full DNMTP program for 16 days, until they had successfully learnt the task to 90% accuracy. After this, the delay program was introduced, as described above for the DMTP task. The delays occurred prior to the simultaneous illumination of lights, animals were tested on this task for 10 days.

7.3.5. Statistical Analysis.

Statistical analyses were conducted in IBM SPSS Statistics 20 Software for windows. Two or three way analyses of variance (ANOVA) were conducted with daily test session, task reversals, and choice delay interval as repeated measures and genotype and apparatus configurations as between subject factors. Where significance was found *post-hoc* tests with Bonferroni corrections were applied to identify the locus of effects and their interaction(s).

7.4. Results.

7.4.1. Optimisation of the DMTP and DNMTP protocols in the 9-hole and 'Skinner-like' operant box.

For comparison between the conventional 9-hole and 'Skinner-like' configurations of the operant apparatus, response accuracy was used as the principle variable used to determine which was the more effective for learning. The number of trials started was used to provide a supplementary measure of task compliance and motivation within the different configurations. The

sensitivity of the two configurations to detect working memory effects was measured upon the introduction of delays into the testing schedule. These measures were assessed for both the DMTP and DNMTP tasks.

7.4.1.1. DMTP acquisition in the 9-hole and 'Skinner-like' operant box configurations.

All C57BL/6J mice learned to acquire the DMTP task in both apparatus configurations, performing at close to chance (50%) levels at the start of training and approaching 70-80% levels of accuracy after 8 days of training. Nevertheless, the mice trained in the Skinner-like configuration performed at higher levels overall, exhibiting more rapid learning, higher levels of accuracy overall, and completing more trials per session, in comparison to those trained in the conventional 9-hole configuration (Figure 7.3A, Accuracy; $F_{1,27} = 16.93$, $p < 0.001$; Figure 7.3C, Trials initiated; $F_{1,27} = 34.69$, $p < 0.001$).

Upon the introduction of delays into the DMTP testing schedule a significant interaction between delay length and apparatus configuration was found (Figure 7.3E, Delay x Apparatus; $F_{2,54} = 8.64$, $p<0.001$). *Post-hoc* analysis revealed significantly greater response accuracy for the Skinner-like configuration than in the 9-hole configuration specifically at the 2s delay length ($p<0.001$), whereas all mice were approaching chance levels of performance (close to 50%) at the longer 10s delay length.

7.4.1.2. DNMTP reversal learning acquisition in the 9-hole and 'Skinner-like' operant box configurations.

Upon reversal of the DMTP task rule to DNMTP all animals initially performed well below chance, perseverating on the old rule with a 20-30% level of accuracy, but then progressively improved performance over 10 days of training and were able to learn the new rule to 70-80% level of accuracy over 15 days of training (Figure 7.3B). Although it appeared to be a somewhat lower level of

performance asymptote in the Skinner-like configuration, perhaps reflecting the heightened learning of the preceding DMTP task, this difference failed to achieve significance (Figure 7.3B, Accuracy; $F_{1,27} = 4.13$, $p = 0.052$), nor were there any differences in the total number of trials started (Trials; Figure 7.3D, $F_{1,27} = 2.00$, p = n.s.), which was seen in the DMTP version of the task (Figure 7.3C, Trials initiated; $F_{1,27} = 34.69$, $p < 0.001$). Nevertheless, upon the introduction of delays, the Skinner-like configuration revealed a significantly higher level of performance at the intermediate 2s delay (Delay; Figure 7.3F, $F_{2,54} = 11.74$, $p<0.001$).

The data presented (Figure 7.3) in this comparison study of operant apparatus configurations, therefore suggests that the results obtained from the DMTP and DNMTP tasks differ depending on the configuration of the operant apparatus used. Specifically animals learn the DMTP task to a higher level of response accuracy in a comparatively shorter time when the 'Skinner-like' configuration of the operant apparatus was used. Upon introduction of delays into both the DMTP and DNMTP tasks, significant differences in response accuracy suggest that a higher level of performance can be achieved using the 'Skinner-like' apparatus configuration. Thus, although it is equally feasible to use the DMTP and DNMTP tasks in both the conventional 9-hole and 'Skinner-like' configurations of the operant apparatus, animals learned the DMTP task more rapidly and efficiently in the 'Skinner-like' configuration. Therefore, for practical purposes, the 'Skinner-like' configuration of the apparatus is the more efficient to yield robust results and consequently is the version we have selected for testing the DMTP and DNMTP tasks in the $Hdh^{Q111/+}$ mouse model of HD.

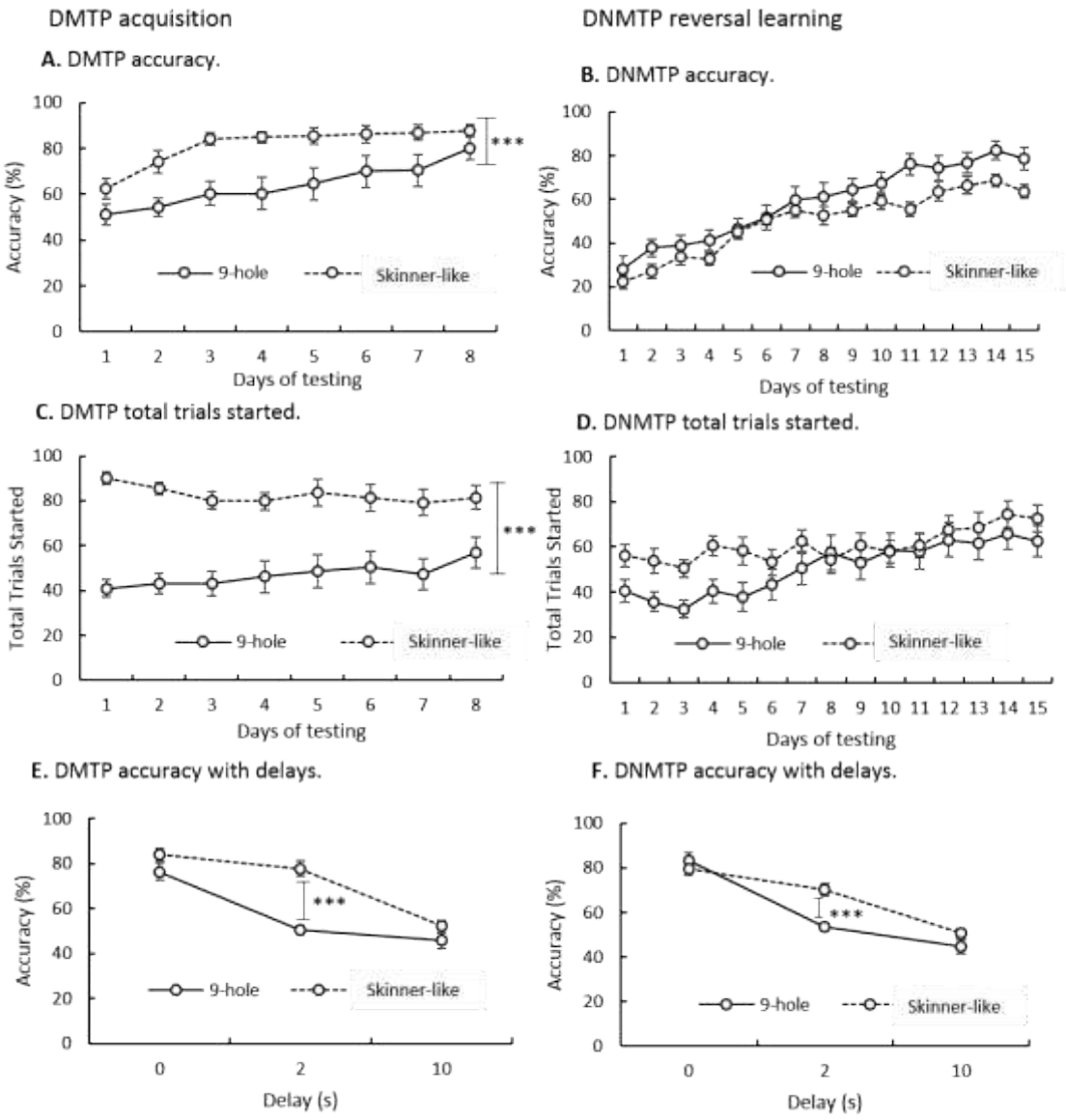

Figure 7.3. A comparison of delayed matching to position (DMTP) (Figures A, C and E) and non-matching to position (DNMTP) tasks (Figures B, D & F) in the 9-hole operant apparatus and Skinner operant apparatus. A. DMTP total trials started indicated significantly more trials were started in the Skinner operant apparatus. **B.** DNMTP total trials started indicated no significant differences between operant apparatus. **C.** DMTP responding accuracy indicated animals were able to reach a higher level of responding in the Skinner operant apparatus. **D.** DNMTP responding accuracy indicated no significant differences between operant apparatus. **E.** DMTP responding accuracy with delays indicated animals were able to reach a higher level of responding in the Skinner operant apparatus, specifically at the 2 second delay length. **F.** DMTP responding accuracy with delays indicated animals were able to reach a higher level of responding in the Skinner operant apparatus, specifically at the 2 second delay length. Error bars indicate ± standard error of the mean. *** $p<0.001$.

7.4.2. DMTP and DNMTP results in $Hdh^{Q111/+}$ animals.

To determine the suitability of the DMTP and DNMTP tasks for use in determining behavioural deficits in the Hdh^{Q111} mouse model of HD, performance was measured for acquisition of the DMTP task and three subsequent reversal phases of the DMTP and DNMTP tasks. Response accuracy was used to determine learning of the task, the total number of trials initiated was used to determine task compliance and the introduction of delays into testing was used to determine working memory performance.

7.4.2.1. Acquisition of the DMTP task reveals significant deficits in learning the DMTP task in the $Hdh^{Q111/+}$ mouse model of HD.

Initial acquisition of the DMTP operant task demonstrated that $Hdh^{Q111/+}$ animals had a significant deficit in acquiring the DMTP task as measured by decreased response accuracy and reduced levels of trial initiations (Figure 7.4A, Accuracy; $F_{1,19} = 5.79$, $p<0.05$, and Figure 7.4B, Trials; $F_{1,19} = 9.41$, $p<0.01$). When delays were introduced $Hdh^{Q111/+}$ animals continued to perform less accurately than wild type controls at all delay lengths (Figure 7.4C, Genotype; $F_{1,19} = 13.53$, $p <0.01$).

7.4.2.2. The DMTP and DMTP tasks reveal significant reversal learning and deficits in the $Hdh^{Q111/+}$ mouse model of HD.

Upon reversal of the initial DMTP task, to DNMTP, then to DMTP and then finally back to DNMTP the $Hdh^{Q111/+}$ animals continued to exhibit marked deficits in response accuracy irrespective of trial conditions (Figure 7.4A, Accuracy; $F_{1,19} = 11.42$, $p<0.01$), suggesting a consistently significant impairment in task acquisition, reversal learning and asymptotic performance in comparison to wild type animals. Similarly the $Hdh^{Q111/+}$ animals consistently exhibited a reduced rate of trial initiation in the reversal tasks (Figure 7.4B, Trials; $F_{1,19} = 12.43$, $p<0.01$), and unsurprisingly, the introduction of the delays

further exacerbated their deficits at all delay lengths in comparison to wild type animals (Figure 7.4C, Delay; $F_{1,19} = 12.83$, $p<0.01$).

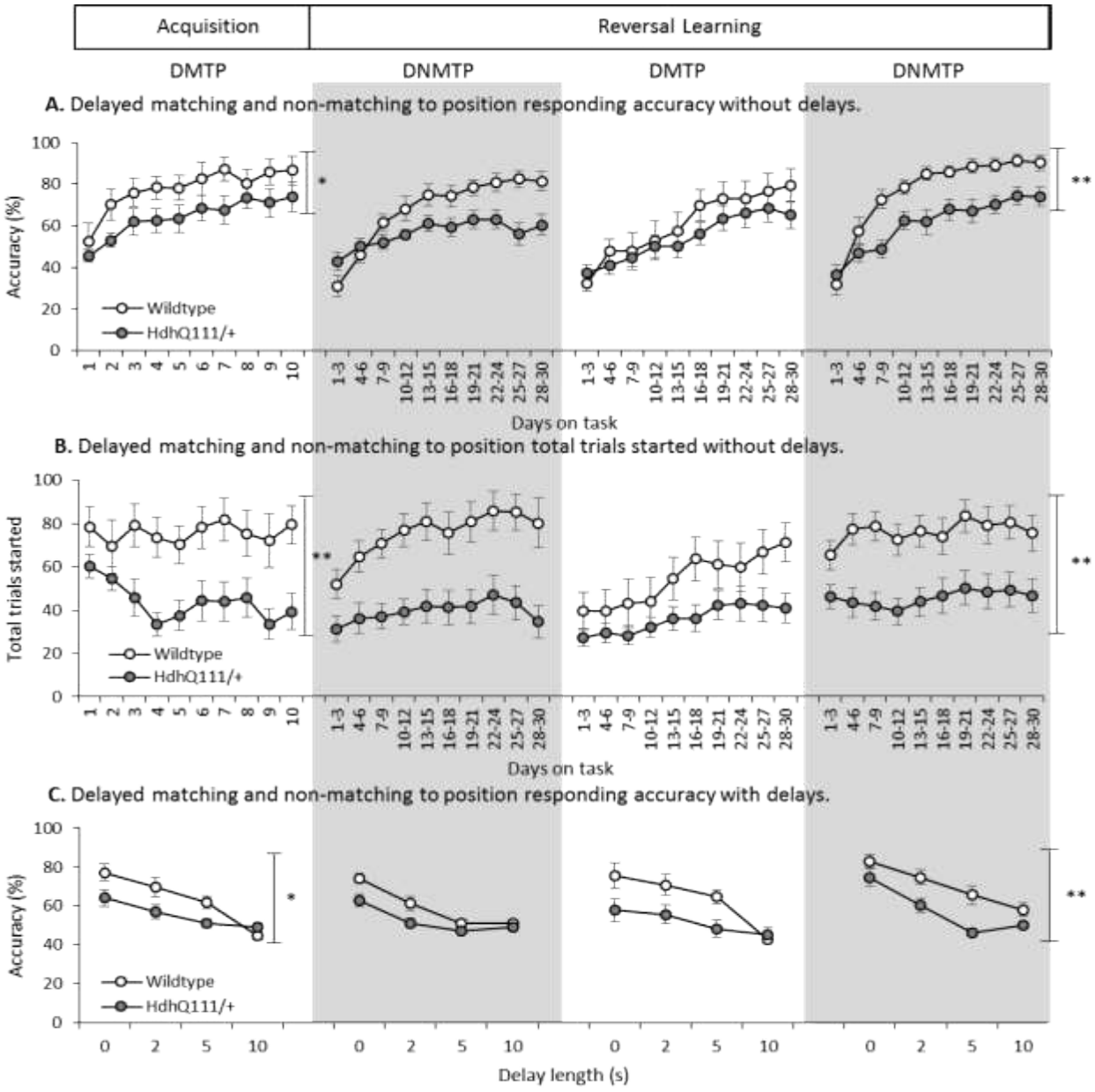

Figure 7.4. Delayed matching to position (DMTP) and non-matching to position (DNMTP) results for Hdh^{Q111} animals. A. DMTP and DNMTP responding accuracy without delays. Hdh$^{Q111/+}$ animals were significantly less accurate in the acquisition phase of the DMTP task. During the three reversal learning tasks Hdh$^{Q111/+}$ animals performed significantly less accurately across all tasks than wild type animals. **B.** DMTP and DNMTP total trials started without delays. Hdh$^{Q111/+}$ animals initiated significantly fewer trials in the acquisition phase of the DMTP task than wild type animals. During the three reversal learning Hdh$^{Q111/+}$ animals initiated significantly fewer trials than wild type animals across all three reversal learning tasks. **C.** DMTP and DNMTP responding accuracy with delays. Upon the introduction of delays into the DMTP and DNMTP tasks in the acquisition phase, Hdh$^{Q111/+}$ animals were significantly less accurate than wild type animals. In the three reversal learning tasks, Hdh$^{Q111/+}$ animals performed significantly less accurately across all delay lengths than wild type animals. For the DMTP and DNMTP tasks n=21 animals, 12 Hdh$^{Q111/+}$ and 9 wild type. Hdh^{Q111} animals began operant testing at 8 months of age. Error bars indicate ± standard error of the mean. * $p<0.05$, ** $p<0.01$.

7.5. Discussion

We have shown that significantly different results are produced when DMTP and DNMTP protocols are performed in different configurations of operant apparatus. Although the DMTP and DNMTP tasks are viable in both conventional 9-hole and 'Skinner-like' operant configurations, acquisition of the DMTP task is significantly faster in the 'Skinner-like' operant configuration. Furthermore, the two configurations have comparatively different levels of sensitivity to detect delay-dependent deficits once delays are introduced into the DMTP and DNMTP testing schedules.

The significant differences demonstrated between the two configurations of the operant apparatus, specifically that the 'Skinner-like' configuration yields more robust results than the conventional 9-hole configuration, provide interesting guides to be considered in the design of operant tasks for cognitive testing in mouse models of neurological diseases. The finding that upon the introduction of delays into the DMTP and DNMTP tasks animals are able to reach a higher accuracy in the 'Skinner-like' operant configuration may be explained by the relative distance between the final response location and the reward location. In the 'Skinner-like' configuration of the DMTP and DNMTP tasks, the reward magazine is located 20mm laterally from the response stimulus light. Whereas, in the conventional 9-hole configuration the reward magazine is located at the opposite side of the operant box, 14cm away from the 9-hole array where the response stimulus lights are located. Therefore the delay between the final response and the collection of the associated reward in the 9-hole configuration of the tasks may weaken the associative strength of the reward and related acquisition of response, resulting in slower learning. This distance also increases the time for each trial to be completed resulting in fewer trials over a set session period (of 30 minutes in the present study). Although the 9-hole operant apparatus configuration offers a greater degree of flexibility and variability in the operant tasks which are able to be performed, for the DMTP and DNMTP

protocols described here, the 'Skinner-like' configuration is more sensitive for performing these particular response choice and execution rules.

Furthermore, in comparison to a previous study conducted in mice which used a traditional Skinner operant box configuration with retractable levers to complete the DMTP and DNMTP tasks (Estapé and Steckler 2001), wild type animals in the study presented here were able to achieve similar levels of accuracy using a 'Skinner-like' configuration of the task which utilised the species-prepotent nose hole pokes to respond. It is not only more economical to modify existing operant boxes in this way to include a 'Skinner-like' configuration, but the nose poke response holes utilised in this study also provide a naturalistic type of response for mice, who may be impaired in their motor function which may affect the ability to press a lever. However, this is at the expense of less salient response options that are provided with retractable levers.

The use of mediating behaviours to gain an advantage in accurately responding in delayed matching tasks has previously been shown in rats (Dunnett 1985; Chudasama and Muir 1997) and pigeons (Blough 1959). In this case animals were not observed during the task and thus we cannot conclude that the use of a mediating behaviour conferred any specific advantage in either of the operant configurations. However, in future iterations of the task mediating behaviours may be overcome by requiring animals to perform a specific behaviour such as nose poking into the reward magazine during the delay to reduce the opportunity of employing a mediating strategy (Dunnett 1985; Bushnell 1990).

When the Hdh^{Q111} mouse model of HD was tested in the 'Skinner-like' configuration on the DMTP and DNMTP tasks, significant deficits were seen in the ability of $Hdh^{Q111/+}$ animals to acquire and perform the DMTP task. Furthermore, when the tasks were reversed, significant deficits were seen in $Hdh^{Q111/+}$ animals in terms of response accuracy and the number of trials initiated. Upon the introduction of delays into both the DMTP and DNMTP tasks, a clear effect of delay was demonstrated in all manipulations of the tasks.

Although, $Hdh^{Q111/+}$ animals were significantly less accurate than wild type animals at all delay lengths including, at the zero delay, thus suggesting a reduced ability to perform the tasks accurately. Nevertheless, we cannot conclude that the Hdh^{Q111} mice exhibit a specific deficit in working memory performance, since they were impaired at all delays including the shortest, at which the memory load is least. Rather the profile of impairments suggests a more executive deficit in learning and performing the choice response rule itself, as well potentially of disturbances in response initiation and motivation.

The reduced ability to acquire, perform and initiate trials in the DMTP and DNMTP tasks may suggest an apathetic phenotype or lack of motivation in $Hdh^{Q111/+}$ mice as suggested by the fewer number of trials performed. Behavioural deficits of this type have previously been seen in people with HD (Rosenblatt and Leroi 2000; Paulsen *et al.* 2001a; Baudic *et al.* 2005), although additional behavioural tests will need to be conducted to determine the specificity of deficits within this domain if this is the case in this mouse model. Alternatively, it may be that the increased number of trials initiated by the wild type animals translates to a practice effect on the performance measure. Therefore, among other variables, future iterations of the task should consider using a set number of trials per session rather than a fixed time duration to overcome this potential confound.

The DMTP and DNMTP reversal learning results demonstrate that wild type animals initially perseverate more on the incorrect response from the previous manipulation as signified by their lower initial baselines on the reversal switch. This was expected as these animals have clearly learnt the previous manipulation of the task and formed a stronger association in learning the rule of the task, than the $Hdh^{Q111/+}$ mice. The $Hdh^{Q111/+}$ animals appear to acquire the task less well, but upon reversal of the task responding accuracy was higher than in wild type animals, this may be due to a general impairment in rule learning of these tasks. Overall the ability of the $Hdh^{Q111/+}$ animals to learn the reversal task was decreased in comparison to wild type animals. The reversal learning deficits

associated with $Hdh^{Q111/+}$ animals in comparison to wild type animals may be reflective of perseverance and behavioural inflexibility which have been previously described in people with HD (Craufurd *et al*. 2001; Hamilton *et al.* 2003; Ho *et al.* 2003; Thompson *et al.* 2014). Therefore, it is possible that $Hdh^{Q111/+}$ animals may be able to perform the task to the same level as wild type animals, although they may take a significantly longer time to do so. Although this suggestion is unlikely as $Hdh^{Q111/+}$ animals seem to reach a plateau in both responding accuracy and the number of trials started over the 30 days of testing on each reversal manipulation.

The DMTP and DNMTP task protocols presented here therefore provide a highly sensitive, robust and reproducible method to test spatial learning and its reversal and short-term memory function in mice. The results demonstrate that this method of behavioural testing provides a valid and robust test of cognition, executive function and working memory for use in murine models of neurodegenerative diseases, including HD. The DMTP and DNMTP operant tests are also suitable to test pharmacological therapeutic interventions in mouse models of HD and are translatable to mouse models of other neurological diseases.

Chapter 8 : General Discussion

The aims of this thesis were to understand the development of the neuropathological and behavioural changes in the $Hdh^{Q111/+}$ mouse model of HD. Consequently, we aimed to use these results to determine how accurately the $Hdh^{Q111/+}$ mouse model replicates the human condition. We further aimed to develop novel tests for use in genetically modified mouse models to probe specific behavioural deficits that are associated with the neurological diseases, including HD.

8.1. Summary of Findings

$Hdh^{Q111/+}$ mice were shown to demonstrate: reduced striatal volume, loss of DARPP-32 staining within the striatum and the progressive formation of NIIs within the striatum. Weight loss was shown to be progressive in $Hdh^{Q111/+}$ animals, from 11 months of age, in relation to wild type controls. Motor dysfunction in balance beam performance was shown in $Hdh^{Q111/+}$ mice from 9 months of age and decreased latency to fall from the rotarod was also demonstrated, although this was not seen to be progressive over time. Operant testing revealed specific deficits including: reduced FR responding, reduced PR responding, reduced 5-CSRTT accuracy, increased 5-CSRTT response time, increased 5-CSRTT timeouts, decreased S1 and S2 accuracy in SILT, increased S1 and S2 response times in SILT. Operant 5-CSRTT training was used successfully as a cognitive training intervention to demonstrate improved attentional and motor performance, as measured by 5-CSRTT accuracy and response times, in $Hdh^{Q111/+}$ animals. Finally, a novel DMTP and DNMTP operant task was developed and tested in $Hdh^{Q111/+}$ mice to reveal significant reversal learning deficits.

8.2. Does the Hdh$^{Q111/+}$ mouse model represent the human condition of HD?

Hdh$^{Q111/+}$ mice were shown to demonstrate specific neuropathological hallmarks which reflect the human condition of HD, including: reduced striatal volume (Vonsattel *et al*. 1985; Aylward *et al*. 2004), loss of DARPP-32 stained MSNs (Deng *et al*. 2004; Starr *et al*. 2008) and the formation of overt NIIs within the striatum (Vonsattel *et al*. 1985; DiFiglia *et al*. 1997; Gutekunst *et al*. 1999; Sieradzan *et al*. 1999) (as shown in Chapter 3).

Significant motor dysfunction was seen in Hdh$^{Q111/+}$ animals in balance beam and rotarod performance (as shown in Chapter 4). However, the motor abnormalities demonstrated were relatively subtle in comparison to the obvious chorea and jerking movements which are observed in the later stages of the human condition (Huntington 1872; Thompson *et al*. 1988; Berardelli *et al.* 1999a). Deficits in the initiation of movement in balance beam, were shown in Hdh$^{Q111/+}$ mice from 9 months of age, this may be reflective of the akinesia (van Vugt *et al*. 1996), which is shown in the human condition. Although, the other more obvious motor dysfunctions such as hypokinesia, bradykinesia (Thompson *et al*. 1988), dystonia, rigidity, tics and tremor (Hefter *et al*. 1987b; Phillips *et al*. 1996; Berardelli *et al*. 1999b; Louis *et al*. 1999) were not evident throughout this behavioural characterisation. The motor abnormalities observed in Hdh$^{Q111/+}$ mice are therefore likely to reflect subtle motor abnormalities which occur early in the human disease, as previously observed in both PREDICT-HD and TRACK-HD studies (Paulsen *et al*. 2008b; Biglan *et al.* 2009; Tabrizi *et al.* 2009; Tabrizi *et al.* 2011; Tabrizi *et al.* 2013).

The cognitive phenotype of the Hdh$^{Q111/+}$ mouse model (as shown in Chapter 5) demonstrated specific cognitive deficits in operant tasks (including; FR, PR, 5-CSRTT and SILT) at 18 months of age. However, these deficits were not typically present until the later stages of the disease progression. Therefore, it could be concluded that these deficits were not present in the absence of motor dysfunction (as shown in Chapter 4) and therefore are not fully representative of

the subtle cognitive deficits that precede profound motor dysfunction in the human condition (Diamond *et al*. 1992; Lawrence *et al.* 1998b; Lawrence *et al.* 1998c; Kirkwood *et al.* 2000; Lemiere *et al.* 2004; Duff *et al.* 2007; Robins Wahlin *et al*. 2007; Paulsen *et al.* 2008b; Tabrizi *et al.* 2009; Tabrizi *et al.* 2011; Tabrizi *et al.* 2013).

There is no rodent test battery currently available that can fully probe all of the symptoms associated with the human condition of HD. However, the experiments conducted in this thesis have revealed that the $Hdh^{Q111/+}$ mouse model replicates some aspects of the human condition in terms of: the neuropathology (reduced striatal volume, reduction of MSNs and NII formation), motor abnormalities (in rotarod and balance beam) and cognitive dysfunctions (FR, PR, 5-CSRTT and SILT) exhibited. Therefore, the $Hdh^{Q111/+}$ mouse model of HD is an accurate representation of the early stages of the human condition of HD.

8.3. How does the $Hdh^{Q111/+}$ mouse model compare to other mouse models of HD?

When mouse models of HD are compared in terms of behaviour and neuropathology, differences in background strain should be carefully considered, as these differences have previously been shown to affect the behavioural and neuropathological phenotype observed (Brooks *et al.* 2004; Lloret *et al*. 2006; Van Raamsdonk *et al*. 2007). However, $Hdh^{Q111/+}$ mice demonstrate a neuropathological and behavioural phenotype which is subtle, certainly in comparison to other transgenic mouse models such as the R6/1 (Mangiarini *et al.* 1996; Hansson *et al.* 1999) and R6/2 models (Mangiarini *et al*. 1996; Morton *et al*. 2000; Meade *et al*. 2002), which demonstrate an extremely rapid and aggressive disease progression. The phenotype of the $Hdh^{Q111/+}$ mouse is subtle and comparatively similar to those previously shown in other knock-in mouse models of HD with similar trinucleotide repeat lengths; such as Hdh^{Q140} (Menalled *et al*. 2003; Hickey *et al*. 2008; Rising *et al*. 2011) and Hdh^{Q150} (Lin *et al*. 2001; Woodman *et al*. 2007; Brooks *et al*. 2012d)

animals. However, zQ175 mice demonstrate an earlier onset of disease progression in both motor dysfunction and neuropathology in comparison to $Hdh^{Q111/+}$ mice (Heikkinen *et al*. 2012; Menalled *et al*. 2012; Smith *et al*. 2014), which can be attributed to the longer CAG trinucleotide repeat length. Furthermore, the nature of the genetic construct in each mouse model is an important consideration in the behavioural and neuropathological results observed. For example, in the knock-in mouse models, Hdh^{Q150} (Lin *et al.* 2001) and zQ175 animals (Menalled *et al*. 2012) contain an inserted construct with an expanded CAG repeat. Whereas, the Hdh^{Q92} and Hdh^{Q111} mouse models contain an insertion which includes an expanded CAG repeat and part of the first intron, which may significantly alter the splicing of the *mHtt* gene and subsequent formation of mHtt protein fragments, particularly small N-terminal fragments, which have previously been shown to be particularly pathogenic (Sathasivam *et al.* 2013). Therefore, the nature of the genetic construct which is inserted into the mouse model is an important consideration in the comparison of different models.

Overall, in comparison to other mouse models of HD, the $Hdh^{Q111/+}$ mouse model reflects a subtle behavioural and neuropathological phenotype which may be representative of the early stages of the human condition.

8.4. Is the $Hdh^{Q111/+}$ mouse model of HD suitable to test therapeutics?

The $Hdh^{Q111/+}$ mouse model has been shown to be an accurate and representative model in recapitulating some of the neuropathological, motor and cognitive symptoms of HD. Therefore, the next step to consider is the use of the $Hdh^{Q111/+}$ mouse model to test therapeutic interventions, with the hope of improving the associated disease symptoms. Thus, different types of therapeutic interventions will be considered for their suitability for use in the $Hdh^{Q111/+}$ mouse model in the light of the neuropathological and behavioural results observed.

The relatively subtle behavioural and neuropathological phenotype observed in the $Hdh^{Q111/+}$ mouse model means that it could be used to trial novel therapeutic

interventions that are targeted to treat early stage HD. However, due to the relatively slow disease progression observed, it may be more appropriate and practical to use a transgenic mouse model, or a knock-in model with a longer trinucleotide repeat length to represent the later stages of the human condition of HD. Although, it should be noted that the nature of the genetic construct in the $Hdh^{Q111/+}$ mouse model and other knock-in mouse models is genetically more similar to the human condition than transgenic mouse models, therefore knock-in mouse models are more genetically representative of the human condition.

The neuropathology of the $Hdh^{Q111/+}$ mouse model of HD demonstrated in Chapter 3 only considered the striatum in stereological quantification of mHtt inclusion pathology. Although, mHtt inclusions were visible in other brain regions, these were not stereologically quantified and did not appear to be widespread until late in the disease progression. Therefore, particularly early within the disease progression of the $Hdh^{Q111/+}$ mouse, the neuropathology is relatively focused to the striatum. Thus, the $Hdh^{Q111/+}$ mouse model may be a suitable mouse model for trialling cell transplantation directly into the striatum as a therapeutic intervention. Although transplantation studies have been used to successfully demonstrate graft induced functional benefit in HD rat lesion studies (Sanberg *et al.* 1986; Isacson *et al.* 1987; Bachoud-Lévi *et al.* 2000; McBride *et al.* 2004; Ryu *et al.* 2004; Vazey *et al.* 2006) the development of similar transplantation protocols in mice has been troublesome. The few transplantation studies in mouse models of HD have generated considerably variable results, which often show only modest functional recovery after transplantation (Dunnett *et al.* 1998; Johann *et al.* 2007). Therefore, transplantation in HD mice requires considerably more investigation before it can be used as an effective treatment option in any mouse model of HD. Furthermore, the topic of cell transplantation, including that of human fetal tissue and stem cells transplantation, has numerous practical, ethical and moral implications which are important to consider before they are used as therapeutic treatment options.

The use of both therapeutic and transplantation interventions has been discussed in relation to the $Hdh^{Q111/+}$ mouse model of HD. However, in Chapter 6, a non-pharmacological intervention of cognitive training was explored and demonstrated positive results which improved both cognitive and motor function in $Hdh^{Q111/+}$ mice. The cognitive training results are supported by previous findings in a transgenic mouse model of HD (Wood *et al*. 2011), although the nature of the cognitive training conducted in this experiment differed, as a novel maze was used. Therefore, the results shown in Chapter 6 demonstrate that the $Hdh^{Q111/+}$ mouse model of HD is suited to exploring the effectiveness of non-pharmacological therapeutic interventions such as cognitive training. Furthermore, other non-pharmacological interventions such as exercise have been shown to improve disease symptoms in both mouse models of HD (Kohl *et al.* 2007; Van Dellen *et al.* 2008; Harrison *et al*. 2013) and in human HD patient studies (Busse and Rosser 2007; Busse *et al*. 2008b; Khalil *et al*. 2012; Busse *et al*. 2013; Khalil *et al*. 2013). Therefore, it may well be the case that non-pharmacological interventions and lifestyle changes such as cognitive training or exercise therapies can be implemented in addition to therapeutic interventions such as transplantation to further improve functional benefit to patients, and the $Hdh^{Q111/+}$ mouse model is a suitable mouse model to test this suggestion.

8.5. Will a single mouse model of HD fully recapitulate disease symptoms?

The single genetic mutation that is the primary cause of HD (MacDonald *et al*. 1993) may suggest that the generation of a single animal model of the disease which accurately reflects the human condition would be relatively simple. Knock-in models of HD such as the $Hdh^{Q111/+}$ mouse model are the most reflective models of the human condition, in that they express the CAG trinucleotide repeat in the appropriate genomic and proteomic context to reflect the human condition. However, due to the comparative life span of human patients and mice, a mouse with the same number of CAG trinucleotide repeats as a human patient will not reflect the neuropathology or behavioural symptoms

of HD. Therefore, the CAG repeat is expanded further in mouse models of HD, in comparison to humans, to equate for the differences in life span. The $Hdh^{Q111/+}$ mouse model appears to reflect a subtle phenotype which models the early symptoms of the disease and is comparable to HD patients who are at the early stages of HD. In order to model the more advanced stages of HD, knock in mouse models with larger CAG repeat lengths, such as zQ175 or Hdh^{Q250} mice, could be used, but they do not appear to produce the degree of behavioural and neuropathological dysfunction which is reflected in human patients. Therefore, it may be the case that knock-in mouse models with comparatively large CAG repeat lengths still do not accurately represent the later stages of the human condition. Thus, in order to model the neuropathology and behaviour of patients in the later stages of the disease it may be more appropriate to use transgenic mouse models of the disease, although they are not as genetically similar to the human condition as knock-in mouse models. Therefore, the animal model chosen to be most representative of the human condition may well depend on which stage of HD the researcher requires to be modelled as well as the mechanism of therapy that they are investigating, for example: neuroprotective, regenerative or symptomatic treatments.

8.6. Theoretical and Practical Considerations

The practical logistics of running longitudinal behavioural studies (such as those in Chapters 4 & 5) can present several problems, including housing and handling large cohorts of mice over extended time periods. Studies of this longitudinal design have considerable financial and ethical implications; thus, the sample size used in the longitudinal experiments presented in Chapter 4 is low (n = 7 $Hdh^{Q111/+}$). Due to the complexity of multifactorial analyses conducted for the behavioural results, some effects, are unlikely to be drawn out due to the small degree of statistical power. This was the case for some findings, in that although a clear main effect of genotype was observed, no interaction between age and genotype was found. Although restricted analyses of the data can be performed, to demonstrate significant genotype differences at specific ages, this does not

reflect the longitudinal nature of the experimental design and cannot be used to determine any progressive behavioural changes over time.

An alternative strategy for conducting experiments assessing longitudinal characteristics would be to utilise small cohorts of mice to provide early, mid and late time points, as well as one cohort of animals which are tested throughout the operant test battery (Brooks *et al*. 2012a; Brooks *et al.* 2012d, c; Brooks *et al.* 2012f; Brooks *et al.* 2012h). Thus, histological samples could be taken progressively throughout the test battery, rather than just at the end as in traditional longitudinal designs. However, this alternative method of performing longitudinal characterisations also has drawbacks, with some animals demonstrating practice or training effects, due to the nature of the experimental design. Although it would be ideal to use separate large cohorts of animals at each experimental time point, the practical issues of generating and testing cohorts of animals in this way make this approach extremely difficult.

The way in which animals are housed can have a significant impact on the behavioural results obtained when characterising mouse models. Social isolation, which may be caused by separating mice that fight while under restriction, can significantly alter behaviour; increasing aggression, anxiety, depression and irritability (Lagerspetz and Lagerspetz 1971; Brain and Benton 1983; Rodgers and Cole 1993; Guo *et al.* 2004; Koike *et al.* 2009; Ma *et al.* 2011). All of these behaviours have previously been demonstrated in the human condition of HD (Burns *et al*. 1990; Craufurd *et al*. 2001; Paulsen *et al*. 2001b; Slaughter *et al*. 2001a; Duff *et al*. 2007; Reedeker *et al*. 2012; van Duijn *et al*. 2013). Thus, housing conditions may impact upon the behavioural results observed and it is important to consider that singly housing animals may increase the prevalence of certain behaviours which are already shown in the human condition of HD.

The nature of the animal housing conditions are also a fundamental consideration in the interpretation of the behavioural results. The animals used in this thesis

were housed with the addition of a single cardboard tube and a chew stick, these items could be considered environmental enrichment, and, indeed, that is the rationale for their use. Environmental enrichments such as the introduction of additional items to the cage, has been shown to slow disease progression in other mouse models of HD (Hockly *et al*. 2002; Glass *et al*. 2004; Spires *et al.* 2004; Lazic *et al.* 2006), but this is yet to be investigated in knock-in mouse models including the $Hdh^{Q111/+}$ mouse model. Although, the housing conditions were kept consistent across all of the studies presented in this thesis, this will not be the case throughout the literature and, therefore, this should be considered when comparing the behavioural results observed in different HD mouse models. Although one would ideally wish to keep environmental enrichment to a minimum in characterisation studies; environmental enrichments such as those used previously (Hockly *et al*. 2002; Glass *et al*. 2004; Spires *et al*. 2004; Lazic *et al*. 2006) could be utilised in the future as a means of therapeutic intervention in the $Hdh^{Q111/+}$ mouse model of HD.

The water restriction regime imposed for the operant testing conducted in Chapters 5, 6 and 7 was required to ensure animals were motivated to respond to similar degrees in the operant boxes for the associated reward. In comparing operant testing results in HD mouse lines, the type of restriction regime imposed is an important experimental factor in the motivation of operant learning and so is an important consideration in the interpretation of the results. While the use of food restriction in operant testing of mice is common (Trueman *et al*. 2007, 2008b; Trueman *et al*. 2009b; Brooks *et al*. 2012h; Trueman *et al*. 2012b); water restriction has been shown to provide more stable motivation and to be more tolerable than food restriction in C57BL/6J mice (Tucci *et al.* 2006), thus water restriction was used in this study to comply with legal requirements and for reasons of experimental consistency. However, it should be noted that the level and implementation of water restriction imposed in operant studies can vary considerably between studies, often depending on the relevant legal requirements under which the study is conducted.

In particular, dietary restriction has been shown to cause epigenetic changes in mice (Lee *et al.* 1999; Chouliaras *et al.* 2011; McKay and Mathers 2011). Food restriction has previously been shown to slow HD disease progression and extend life span in the HD-N171-82Q transgenic mouse model (Duan *et al*. 2003a). Of particular interest is the activation of the histone deacetylase (HDAC) Sirt-1, which causes longevity and increased life span in mice (Wakeling *et al.* 2009) and has been shown to be neuroprotective in the N171-82Q mouse model of HD (Jiang *et al.* 2012). Furthermore, dietary restriction has also been shown to have more general neuroprotective effects in mice; increasing levels of endogenous BDNF (Duan *et al.* 2001a; Duan *et al.* 2001b; Duan *et al.* 2003b) and enhancing neurogenesis (Mattson 2000; Lee *et al.* 2002; Bondolfi *et al.* 2004). Although these suggestions are yet to be explored specifically in HD mice. Therefore, it may be the case that in the same way that food restriction may alter HD disease progression, the water restriction imposed in this thesis, may well affect disease progression in the $Hdh^{Q111/+}$ mouse model of HD. Although, these suggestions were not specifically investigated in this thesis and therefore require further experimental exploration.

8.7. Future Directions

In subsequent longitudinal tests (such as those conducted in Chapters 4 & 5) it may be necessary to increase the sample size of animals used in the study, due to the complex multifactorial analyses conducted. However, this will have considerable financial and ethical implications.

Furthermore, imaging studies could be included in future longitudinal studies to further explore the gross neuroanatomical changes that underlie the progression of HD in the $Hdh^{Q111/+}$ mouse model. If imaging studies of this sort were to be undertaken, they could be directly compared to other human HD imaging studies (Thieben *et al*. 2002; Rosas *et al.* 2003; Reading *et al.* 2005; Henley *et al.* 2006; Rosas *et al.* 2008; Henley *et al.* 2009; Tabrizi *et al.* 2009; Hobbs *et al.* 2010; Tabrizi *et al.* 2011), and this would add further clarity to how well the $Hdh^{Q111/+}$ mouse model reflects the human condition. Although, practical difficulties in

scanning small animals and fundamental differences in the neuroanatomy of human and rodent brains should be considered (Flood and Coleman 1988; Parent 1990) in the comparative use of imaging studies.

It became evident that the cleanliness of the animal unit in which the mice were housed, gradually decreased throughout behavioural testing, and animals were increasingly exposed to pathogens including: mouse hepatitis virus, mouse parvovirus, mouse norovirus, mouse adenovirus, intestinal helminths and helicobacter. While we cannot be certain of the exact impact that this had on the behavioural results observed, neuro-inflammation has already been shown to occur in neurodegenerative diseases, including HD (Dalrymple *et al.* 2007; Silvestroni *et al.* 2009; Staal and Möller 2014) and the additional presence of these pathogens can only add to the disease burden which is observed in the $Hdh^{Q111/+}$ mouse model. Therefore, access to an animal holding unit with a comparatively higher health status would be preferable in future studies.

The results presented in Chapter 6, investigating cognitive training as a therapeutic intervention in HD are particularly exciting and further studies could be undertaken based on the results obtained. The demonstration of practice or training effects, due to repeated testing in the longitudinal experimental design has previously been studied in mice (McIlwain *et al.* 2001). In this study significant differences in behavioural phenotypes were demonstrated in rotarod and open field performance in wild type mice who had undergone a motor test battery in comparison to naïve mice. The results of this previous study are therefore directly translatable to the results observed here in Chapter 4, where animals were repeatedly tested in a test battery exploring motor dysfunction over time. Therefore it may be the case that repeatedly testing $Hdh^{Q111/+}$ animals in motor tasks alters the behavioural phenotype observed. Moreover, exercise, which may be considered a form of motor training, has been used previously to improve behavioural symptoms in mouse models of HD, including; the R6/1 (Pang *et al*. 2006; Harrison *et al*. 2013) and R6/2 mouse models (Hockly *et al.* 2002). However, one study in N171-82Q animals showed that exercise may in

fact accelerate the onset of motor symptoms, as measured by rotarod performance (Potter *et al*. 2010). Exercise therapies are yet to be investigated specifically in knock-in mouse models of HD, including the Hdh^{Q111} model. Perhaps most importantly, these observations have significant implications for the HD patient population. It may be the case that HD patients who are particularly active develop motor dysfunctions at later time points in the disease progression than those who are less active. These suggestions are also important to consider in terms of therapeutic interventions, as it has been shown that physical training can improve HD related motor symptoms (Busse and Rosser 2007; Busse *et al*. 2008b; Khalil *et al*. 2012; Khalil *et al*. 2013). Thus, motor training in the $Hdh^{Q111/+}$ mouse model is a research area of future interest.

The results presented in Chapter 6 demonstrate a clear difference in the operant performance of $Hdh^{Q111/+}$ mice who have received previous cognitive training in either an attentional or non-attentional task in comparison to naïve animals. Therefore, completing an operant test battery at a young age affects subsequent operant performance at an older age, which may well mask cognitive deficits that would have otherwise developed if animals were naïve to the task. Although further experiments will need to be conducted to validate the suggestion that cognitive training impacts upon subsequent testing both in other mouse models of HD and in other operant tasks, the results presented in Chapter 6 would suggest that this is the case. Furthermore, in translating this work into the HD patient population, it may be important to consider that patients who are practiced in a particular cognitive skill may develop cognitive dysfunctions later in their disease progression. Cognitive training may therefore prove beneficial in terms of a possible therapeutic intervention. Indeed, cognitive training programmes have been used in other neurodegenerative diseases, such as Alzheimer's disease (Hofmann *et al*. 1996; Davis *et al.* 2001; Farina *et al.* 2002; Clare *et al.* 2003; Clare and Woods 2004) and Parkinson's disease (Sinforiani *et al.* 2004; Sammer *et al.* 2006; París *et al.* 2011; Milman *et al.* 2014) where improvements in both cognitive and motor disease symptoms have been shown.

Therefore, the cognitive training results obtained in Chapter 6 combined with patient studies in other neurological diseases suggest that a cognitive training intervention could offer a viable non-pharmacological therapeutic intervention in the HD patient population, which would be an important area of future translational research.

8.8. Concluding Remarks

The research undertaken in this thesis characterised the neuropathological, motor and cognitive phenotype of the $Hdh^{Q111/+}$ mouse model of HD. Further research was also undertaken to explore a non-pharmacological cognitive training intervention, and the development of a novel operant task. As more mouse models of HD become available; continued research will be required to successfully understand the associated phenotype and, therefore, the advantages and disadvantages of using each mouse model. Furthermore, clinical studies which allow us to understand HD as a neurological condition, which will increasingly provide feedback into the development of novel behavioural tasks, histological stains and molecular techniques which examine these specific functions in animal models. Nevertheless, the findings presented in this Thesis are of vital importance in further understanding that the $Hdh^{Q111/+}$ mouse model is reflective of the early stages of the human condition of HD. Pre-clinical studies such as those described here are of vital importance to HD research. However, the future translation of this pre-clinical work into a clinical setting, should always be an important consideration in the undertaking of any pre-clinical research.

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Appendix

Solutions

ABC solution
5µl A (DAKO)
5µl B (DAKO)
1ml 1% serum in 1X TBS

Acid Alcohol
5ml Glacial Acetic Acid
200ml 95% IMS

Antifreeze solution
5.45g Di-sodium hydrogen orthophosphate
1.57g Sodium di-hydrogen orthophosphate
400ml Distilled water
300ml Ethylene glycol
300ml Glycerol
pH 7.3

Cresyl violet
7g Cresyl violet acetate (Sigma, C5052)
5g Sodium acetate (anhydrous)
1000ml Distilled water
pH 3.5

Paraformaldehyde solution (4%)
90g Di-sodium hydrogen phosphate
45g Sodium chloride
200g Paraformaldehyde (Fisher Scientific)
pH 7.3
Made up to 5L with Distilled water

Pre-wash (0.1M PBS)
18g Di-sodium hydrogen phosphate (dihydrate)
9g Sodium chloride
pH 7.3
Made up to 1L with Distilled water

Quench solution
10ml Methanol (Fisher Scientific)
10ml Hydrogen peroxide (VWR Prolabo BHD)
80ml Distilled water

TNS
6g Trisma base (Sigma)
1000ml Distilled water
pH 7.4

TRIS buffered saline (concentrated stock solution 4x TBS)
48g TRIS base (Sigma)
36g Sodium chloride (Sigma)
1000ml Distilled water
pH 7.3

TRIS buffered saline (working solution 1x TBS)
1: 4 dilution of the concentrated stock solution (above) with distilled water
pH 7.3

TXTBS
250ml 1xTBS
500µl Triton X – 100 (Sigma)
pH 7.4

Sucrose solution (25%)
25g Sucrose (Fisher Scientific)
1000ml Distilled water
pH 7.3