Developing and evaluating behavioural tasks to assess basal ganglia function

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

Susanne Clinch

Supervisors:

Dr Mariah Lelos
Professor Monica Busse
Professor Anne Rosser

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Specimen layout for Declaration/Statements page to be included in a thesis.

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This thesis is dedicated to my dad, Alan Clinch.

You may be out of sight but you are never out of mind.

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….I am one lucky girl

Summary

Huntington's disease (HD) is a primary example of a basal ganglia disorder, from which, medium spiny neurons in the striatum degenerate. As this causes a breakdown in basal ganglia cortico-thalamic circuitry, this leads to a range of symptoms including motor, cognitive and behavioural deficits. One therapeutic option is to replace medium spiny neurons with precursor striatal cells and reconnect the lost circuitry. However, the lack of performance based functional outcome measures for people with HD have made it difficult to assess how the graft affects standards of daily living. Although neuroimaging techniques can be used to quantify the morphological and molecular effects that the intervention has on that brain region, and associated circuitry, an important question still remains, namely whether there has been any effect on functional ability. Using assessments that have high ecological validity, such as dual tasks could be a valuable measure, especially as previous studies suggest that the striatum is required for optimal performance in such tasks. Therefore the focus of this thesis was to design, develop and assess performance based functional tasks that involve the neurocircuity affected in HD; namely the basal ganglia.

The aim of the study in Chapter 2 was to select, develop and evaluate motor-cognitive dual task paradigms for use in people with HD. The findings revealed that the *Step and Stroop* which targeted lower limb function, best distinguished disease stage in HD compared to the other lower limb assessments tested. During this experiment, it became apparent that upper limb assessments for people with HD were particularly limited. Therefore, in Part 2, a new upper limb dual task assessment was developed and called the *Clinch token transfer test* (C3t). The findings revealed that this was sensitive to disease stage and could provide a useful outcome measure for people with HD in the future.

To take the findings from Chapter 2 further, a new, standardised C3t was developed. This version was evaluated, optimised, and then validated in a large group of

people gene positive for HD, and in heathy controls. The findings revealed that the C3t significantly correlated with all the Unified Huntington Disease Rating Scale measures, successfully distinguished between all disease stages, and revealed that the performance in this task was also sensitive to the subtle disease symptoms in the early stages of HD.

As the Stroop task is commonly used in people with HD, the aim of Chapter 3 was to use immediate early gene expression to identify if the striatum was activated during a rodent analogue of the Stroop task. The findings revealed what could be the first in a series of experiments in that, striatal activation significantly correlated with performance in the congruent and the incongruent versions of this test when compared to cage controls.

The findings presented in this thesis support that dual task assessments could have an important role in assessing *function* in HD, which could translate to performance in tasks that affect the standards of daily living. Importantly, as different dual tasks can result in different levels of dual task interference, this suggests that practice effects could affect how sensitive some dual task paradigms are over others. In addition, selecting outcome measures that are specific to the regions affected in HD, in both clinic and in pre-clinical models, will permit sensitive tracking of neurodegeneration, and could also be used to assess the outcomes of therapies that target this specific neural region.

Abbreviations

ACC	Anterior cingulate cortex
ANOVA	Analysis of variance
BAC	Bacterial artificial chromosome
Bl	Baseline
BOLD	Blood-oxygen-level dependent
C3t	Clinch token transfer test
CAPIT-HD	Core Assessment Program for Intracerebral Transplantation
CoV	Coefficient of variance
DAB	3,3'-Diaminobenzidine
DG	Dentate gyrus
DLPFC	Dorsolateral prefrontal cortex
DLS	Dorsolateral striatum
DMS	Dorsomedial striatum
DoDLS	Duration of double limb support
DPX	di-n-butyl phthalate in xylene
DT	Dual task
DTC	Dual task cost
E	Embryonic day
fMRI	functional Magnetic resonance imaging
fNIRS	near infrared spectroscopy
GABA	Gamma-aminobutyric acid
GE	Ganglionic eminence
HD	Huntington's disease
IEG	Immediate early gene
IL	Infralimbic cortex
LL-FDI	Late Life Functional Disability Instrument
LVF	Letter Verbal Fluency
MBT	Moneybox test
mHtt	mutant Huntingtin
MSN	Medium spiny neuron
NINDS	Neurological Disorder and Stroke
n.s.	Not significant
NMDA	N-Methyl-D-Aspartic acid
PBA	Problem Behaviour assessment
PET	Positron emission tomography
Pre-HD	Pre-manifest
PrL	Prefrontal cortex
rRCT	rodent Response conflict task

RSC	Retrosplenial cortex (a = granular; b=dysgranular)
S	(disease) Stage
SDMT	Symbol Digit Modality test
SEM	Standard error of the mean
SF-12	Short form-12
TFC	(UHDRS) Total Functional Capacity
TH	Tyrosine hydroxylase
TMS	(UHDRS) Total Motor Score
TUG	Timed Up and Go
UHDRS	Unified Huntington's Disease Rating Scale
VS	Ventral striatum
WGE	Whole ganglionic eminence

Contents

Chapter 1

General Introduction

Introduction Summary

The basal ganglia consists of a group of nuclei in the midbrain. The striatum is the largest nucleus of the basal ganglia and receives input from virtually all areas of the cortex (Braunlich and Seger, 2013). This forms a highly organised cortico-basal ganglia circuitry, which is responsible for motor, cognitive and emotionally based behaviours. In Huntington's disease (HD), degeneration of medium spiny neurons (MSNs), the most abundant cell in the striatum, are primarily affected, meaning that the striatum takes a major part of the pathological burden. This leads to a break in basal ganglia circuitry and results in a triad of motor, cognitive and psychiatric symptoms. There is currently no cure for HD. However, there are a number of therapeutic strategies in pre-clinical assessment and very early clinical trials that have the potential to alter the course of the disease. For example, the relatively focal neuronal loss makes cell replacement therapy a promising therapeutic option.

Cell transplantation is used to replace the MSNs lost in HD with another healthy source, typically striatal precursor neurons from foetal tissue. Proof of concept trials in animal models and in people with HD have shown that transplantation using foetal striatal cells can alleviate and/or stabilise some motor and cognitive symptoms associated with HD (Bachoud-Lévi et al., 2000, 2006; Brasted et al., 2000). Importantly, pre-clinical studies have revealed rehabilitative training post transplantation is necessary for optimal graft functionality (Mayer et al., 1992; Brasted et al., 2000; Döbrössy and Dunnett, 2005; Döbrössy et al., 2010). However, such training has not yet been implemented in patients with HD, following cell transplantation (Cisbani and Cicchetti, 2014), and may contribute to the limited clinical improvements.

A further challenge is the lack of translational and functional outcome measures that specifically target the neurocircuitry affected in this population. Such outcome measures are necessary to ensure an optimal cohort is selected for cell transplantation trials, and to detect change over time, whilst also aiding the development of rehabilitative strategies to implement post transplantation.

Current performance based functional outcome measures for HD are limited. Many of these are rated on an ordinal scale, which can result in observer bias. This also limits the sensitivity when measuring change in disease symptoms over time. Performance based functional outcome measures provide a fundamental link for the researcher/clinician between performance in assessments at clinic and how these relate to tasks at home. Dual tasks are a primary example of an activity performed on a daily basis, which involves performing two tasks simultaneously. This is a reported deficit in people with HD. An explanation for this is that this population have limited attentional capacity, meaning they have limited resources that can be allocated to more than one task. Previous studies suggest that the striatum might be implicated in dual task performance, by prioritising certain behaviours and automating others (Yogev-Seligmann, Hausdorff and Giladi, 2008; Ashby, Turner and Horvitz, 2010; Kim and Hikosaka, 2015). This suggests that dual tasks could provide a useful performance based functional outcome measure for people with HD. The possibilities for such tacks includes, tracking disease progression in HD, a functional outcome measure following interventions such as neural transplantation, and for moulding the development of rehabilitative strategies to help reconstruct basal ganglia circuitry post transplantation.

There are numerous avenues for exploration in the development of therapeutics for HD; the focus in this thesis is cell transplantation, and so this introduction is divided into four parts:

- *Part 1: The Basal ganglia and Huntington's disease*
- *Part 2: Cell transplantation*
- *Part 3: Outcome measures used in Huntington's disease*
- *Part 4: Outcome measures used to assess cell transplantation in Huntington's disease*

1.1 Part 1: The Basal Ganglia and Huntington's disease

1.1.1 The Basal ganglia

The basal ganglia consists of a group of nuclei embedded in the midbrain, which includes the striatum (caudate and putamen in primates), globus pallidus (externa and interna), the subthalamic nucleus and the substantia nigra (pars reticula and pars compacta (Figure 1). The function of the basal ganglia was originally based on clinical observations that showed lesions to the putamen, globus pallidus and subthalamic nucleus resulted in Parkinsonian motor disturbances and dystonia (Motility, 1925). Thus, many early researchers proposed the function of the cortico-basal ganglia relationship was primarily for motor control (reviewed in: Braunlich and Seger, 2013). However, additional studies revealed that lesions to regions of the basal ganglia resulted in many of the same cognitive deficits as those with damage to the frontal regions of the brain (Leisman, Braun-Benjamin and Melillo, 2014). It is now known that the basal ganglia forms both closed loop and open loop circuitry that involves several brain regions, including the cerebral cortex, the thalamus and the cerebellum (Hélie, Ell and Ashby, 2015).

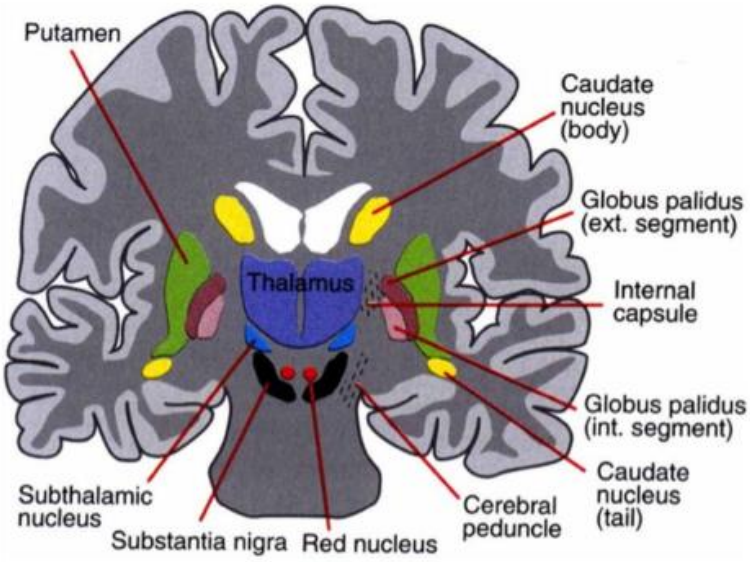


Figure 1: The basal ganglia nuclei in primates. In the rodent brain, the caudate and putamen are contiguous and referred to as the striatum. This figure was reproduced from: (Leisman, Braun-Benjamin and Melillo, 2014).

One of the primary roles of the basal ganglia is to facilitate and inhibit patterns of cortical activity (Braunlich and Seger, 2013; Hélie, Ell and Ashby, 2015), which is done through a cortico-basal ganglia-thalamo cortical loop. Within this circuitry, five segregated but partially overlapping parallel loops have been identified, where the basal ganglia receives inputs from separate cortical regions including the prefrontal, cingulate cortices, orbitofrontal, the supplementary motor area and the frontal eye fields (Leisman, Braun-Benjamin and Melillo, 2014). The loops involving these regions have been supported by studies using retrogradely transported virus particles (Kelly and Strick, 2004) and behaviourally are associated with motor, associative (cognitive), and limbic (emotional) domains (Alexander, DeLong and Strick, 1986). As a result, a break in the basal ganglia circuitry can cause widespread symptoms, including dysregulation of mood, cognition and motor function.

The basal ganglia has evolved over time and consists of the paleostriatum (globus pallidus), and the neostriatum (known as the caudate and the putamen in primates). In rodents the caudate and putamen are contiguous and instead referred to as the dorsomedial and the dorsolateral striatum (Braunlich and Seger, 2013). In primates, the ventral striatum consists of the ventral portions of the caudate and putamen, whereas in rodents, this consists largely of the nucleus accumbens. For the entirety of this thesis, the caudate and putamen will be collectively referred to and specified as the dorso medial/lateral or ventral striatum.

The striatum is the largest nucleus of the basal ganglia and receives topographically organized fibres from almost the entire neocortex (Alexander, DeLong and Strick, 1986). The organisation of the striatum can be divided into distinct compartments; the matrisomes and the striosomes, also known as the patch and matrix (Reinius *et al.*, 2015). These can be defined by the neurochemical make-up and connections (Huot and Parent, 2007), where the matrisome receives input primarily from associate and sensorimotor cortex, whereas the striosome receives input from the limbic regions (Reinius *et al.*, 2015).

The striatum consists of predominantly one neuronal type, named medium spiny neurons (MSNs). MSNs are inhibitory and use γ-aminobutyric acid (GABA) as their neurotransmitters, and in rodents, consist of approximately 95-98% of the neuronal population (Dubé, Smith and Bolam, 1988; Reiner *et al.*, 1988; Kravitz, 2009). The remaining striatal makeup are interneurons, which can be classified based on their morphology, behaviour and histological properties. These receive both glutamatergic and dopaminergic inputs and consist of cholinergic interneurons and three different types of aspiny GABAergic interneurons (Huot and Parent, 2007).

1.1.1.1 The direct and indirect pathways of the basal ganglia

The direct, indirect and hyperdirect pathways form three cortical routes which are primarily responsible for forming the motor circuit, of which the striatum is integral. These pathways exert opposing influences on the excitation of the thalamus to convert information to control movement into a highly organised motor program (Figure 2). Striatal MSNs form two populations which define the direct and indirect pathways, which are dependent on the dopaminergic receptors they possess (Galvan *et al.*, 2012), and their efferent projections to either the globus pallidus interna or externa (Albin, Young and Penney, 1989; Delong, 1990). Output projections from the striatum to the globus pallidus interna/substantia nigra reticula represent the direct, monosynaptic pathway and express D1 dopamine receptors, substance P and dynorphin (Galvan *et al.*, 2012). Activation of this pathway is inhibitory which lowers the threshold required to excite the thalamus, which leads to hyperstimulation and movement activation. MSNs that form the indirect pathway possess D2 receptors and encephalin. In this pathway, the globus pallidus externa and the subthalamic nucleus inhibits striatal output to the globus pallidus interna and substantia nigra pars reticula, which increases the threshold required to excite the thalamus, leading to movement inhibition. The third pathway is the hyperdirect pathway. These projections are excitatory and bypass the striatum, projecting straight to the subthalamic nucleus, and then the globus pallidus interna and substantia nigra pars reticula. This lowers the excitatory outputs from the thalamus and leads to movement activation. As the hyperdirect pathway has a greater inhibitory effect on the cortex than

both the direct and indirect pathways, this route may play a role movement initiation (Nambu, Tokuno and Takada, 2002).

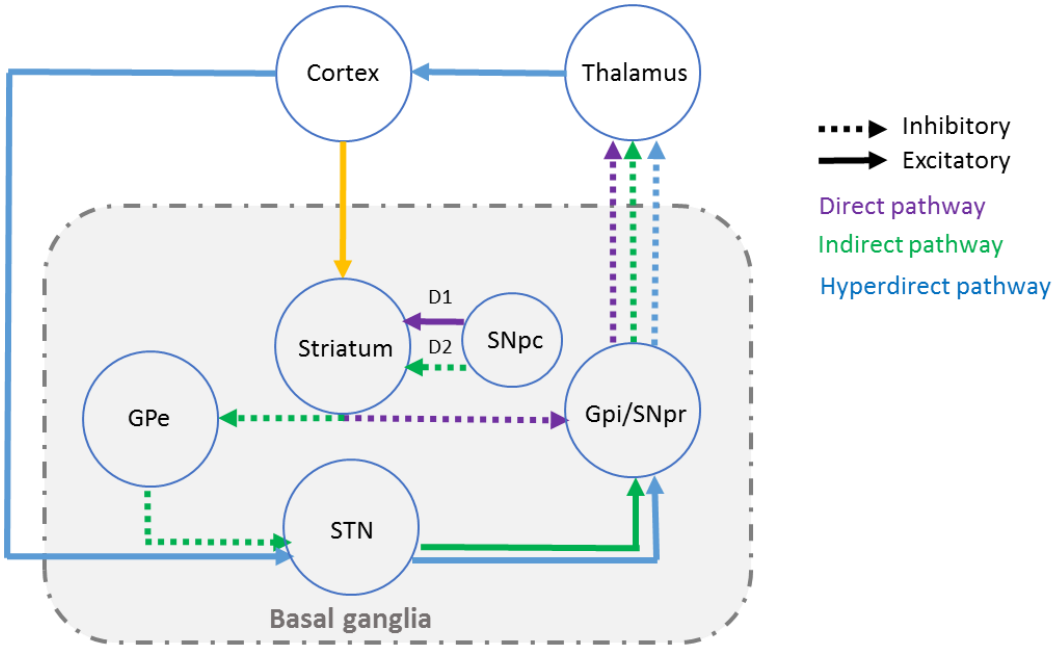


Figure 2: The direct, indirect and hyperdirect pathways are the three major pathways that involve the basal ganglia circuitry (basal ganglia nuclei inside the grey box). The cortex projects afferents to the striatum which are excitatory (indicated in the diagram by the bold yellow line). The SNpc releases dopamine which either binds to the D1 receptors and excite the neurons of the direct pathway (purple), or the D2 receptors which inhibit the neurons of the indirect pathway (green). Afferents from the striatum are inhibitory (GABAergic) as indicated by the dotted arrows. The hyperdirect pathway (blue) is excitatory (glutamatergic) and bypassess the striatum from the cortex to the STN, to the Gpi/SNr and the thalamus. Where GPe, globus pallidum externa; GPi, globus pallidum interna; STN, subthalamic nucleus; SNpr, substantia nigra pars reticula; substantia nigra pars compacta, SNpc.

The parallel structure of the direct and indirect pathways were recently debated since it was revealed that a subpopulation of MSNs (~6%) distributed throughout the striatum, coexpressed both D1 and D2 receptors (Perreault *et al.*, 2012; Calabresi *et al.*, 2014; Gagnon *et al.*, 2017). Although the function of these neurons remains elusive, it was suggested that the direct and indirect pathways might communicate information bidirectionally (Perreault *et al.*, 2012), proposing that the pathways may not be as segregated as the original model suggests.

1.1.2 General overview of Huntington's disease

Huntington's disease (HD) is a prime example of a basal ganglia disorder, as degeneration of MSNs in the striatum take the major pathological burden, leading to a breakdown of cortico-basal ganglia circuitry. HD was originally described by George Huntington in 1872 and later termed Huntington's chorea (Huntington, 1872). Although he denoted emphasis on the motor aspects of the disease, he also recognised sufferers having a "tendency for insanity and suicide" (Huntington, 1872). It is now accepted that these form some of the behavioural abnormalities that manifest as part of HD, with cognitive deficits also being a prominent feature (reviewed in: Paulsen, 2011). As a result, people with HD present with a triad of motor, cognitive and behavioural symptoms.

People positive for the HD gene have a 50% chance of passing the disease to their children. In 1983, it was revealed that the genetic locus of HD is localised to Chromosome 4 (Gusella *et al.*, 1983). This was followed by a large collaborative effort that in 1993, revealed IT15 as the autosomal dominant gene that causes the disease, which is now known as the Huntingtin gene (MacDonald *et al.*, 1993). This is explained in more detail in Section 1.1.3 (page 8).

The discovery of the Huntingtin gene has meant that an accurate diagnosis for HD is now available. This has led to improved accuracy in epidemiology studies and inevitably an increased prevalence. Over the last 25 years records of people living with HD in the UK rose from an average of 5.3 people per 100,000 in 1990, to 12.3 in 2010 (Evans *et al.*, 2013). Prevalence rates in Caucasian populations such as Western Europe, North America and Australia, have risen from 15-20% per decade, compared to Asia where reported rates have not increased (Rawlins *et al.*, 2016) and were previously reported as low as 0.4 per 100,000 (Pringsheim *et al.*, 2012).

People who carry the HD gene (termed gene positive) can begin showing symptoms at any age, although this is most commonly between 30 and 55 years of age (Figure 3). As symptoms worsen, so does function and quality of life (Helder *et al.*, 2001; Ready *et al.*, 2008; Ho *et al.*, 2009) and loss of independence is the primary predictor of

nursing home admission (Wheelock *et al.*, 2003). Death occurs approximately 20 years after motor onset (Bates *et al.*, 2015) and, although the cause of death is not fully understood, it is usually a result of symptom complications, such as falling, malnourishment and pneumonia (Walker, 2007). A small number of gene carriers develop juvenile HD, in cases where symptom onset occurs before the age of 20 years (Walker, 2007). The phenotypic profile of juvenile HD differs from adult onset HD as the movement disorder is predominantly bradykinetic and rigid in nature rather than choreic (Douglas *et al.*, 2013). Current treatments for HD are symptomatic only and are limited to treating symptoms such as chorea and depression (Mestre and Ferreira, 2012).

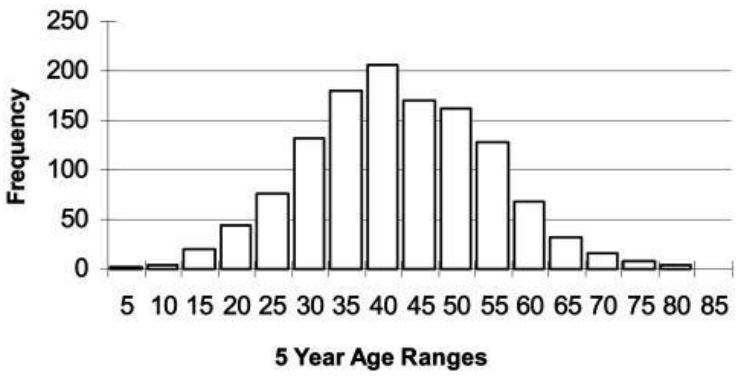


Figure 3: The age of onset in Huntington's disease. This typically peaks mid-life but can span from 2 to 85 years of age. This image was reproduced from (Myers, 2004).

1.1.3 HD Genetics

HD is caused by a mutation in the Huntingtin gene, which is located on the short arm of chromosome 4 (MacDonald *et al.*, 1993). This results in an expanded cytosine, adenine, guanine (CAG) polyglutamine repeat, which encodes the protein mutant huntingtin (mHtt). The average person carries between carry 10-29 CAG repeats (Kumar, Kalonia and Kumar, 2010), whereas people with HD carry CAG repeats of more than 35 and have full penetrance with repeats of 40 or more (Walker, 2007). Wild type huntingtin is ubiquitously expressed throughout the body and is essential for embryonic development and in adulthood (Nasir *et al.*, 1995; Schulte and Littleton, 2011; Martin *et*

al., 2015). Although the exact function of the huntingtin protein is not completely understood, previous studies suggest that it is involved in intracellular trafficking, membrane recycling and interacts with transcriptional regulatory proteins (see reviews: Sari, 2011; Schulte and Littleton, 2011; Martin *et al.*, 2015). In HD there is an abundance of evidence to suggest that pathology is caused by a toxic gain of mHtt (Han *et al.*, 2010; Schulte and Littleton, 2011). At the same time it is also recognised that loss of wildtype huntingtin in HD could also lead to cellular dysfunction, exacerbating the neuropathology in HD (Martin *et al.*, 2015).

In HD, the age of disease onset cannot be accurately predicted. However, it is thought that both environmental and genetic factors are involved in determining this. The main driving factor is understood to be the CAG repeat length, which influences as much as 70% variance in age of disease onset (Tabrizi *et al.*, 2012). Previous studies revealed that CAG repeat length inversely correlated with age of onset (Langbehn *et al.*, 2010), and positively correlated with a more aggressive rate of disease progression (Rosenblatt *et al.*, 2012). Therefore, as a way of estimating disease onset, the CAG disease burden score was developed (Penney *et al.*, 1997). This is calculated using the equation: CAG repeat length minus 35.5, multiplied by the subject's age. In this equation, 35.5 is used as a constant where this is the maximal number of CAG repeats that no HD symptoms are likely to develop. This measure was strongly associated with the amount of striatal pathology in the brains of people with HD at autopsy (Penney *et al.*, 1997). As a result, this is widely used as an effective, non-invasive method to estimate disease progression in HD.

1.1.4 Basal ganglia pathology in HD

HD is a multisystem degenerative disease (Ruocco *et al.*, 2006). One of the primary outcomes of mHtt is neuron degeneration, and particularly the MSNs located in the striatum, whereas large cholinergic striatal interneurons are spared (Raymond *et al.*, 2011). It is unclear the exact role of mHtt and why MSNs are particularly susceptible, especially as mHtt is ubiquitously expressed and distributed throughout the cell, including the cytoplasm, axon and synapse (Sari, 2011; Rüb *et al.*, 2016). One explanation for the

striatal susceptibility in HD is the presence of *RASD2*/Rhes, a protein specifically located in the striatum and selectively binds to mHtt (Subramaniam and Snyder, 2011). This interaction enhances sumoylation of mHtt, causing disaggregation and enhanced mHtt toxicity, which could lead to dysfunction and cell death (Cepeda *et al.*, 2007; Subramaniam *et al.*, 2009). Another explanation for striatal sensitivity is post-mitotic CAG-expansion (Swami *et al.*, 2009). As the CAG repeats in the striatum are particularly unstable (Kennedy and Shelbourne, 2000), this means that the number of repeats get progressively longer with increasing cell cycles, leading to increased transcription and translation of mHtt (Swami *et al.*, 2009). This could lead to increased microglia activation, excitotoxity and a decrease in important neurotrophins such as brain derived neurotrophic factor (BDNF) (Ehrlich, 2012; Andre, Carty and Tabrizi, 2016). As the mechanisms whereby mHtt produces dysfunction are not yet completely clear, this means that the development of treatments to address the underlying neuropathology in HD has been slow, and in many cases unsuccessful (Ross *et al.*, 2014; Kumar *et al.*, 2015).

Advances in neuroimaging have extended understanding of the neuropathology underlying HD and how it leads to such widespread symptoms (Dogan *et al.*, 2015). The most prominent changes are evident in the striatum, where people with early to mid-stage HD have reduced putamen and caudate volume by up to 50% and 27% (Niccolini, 2014). Changes in other regions of the HD brain include the pre-frontal cortex, parietal cortex, motor and premotor cortex, amygdala, thalamus and hippocampus (Sari, 2011; Dogan *et al.*, 2015; Novak *et al.*, 2015). Previous studies revealed that people with pre-manifest HD had widespread white matter atrophy 15 years before symptom onset, and reduced blood oxygen level dependent signals between the caudate and the premotor cortex (Unschuld *et al.*, 2012; Niccolini, 2014). Furthermore, it has been reported that people with early manifest HD had an overall loss and a gain in cortico-basal ganglia connectivity compared to controls (Novak *et al.*, 2015). As structural and functional changes in the HD brain are evident before any clinical symptoms have developed (Tabrizi *et al.*, 2013), this suggests that compensatory mechanisms may delay the onset of HD symptoms and increased synaptogenesis and angiogenesis may be a potential coping mechanism early in the disease process (Niccolini, 2014; Drouin-Ouellet *et al.*, 2015).

Neuroimaging studies have also been extremely valuable in understanding and tracking disease progression in HD. Positron electron topography (PET) has been useful in identifying specific targets such as glucose and cerebral blood flow changes in HD, as well as changes in neurotransmitter signalling as a result of altered neuroreceptors (Niccolini, 2014; Pagano, Niccolini and Politis, 2016). For instance, a previous study revealed an increase in GABA receptors in the globus pallidus externa (Glass, Dragunow and Faull, 2000). An explanation for this could be to intensify GABA sensitivity due to degenerating striatal projections. Clinically, this may explain why involuntary movements are one of the first noticeable motor symptoms in HD, as loss of striatal projections could lead to intensified GABA sensitivity through the indirect pathway and disinhibiting the thalamo-cortical response. Overall, neuroimaging techniques have facilitated a greater description and linkage to the structural and molecular changes in the brain and how this translates to the broad disease phenotype associated with HD.

1.1.5 Disease symptoms and progression of Huntington's disease

1.1.5.1 Natural course of HD

In the UK, any person that has a parent with HD or shows symptoms associated with HD has the option to have a genetic test to confirm their prognosis from 18 years of age. People who are gene positive for HD will develop symptoms, although it is not possible to know the exact age at which symptoms will develop. There is often a period of time where the person with HD shows no disease symptoms, which is known as "asymptomatic" or "pre-manifest HD" (Duff *et al.*, 2010). Prodromal HD is when a gene positive person starts to show symptoms or signs relating to HD but does not meet the criteria for 'manifest' (Misiura *et al.*, 2017). Cognitive and behavioural abnormalities are prevalent in the prodromal phases of disease and can occur up to 15 years before motor symptoms manifest (Paulsen *et al.*, 2008). This supports findings that showed caudate and putamen (striatal) changes can begin more than a decade prior to disease onset (Aylward *et al.*, 2004; Paulsen *et al.*, 2006; Tabrizi *et al.*, 2012), and may therefore initiate subtle clinical symptoms. As a result, researchers are keen to develop therapeutics that target this stage of disease before neurodegenerative changes become too extensive and irreversible. However, as few outcome measures are sensitive to subtle changes in people

with pre-manifest HD (Paulsen *et al.*, 2014), this makes it particularly difficult to estimate how close someone at this stage is to manifest onset.

Disease onset in HD is diagnosed clinically when motor abnormalities begin, termed "motor manifest". This diagnosis is usually made by a neurologist who uses a range of HD specific assessments known as the Unified Huntington's disease rating scale (UHDRS) (Kieburtz, 1996). The motor manifest stage can be divided into five disease stages. These stages are arbitrary and based on scores from the UHDRS-Total Functional Capacity (UHDRS-TFC). Stages of HD are described in more detail in Section 1.1.7 (page 15).

The fact that the HD gene is dominant and penetrant has facilitated longitudinal studies such as REGISTRY and ENROLL-HD (https://www.enroll-hd.org/). The aim of these longitudinal, multi-national studies is to optimise methods to assess disease progression in HD and have enabled a greater understanding of the disease phenotype (Orth *et al.*, 2010; CHDI Foundation, 2012). These were initiated to understand disease progression in people with all stages of HD (Orth *et al.*, 2010), and involves annually recruiting and testing any person at risk for HD on a battery assessments. The assessments include those from the UHDRS (Described in Section 1.3, page 27), demographic information, quality of life, medication and HD characteristics. Other longitudinal studies include PREDICT-HD and TRACK-HD. These are two now completed large observational studies that were designed to identify potential outcome measures and biomarkers for people with pre-manifest HD (Biglan *et al.*, 2009; Tabrizi *et al.*, 2012). Ultimately, these studies have allowed better characterisation of HD, putting the field in a good position to develop interventions to improve standards of living (Mestre and Ferreira, 2012; Reilmann, Leavitt and Ross, 2014).

1.1.6 Clinical symptoms

1.1.6.1 Motor

Chorea is the most recognised involuntary movement in people with HD (Albin, Young and Penney, 1989; Joel, 2001). This typically begins with subtle motor signs in the

distal extremities such as the fingers and in small facial muscles, which for bystanders often go undetected (Roos, 2010). Chorea increasingly affects all parts of the body with disease progression and plateaus around the mid-stages of HD. As chorea lessens, dystonia progresses. Dystonia is caused by sustained muscle contractions leading to involuntary twisting in the trunk and limbs (Louis *et al.*, 1999), and is typically present in several body regions (Louis *et al.*, 1999). Towards the advanced stages of disease, more evident motor features of HD include bradykinesia and rigidity (Fenney, Jog and Duval, 2008). Other movement abnormalities that can feature in HD include tics (Roos, 2010) and oculomotor dysfunction (Gajdusek, 1982; Leigh *et al.*, 1983). During the course of HD, people gradually lose the ability to swallow (dysphagia) and articulate words (dysarthria), leading to slurring, respiratory issues and problems with eating (Hartelius *et al.*, 2010; Alves *et al.*, 2016).

1.1.6.2 Cognitive

The cognitive profile in people with HD includes problems with executive function, attention, mental flexibility, emotional recognition, psychomotor function, and learning and memory retrieval which gradually worsen over time (Lawrence *et al.*, 1998; Ho *et al.*, 2003; Georgiou-Karistianis *et al.*, 2013; Ross *et al.*, 2014). Subtle cognitive changes can be detected many years prior to motor onset (Tabrizi *et al.*, 2011) and highly correlate with volumetric loss in the striatum (Aylward *et al.*, 2011). A previous study revealed that 40% of people estimated to be over 14 years from motor onset had problems with episodic memory, executive function and processing speed (Duff *et al.*, 2010). Furthermore, another study found that people with pre-manifest HD had problems with word list learning and odour recognition 15-20 years prior to disease onset (Paulsen *et al.*, 2008). However, as symptom progression at this stage is subtle, this makes symptom changes this makes them difficult to track change over time. A previous study revealed no evidence of cognitive decline in people with pre-manifest HD after 24 months, whereas a significant decline was detected 12 months in people with early manifest HD (Stout *et al.*, 2012). One explanation for the undetectable changes in pre-manifest disease could be that more cognitively demanding tasks are required to measure the subtle cognitive decline associated with this stage of HD (Stout *et al.*, 2012). Overall, cognitive

symptoms are believed to have the greatest impact on the rate of functional decline in HD and appear to place the greatest burden on people with HD and their families (Paulsen, 2011).

1.1.6.3 Behavioural changes

Similar to cognitive abnormalities, behavioural problems are thought to arise potentially decades prior to motor onset (Tabrizi *et al.*, 2011). A previous study evaluated REGISTRY data from 1766 people gene positive with HD and revealed that 87% had some form of behavioural disturbance (Orth *et al.*, 2010). Furthermore, the extent of behavioural symptoms varied from one individual to the next. The most common behavioural disturbances include depression, irritability, obsessiveness and apathy (Marder *et al.*, 2000; Craufurd and Snowden, 2002; van Duijn *et al.*, 2014). Depression and suicidal ideation are frequent in people gene positive with HD, affecting up to 60% of individuals (Orth *et al.*, 2010; van Duijn *et al.*, 2014). A prominent and progressive behavioural feature in HD is apathy, which can be defined as "disengagement with passivity and loss of enthusiasm, interest, empathy and interpersonal involvement" (Marin, 1991), and affects about of 48% of the HD population (Kingma *et al.*, 2008; Tabrizi *et al.*, 2013; van Duijn *et al.*, 2014).

1.1.6.4 Function and quality of life

Health related quality of life can be defined as the "optimum levels of physical role (e.g., work, carer, parent) and social functioning, including relationships and perceptions of health, fitness, life satisfaction and well-being" (Bowling, 2005). Given the progressive nature of HD, this gradually results in reduced quality of life (Ho *et al.*, 2004, 2009). A previous study recruited 77 people with all stages of HD and revealed that they had severe impairments in domains that included work and alertness, forgetfulness, attentional and problem-solving (Helder *et al.*, 2001). In addition, the same study revealed that 51% of people had retired from work, a factor which increases self-esteem in people that suffer from a mental illness (Van Dongen, 1996). Other problems that affect performance in common daily tasks include planning and inhibition, managing finances,

shopping and dividing attention between multiple tasks (Helder *et al.*, 2001; Delval, Krystkowiak, Delliaux, Dujardin, *et al.*, 2008).

The primary cause of decreased quality of life is unknown, where one study found that depressive mood and functional incapacity were the greatest contributors (Ho *et al.*, 2009), whereas another study revealed that neuropsychiatric symptoms were not associated with patient or caregiver quality of life (Ready *et al.*, 2008). The methodological differences between these studies make them difficult to compare as the latter used combined self- and caregiver- reports to analyse patient quality of life, whereas structured questionnaires that were given to the patient was used in other studies (Ho *et al.*, 2004, 2009). As a result, a more extensive study used multiple quality of life assessments, as well as cognitive tests for executive function and the UHDRS motor score (Eddy and Rickards, 2013). They found that apathy and responses related to daily activities that require executive dysfunction, such as performing two tasks simultaneously, best related to quality of life. These findings suggest that factors that affect quality of life in HD are multidimensional. Furthermore, interventions that address functions that relate to daily activities could lead to the most significant positive changes in wellbeing (Eddy and Rickards, 2013).

1.1.7 Assessing disease stage in HD

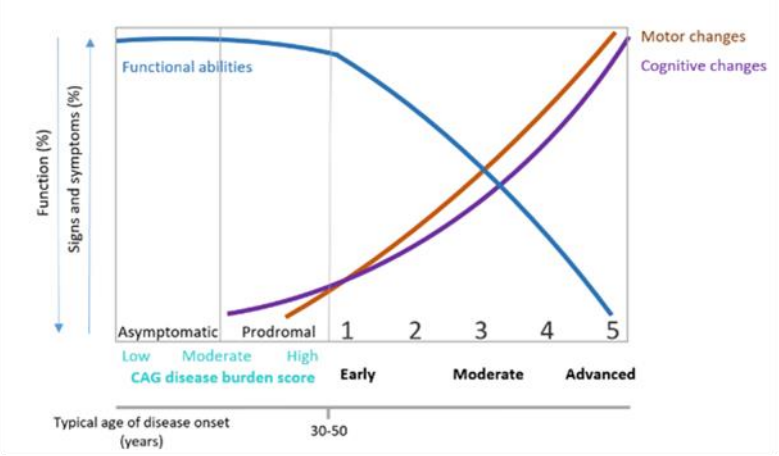
There is no defined way to assess disease stage in people with HD. A common method used to identify disease stage is using assessments from the Unified Huntington's disease rating scale (UHDRS). This consists of a group of six standardised assessments used to determine the range of clinical features associated with HD, and includes a motor, cognitive, functional capacity, behavioural, functional assessment and an independence scale (Kieburtz, 1996). In particular, the UHDRS total motor score (UHDRS-TMS) and the total functional capacity (UHDRS-TFC) are most frequently used to identify disease stage. Although these are described individually in Section 1.3 (page 27), in brief, the UHDRS-TMS is performance based, whereas the UHDRS-TFC is a questionnaire. Both of these are rated on an ordinal scale by a neurologist and each summed to give an overall score. A greater score in the UHDRS-TMS equates to more advanced motor symptoms (Ranges

from 0 (no symptoms) to 124 (advanced)), whereas a lower UHDRS-TFC score equates to more advanced functional difficulties (Ranges from 0 (functional incapacity) to 13 (normal function)). Some researchers use the UHDRS-TFC score to categorise subjects into 5 disease stages, where stage 1 is the earliest disease stage and stage 5 is the most advanced (

Figure 4a) (Shoulson and Fahn, 1979; Enrolled, 2006; Paulsen *et al.*, 2010). However, as people in the early disease stages manifest very subtle clinical symptoms, the questions in the UHDRS-TFC may be too vague to capture functional decline (Paulsen *et al.*, 2010). To increase sensitivity, some researchers use both the UHDRS-TMS and the UHDRS-TFC to define people with pre-manifest HD, where a UHDRS-TFC score equal to 13 and a UHDRS-TMS score less than 5 defines people with pre-manifest HD, and people with a UHDRS-TMS score greater than 5 are classed as manifest (Busse *et al.*, 2014) (

Figure 4b). Again, this method of grouping people with HD is not ideal, as this means that the manifest group ranges from people with early HD to advanced, but gives no indication of any stages in between. Another alternative to understanding stage of disease is to use the CAG disease burden score (Zhang *et al.*, 2011) (refer to Section 1.1.3; page 9). This calculation uses the subject's ages and CAG repeat length and can be used estimate how close someone with pre-manifest HD is to developing manifest symptoms.

a)



b)

Stage of disease	General features	TFC score
Pre-manifest	No clinically detectable symptoms: the patient has had a positive gene test confirming that they will develop the disease, but is still fully functional and independent at home and work.	13
Stage 1	Mild impairments: the patients maintain their independence in performing activities of daily living (ADL), domestic chores and managing finances. They may still be employed.	11-13
Stage 2	The patients maintain their independence in performing ADL and domestic chores. They may need slight assistance in managing finances. Capacity for normal job is reduced.	7-10
Stage 3	Patients need minimal assistance in performing ADL and domestic chores. They need major assistance in managing finances and capacity for normal job is obviously reduced.	3-6
Stage 4	Patients need major assistance in performing ADL, domestic chores and managing finances.	1-2
Stage 5	Patients need complete assistance with ADL, domestic chores and managing finances. Twenty four hour supervision at home or facility placement might also be required.	0

Figure 4: Figure a is adapted from (Ross *et al.*, 2014)*, which shows the progressive disease symptoms and different disease stages in HD. No changes are typically detected in asymptomatic/pre-manifest subjects (low CAG disease burden score). As neurodegeneration progresses, subtle cognitive and almost undetectable motor changes manifest (prodromal). Functional abilities remain fairly stable until the motor manifest stage where they more rapidly decline. Other symptoms include behavioural problems. However these tend to be more variable from one individual to the next. Manifest stages of HD can be classified using the UHDRS-TFC assessment, that was developed by (Shoulson and Fahn, 1979) (Figure b). Throughout these stages, motor and cognitive abilities progressively worsen leading to the requirement of full time care and support during the advanced stages of HD (stage 5).*

1.1.8 Animal models of HD

Genetic and lesion models have been crucial in understanding disease mechanisms that cannot ethically be studied in people, such as the development of new treatments and sensitive tracking of HD pathology. There is currently no perfect HD animal model, as each elicits different behavioural phenotypes linked to the disease.

1.1.8.1 Lesion models of HD

The first model for HD was generated in 1976 (Coyle and Schwarcz, 1976) and was developed on the basis that glutamate is the major excitatory corticostriatal neurotransmitter (McGeer *et al.*, 1977). Glutamate release and N-methyl-D-aspartate (NMDA) receptor hypersensitivity causes striatal cell death, largely due to an excessive calcium influx, triggering apoptotic pathways (Watkins and Jane, 2006). Thus, the initial hypothesis for neurodegeneration in HD was excitotoxicity (DiFiglia, 1990). One of the first HD models that supported this theory was an intra striatal injection of kainic acid, a potent analogue of glutamate with pronounced selectivity for MSNs (Coyle and Schwarcz, 1976). However, as kainic acid destroys both GABAergic and cholinergic neurons, quinolinic acid is currently the excitotoxin of choice. This has a high affinity for NMDA receptors positioned on the post-synaptic membrane of striatal specific projection neurons (MSNs).

Quinolinic acid better replicates the neuropathology of HD due to the sparing of the striatal interneurons and increased sensitivity for MSNs (Schwarcz, Whetsell and Mangano, 1983; Cepeda *et al.*, 2010). The impact of the toxin can be controlled by giving unilateral or bilateral lesions and modifying the toxin dose, infusion rate, injection placement and number of injection sites. The benefit of using a unilateral lesion means that the intact striata can be used as a histological control. For example, in skilful grasping tasks, rats with unilateral lesions continue performing accurately using the paw ipsilateral to the lesion, whereas performance using the paw contralateral to the lesion is severely impaired. Unilateral lesions are particularly useful for assessing certain behaviours in

transplantation studies as only one unilateral graft is required to observe functional improvement, for example in a grasping task (Döbrössy and Dunnett, 2003), compared to two, which are required in some cognitive tasks (Dunnett and White, 2006). Although lesion models do not necessarily improve the understanding behind the natural disease course, as neurodegeneration using this method is not progressive, they remain a valuable model to study striatal specific behaviours and the development and optimisation of treatments such as cell transplantation.

1.1.8.2 Genetic models of HD

The discovery of the huntingtin gene spurred the development of genetically modified animal models for HD. These have provided an understanding behind the neuropathological mechanisms that underlie HD (Kumar, Kalonia and Kumar, 2010). One noticeable difference between the human condition and animal models is that many of the mouse models carry much longer CAG repeats in order to show a HD phenotype, which is probably due to the shorter life span of rodents. Therefore, a number of mouse models have been developed with different CAG lengths to characterise early symptoms, and more aggressive forms (that have longer CAG repeats) that are associated with later clinical onset in HD. Detailed reviews on animal models used for HD are reviewed by (Ramaswamy, McBride and Kordower, 2007; Kumar, Kalonia and Kumar, 2010). In brief, mouse models fall into one of three main categories. Firstly; the generation of fragment models, where mice carry a small fragment of the human huntingtin gene that causes an expanded CAG repeat. This has resulted in four R6 lines that carry between 115-156 CAG repeats and show accelerated and severe disease progression. Secondly; insertion of the full length human huntingtin gene carried in a yeast or bacterial artificial chromosome. These present slower disease progression more representative of the human condition. Thirdly; knock-in models, where an expanded CAG repeat is inserted into the existing huntingtin homologue (Ramaswamy, McBride and Kordower, 2007; Kumar, Kalonia and Kumar, 2010). It should be noted that the type of mouse model selected for research depends very much on the experimental question and the disease stage targeted. Therefore, there is now a need to develop animal models that more closely depict the natural progression in HD, allowing for better direct comparisons in clinic and the

laboratory, and the ability to track neuropathological features in longitudinal studies (Kumar, Kalonia and Kumar, 2010).

1.2 Part 2: Cell transplantation in Huntington's disease

There is currently no cure for people with HD. Current medication is prescribed to ameliorate downstream symptoms, such as motor abnormalities and depression (albeit with limited success) (Mestre and Ferreira, 2012). However, there is now a drive to develop therapeutics that target the specific neuropathological hallmarks identified in HD, as a way of preventing disease onset and slowing disease symptoms. This has included potential treatments looking at reducing excitotoxicity, targeting mHtt aggregates, mHtt gene silencing, and drugs for mitochondrial dysfunction (Kumar *et al.*, 2015). One potential disease modifying treatment is cell transplantation, which can be used to replace degenerating MSNs with a healthy MSN source. Whilst it is acknowledged that both foetal tissue and stem cells have been used for transplantation therapy in HD (reviewed in: Perrier and Peschanski, 2012; Dunnett and Rosser, 2014; Rosser and Svendsen, 2014; Kumar *et al.*, 2015), cell transplantation using foetal tissue will be the focal therapeutic referred to throughout this section.

1.2.1 What is cell transplantation and what has been done in HD?

Cell transplantation is a promising therapy for people with HD. The aim of this surgical procedure is to replace the degenerating striatum with a high proportion of healthy MSNs that will integrate, form synapses and in time reconnect lost striatal circuitry (Figure 5) (Döbrössy and Dunnett, 2005a; Kendall et al., 1998). In the majority of preclinical and clinical trials, the donor cells of choice have been the whole ganglionic eminences (WGE) dissected from foetal tissue, as this collective region gives rise to the highest proportion of DARPP-32 positive cells (a marker used to stain for MSNs) (Watts, Dunnett and Rosser, 1997). Transplanting WGE produces regions in the graft referred to as P-zones, which upon histological staining using striatal specific markers, results in dense, patchy regions (Graybiel, Liu and Dunnett, 1989). Furthermore, the amount of P-zones present are proportional to the extent of functional recovery (Nakao *et al.*, 1996). In previous studies, the greatest P-zones were evident in WGE, followed by lateral GE grafts, and then medial GE grafts (Pakzaban *et al.*, 1993; Watts, Dunnett and Rosser, 1997;

Olsson, Björklund and Campbell, 1998). Although the lateral GE is understood to be the primary site of MSN generation (Olsson, Björklund and Campbell, 1998), these findings suggest that both the medial and lateral WGE are required to develop a mature striatal phenotype.

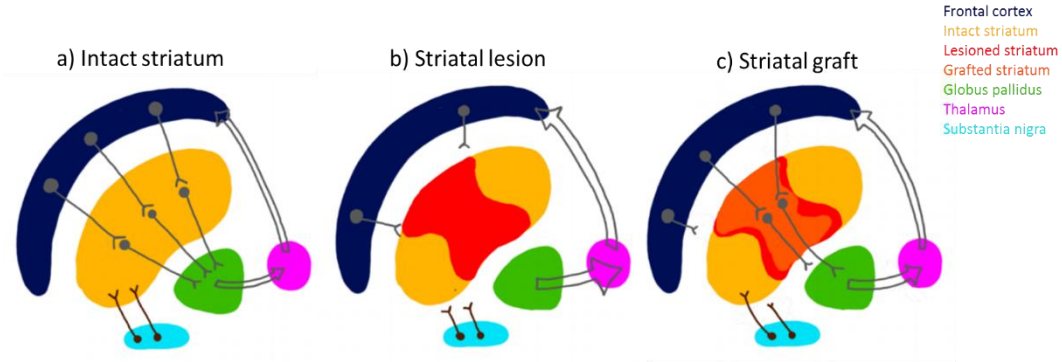


Figure 5: A schematic to illustrate the striatal circuitry in the intact (a), lesioned (b) and grafted (c) rodent brain. Efferent GABAergic projections from the cortex and the substantia nigra to the striatum, and afferents from the striatum to the globus pallidus degenerate in the lesioned striatum. This breaks the cortico-basal ganglia-thalamo circuitry. Following a striatal graft, afferent and efferent projections begin to reform, but not to the same extent as a control (a).

Another important consideration, when preparing for transplantation, is the age of the embryonic tissue used. In development, the WGE is first seen at embryonic age (E) 10.5 in rats and 42 days post conception in the human foetus (Pauly *et al.*, 2012). Striatal neuronal sub populations are generated through several developmental waves, where each wave binds the cells closer striatal phenotype (Schackel *et al.*, 2013). To understand the optimal donor age for transplantation, a previous study grafted lesioned rats with E14 or E16 WGE, lateral GE or medial GE tissue (Watts, Brasted and Dunnett, 2000). They revealed that using E14 WGE generated the largest volume of striatal grafts. This supports another study which transplanted different donor age WGE (E13, E14, E15) into lesioned rats (Schackel *et al.*, 2013), and found that E14 WGE transplants contained the largest volume of striatal grafts, but E13 WGE contained the largest P-zones. As all transplanted rats showed behavioural recovery, the higher percentage of DARPP-32 in E13 suggests that a younger age may have the greatest potential when translating cell transplantation into clinic.

Once the donor tissue is dissected, the next stages of the transplantation procedure is to prepare the tissue into a dissociated cell suspension and, using stereotaxic injection, transplant this homotopically into the diseased striatum. This results in functional afferent, efferent and synaptic anatomical connections with the host brain (Wictorin *et al.*, 1992; Clarke and Dunnett, 1993; Chin *et al.*, 1999), and over time, this theoretically gives the striatum a platform to bridge the missing circuitry in HD.

Much of the transplantation research to date has been performed in rats and primates that have sustained excitotoxic lesions to the striatum (Walsh *et al.*, 1988; Mayer *et al.*, 1992; Kendall, Rayment and Torres, 1998; Nakao and Itakura, 2000; Döbrössy and Dunnett, 2006; Pauly *et al.*, 2012). Such models are beneficial as the relevant cell loss is specified, and also, striatal specific behaviours can be assessed pre and post transplantation to test graft functionality. Lesion models also better replicate the neurodegeneration in the human condition compared to transgenic mouse models of HD. The first transgenic transplantation study was performed by Dunnett and colleagues in the R6/2 line (Dunnett *et al.*, 1998). They revealed that striatal grafts survived, however functional improvements were modest and did not significantly improve behaviour. One explanation for this could be because the R6/2 mouse model was used, which develops a particularly accelerated and aggressive disease phenotype. Furthermore, pathology in genetically modified mouse models is not striatal specific and is far more widespread (Morton *et al.*, 2001). This suggests that a striatal transplant may not be sufficient to ameliorate the behavioural deficits observed in current transgenic mouse models. Although there is a risk of non-specific damage using the cannula to lesion animals, the excitotoxic lesion model remains the model of choice for transplantation studies.

Promising cell transplantation results in pre-clinical models of HD (Wictorin, 1992; Dunnett, 1995) led to the first clinical trial in 4 patients in 1992 (Sramka *et al.*, 1992), followed by two clinical trials in the mid and late nineties (Madrazo *et al.*, 1995; Philpott *et al.*, 1997; Kopyov *et al.*, 1998). Since then several other studies have taken place across the world, with some suggestion of improvement in elements of the UHDRS, albeit modest (Philpott *et al.*, 1997; Kopyov *et al.*, 1998; Bachoud-Lévi *et al.*, 2000, 2006; Hauser *et al.*, 2002; Rosser *et al.*, 2002; Keene *et al.*, 2007; Reuter *et al.*, 2008; Gallina *et al.*, 2010),

as well as motor and cognitive improvement and stabilization over 5-6 years (Bachoud-Lévi *et al.*, 2006; Reuter *et al.*, 2008). A number of factors have made previous HD transplantation trials difficult to compare; in particular heterogeneity in study design, surgical procedure and assessment of efficacy. Such differences range from the age of the donor embryo and number of embryos transplanted to the use of immunosuppression and the size of the cannulae used for transplantation. In summary, the optimal method for cell transplantation is yet to be determined (Freeman *et al.*, 2011; Cisbani and Cicchetti, 2014).

Post mortem studies have also provided an insight into the composition of the graft, including the cell content of the graft, the impact of the immune system on the graft, whether mHtt accumulated in the graft, and graft-host integration (Cisbani and Cicchetti, 2014). Findings from previous studies revealed that transplants were capable of making afferent connections after they showed positive staining for tyrosine hydroxylase (Freeman *et al.*, 2000; Keene *et al.*, 2007, 2009; Capetian *et al.*, 2009; Cicchetti *et al.*, 2009; Cisbani *et al.*, 2013). In addition, other studies revealed that grafts stained positively with the MSN marker, DARPP-32 in people that died between 6 months to 6-7 years post transplantation (Freeman *et al.*, 2000; Keene *et al.*, 2007; Capetian *et al.*, 2009), but to date not in older grafts (9-12 years) (Cicchetti *et al.*, 2009; Keene *et al.*, 2009; Cisbani *et al.*, 2013). This suggests that the HD graft environment could have a negative impact on the graft survival over time. This is supported by other findings that showed an increased immune reaction (Freeman *et al.*, 2000; Keene *et al.*, 2007; Capetian *et al.*, 2009; Cicchetti *et al.*, 2009) and some evidence of mHtt accumulation in the grafts (Freeman *et al.*, 2000; Keene *et al.*, 2007; Cicchetti *et al.*, 2009). Furthermore, as microglia increases with age, this could also have an effect on graft survival (Luo, Ding and Chen, 2010). The age of graft recipients in previous trials were very different (they ranged from 29-64 years of age), and the disease duration in patients ranged from 2-17. As MSNs progressively regress in people with HD (Han *et al.*, 2010), transplanting into an advanced patient could make it harder for the graft to integrate with host striatum, and limit the graft potential. To summarise, this evidences the need for standardised transplantation procedures and methodology to allow for accurate comparisons between studies. In addition, it suggests

that people that are younger and in the earlier stages of disease could be the target population to benefit from cell replacement therapies.

1.2.2 Learning to use the graft in pre-clinical models

Numerous studies have revealed that grafts have the ability to alleviate a broad range of motor and cognitive symptoms resulting from excitotoxic lesions in rodents and non-human primates (for reviews see: Nakao, 2000; Döbrössy and Dunnett, 2005; Dunnett and Rosser, 2007). However, graft survival and anatomical integration alone is not *sufficient* to achieve such improvements. Thus, for optimal graft functionality, extended striatal specific behavioural training may be required to allow the recipient to *'learn to use the transplant'* (Dobrossy and Dunnett 2005a; Dobrossy and Dunnett 2003; Dunnett and White 2006; Mayer et al. 1992; Brasted et al. 1999). This concept was first introduced by Mayer and colleagues (Mayer et al., 1992) who found that after intra-striatal grafting of foetal tissue, striatally-dependent behaviours need to be re-established through targeted training, which requires appropriate integration of the graft into the host tissue for circuit reconstruction. For example, previous studies revealed that lesioned rats which received striatal grafts, and then striatal specific training, gradually improved behavioural performance to a level that was significantly better than lesion only rats, and a similar level as controls (Brasted *et al.*, 1999a; Brasted *et al.*, 2000; Dobrossy and Dunnett, 2003; Dunnett and White, 2006). Other factors that affect graft functionality and integration includes the *type* of pre-training received prior to transplantation and the extent of environmental enrichment received post transplantation (Döbrössy and Dunnett, 2005; Pauly *et al.*, 2012). Environmental enrichment generally refers to stimulating living conditions that promote more social interaction through play and motor activity, compared to standard housing conditions that are relatively impoverished, standard cages (Rosenzweig *et al.*, 1978). In an experimental setting, enriched housing is generally more complex compared to standard laboratory cages with the addition and regular changing of toys, tunnels, running wheels, nesting material, as well as bigger cages and larger group sizes permitting more frequent and varied social interactions. Previous studies revealed that animals living in an enriched environment, post transplantation, had larger grafts and contained neurons with greater spine density (Döbrössy and Dunnett,

2008). These effects were also time-dependent whereby animals in full time environmental enrichment had the largest grafts compared to those given daily exposure for one hour. In summary, previous research suggests that conditions post transplantation, such as environmental enrichment and striatal-specific training is necessary for optimal graft morphology and functionality.

1.2.3 Learning to use the graft in people with HD

The implication of '*learning to use the transplant*' in human transplant trials is that graft functionality may be enhanced by striatal-specific training. This may be improved further with environmental stimulation, suggesting that it may be important to combine transplantation with a carefully developed rehabilitation program. Although the benefits from learning to use the transplant have consistently shown positive results in preclinical models (See previous section), clinical trials have not, thus far, adopted this strategy post transplantation. In fact, no strategy has been developed for people with HD following transplantation, other than return visits to perform various outcome measures to (Quinn *et al.*, 1996). Similar to the grafting studies previously discussed in pre-clinical models, previous studies in people with HD have shown that rehabilitative interventions alone could benefit functional mobility (Busse and Rosser, 2007; Quinn, Hamana, *et al.*, 2016; Quinn, Trubey, *et al.*, 2016). Such strategies could be adopted in people with HD to encourage graft-host interaction post transplantation, with the aim of translating the *learning to use the transplant* concept from the laboratory, into clinic.

1.3 Part 3: Outcome measures used in HD

Assessing disease stage, symptoms and progression using valid and reliable outcome measures is crucial to characterise clinical phenotype and to accurately assess interventions. The following section describes the importance of disease-specific outcome measures and the methods that should be considered when these are selected for a study or a clinical trial, and indeed for evaluation of cell transplantation. This section also describes the commonly used outcome measures to assess the symptoms and disease progression in people with HD. Specific outcomes measures used for cell transplantation are reviewed in Part 4.

1.3.1 What are outcome measures?

Outcome measures provide a way of assessing a clearly defined construct or concept. These are recognised as the most credible characteristics to translate how a person functions and feels, can be used as a way to monitor the progression and severity of disease symptoms over time, and to interpret the results of a clinical trial (Fleming and Powers, 2012; Iansek and Morris, 2013). Selecting an appropriate outcome measure(s) for an intervention is crucial. An outcome measure that is not specific to the population or the construct of interest can result in outcomes being missed or misinterpreted. A number of theoretical and practical considerations can guide the selection of suitable outcome measures. For instance, it is important that the outcome measure used has established clinometric properties; a term used to describe the overall quality of an outcome measure, such as validity, reliability and responsiveness (Iansek and Morris, 2013). Validity is used to ensure the outcomes from an assessment or clinical trial measure what they intend to measure, and can be divided into internal, external and test validity (Iansek and Morris, 2013). Internal validity is more commonly used following clinical trials to establish if any other factors could have led to the outcomes shown (See Figure 6). External and test validity are used to ensure an outcome measure is appropriate for the purpose intended. Reliability is used to assess the consistency of an assessment (Kimberlin and Winterstein, 2008). This includes participant consistency (whether the test score

varies in a short space of time when all other factors remain consistent) and rater reliability (whether the rater gives the same score when all other factors remain consistent) (Pin, 2014), which are more applicable to assessments that are rated subjectively. Another consideration when selecting an outcome measure is if the results are prone to floor and ceiling effects. An example of a floor effect is when a test is too difficult for a population, resulting in the poorest possible performance. A ceiling effect occurs when a test is too easy, leading to close to maximum scores. The risk of floor and ceiling effects means that they are insensitive to either further deterioration in performance (floor effect), or improvement (ceiling effect) over time (Rasmussen *et al.*, 2001). Other factors that should be considered when selecting an appropriate outcome measure in clinical research are presented in (Table 1).

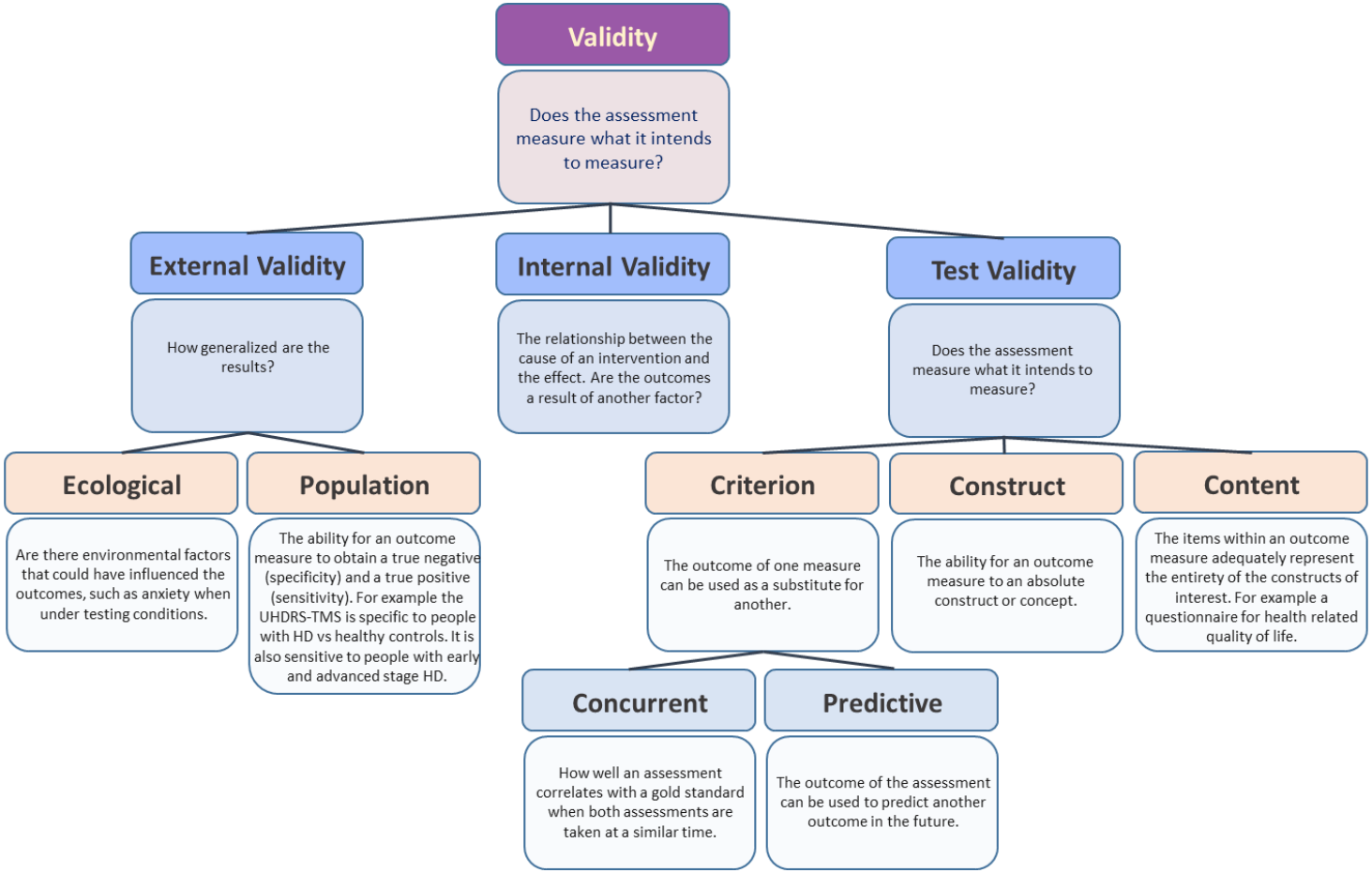


Figure 6: The different types of validity used to establish how well outcomes used for research or a clinical trial measures what it intends to measure. For a review see (Kimberlin and Winterstein, 2008).

Table 1: *Considerations to ensure that an appropriate outcome measure is selected for purpose.*

Primary consideration	Relevant questions
Why is the outcome measure being used?	To identify the impact of specific symptoms on the individual?
	To establish a baseline measure to monitor change over time?
	To evaluate the response to an intervention?
	To diagnose a patient?
What is being measured?	Impairments of body structure and function?
	Activity limitations?
	Participant restrictions?
	Quality of life?
Have the clinometric properties of this tool been assessed in a sample of people similar to those in this study?	Do the study samples have the same movement disorder/condition?
	Was the assessment performed in people of a similar ethnicity?
	Is the study sample similar in disease severity?
	Is the study sample similar in disease specific factors that are considered important. For example, level of mobility, age, presence of co-existing medical problems
Is the outcome measure reliable?	Are the assessment results consistent at a participant level?
	Are the assessment results consistent at a rater level?
Is the outcome measure valid?	Has the assessment been tested for criterion, construct and content validity in a population similar to the population of interest?
	What was the sample size of the previous studies?
	Are the participants previously tested similar to the population of interest demographically? For example, age, weight, education.
Is the outcome measure responsive to change?	Is there a known minimum clinically important difference?
Is there a financial consideration?	Is there a cost for the assessment?
	Is a licence needed for the assessment?
	Is equipment needed?
Implementation: resources	Is special equipment needed?
	Is more than one researcher/clinician required to carry out the assessment?
	Is there enough space available?
	Does the assessment take a long time to set up?
Implementation: client and carer	How much time is needed to complete the assessment?
	How difficult is the assessment?
	Is it realistic to ask the participant to return to redo the assessment?
	Does the participant need privacy when doing the assessment?
	Does the participant require a translator?
For patient-reported outcome measures (questionnaires)	Does the assessment need to be done face to face with the researcher/clinician or can it be sent to the participant to do at home?
	Does the assessment include questions the participant my feel sensitive answering?
	Could items within the questionnaire be interpreted differently due to differences in language/ethnicity?
	Is the questionnaire written in a language that can be easily understood by the participant?
Cognitive/Behavioural/motor fluctuations	Does the assessment account for fluctuations in cognitive/behavioural or motor symptoms. For example a change in medication.
	Does the assessment need to be performed a certain time of day to account for periods where for example, medication may be waring off.
Interpretability	Is the outcome of the assessment in a language that is useful to the clinician and researcher?
	Can the outcome of the assessment be interpreted efficiently?
	Are there normative data for healthy people of similar ages?
Data storage	If the outcome measure is provided online, where is the data stored and can all elements be accessed?

This table was adapted for use from (Iansek and Morris, 2013).

1.3.2 Outcome measures used in people with HD

1.3.2.1 Outcome measures to assess motor symptoms

The UHDRS-TMS is the current gold standard for assessing motor severity in people with HD and is the most commonly used outcome measure for this population (Bilney, Morris and Perry, 2003). This covers a broad range of motor domains including bradykinesia, chorea, dystonia, rigidity, gait and oculomotor function. The limitations of this assessment are acknowledged in a number of papers (Tabrizi *et al.*, 2009; Bechtel *et al.*, 2010; Reilmann *et al.*, 2011), with the main problem being the way performance is rated. In this assessment, subjects are rated by a neurologist, where each item is scored on a five point rating scale (0 = normal and 4 = severe impairment) and summed, equating to a score out of 124. Due to this subjective scoring method this limits inter rater reliability. Furthermore, previous studies revealed that the UHDRS-TMS led to floor and ceiling effects in people with early and advanced stages of HD (Hogarth *et al.*, 2005; Tabrizi *et al.*, 2011; Youssov *et al.*, 2013). This suggests that additional motor assessments should be used alongside the UHDRS-TMS to sensitively capture people with all stages of HD.

The Quantitative (Q)-motor assessments provide quantitative motor feedback via a force based transducer (Reilmann *et al.*, 2013). These were originally developed for the HD population to overcome the limitations that arise using the UHDRS-TMS (Reilmann, 2012). The Q-motor assessments include finger tapping (Bechtel *et al.*, 2010), tongue force (Reilmann, Bohlen, Klopstock, Bender, Weindl, Saemann, Auer, E. Bernd Ringelstein, *et al.*, 2010) and grip force tests (Reilmann, Bohlen, Klopstock, Bender, Weindl, Saemann, Auer, Erich B. Ringelstein, *et al.*, 2010) and have proven their ability to distinguish between people with pre-manifest HD and both healthy controls and manifest people with HD (Tabrizi *et al.*, 2009; Reilmann, 2012). Although these assessments require specialist equipment, they are scored objectively, and therefore may be more sensitive than subjective scoring methods used for the UHDRS assessments. This makes them a useful biomarker to track subtle motor changes that otherwise go unnoticed using ordinal based measures (Reilmann, 2012). Furthermore, a previous study revealed that Q-motor performance highly correlated with caudate and putamen volume reduction (Scahill *et*

al., 2013), presenting a link between brain atrophy and some of the earliest clinical symptoms in HD.

1.3.2.2 Outcome measures to assess mobility and balance

The *Berg Balance Scale* (Berg *et al.*, 1992) and *Tinetti Mobility Test* (Tinetti, Williams and Mayewski, 1986) are consistently used as outcome measures to assess mobility and balance following a physical intervention in people with HD (Quinn *et al.*, 2013; Busse *et al.*, 2014). However, as they were originally developed for the elderly, these include a number of redundant items, such as sitting unsupported (Busse *et al.*, 2014), which makes their use unnecessarily time consuming. Furthermore, the lack of item specificity means that many items are too easy for people with early-mid stage HD, which combined with the ordinal rating scales, frequently results in both floor and ceiling effects (Quinn, Khalil and Dawes, 2013; Busse *et al.*, 2014) and limits the ability to measure meaningful change over time.

1.3.2.3 Outcome measures to assess cognition

Cognitive function in HD is commonly evaluated using the UHDRS cognitive test, which includes three executive function tests: the letter fluency test (LVF), Symbol Digit Modalities test and the Stroop test (Kieburtz, 1996). These are tests that tap into executive function abnormalities, an umbrella term for processes such as task-switching, planning, and working memory (Elliott, 2003).

The LVF test requires the spontaneous generation of words beginning with a given letter (Benton, 1968). Typically, subjects are asked not to repeat themselves or say any pronouns. This tests attention, inhibition and retrieval processes which heavily rely on the fronto-striatal and temporal regions that are known to degenerate in HD (Ho *et al.*, 2002). LVF tasks have been well validated in this disease population, revealing that people with HD consistently recite significantly less words in LVF tasks compared to healthy controls (Butters *et al.*, 1986; Hodges, Salmon and Butters, 1990; Rosser and Hodges, 1994).

However, as lifestyle factors could affect the performance in this task, such as education, hobbies (e.g. reading) and type of job, this could improve the baseline score, suggesting that a participant is earlier in the disease stage than they actually are. This should therefore be considered when interpreting LVF results.

The Symbol digit modality test involves translating geometric shapes into numbers by following a number-shape reference key (Smith, 1968). This is used to assess perceptual speed, motor speed, visual scanning and visual tracking. A previous study revealed that this test significantly distinguished between healthy controls and people with pre-manifest HD (Tabrizi *et al.*, 2013). In addition, another study revealed that performance in the Symbol digit modality test significantly deteriorated in people with manifest HD over a period of 16 months (Beglinger *et al.*, 2010), suggesting this may be a useful measure to track change over time. However, as subjects are required to translate their answers onto paper using a pencil, motor symptoms could also affect performance in this test as this test. Therefore, defining how much the Symbol digit modality test assesses cognition over motor ability can be challenging.

The Stroop task requires a participant to read colour-words (congruent) or name the coloured ink of colour-words (incongruent) (Stroop., 1935). The simpler, congruent task is where colour-words are written in their coloured ink (e.g. BLUE written in the colour blue). The complex task involves saying the coloured ink which is incongruent to the colour-word (e.g. BLUE is written in the colour yellow). As reading words is more common in everyday life than acknowledging the coloured ink of the word, responses in the incongruent task are often slower, leading to lower performance accuracy (MacLeod and MacDonald, 2000). This task is described in more detail in Chapter 2 and Chapter 3.

Increased understanding regarding the cognitive symptoms associated with HD means that a larger HD-specific, cognitive assessment battery was recently developed and was called the HD Cognitive assessment battery (Stout *et al.*, 2014). This consists of six cognitive assessments that assess attention, processing speed, visuospatial processing, timing, emotion processing, memory, and executive function. The aim is for this to

become a standardised cognitive battery for people with HD, to allow for accurate comparisons between sites and treatments for future clinical trials.

1.3.2.4 Psychiatric/behavioural outcome measures

The *Problem Behaviour Assessment* is a questionnaire used to assess the behavioural symptoms in people with HD, including apathy, depression and irritability (Craufurd, Thompson and Snowden, 2001). In a previous systematic review, this was reported as the recommended method to assess behavioural symptoms in people with HD (Carlozzi *et al.*, 2014). Furthermore, additional studies have revealed this is valid, reliable and responsive to change over time in people with HD (Kingma *et al.*, 2008; Thompson *et al.*, 2012).

1.3.2.5 Function and Quality of life assessments

The current lack of treatments available for HD means that disease symptoms gradually worsen and negatively impact quality of life (Ho *et al.*, 2004). As a result, functional and quality of life assessments are crucial to gain an understanding about how standards of living change as HD progresses, and also following an intervention.

Functional capacity in HD is commonly rated using the UHDRS-TFC questionnaire. This is used to understand how people with HD manage their work, finances, daily living, domestic chores and their care arrangements (Shoulson and Fahn, 1979). Answers from the patient are rated by a neurologist on an ordinal scale ranging from 0 (unable) to 3 (normal function). These are summed to produce an overall score, where 13 equates to normal function and 0 equates to severe functional incapacity. As mentioned in Section 1.1.7, this measure is also frequently used to class people into different disease stages. However, given that this scoring method is arbitrary, alternative measures are also required when determining disease stage in HD.

The Short Form-36 (SF-36) and the SF-12 assessment is one method used to measure quality of life in people with HD (Ware, Kosinski and Keller, 1996). This requires subjects to rate 36 or 12 items on an ordinal scale based on how they have been feeling and how this has affected performing social and physical activities. Both surveys have shown to be valid and reliable tools used for the HD population (Ho *et al.*, 2004), and in particular have implicated functional ability as a key factor that impacts health related quality of life (Ho *et al.*, 2009).

The functional component of the *Late-Life Functional Disability Instrument* (LL-FDI) is a 32 item questionnaire that focuses on common daily activities, including how the person manages when using kitchen utensils to prepare meals, and getting into and out of a car (Haley *et al.*, 2002). Questions are scored on an ordinal scale ranging from no difficulty (=0) to cannot do (=5). The novel component of this questionnaire is that it can be subcategorised into items involving upper extremity function and, basic and advanced lower extremity function, where each domain is scored and transformed; resulting in linear scaled scores from 0 to 100. Although the LL-FDI was originally developed and validated in community dwelling older adults (Sayers *et al.*, 2004), the items within the questionnaire are applicable to any person with or at risk of reduced functional mobility. The LL-FDI has not yet been evaluated in HD. However, as this assessment focuses on a range of common daily activities, and scoring allows disease progression to be sensitively tracked, the LL-FDI may be a useful measure of functional impairment in this disease population.

A limitation of giving questionnaires about general function to people with HD is that there can be discrepancies between what the patient reports and what they can actually do. Therefore, the *Physical Performance test* has previously been used to measure functional performance in people with HD (Reuben and Siu, 1990; Quinn *et al.*, 2013; Busse *et al.*, 2014). This uses time as a primary measure to rate activities such as '*put on and removing a jacket*' and '*pick up a penny from the floor*'. A previous study revealed that the *Physical Performance test* had excellent reliability and low minimal detectable change across disease stage in HD (Quinn et al. 2013). However, a limitation of this assessment is that 2 of the 9 items require the participant to climb a flight a stairs,

which is not always possible in a clinical setting. Furthermore, although the *Physical Performance test* adopts a quantitative scoring method, timing categories were devised for use in the elderly (Reuben and Siu, 1990). As a result, this reduces the sensitivity of this assessment across people with different stages of HD (Quinn et al. 2013).

1.4 Part 4: Outcome measures to assess graft functionality in HD

Well designed, sensitive outcome measures and clinical tools are required to assess the effectiveness of HD clinical trials, such as cell transplantation. Furthermore, selecting outcome measures that test behavioural functions that target the neurocircuitry effected in HD, will permit sensitive tracking of neurodegeneration, and could also be used to assess the outcomes of therapies that target this specific neural region. As a primary goal of cell transplantation in HD is to improve standards of living, this suggests that performance based outcome measures could be a useful way to translate assessment outcomes at clinic to common behavioural tasks performed at home. Therefore, this section begins with a review on previously used outcome measure for transplantation studies in HD, and then focuses on functional tasks that could be used to assess the effectiveness of cell transplantation in clinical trials, such as dual task paradigms.

1.4.1 CAPIT-HD

The Core Assessment Protocol for Intrastriatal Transplantation in Huntington's disease (CAPIT-HD) was originally developed as a way to standardise the outcome measures used pre and post transplantation (Quinn *et al.*, 1996). This is typically used before transplantation and 6-12 months post-surgery, from which it assesses a wide range of outcome measures, including; motor, cognitive, psychiatric function, neuropsychological and neuropsychiatric tests, as well as undergoing magnetic resonance (MRI) and positron emission tomography (PET) imaging (Quinn *et al.*, 1996; Kopyov *et al.*, 1998; Bachoud-Lévi *et al.*, 2000; Hauser *et al.*, 2002; Rosser *et al.*, 2002). Although the development of CAPIT-HD represented the standard baseline of current knowledge, a wealth of research has since improved our understanding behind the neuropathology and the clinical symptoms seen in people with HD. As a result, it is important that CAPIT-HD is updated to ensure that the research advancements made over the last two decades result in more sensitive assessments that better capture disease progression, and any changes that result from cell transplantation.

1.4.2 Neuroimaging post transplantation

Neuroimaging is routinely used to assess graft placement, graft volume and to quantify morphological and metabolic changes. Techniques such as MRI, PET and single photon emission computed tomography are routinely used as part of CAPIT as a way of tracking and assessing graft morphology, graft function, graft-host integration and circuit reconstruction (Quinn *et al.*, 1996; Rosser and Bachoud-Lévi, 2012). Such techniques revealed that the minimum follow up time to allow the graft to mature, to the point of exhibiting functional signs, that can be attributable to grafted connections, is estimated as 12-24 months (Rosser and Bachoud-Lévi, 2012). Similar to pre-clinical studies, this suggests that graft integration is gradual. However, whether or not this improvement could be optimised or appear sooner than 12-24 months using the *'learning to use the transplant'* concept, is unknown.

Although neuroimaging is beneficial to demonstrate graft survival, graft placement and graft function, it is not currently an informative method to determine neuronal type (proportion of striatal to non-striatal neurons), or the functional impact of the graft on behaviour. As a result, neuroimaging, as well as appropriate clinical outcome measures and post-mortem studies are fundamental to gain a true understanding behind how the graft develops and if this improves the standards of daily living in HD patients.

1.4.3 What outcome measures are missing in transplantation trials?

Since individual UHDRS items do not necessarily test known striatal dependent behaviours, using UHDRS assessment(s) as a primary outcome measures in transplantation trials risks rating functions reliant on compensatory neural networks that bypass the striatum, rather than directly testing the effects of the transplanted tissues on their primary target i.e., the striatal circuitry itself. In addition, it is critical to assess not only graft integration, but to include outcome measures representative of a variety of domains in relation to function.

1.4.3.1 Functional assessments

Whereas the majority of outcome measures are performed in a laboratory setting, there are few performance based assessments available to understand *how* people with HD function in everyday life. Such tasks are required to improve the ecological validity of currently used outcome measures.

Assessing lower limb function in HD

Gait, sitting, standing and stepping are different examples of lower limb functional tasks that are used in people with HD. Gait is assessed using the UHDRS-TMS, and is purely observational. More sensitive measures of gait impairment have been observed using objective measures, such as the GaitRite and motion analysis (Rao, Quinn and Marder, 2005a; Delval, *et al.*, 2008; Grimbergen *et al.*, 2008; Rao *et al.*, 2008a, 2011; Dalton, *et al* 2013). The GaitRite is an electronic walkway that was found to be a reliable and valid method to measure gait parameters in HD (Hausdorff *et al.*, 1998; Rao *et al.*, 2008). In a longitudinal study, 10 pre-manifest people with HD were recruited to perform gait analysis tests at baseline and after 1 and 5 years (Rao *et al.*, 2011). They found that step length reduced and swing time increased 1 year after baseline, whereas no changes were detected using the UHDRS-TMS gait item. Previous findings also revealed that the GaitRite was sensitive to changes in pre-manifest people with HD and controls, and revealed that the pre-manifest group had decreased gait velocity and stride length, and an increased time in double limb support, with a more variable stride length and step time (Rao *et al.*, 2008). These findings are consistent with other studies that used the GaitRite, as well as other objective methods to analyse gait, such as inertial measurement units worn on the trunk whilst walking (Dalton, Khalil, Busse, Rosser, van Deursen, *et al.*, 2013; Collett *et al.*, 2014; Danoudis and Iansek, 2014). To summarise, these findings suggest that gait variability is a common feature in HD. Furthermore, the use of objective measures to analyse gait parameters are likely to reveal more subtle changes in people with pre-manifest HD that cannot be detected using the UHDRS-TMS.

People with HD find it increasingly difficult to step as the disease progresses, whether that is going up and down the stairs or clearing an obstacle. This requires both strength and balance, which are features known to deteriorate in people with HD (Panzera *et al.*, 2011). Such deficits are reflected in stepping tasks in people with HD, as a previous study revealed that manifest subjects were generally slower performing a stepping task than healthy controls (Goldberg *et al.*, 2010). This slowness could be due to a problem automating movements, such as stepping, and/or a problem with timing control, both of which are regulated through basal ganglia thalamo-cortical motor circuits (Kim and Hikosaka, 2015; Dudman and Krakauer, 2016) and could explain why people with HD have problems performing such tasks. Stepping is reviewed in more detail in Chapter 2 (page 58).

The *Sit to stand* (Rikli and Jones, 1999) and the *Timed up and go* (TUG) (Dorman, 2009) both require lower limb strength to stand up and sit down to and from a sitting position. The difference between the two tests being, the *sit to stand* requires the subject to repeatedly stand up and sit down from a chair over a set period of time, whereas the TUG is timed, and requires the subject to start by sitting, walk 3 metres, turn and return back to the chair, finishing by sitting. The TUG appears to be used more often than S*it to Stand*. This may be because floor effects have been reported in the *Sit to Stand* in a previous study (Khalil *et al.*, 2010), and could be because, similar to the *Sit to Stand,* the TUG includes sitting and standing as well as a walk and turn. A previous study revealed that performance in the TUG deteriorated with HD disease progression, where it took longer for people to complete the task in people with more advanced disease stages compared to healthy controls and those in early disease stages (Rao *et al.*, 2009; Quinn *et al.*, 2013). This suggests that the multiple constructs required to perform the standing, sitting and turn required in the TUG is sensitive to disease progression. This is described in more detail in Chapter 2 (page 57).

Assessing upper limb function in HD

Performance based outcome measures used for people with HD commonly focus on lower limb function (Quinn *et al.*, 2013; Busse *et al.*, 2014). However, another

important motor impairment seen in this population is the loss of upper limb dexterity, which can have a major impact when performing daily activities (Quinn, Busse and Dal Bello-Haas, 2013). The combination of the voluntary and involuntary symptoms associated with HD means could explain why people with HD have altered arm trajectories, grasp impairments, accuracy, problems with timing and hand rotation when compared to healthy controls (Quinn *et al.*, 2001; Fenney *et al,* 2008; Reilmann, *et al.*, 2010; Klein *et al.*, 2011a). Problems with upper limb movement is also observable in pre-manifest subjects, where this population were slower performing a speeded metronome finger tapping task (Bechtel *et al.*, 2010) and had more variable grip force (Rao, Gordon and Marder, 2011) compared to healthy controls. This demonstrates the importance of using objective, quantifiable instrumentation to capture subtle movement abnormalities that might not be captured when using subjective rating scales. Other upper limb assessments that have been used in people with HD are reviewed in Chapter 2 (Table 13). Given that many of these are limited due to the subjective rating scale, or because they were developed for another population, and so they do not capture all symptoms seen in HD, this suggests that an assessment that rates functional grasping and dexterity using quantitative feedback could provide a useful measure to assess upper limb function in people with HD.

Dual tasking

Dual or multitasking is the performance and synchronisation of multiple activities. There is no set definition for dual tasking, but it can be described as; dividing attention between two or more tasks performed simultaneously with distinct, dissociable goals (McIsaac, Lamberg and Muratori, 2015). Examples of dual tasking include, walking whilst texting, or driving whilst talking, with the main purpose being to be maximise efficiency. Furthermore, the type of tasks combined as well as the task complexity will impact performance, which is exacerbated in people with a neurological disorder such as HD (Delval, Krystkowiak, Delliaux, Dujardin, *et al.*, 2008; McIsaac, Lamberg and Muratori, 2015; Vaportzis *et al*, 2015b). In many cases, for people with no neurological disorder, one task becomes automated, such as walking or balance, freeing the cortical regions to attend to the secondary task (Saling and Phillips, 2007). To our knowledge, twelve

previous studies have investigated dual task performance in HD, which are presented in Table 2. These consistently reveal that dual task ability is compromised in people with HD, leading to deterioration in one or both tasks performed, which is known as 'dual task interference' (McIsaac, Lamberg and Muratori, 2015). One explanation for increased dual task interference in HD patients compared to controls is that automatic tasks could become attention demanding, resulting in a demand for greater cognitive resources (Saling and Phillips, 2007; Kelly, Eusterbrock and Shumway-Cook, 2012). In a previous study, walking was combined with a secondary motor task (carrying four glasses on a tray) or a cognitive task (counting backwards) (Delval, Krystkowiak, Delliaux, Dujardin, *et al.*, 2008). This revealed that gait parameters including; walking speed, cadence and stride length, reduced in both dual tasks compared to baseline, whereas no dual task interference was observed in healthy controls. Furthermore, people with HD were even more impaired in the motor-cognitive task (walking whilst counting backwards) than the motor-motor dual task (walking whilst carrying a tray), suggesting that motor-cognitive tasks might be more attentionally demanding than motor-motor tasks. Another study revealed no difference in a motor-cognitive task, which used a zig-zag drawing test combined with a simple cognitive task (Georgiou *et al.*, 1997). This suggests that the complexity of the secondary cognitive task could play an important role when designing dual task assessments and could have an affect on dual task interference (McIsaac, Lamberg and Muratori, 2015).

Multiple theories have been developed to explain performance deterioration in attentional demanding tasks. The first was the *bottleneck theory*, which proposed that in a given scenario where multiple stimuli are presented, information serially enters the brain (Broadbent, 1966). This is held long enough that a primary stimulus is selected and attended to, whilst the others are filtered out, and suggests that stimuli never run in parallel, meaning a performance decrement always occurs. However, although dual task interference is common, previous studies reveal that this is not always the case (reviewed in: Saling and Phillips, 2007). The *limited capacity theory* resolves this problem as it suggests there is an attention pool with limited capacity for processing information (Kahneman, 1973). Therefore, attention can be allocated to one complex task or divided between multiple tasks concurrently. If the tasks do not require the full capacity, both are

performed simultaneously and do not result in dual task interference. The *multiple resources theory* is more task dependent and postulates that there are multiple resource pools each with a limited capacity (Wickens, 2002). Thus, a motor-motor dual task will consume attention from the same pool and will theoretically be more difficult than for instance an auditory-visual task or a cognitive-motor task, which would require attention from different pools. However, using the study previously described by Delval and colleagues as an example; the motor-cognitive task resulted in greater dual task interference than motor-motor (Delval, Krystkowiak, Delliaux, Dujardin, *et al.*, 2008), suggesting that motor-cognitive modalities are not completely separate (Vaportzis *et al.*, 2014).

The problem with these theories is that they do not explain skilled motor or automatic behaviours. These are often performed faster than tasks that require information processing (Saling and Phillips, 2007). As dual tasking does not always result in increased neural activity, it suggests that automaticity may employ a different neural network which, given that the basal ganglia is implicated in automatic, habitual tasks, suggests that it could be involved in automating aspects of dual task performance (Saling and Phillips, 2007; Ashby, Turner and Horvitz, 2010; Kim and Hikosaka, 2015). Therefore, it may be that with increasing basal ganglia circuit degeneration, the difficulties in multi-tasking that are experienced by people with HD may be two-fold. Not only do they have a limited attentional capacity, but they may also have difficulty carrying out simple, automatic tasks, such as walking whilst talking.

Table 2: Dual task studies performed in HD to date

Study reference	Dual task testing domains	Dual task method	Population tested	Main findings
(Brown, Jahanshahi and Marsden, 1993)	motor-motor	Subjects had to tap a button with one finger and perform the Perdue pegboard test with the other	People with all stages of HD: n=6 (a mean of 4-7 years of disease duration) and n=12 healthy controls, n=7 people with PD, n=7 people with cerebellar degeneration	The HD group was significantly slower than controls performing the pegboard test and tapping in the dual task. There was no significant difference between the disease groups although people with HD generally performed better than the PD group and people with cerebellar degeneration.
(Dujardin *et al.*, 2013)	visual-acoustic task (and a motor element)	Subjects had to press a button on a keyboard when four 'x' that were superimposed on a 16 dot matrix, made a square. At the same time subjects listened to high and low pitched alternating tones. If the tone was the same pitch as the previous then the subject had to react by pressing a button on a keyboard	People with all stages of HD: n=20 (the average duration of disease 3.8 ± 0.62) and n=27 healthy controls	The HD group made significantly more errors and omissions than controls in both baseline and the dual task condition.

(Georgiou *et al.*, 1997)	motor-cognitive	Subjects had to draw zig-zags to markers. The markers were printed close together (small zig zags) or far apart (large zig zags). They did this with and without performing a digit span task	12 people with HD with a mean duration of 3.12 (SD, 2.58) and TFC stage 1-3. 12 age matched controls	HD subjects were generally slower drawing longer zig-zag strokes and were slower decelerating strokes in the baseline and dual tasks. The concurrent task was associated with shorter movement times compared to baseline in the HD group. More digit span errors were made in the HD group compared to controls, especially when drawing the longer strokes
(Müller *et al.*, 2002)	visual-acoustic task (and a motor element)	The same as dual task as Sprengelmeyer	People with mid stage HD: n=13 (average TFC 8.08 ± 0.89) and n=13 age matched controls	No baseline data. People with HD took significantly longer to respond and made significantly more errors and omissions compared to healthy controls in the dual task condition.
(Delval, Krystkowiak, Delliaux, Dujardin, *et al.*, 2008)	motor-cognitive and motor-motor	Walk with tray, and walk whilst counting backwards	Early to mid-stage HD: n=15 (TFC range 4-12) and n=15 healthy controls	The motor-cognitive dual task resulted in a greater performance decline than the motor-motor dual task
(Delval, Krystkowiak, Delliaux, Blatt, *et al.*, 2008)	motor-cognitive and motor-motor	Walking to a metronome whilst either carrying a tray or counting backwards	Early to mid-stage HD: n=15 (TFC range 9 ± 2.4) and n=15 healthy controls	There was a trend towards improvement when subjects walked to a metronome, although this was not significant.

(Mazzoni and Wexler, 2009)	auditory-motor	Subjects were asked to make dual explicit and dual implicit reaching actions. After hearing a tone, subjects used their finger to guide a cursor to a target in a location previously seen or reflected at a different angle.	Asymptomatic HD: (n=13) and n=13 healthy controls	People with HD were impaired in the dual task conditions compared to controls
(Thompson *et al.*, 2010)	motor-motor	Finger tapping using one and both hands to a metronome	Early-mid stage HD: n=14 (TFC stage 8.9 ± 1.9) and n=14 healthy controls	In the HD group there was greater tapping variability when two hands were used compared to one, whereas the opposite pattern was true for controls.
(Vaportzis *et al.*, 2014)	motor-cognitive	serial subtraction in 2s or 3s whilst circle tracing	All stages of HD: n=15 (range 3-13) and n=15 healthy controls	Simple tasks placed greater attentional demands on HD participants compared with controls.

(Vaportzis, Georgiou-Karistianis, Churchyard and Julie C. Stout, 2015)	auditory-motor	Subjects performed a letter cancellation task where they had to identify the letter 'O' amongst easy and hard distractor letters. Whilst doing this they were asked to count the number of high pitched tones presented on their own (easy) and with low pitched tones (hard)	Early-mid stage people with HD: n=14 (Mean TFC 10.1 ± 3.04) and n=14 ages matched healthy controls	The HD group were not significantly slower or less accurate compared to controls. Dual task costs were significantly greater in people with HD relative to controls in terms of time to complete the dual task. People with HD with greater cognitive impairment were significantly slower performing the hard dual task relative to the easy.
(Vaportzis, Georgiou-Karistianis, Churchyard and Julie C Stout, 2015)	cognitive-motor	Subjects performed an easy and a hard choice reaction time task (selecting 4 or 5 target letters over non-target letters) whilst reciting an easy and hard digit forward and digit backward task.	Early-mid stage HD: n=13 (Mean TFC 10.08 ± 3.17) and n=13 healthy controls.	The HD group were significantly slower than the control group in the dual task but not less accurate. In the hard dual task, people with HD were significantly less accurate than healthy controls.
(Fritz *et al.*, 2016)	motor-cognitive	Walk and talk. Subjects walked and recited the alphabet and every other letter of the alphabet	Early to mid-stage: n=32 (n=16 UHDRS-TMS<35 and n=16 UHDRS-TMS >35)	Gait speed declined under simple and complex dual task conditions. The simple walking whilst talking task correlated with the UHDRS-TMS but not the UHDRS-TFC. The opposite pattern was observed for the complex walking whilst talking task.

Neural regions implicated for dual task performance

The neural regions responsible for dual tasking is variable and seems to depend largely on the dual task paradigms employed (Wu and Hallett, 2008). The brain regions most commonly associated with dual task processing include the prefrontal cortex (PFC), specifically the anterior cingulate cortex and the infralimbic cortex, (Kondo, Osaka and Osaka, 2004; Manuscript, 2013; Ohsugi *et al.*, 2013; Watanabe and Funahashi, 2014), as well as the precuneus (Wenderoth *et al.*, 2005; Wu *et al.*, 2013), left inferior frontal sulcus (Swick, Ashley and Turken, 2008), and sub-regions of the cerebellum (Wu *et al.*, 2013). These studies have led to contrasting results, where some studies revealed that the regions required for single task performance were more strongly activated during dual task performance (Nijboer *et al.*, 2014; Chan, Kucyi and DeSouza, 2015), suggesting greater attentional load. Conversely, other studies revealed that regions required for single task performance were less activated during the dual task condition, which could suggest limited attentional resources (Just *et al.*, 2001; Chan, Kucyi and DeSouza, 2015).

Models of basal ganglia thalamo-cortical circuitry suggest that cortico-striatal cross talk could influence dual tasking. In the healthy brain, the prefrontal cortex and the basal ganglia closely interact (Leisman, Braun-Benjamin and Melillo, 2014). Thus, one explanation for difficulty in dual tasking in people with HD could be because the anatomical and functional connections between such brain regions become dysfunctional and degenerate (Cepeda *et al.*, 2007), meaning cross talk is limited and resulting in reduced attentional capacity. Furthermore, reported deficits in resource allocation (Georgiou *et al.*, 1997), attentional set shifting (Lawrence *et al.*, 1998) and fixed, automatic behaviours (Thompson *et al.*, 2010) are likely to affect performance when carrying out multiple tasks simultaneously, which also to some extent all require basal ganglia function.

Anatomically, automatic behaviours result in reduced neural activity between brain regions involved in task performance, tighter connections and greater efficiency between these regions (Wu and Hallett, 2008). Automatic (also referred to as 'habitual') behaviour is a dynamic process known to depend on basal ganglia circuitry and in particular the striatum (Ashby, Turner and Horvitz, 2010). Automatic behaviours emerge

from initial learning of planned actions in response to a stimulus. With repetition, this results in an ingrained memory that has potential to dominate other alternative outcomes. In a series of pioneering pre-clinical studies that were performed in lesioned rats, Yin *et al* identified regions of the striatum specifically involved in the management of goal directed (action-outcome) responses and habit (i.e. automatic) formation (Yin, Knowlton and Balleine, 2004). They found that the neural processes utilized could be manipulated from learned outcome value, showing that rats with lesions to the dorsolateral striatum were unable to form habitual behaviour, but maintained goal directed behaviour (Yin et al. 2004; Yin, Knowlton, et al. 2005; Yin, Ostlund, et al. 2005). To summarise, this suggests that intact striatal function could be required to form habitual (i.e. well –practiced, automatic tasks), that require relatively little attentional resources. As this is mediated by the basal ganglia, this could lead to increased dual task interference in people with HD compared to healthy controls (Yogev-Seligmann, Hausdorff and Giladi, 2012; Wu *et al.*, 2013)

1.5 Thesis aims, objectives and hypothesis

The overall aim of the studies presented in this thesis were to develop and evaluate outcome measures that target the neurobiology that is affected in Huntington's disease. Although numerous outcome measures are available, they are limited as they do not capture people with all stages of disease. Furthermore, as questionnaires are commonly used to measure functional changes in this population i.e. behaviours that translate to daily activities, performance based functional outcome measures are required to directly test behaviours that involve basal ganglia circuitry. This is particularly important to assess the effectiveness of therapies that directly target the basal ganglia, such as cell transplantation. For this reason, it is also important that outcome measures used in pre-clinical models tap into the circuitry affected in HD so Phase 1 clinical trials can be accurately assessed.

In this thesis, the first objective was to develop and assess dual task paradigms for use in people with HD. Dual task paradigms were selected due to their high ecological validity, but also because people with HD are deficient when performing two tasks simultaneously, and, this could be due to a breakdown in cortico-striatal cross talk as HD progresses. A second objective was to evaluate the performance of rats in a rodent analogue of the Stroop task. Although the striatum has been implicated for successful performance in the human Stroop task, whether striatal regions were required to perform aspects of the same task in rodents is currently unknown.

Chapter 2

Optimising dual task assessments in Huntington's disease

Chapter Summary

Current functional assessments for people with HD are limited. These are either questionnaire based, or they were developed for another disease population, which makes them insensitive to people with HD and difficult to track change over time. The aim of the experiments in this Chapter was to develop a functional, performance based assessment for people with HD, which consisted of behavioural tasks that involve the basal ganglia circuitry, and more specifically the striatum. Such tasks are particularly important when assessing disease progression, but also to assess the effectiveness of potential therapeutics that directly target the striatum, such as cell transplantation. Given that people with HD have difficulties performing more than one task simultaneously (See Chapter 1, page 41), and striatal involvement is implicated in such tasks, a range of dual task assessments were identified, developed and tested in people gene positive with HD. The dual tasks in Part 1 of this study targeted lower limb function. Since current upper limb assessments are not applicable to a disease population with a broad phenotype, the study in Part 2 involved the design and development of a new upper limb, dual task assessment.

2.1 Introduction

There are currently no disease modifying treatments for people with HD; however, advances in regenerative medicine and drug trials are rapidly changing prospects. In anticipation of impending clinical trials, there is an imperative for well-defined clinical endpoints (outcome measures). Such assessments are required to detect symptom change over time following an intervention. Using cell transplantation as an example, the main aim is to reconnect lost basal ganglia circuitry anatomically and restore lost functional connectivity. Where neural circuitry is restored this should be reflected in functional improvement in the patient's daily life. However, a major challenge is the lack of outcome measures that can be used to measure the extent of graft functionality and the resulting impact on daily function. From here on in, when referring to function it is important to clarify this is in relation to activities of daily living. There is no one definition for function. Previous definitions include "those activities identified by an individual as essential to support physical, social, and psychological well-being and to create a personal sense of meaningful living" and "any movement at the level of the person that is task related, goal oriented, environmentally germane and involves the integration of multiple body systems and structures." (Reiman and Manske, 2009).

A list of recommended outcome measures for the HD population was previously established by The National Institute for Neurological Disorder and Stroke (NINDS) (Grinnon *et al.*, 2012). This was important to improve and promote data quality and sharing in research and clinical practice. Through this work, it became apparent that all functional outcome measures were questionnaire based. Such questionnaires are often rated on an ordinal scale, which makes them vulnerable to observer bias and reduced sensitivity. Thus, to sensitively track change over time, it is important that scoring methods are consistent across raters (Hobart, Freeman and Thompson, 2000), which can be difficult using descriptive, ordinal scoring methods as it requires ongoing rater training. In addition, self-reported questionnaires are not always a true reflection of people with HD and their capabilities, given that this population are not always

aware of the extent of their symptoms (Snowden *et al.*, 1998; Hoth *et al.*, 2007; Sitek *et al.*, 2013; McCusker and Loy, 2014). In a previous study, symptoms reported by people with HD significantly differed from gene negative companions in motor, cognitive and emotional control (Hoth *et al.*, 2007). These findings also supported another study that revealed no relationship between self-reported motor and cognitive function, and objective outcomes used to assess these domains. To overcome this problem, performance based functional outcome measures can be used. This includes assessments such as the *Physical performance test,* which consists of 9 items which relate to daily activities, such as '*putting on a coat'* and *'standing balance'* (described in Chapter 1, page 35; Mathias et al., 1986; Reuben and Siu, 1990). However, as this test was originally developed for the elderly, it means that some of the assessment items are not applicable for a broad disease phenotype, such as HD (Quinn et al., 2013). Although the *Physical performance test* is quantitatively assessed using a time based measure, time ranges are formed into categories that are not applicable for people with HD, meaning sensitive data is lost. There is also a general assumption that faster performance, in assessment items is better, which for HD is not always the case. For instance, a previous study revealed that people with a greater UHDRS total motor score (UHDRS-TMS) (indicative of greater motor impairment) performed the Timed up and Go (this involves standing from sitting, walk 3 metres and back), faster than those with a lower UHDRS-TMS (Quinn et al., 2013). This suggests that precipitous movement caused by involuntary deficits may aid speed in some cases, leading to a faster performance time. As quality and accuracy of the movement is not reflected when time is the only rating measure, this suggests a score combining time with test accuracy may better reflect performance.

The next question is how best to develop paradigms that measure functional improvement following complex interventions such as cell transplantation. Given that the expectation is that the graft will restore functional striatal pallidal connections with subsequent restoration of connectivity in frontostriatal loops, it is important to understand the behaviours attributed to this circuitry. For instance, intact frontostriatal circuitry is required when two behaviours are required to run in parallel, so called "multi-tasking" (Bloem *et al.*, 2006; Yogev-Seligmann, Hausdorff and Giladi,

2008; Isoda and Hikosaka, 2011). The ability to dual task or multitask can be defined as the simultaneous performance of two or more activities with distinct goals (McIsaac, Lamberg and Muratori, 2015). This ability is important to maintain a functional and an independent lifestyle (Foley, Kaschel and Sala, 2013). Previous reports suggest that dual tasking is a common difficulty in people with HD in day to day life (Craufurd and Snowden, 2002), which often results in decreased performance in one or both tasks. (Delval, Krystkowiak, Delliaux, Dujardin, *et al.*, 2008; Thompson *et al.*, 2010; Vaportzis *et al.*, 2014; Fritz *et al.*, 2016) and is also associated with increased risk of falling (Grimbergen *et al.*, 2008). This suggests that dual tasking may be a useful means by which to assess function and track disease progression in HD.

Optimal dual task performance is achieved if one behaviour can be automated, whilst the other requires attention, such as walking whilst talking. This allows familiar behaviours to be performed with minimal attention, freeing limited attentional resources for other tasks (Thompson *et al.*, 2010). In many ways, this makes life more efficient when performing routine activities. Typically, people with HD perform tasks in a serial, goal directed fashion which is slower and not automatic, and limits attentional resources that could otherwise allocated to other tasks (Bloem *et al.*, 2006; Redgrave *et al.*, 2010; Thompson *et al.*, 2010). Furthermore, performance deterioration is even more obvious when the secondary task is more complex (Vaportzis *et al.*, 2014), suggesting that increased cognitive load may stress cortico-basal ganglia circuitry further, resulting in performance decline (Fritz *et al.*, 2016). This decline is known as dual task interference, and is calculated by measuring the percentage change from baseline to dual task performance (known as dual task cost) (Friedman *et al.*, 1982; Fritz *et al.*, 2016). As well as the complexity of the secondary task being an important factor in dual task development, a previous study revealed that motor-cognitive dual tasks (walking whilst performing mental arithmetic) resulted in greater dual task interference compared to motor-motor dual tasks (walking whilst carrying a tray (Delval, Krystkowiak, Delliaux, Dujardin, *et al.*, 2008). This suggests that the neurodegeneration in HD may be more sensitive to motor-cognitive performance compared to motor-motor.

A limitation in previous dual task studies is that people with HD were not grouped based on disease stage, but instead by pre-manifest and manifest HD (Delval, Krystkowiak, Delliaux, Blatt, *et al.*, 2008; Delval, Krystkowiak, Delliaux, Dujardin, *et al.*, 2008; Thompson *et al.*, 2010; Vaportzis *et al.*, 2014; Vaportzis, Georgiou-Karistianis, Churchyard and Stout, 2015). As a result, whether or not people with different stages of manifest HD have different coping strategies when performing dual tasks is unknown. Therefore, the aim of this study was to select, develop and evaluate motor-cognitive dual task assessments that were sensitive to the deficits associated with all stages of HD and could be used as a functional outcome measure for HD in the future.

Using the clinometric table presented in Chapter 1 (Table 1), the following criteria for each dual task assessment were:

- sensitive to the functional deficits seen in HD
- no longer than 10-15 minutes to carry out
- easy to administer
- Measureable using time and/or other quantified method

This chapter includes findings from two clinical studies. Part 1 describes the rationale and testing of three lower limb dual task assessments. Part 2, was stimulated by the thinking that developed whilst running Part 1, but was also largely performed in parallel. It describes the design, development and testing of a new upper limb dual task assessment. The study was conducted as part of one protocol in the same setting. Therefore, the methodology is described in detail in Part 1 and referred to where necessary in Part 2. A discussion is presented following Part 1 and Part 2, with final conclusions and future work presented at the end of the chapter.

2.2 Part 1

The aim of this study was to select and develop motor-cognitive dual task assessments, sensitive to the deficits associated with HD. These were then tested in a cohort of people gene positive with HD.

2.2.1 Rationale and study design

Analysis of the literature for dual tasks previously used in people with HD (Chapter 1: Table 1) revealed that the only the only lower limb motor task previously tested in HD is walking (Delval, Krystkowiak, Delliaux, Blatt, *et al.*, 2008; Delval, Krystkowiak, Delliaux, Dujardin, *et al.*, 2008; Fritz *et al.*, 2016). For the cognitive components, secondary tasks previously tested include; serial subtractions (Delval, Krystkowiak, Delliaux, Blatt, *et al.*, 2008; Delval, Krystkowiak, Delliaux, Dujardin, *et al.*, 2008; Vaportzis *et al.*, 2014), digit span (Georgiou *et al.*, 1997; Vaportzis, Georgiou-Karistianis, Churchyard and Stout, 2015) and reciting the alphabet (Fritz *et al.*, 2016). As the specific motor vs cognitive tasks used can lead to different amounts of dual task interference (Carmela *et al.*, 2017), the next stage was to develop several different motor-cognitive dual tasks to see which best distinguished between disease stage. One dual task was taken from the literature (*Walk and talk*); the other two (*Timed up and go and letter verbal fluency (TUG and LVF), and the Step and Stroop*) combined two existing tasks to form a new dual task.

2.2.1.1 Selection of motor and cognitive items for the dual tasks

Three motor-cognitive dual tasks were taken forward for testing in people with HD. An explanation as to why each dual task was selected/developed is given below. The dual task procedure for each test can be located in the Methods (Section 2.2.2.4).

2.2.1.2 Walk and Talk

The *Walk and talk* is an established dual task that requires a participant to walk whilst reciting the alphabet (simple) or every other letter of the alphabet (complex) (Verghese *et al.*, 2002). This was previously tested in 32 people with early to mid-stage HD, and revealed that performance in both tasks decreased in the dual task condition compared to baseline, suggesting mutual interference (Fritz *et al.*, 2016). As they measured time taken to walk 40 feet and back, another more sensitive method to analyse gait is motion analysis or the GaitRite electronic walkway. These were previously validated in HD and revealed that people with HD walked slower and had decreased, velocity and cadence and increased stride variability and duration of double limb support compared to controls (Hausdorff *et al.*, 1998; Delval, Krystkowiak, Delliaux, Blatt, *et al.*, 2008; Delval, Krystkowiak, Delliaux, Dujardin, *et al.*, 2008; Rao *et al.*, 2008). Furthermore, motion analysis revealed that these parameters got worse when walking was combined with a secondary task (Delval, Krystkowiak, Delliaux, Blatt, *et al.*, 2008; Delval, Krystkowiak, Delliaux, Dujardin, *et al.*, 2008). As the only gait measure in the previous *Walk and talk* test was time, it is possible other gait parameters changed in the dual task but were undetected. Therefore, in this study we used the same *Walk and talk* employed by (Fritz *et al.*, 2016), but used the GaitRite system to see if additional gait parameters changed in the dual task condition.

2.2.1.3 The Timed Up and Go and Letter Verbal Fluency

The *Timed Up and Go* (TUG) and the L*etter Verbal Fluency* (LVF) are two well established outcome measures that are regularly used in people with HD. These were previously combined to form a dual task and tested in the elderly (van Iersel, 2007). However, the *TUG and LVF* dual task has never been tested in people with HD.

The TUG is a measure of dynamic and static balance (Christofoletti *et al.*, 2014), which times participants to stand from sitting position, walk 3 metres, turn around a cone and finish by sitting. This outcome measure has been well validated in people with HD (Busse, Wiles and Rosser, 2009; Busse *et al.*, 2014) and, along with the *Sit to Stand test*

(the participant stands from sitting repeatedly over 30 seconds), these form the only performance based functional assessments tested in ENROLL-HD (See Chapter 1, page 12 for information on the ENROLL-HD study). Similar to the *Sit to Stand,* the TUG consists of a standing and sitting element, but it also includes a walk and a turn. As the TUG relates to a range of motor tasks that are commonly performed on a daily basis (sit, standing, walking and turning), and as it consistently correlates with UHDRS measures (Quinn *et al.*, 2013; Busse *et al.*, 2014), whereas the *Sit to Stand* does not (Khalil *et al.*, 2010), the TUG was selected over the *Sit to Stand* to form the motor item of a dual task.

Letter verbal fluency (LVF) tasks involve recalling words that begin with a given letter, and are considered an executive function task (Shao *et al.*, 2014). The participant must supress the tendency to say names of people or places, whilst also using working memory so that previously said words are not repeated. This outcome measure forms one of the UHDRS cognitive assessments and is used in the ENROLL-HD, along with categorical verbal fluency. In categorical verbal fluency, the participant is asked to say as many words as possible that relate to a given category, such as animals. Arguably, the latter assessment may better relate to functional tasks required on a daily basis, such as forming a shopping list. However, it is understood that the LVF may tap more into the frontostriatal circuitry whereas performance in categorical tasks requires more temporal lobe function (Rosser and Hodges, 1994; Ho *et al.*, 2002). Due to the direct neuroanatomical relevance to HD, the LVF was selected for this study and was combined with the TUG.

2.2.1.4 The Step and Stroop

The *Step and Stroop* are two individual tests, which for this study were combined to form a new dual task. To our knowledge, this has never been tested in any population.

The ability to step is an important function of daily living, from avoiding obstacles to going up a flight of stairs. A previous study, tested the ability of fourteen symptomatic people with HD to step as quickly as possible from one sensored foot pad to another following an auditory cue (Goldberg *et al.*, 2010). This revealed that people with HD

reacted significantly slower than healthy controls. Although this could suggest that stepping is a problem for this population, stepping ability is not currently assessed in the UHDRS or in ENROLL-HD. Stepping is however tested in one item of the *Physical Performance test*, which requires participants to climb a flight of stairs (Reuben and Siu, 1990). However, as previously mentioned, the *Physical Performance test* was developed for the elderly meaning that the categorical time ranges used to assess performance are not sensitive to people with HD. In addition, climbing a flight of stairs is not always possible in a clinical setting, meaning it is difficult to assess. As stepping on the spot requires little clinical space, stepping onto and off an aerobic step was selected to form the motor component of a dual task.

The Stroop task is often employed to test attention and forms part of the UHDRS cognitive battery (Kieburtz, 1996). This requires participants to read colour words presented on paper written in their coloured ink (e.g. BLUE in the colour blue) or say the coloured ink of colour words which are incongruent (e.g. BLUE in the colour red) (Bullard *et al.*, 2012). The Stroop task is one of the few cognitive tasks that can measure cognitive decline in HD and significantly correlates with striatal degeneration (Sanchez-Pernaute *et al.*, 2000). To see how participants responded to the Stroop task when combined with a motor test, the Step and Stroop was developed. The Stroop task is traditionally performed whilst sitting and presented on a piece of paper. However, this presentation of the Stroop task would restrict its use with a lower limb motor task. Therefore, the Stroop task in this study was shown as a PowerPoint presentation on a laptop. This provided a display which could be reliably viewed by the patient whilst they performed the stepping task.

2.2.2 Methods

2.2.2.1 Design

This was an observational, cross sectional study. People positive for the HD gene, were recruited to perform a maximum of three dual task assessments. Initial evaluation of the dual task results revealed the *Step and Stroop* to be most sensitive to disease stage and so this was tested in a larger cohort of patients.

2.2.2.2 Setting

People with HD were recruited from the South Wales research and management clinic. Recruitment began in January 2015 and continued to January 2017. Ethical approval was obtained from South East Wales Research Ethics Committee (REC reference: 14/WA/1195). Many patients attending the HD clinic were already enrolled in the ENROLL-HD study (04//WSE05/89), and so their disease progression had been followed longitudinally, some for several years. One of the optional components within the ENROLL-HD study was permission by participants to be contacted about other additional and affiliated HD research projects. In consenting to be enrolled in the ENROLL-HD study, participants also gave their permission for their unidentifiable data to be accessed by researchers conducting other HD related research.

Potential participants were approached at the beginning of clinic, and, if interested, were given an information sheet on the study. Participants were given as much time as needed to decide if they wanted to take part. If willing, participants signed a consent form prior to participation, and also had the option to take part in the study at a future clinic visit.

2.2.2.3 Participants

Participant data recruited for this study are presented in the Results of this chapter (page 68) and the number of people that performed each dual task item is presented in Appendix 1.

Inclusion criteria

1) Must be confirmed to carry the HD gene through genetic testing
2) Must be 18 years or above
3) Must be enrolled in the Registry/ENROLL-HD study

Exclusion criteria

1) The inability to approve consent
2) Comorbid condition that has the potential to confound the results of the study

2.2.2.4 Dual task procedures

A brief description of the *Step and Stroop*, the *TUG and LVF* and the *Walk and talk* is described below. Each dual task assessment consisted of assessment items. A full description of the testing procedures can be found in Appendix 2. Following initial observation of dual task performance, a performance threshold was set for each assessment item so participants had to 'pass' set criteria to proceed to the simple and complex dual tasks (Appendix 2).

Walk and talk

This consisted of 5 assessment items: walk baseline, alphabet simple baseline, alphabet complex baseline, *walk and talk* (alphabet) simple, *walk and talk* (alphabet) complex (Figure 7). Participants were first asked to walk for 30 seconds along a GaitRite mat. Next, participants were timed to recite the alphabet as quickly as possible (alphabet baseline simple) and then say every other letter of the alphabet (alphabet baseline complex). For the dual task, participants were asked to walk for 30 seconds whilst performing the alphabet simple (*Walk and talk* simple) and then the alphabet complex tasks (*Walk and talk* complex). Spatiotemporal parameters recorded for each walking test related to speed (velocity (m/s), cadence(steps/min)), balance (duration of double limb support (s)) and stride variability (coefficient of variation for stride length) (Rao *et al.*, 2009). The number of correct letters said per second were calculated for each alphabet test.

Figure 7: Walk and talk setup. Participants walked up and down a GaitRite mat whilst reciting the alphabet (dual simple) and then every other letter of the alphabet (dual complex).

The TUG and LVF

This consisted of 5 assessment items: TUG baseline, LVF simple baseline, LVF complex baseline, *TUG and LVF* simple, *TUG and LVF* complex. Participants were timed to stand from sitting, walk 3 metres, turn around a cone and walk back, finishing by sitting in a chair (TUG baseline). Next participants were given 30 seconds to recite as many different words they could think of that began with R (LVF simple) and N (LVF complex). The simple and complex letters were selected according to (Fu et al., 2002), and differed from the letters used in the ENROLL-HD battery to reduce the chances of practice effects. Participants were asked not to repeat themselves or say the names of people or places. The dual tasks involved simultaneous performance of the *TUG and LVF* simple and LVF complex. The time taken to perform the TUG and the number of correct answers given were recorded.

The Step and Stroop

The *Step and Stroop* consisted of 6 assessment items: stepping baseline, colour and word reading test, Stroop simple baseline, Stroop complex baseline, *Step and Stroop* simple, *Step and Stroop* complex (Figure 8). Participants were first asked to step onto and off an aerobic step as quickly as possible for 45 seconds (step baseline). The colour and word reading test was performed next to ensure participants could distinguish between the colours and colour-words: yellow, pink and grey. Originally the traditional colours: red, blue and green were used in this assessment (Bullard *et al.*, 2012). However as these same colours are used as part of the ENROLL-HD battery, which the participant performed earlier that day, the colours were changed to yellow, pink and grey, to reduce the chances of practice effects. These have not been used in previous Stroop tasks, however they were selected as they are not colours that are sensitive to people that are colour blind.

For the Stroop simple, the participant had to name colour words, which were written in their coloured ink (e.g. the word PINK written in the colour pink; Stroop simple baseline). Participants were next asked to say the coloured ink of the words, which were incongruent to the colour-word (e.g. the word PINK written in the colour yellow). For the

dual tasks participants were asked to perform the simple and complex Stroop tasks whilst stepping for 45 seconds. The number of steps and/or correct answers were recorded for each test. The Stroop tasks were presented via a PowerPoint presentation positioned on a table in front of the participant. Four colour options were presented vertically on each slide. As soon as the participant gave their fourth answer, the researcher changed the slide using a USB pen revealing a new colour sequence.

Figure 8: Step and Stroop setup. Participants stepped up and down off the step whilst reading colour-words (Stroop simple) and the coloured ink of the words (Stroop complex) presented via a PowerPoint presentation.

2.2.2.5 Rating participant performance

To quantify participant performance, all assessments were video recorded, allowing an accurate timing measure. To calculate the dual task cost the following equation was used (Friedman *et al.*, 1982) :

$$\left(\frac{\text{The difference between baseline and dual task performance}}{\text{Baseline performance}} \right) \times 100 = \text{Dual task cost}$$

For example, if participants performed the TUG in 15 seconds in the TUG baseline and then took 20 seconds to perform the TUG in the *TUG and LVF* dual simple, the difference of 5 seconds was taken and divided by 15 (the TUG baseline), and then multiplied by 100. This would give 33.33% cost in the dual task relative to baseline performance. This percentage difference could also be negative if dual task performance was better than baseline.

2.2.2.6 Additional data collected

As part of the ENROLL-HD assessment, participants had already supplied demographic data, current medications, and were assessed on the UHDRS assessments, including the UHDRS-TMS, UHDRS-TFC, UHDRS Cognitive score, UHDRS Functional Assessment scale, UHDRS independence scale, Short Form-12 (SF-12) (See Section 1.3.2.5) and Problem Behaviours Assessment (PBA) (See Section 1.3.2.4). Performance in many of these assessments change little over a period of weeks, thus it was decided that these did not need to be repeated if the participant had been assessed for ENROLL-HD within six weeks.

2.2.2.7 Statistics

SPSS version 20 (PASW) (IBM Corporation, USA) was used to evaluate the results of the study.

The following variables were recorded for each dual task:

- *Walk and talk:* Velocity (m/s), cadence(steps/min), duration of double limb support (s) and coefficient of variation (CoV) for stride length (Rao *et al.*, 2009). The number of correct answers given per second for the simple and complex alphabet tasks.
- *TUG and LVF:* Time taken to perform the TUG and the number of correct answers given per second for the simple and complex LVF.
- *Step and Stroop:* The number of steps performed in 45 seconds and the number of correct answers given in 45 seconds.

Participant data were grouped in two ways: i) by group [pre-HD, TMS ≤ 5 and TFC = 13; Manifest, TMS>5 and TFC<12); ii) TFC disease stage (TFC scores 11–13 = stage 1 (earliest symptomatic stage); 7–10 = stage 2; 3–6 = stage 3; 1–2 = stage 4; 0 = stage 5 (most advanced stage). For the *TUG and LVF,* and the *Walk and talk*, stages 3 and 4 were combined (stage 3,4) due to the small sample sizes. Furthermore, for the *Step and Stroop,* stages 4 and 5 were combined again due to the small sample size recruited (stage 4,5).

All graphical data are presented as performance means and standard error of the mean (SEM). Performance results between Pre-HD and manifest groups were compared using an independent t-test, using time taken (TUG), the number of correct responses (LVF, Stroop tasks), alphabet rate, and walking parameters as dependent variables.

Comparisons between TFC stages were made for all dual task items using analysis of variance (ANOVA). TFC stage (stage 1, stage 2, s3,4) for the *TUG and LVF* and *Walk and talk* dual tasks, and (stage 1, stage 2, stage 3, s4,5) for the *Step and Stroop* were used as independent variables. Dependent variables were the same as those used for the t-test described in the above paragraph.

A two way repeated ANOVA was used to identify if there was an interaction between item performance (within subject factor) and TFC disease stage (between subject factor). A Bonferroni post-hoc was used to account for multiple comparisons if significance returned being $p < 0.05$.

A Pearson correlation was performed to identify the relationship between each dual task item for the *Walk and talk*, *TUG and LVF*, *Step and Stroop* with UHDRS assessment scores (UHDRS-TMS, TFC, functional assessment score, independence scale and cognitive measures from the UHDRS LVF, Stroop task and symbol digit), as well as the quality of life SF-12 mental and physical summaries, and the *Problem behaviour assessment* to measure apathy and executive function scores. The CAG disease burden score was also measured ($(CAG_n - 35.5)*Age$) (Penney *et al.*, 1997) as an indicator of disease progression (See Section 1.1.7).

2.2.3 Results

2.2.3.1 Participants

Demographic data from participants that performed the first item of each dual task assessment is presented in Table 3. Sixty-six people with HD consented to take part in this study, from which twenty-eight people attempted all three dual task assessments. Thirty-one people attempted the *TUG and LVF*, from which seventeen completed the dual task assessment. Thirty-one people attempted the *Walk and talk* from which eighteen completed the assessment. Fifty-four people attempted the *Step and Stroop* from which forty eight people completed the assessment. Because of the set criteria developed for each dual task, a different number of people completed each assessment item. The number of people that completed each of these is presented in Appendix 1.

For the *TUG and LVF*, and *Walk and Talk* people in stage 3 and 4 were combined given the limited number of people recruited in stage 4 (n=1). No people in stage 5 were recruited. Final TFC disease stage groups for the *TUG and LVF*, and *Walk and Talk* were stage 1, stage 2, stage 3,4. A larger cohort of people with HD were recruited for the *Step and Stroop* and therefore more people in stage 4 were recruited (n=3). Data from people in stage 4 and 5 were combined given the limited number of people recruited in stage 5 (n=1). Final TFC disease stage groups for the *Step and Stroop* were stage 1, stage 2, stage 3, stage 4, 5.

There was a significant age difference in the Pre-HD and Manifest group for the *Step and Stroop,* and also between TFC groups for the *Walk and talk, TUG and LVF* and the *Step and Stroop* (analysis presented in Table 3). The number of years in education was significantly different between people in stage 3 and stage 4,5 for the *Step and Stroop,* but not for any other dual task assessment. Body mass index significantly differed between stages in the *Step and Stroop,* but for no other dual task assessment.

Table 3: Chapter 2, Part 1: Dual task demographic information

		Pre-HD	Manifest	Group differences	TFC disease stage				Group differences
					1	2	3,4	4,5	
Walk and talk	**n (male:female)**	n=4 (3:1)	n=27 (15:12)	/	n=13 (8:5)	n=5 (2:3)	n=15 (9:6)		/
	Age	38.25 (8.54)	53.15 (15.44)	t (29) = -1.982, p = n.s.	39.08 (9.22)	52.8 (15.39)	61.07 (11.19)		F (2,28) = 13.77, p < 0.001 (s1 vs s3 and s2)
	TMS	0.75 (0.96)	48.48 (26.02)	t (30) = -3.542, p < 0.001	12.92 (12.12)	41.6 (7.09)	67.79 (18.12)		F (2,29) = 69.97, p < 0.001 (s1 vs s2 and s3; s2 vs s3)
	TFC	13 (0)	7.15 (3.92)	t (30) = 2.731, p < 0.01	12.5 (0.8)	8.4 (1.34)	3.79 (0.97)		F (2,29) = 307.52, p < 0.001 (s1 vs s2 and s3; s2 vs s3)
	Functional scale	25.00	16.96 (16.76)	t (29) = 2.253, p < 0.05	24.25 (1.14)	21.8 (0.45)	10.85 (3.46)		F (2,28) = 122.7, p < 0.001 (s3 vs s1 and s2)
	Independence scale	100.00	73.8 (18.16)	t (28) = 2.69, p < 0.05	96.25 (6.44)	80 (0)	59.23 (10.17)		F (2,27) = 76.78, p < 0.001 (s1 vs s2 and s3; s2 vs s3)
	CAG disease burden score	214.88 (45.86)	412.63 (84.39)	t (28) = -4.261, p < 0.001	300.21 (88.84)	405.2 (70.96)	458.42 (67.47)		F (2,27) = 16.12, p < 0.001 (s1 vs s3)
	Years of education	13 (1)	12.72 (4.27)	t (29) = 1.862, p = n.s.	11.89 (3.44)	14 (5.66)	13.3 (4.37)		F (2,28) = 0.13, p = n.s.
	BMI	32.34 (8.24)	26.73 (4.89)	t (29) = 1.838, p = n.s.	29.52 (6.37)	24.81 (6.07)	26.84 (4.44)		F (2,28) = 1.42, p = n.s.
TUG and LVF	**n (male:female)**	n=4 (3:1)	n=26 (16:11)	/	n=12 (8:4)	n=5 (2:3)	n=14 (9:5)		/
	Age	38.25 (8.54)	53.56 (14.92)	t (29) = -1.982, p = n.s.	40 (8.66)	52.8 (19.29)	61.07 (11.19)		F (2,28) = 10.04, p < 0.001 (s1 vs s3)
	TMS	0.75 (0.96)	48.26 (26.33)	t (30) = -3.713, p < 0.001	12.42 (12.1)	41.6 (7.09)	67.79 (17.12)		F (2,29) = 67.92, p < 0.001 (s1 vs s2 and s3; s2 vs s3)
	TFC	13 (0)	7.22 (4.01)	t (30) = 2.844, p < 0.01	12.67 (0.65)	8.4 (1.34)	3.79 (0.97)		F (2,29) = 301.28, p < 0.001 (s1 vs s2 and s3; s2 vs s3)
	Functional scale	25.00	17.07 (6.87)	t (29) = 2.271, p < 0.05	24.5 (0.9)	21.8 (0.45)	10.85 (3.46)		F (2,28) = 127.11, p < 0.001 (s1 vs s3; s2 vs s3)
	Independence scale	100.00	74.2 (18.63)	t (28) = 2.764, p < 0.01	97.08 (6.2)	80 (0)	59.23 (10.17)		F (2,27) = 78.2, p < 0.001 (s1 vs s2 and s3; s2 vs s3)
	CAG disease burden score	214.88 (45.86)	413.58 (84.85)	t (28) = -4.268, p < 0.001	286.83 (80.8)	405.2 (70.96)	458.42 (67.47)		F (2,27) = 19.53, p < 0.001 (s1 vs s2 and s3)
	Years of education	13 (1)	12.94 (3.99)	t (29) = 0.371, p = n.s.	12.33 (2.78)	14 (5.66)	13.3 (4.37)		F (2,28) = 0.502, p = n.s.
	BMI	32.34 (8.24)	26.48 (4.73)	t (29) = 1.838, p = n.s.	29.01 (6.34)	24.81 (6.07)	26.84 (4.44)		F (2,28) = 1.191, p = n.s.
Step and Stroop	**n (male:female)**	n=8 (6:2)	n=49 (30:19)	/	n=22 (14:8)	n=14 (8:6)	n=18 (13:5)	n=3 (1:2)	/
	Age	35.88 (6.33)	53.11 (14.23)	t (54) = -3.284, p < 0.01	35.67 (11.11)	40.69 (9.43)	58.25 (15.14	55.11 (12.84)	F (3,52) = 6.147, p < 0.001 (s1 vs s2 and s3)
	TMS	1.13(1.13)	44.56 (25.26)	t (54) = -4.7, p < 0.001	11.04 (10.56)	42.92 (15.83)	59.67 (22.55)	65.67 (13.32)	F (3,52) = 28.63, p < 0.001 (s1 vs s2,s3,s4,5 and s2 vs s3)
	TFC	12.88 (0.35)	7.43 (3.82)	t (54) = 3.828, p < 0.001	12.68 (0.64)	8.53 (1.33)	4.44 (1.15)	1.33 (1.15)	F (3,52) = 247.74, p < 0.001 (all stages s.d.)
	Functional scale	24.88 (0.35)	17.78 (6.18)	t (52) = 3.113, p < 0.01	24.64 (0.73)	20.46 (3.15)	12.13 3.74)	14 (1.73)	F (3,50) = 72, p < 0.001 (all stages s.d. except s3 vs s4)
	Independence scale	99.38 (1.77)	76.98 (16.62)	t (50) = 3.651, p < 0.001	97.27 (6.12)	79.17 (8.48)	65 (10.64)	60 (14.14)	F (3,48) = 45.1, p < 0.001 (all stages s.d. except s3 vs s4)
	CAG disease burden score	225.06 (42.93)	397.86 (88.36)	t (52) = -5.563, p < 0.001	294 (83.96)	406.67 (75.41)	446.47 (84.22)	440.33 (10.6)	F (3,50) = 12.49, p < 0.001 (s1 vs s2,s3,s4,5)
	Years of education	13.14 (2.79)	11.69 (3.44)	t (42) = 0.79, p = n.s.	12.74 (2.51)	11.11 (2.62)	13.38 (3.93)	7.33 (5.69)	F (3,40) = 3.39, p < 0.05 (s3 vs s4,5)
	BMI	30.86 (6.01)	26.89 (5.37)	t (48) = 2.441, p < 0.05	27.79 (5.58)	26.54 (4.32)	26.57 (4.77)	29.1 (5.37)	F (3,52) = 28.63, p < 0.001 (all stages s.d. except s4 vs s3 and s2)

All values are shown mean (Standard deviation) for those recruited in the baseline motor dual tasks. Groups were categorised as pre-HD (total functional capacity (TFC) = 13, Unified Huntington's disease total motor score (TMS) <5), and manifest (all symptomatic stages). For the TUG and LVF and walk and talk, the manifest group was further delineated by TFC disease stage (stage 1, TFC =11-13; stage 2, TFC=7-10; stage 3,4, TFC=1-6. For the Step and Stroop manifest groups were split so (stage 1, TFC =11-13; stage 2, TFC=7-10; stage 3, TFC=3-6; stage 4,5, TFC=0-2). A t-test was used to compare groups differences between pre-HD with manifest subjects, where the t (degrees of freedom) = t value. An ANOVA was used to compare TFC group differences, where F (error, degrees of freedom) =F value. A Bonferroni post-hoc was used if significance was met, where p < 0.05. Demographic data was missing from a maximum of two participants in the Walk and talk, and the TUG and LVF. Data was missing from a maximum of twelve participants in the Step and Stroop. TFC = Total Functional Capacity; TMS= total motor score; BMI = body mass index.

2.2.3.2 Walk and talk

For clarity, a reference of the *Walk and talk* assessment items are presented in *Table 4*:

Table 4: Walk and talk assessment procedure

	Test order	Dual task item	Brief procedure
Walk and talk	1	Walk baseline	Walk for 30 seconds
	2	Alphabet baseline simple	Recite the alphabet
	3	Alphabet baseline complex	Recite every other letter of the alphabet
	4	Walk and talk simple	Walk whilst reciting the alphabet simple (30 seconds)
	5	Walk and talk complex	Walk whilst reciting the alphabet complex (30 seconds)

Measures reported include the velocity, cadence, duration of double limb support and the stride length CoV for items 1, 4 and 5; the number of correct letters of the alphabet recited per second for items 2-5.

Walk and talk performance scores and statistical outcomes between Group and TFC disease stage are presented in Table 5.

The results revealed no significant difference between pre-HD and manifest participants for any walking measure during the baseline or dual simple and dual complex tasks. For the alphabet tests, pre-HD participants recited significantly more correct letters per second than manifest in the alphabet complex baseline but not for the dual complex, dual simple or baseline simple tasks.

When participants were grouped based on TFC stage, people in stage 1 had a significantly greater velocity and a significantly smaller stride length CoV than stage 3,4 in the baseline and dual simple task (Figure 9a-d). There was no significant difference for cadence between disease stage in any assessment item and no significant difference for any walking measure in the dual complex task. For the alphabet tasks participants in stage 1 said significantly more correct letters of the alphabet per second compared to participants in stage 2 and stage 3,4 in the alphabet baseline simple and dual simple task (Figure 9e). There was no performance difference between disease stage for the complex baseline or dual tasks.

Table 5: Walk and talk performance scores

Walk and talk	Pre-HD	Manifest	t-test results		TFC stage			ANOVA results		Pairwise compairsons
			t value (df)	p value	Stage 1	Stage 2	Stage 3,4	F value (df, error)	p value	
Velocity bl (m/s)	131.85 ± 8.51	103.41 ± 7.22	$1.453_{(30)}$	p = n.s.	131.71 ± 8.28	113.76 ± 13.53	81.56 ± 7.98	$9.659_{(2,29)}$	p < 0.01	s1 vs s3 p<0.001
Velocity dual simple (m/s)	133. 78 ± 7.5	102. 18 ± 7.24	$1.734_{(26)}$	p = n.s.	129.45 ± 7.32	107.08 ± 11.81	76.93 ± 8.35	$11.185_{(2,25)}$	p < 0.001	s1 vs s3 p<0.001
Velocity dual complex (m/s)	123.68 ± 10.23	102.1 ± 9.31	$1.134_{(17)}$	p = n.s.	114.18 ± 9.75	103.05 ± 16.89	81.27 ± 19.5	$1.168_{(2,16)}$	p = n.s.	/
Cadence bl (steps/minute)	110.23 ± 3.79	109.53 ± 3.7	$0.07_{(30)}$	p = n.s.	113.87 ± 5.18	106.62 ± 8.36	106.73 ± 5	$0.568_{(2,29)}$	p = n.s.	/
Cadence dual simple (steps/minute)	112.38 ± 2.99	108.29 ± 4.79	$0.341_{(26)}$	p = n.s.	113.43 ± 6.1	99.7 ± 9.84	107.54 ± 6.96	$0.732_{(2,25)}$	P = n.s.	/
Cadence dual complex (steps/minute)	107.83 ± 2.66	97.53 ± 6.08	$0.851_{(17)}$	p = n.s.	105.42 ± 5.93	96.28 ± 10.26	81.4 ± 11.86	$1.713_{(2,16)}$	p = n.s.	/
Duration of double limb support bl (s)	0.31 ± 0.02	1.27 ± 0.75	$-0.486_{(30)}$	p = n.s.	0.281 ± 1.02	1.14 ± 1.84	1.95 ± 0.99	$0.692_{(2,28)}$	p = n.s.	/
Duration of double limb support dual simple (s)	0.3 ± 0.01	0.58 ± 0.26	$-0.435_{(26)}$	p = n.s.	0.29 ± 0.33	0.29 ± 0.53	0.98 ± 0.37	$1.119_{(2,25)}$	p = n.s.	/
Duration of double limb support dual complex (s)	0.33 ± 0.01	0.75 ± 0.36	$-0.594_{(17)}$	p = n.s.	0.79 ± 0.38	0.31 ± 0.65	0.61 ± 0.75	$0.215_{(2,16)}$	p = n.s.	/
Stride length CoV bl	3.11 ± 0.08	16.75 ± 4.18	$-1.215_{(30)}$	p = n.s.	3.62 ± 5.23	12.57 ± 8.43	26.54 ± 5.04	$5.039_{(2,29)}$	p < 0.05	s1 vs s3,4 p<0.05
Stride length CoV dual simple	2.71 ± 0.25	13.38 ± 3.72	$-1.152_{(26)}$	p = n.s.	4.23 ± 4.36	10.85 ± 7.03	22.27 ± 4.97	$3.731_{(2,25)}$	p < 0.05	s1 vs s3,4 p<0.05
Stride length CoV dual complex	5.55 ± 1.64	9.74 ± 2.43	$-0.861_{(17)}$	p = n.s.	9.05 ± 2.61	7.39 ± 4.53	10.03 ± 5.23	$5.039_{(2,25)}$	p = n.s.	/
Alphabet bl simple (letters/s)	2.81 ± 0.33	2.33 ± 0.23	$0.836_{(26)}$	p = n.s.	3.12 ± 0.26	1.97 ± 0.37	1.79 ± 0.28	$6.844_{(2,25)}$	p < 0.01	s1 vs s2 p<0.05; s1 vs s3,4 p<0.01
Alphabet dual simple (letters/s)	1.43 ± 0.3	1.71 ± 0.19	$-0.454_{(20)}$	p = n.s.	2.22 ± 0.21	1.45 ± 0.3	1.09 ± 0.25	$6.266_{(2,19)}$	p < 0.01	s1 vs s3 p<0.01
Alphabet bl complex (letters/s)	0.75 ± 0.08	0.47 ± 0.05	$2.322_{(18)}$	p < 0.05	0.62 ± 0.61	0.44 ± 0.1	0.32 ± 0.12	$2.91_{(2,17)}$	p = n.s.	/
Alphabet dual complex (letters/s)	0.67 ± 0.04	0.41 ± 0.07	$1.383_{(14)}$	p = n.s.	0.53 ± 0.08	0.33 ± 0.12	0.33 ± 0.14	$1.253_{(3,13)}$	p = n.s.	/
Velocity simple dtc (%)	-1.76 ± 3.33	6.86 ± 1.46	$-0.435_{(26)}$	p < 0.05	1.894.2	7.33 ± 3.04	9.93 ± 2.15	$4.354_{(2,25)}$	p < 0.05	s1 vs s3,4 p<0.05
Velocity complex dtc (%)	6.03 ± 6	20.23 ± 4.41	$-0.435_{(17)}$	p = n.s.	14.86 ± 4.87	14.69 ± 8.43	30.17 ± 9.74	$1.048_{(2,16)}$	p = n.s.	/
Cadence simple dtc (%)	-2.08 ± 1.87	1.49 ± 3.26	$-0.435_{(26)}$	p = n.s.	0.37 ± 4.2	6.66 ± 6.77	-1.06 ± 4.79	$0.453_{(2,25)}$	p = n.s.	/
Cadence complex dtc (%)	1.76 ± 4.78	14.31 ± 4.07	$-0.435_{(17)}$	p = n.s.	7.7 ± 4.06	10.64 ± 7.04	28.89 ± 8.13	$2.73_{(2,16)}$	p = n.s.	/
Duration of double limb support simple dtc (%)	-0.99 ± 4.07	76.05 ± 81.31	$-0.435_{(25)}$	p = n.s.	3.07 ± 100.18	-36.16 ± 180.6	184 ± 114.22	$0.42_{(2,24)}$	p = n.s.	/
Duration of double limb support complex dtc (%)	7.25 ± 5.92	99.8 ± 100.37	$-0.435_{(17)}$	p = n.s.	147.28 ± 101.82	-60.86 ± 176.36	0.66 ± 203.64	$0.613_{(2,16)}$	p = n.s.	/
Stride length CoV simple dtc (%)	51.4 ± 38.05	78.23 ± 24.77	$-0.435_{(26)}$	p = n.s.	77.65 ± 33.19	67.72 ± 53.52	73.52 ± 37.84	$0.13_{(2,25)}$	p = n.s.	/
Stride length CoV complex dtc (%)	44.36 ± 37.69	79.38 ± 68.25	$-0.435_{(17)}$	p = n.s.	112.47 ± 70.12	16.31 ± 121.45	-15.61 ± 140.24	$0.467_{(2,16)}$	p = n.s.	/
Alphabet simple dtc (%)	47.62 ± 21.35	22.19 ± 4.87	$-0.435_{(19)}$	p = n.s.	24.4 ± 7.52	24.91 ± 10.63	24.73 ± 9.71	$0.001_{(2,18)}$	p = n.s.	/
Alphabet complex dtc (%)	76.94 ± 3.89	81.46 ± 2.66	$-0.435_{(13)}$	p = n.s.	79.23 ± 3.42	82.62 ± 4.84	82.85 ± 5.59	$0.244_{(2,12)}$	p = n.s.	/

The mean test scores are presented and recorded to 2 decimal places ± SEM. Groups were categorised as pre-HD (total functional capacity (TFC) = 13 and Unified Huntington's Disease total motor score <5) and manifest (all symptomatic stages). The manifest group was further delineated by TFC disease stage (stage 1, TFC =11-13; stage 2, TFC=7-10; stage 3,4, TFC=1-6). All measures in light blue are spatiotemporal measures recorded from the GaitRite. For velocity, cadence and duration of double limb support, a lower measure is indicative of worse performance. For stride length CoV, a higher measure is indicative of worse performance.

The alphabet scores are highlighted in dark blue as the number of correct letters of the alphabet recited per second. A lower score is indicative of worse performance. Dual task cost is shown in orange and presented as a percentage where a lower score is indicative of better performance. A positive percentage is indicative of slower or worse dual task performance relative to baseline, whereas a negative dual task cost is indicative of better performance in the dual task compared to baseline. Significance was set so $p < 0.05$ and followed by a Bonferroni post-hoc test.
Where TFC= total functional capacity; ANOVA = analysis of variance; bl = baseline; CoV = coefficient of variation; dtc = dual task cost; n.s. = not significant; df = degrees of freedom.

For dual task cost, stage 3,4 presented greater velocity costs from baseline to the dual task condition in the *Walk and talk* simple compared to stage 1 (

Figure 10a). There were no differences for any other *Walk and talk* dual task item between disease stage.

Results from a two way repeated measures ANOVA are presented in Table 6. This revealed that Stride length CoV in the Stage 3 group was better (significantly less) in the dual complex relative to baseline performance. Performance in no other measure from the *Walk and talk* significantly differed from the baseline to the dual task.

Table 6: Walk and talk two way repeated measures ANOVA

	Dual task item	Baseline vs dual simple	Stages	Baseline vs dual complex	Stages
Walk and talk	Velocity	$F_{(2,25)}$=1.556, p = n.s.	/	$F_{(2,16)}$=0.572, p = n.s.	/
	Cadence	$F_{(2,25)}$=0.474, p = n.s.	/	$F_{(2,16)}$=2.805, p = n.s.	/
	Duration of double limb support	$F_{(2,24)}$=1.495, p = n.s.	/	$F_{(2,15)}$=1.764, p = n.s.	/
	Stride length CoV	$F_{(2,25)}$=0.073, p = n.s.	/	$F_{(2,16)}$=3.974, p < 0.05	Stage 3: p<0.05
	Alphabet	$F_{(2,18)}$=0.807, p = n.s.	/	$F_{(2,13)}$=0.562, p = n.s.	/

This table presents results from a two way repeated measures ANOVA. If this returned as significant (where p < 0.05), a Bonferroni post-hoc was used to identify which disease stage this was relevant to. This is presented in the 'Stages' column. The results in this table and in Table 5 revealed that stage 3 had a significantly lower stride length coefficient of variance (CoV) in the dual complex relative to baseline. No other results were significant (n.s.). Significance was met so p < 0.05.

2.2.3.3 TUG and LVF

For clarity, a reference of the TUG and LVF test items are presented in Table 7:

Table 7: TUG and LVF assessment procedure

	Test order	Dual task item	Brief procedure
TUG and LVF	1	TUG baseline	Stand from sitting, walk 3 metres, turn, walk back and sit down
	2	LVF simple	Say words beginning with the letter R
	3	LVF complex	Say words beginning with the letter N
	4	TUG and LVF simple	Perform the TUG whilst doing the LVF simple
	5	TUG and LVF complex	Perform the TUG whilst doing the LVF complex

Measures reported includes the time taken to perform the TUG for items 1, 4 and 5. The number of correct words said per second is recorded for items 2-5. TUG = timed up and go; LVF = letter verbal fluency.

TUG and LVF performance scores and statistical outcomes between Group and TFC disease stage are presented in Table 8.

There was no difference in TUG performance time between pre-HD and manifest participants in the baseline or dual simple and dual complex tasks. For the LVF, pre-HD participants said significantly more correct LVF answers per second than Manifest in the LVF simple baseline test. Although pre-HD participants generally said more correct answers per second than Manifest this did not reach significance for the LVF complex baseline or dual simple and dual complex tasks.

When data was grouped by TFC stage, participants in stage 1 performed the TUG baseline, dual simple and dual complex tasks significantly faster than participants in stage 3 (Figure 9f). For the LVF, participants in stage 3,4 said significantly more correct words per second than stage 1 in the LVF dual simple task (

Figure 10g). There was also a significant effect of disease stage for the LVF simple baseline task, but following a Bonferroni post-hoc, this revealed no differences between any particular disease stage. There was no difference between disease stage for the LVF baseline complex or LVF complex dual task.

Table 8: TUG and LVF performance scores

TUG and LVF	Pre-HD	Manifest	t-test results		TFC stage			ANOVA results		Pairwise compairsons
			t value $_{(df)}$	p value	Stage 1	Stage 2	Stage 3,4	F value $_{(df, error)}$	p value	
TUG bl (s)	9.57 ± 0.82	15.05 ± 2.23	$-0.919_{(30)}$	p = n.s.	9.04 ± 2.97	12.05 ± 4.2	19.97 ± 2.75	$3.85_{(2,29)}$	p < 0.05	s1 vs s3,4 p<0.05
TUG dual simple (s)	11.04 ± 1.13	17 ± 1.9	$-1.34_{(23)}$	p = n.s.	10.63 ± 1.73	13.61 ± 2.56	24.02 ± 1.91	$14.06_{(2,22)}$	p < 0.001	s1 vs s3,4 p<0.001; s2 vs s3 p<0.01
TUG dual complex (s)	12.41 ± 1.88	16.93 ± 2.66	$-0.736_{(16)}$	p = n.s.	10.39 ± 2.84	12.66 ± 3.76	23.98 ± 2.84	$6.27_{(2,15)}$	p < 0.01	s1 vs s3,4 p<0.05
LVF bl simple	0.29 ± 0.04	0.16 ± 0.02	$2.894_{(25)}$	p < 0.01	0.23 ± 0.03	0.13 ± 0.04	0.15 ± 0.03	$3.63_{(2,24)}$	p < 0.05 $^{\$}$	n.s.
LVFdual simple	0.38 ± 0.03	0.31 ± 0.04	$0.739_{(23)}$	p = n.s.	0.43 ± 0.05	0.31 ± 0.07	0.19 ± 0.52	$5.63_{(2,22)}$	p < 0.05	s1 vs s3 p<0.01
LVF bl complex	0.17 ± 0.03	0.15 ± 0.02	$0.414_{(19)}$	p = n.s.	0.16 ± 0.02	0.14 ± 0.32	0.16 ± 0.02	$0.11_{(2,18)}$	p = n.s.	n.s
LVF complex dual	0.31 ± 0.03	0.26 ± 0.04	$0.548_{(15)}$	p = n.s.	0.33 ± 0.06	0.3 ± 0.07	0.2 ± 0.05	$1.39_{(2,14)}$	p = n.s.	n.s.
TUG simple dtc (%)	15.6 ± 6.48	36.43 ± 9.72	$-0.913_{(23)}$	p = n.s.	21.24 ± 11.48	12.39 ± 17.02	59.09 ± 12.69	$3.371_{(2,22)}$	p = n.s.	/
TUG complex dtc (%)	20.19 ± 13.2	28.44 ± 13.18	$-0.261_{(23)}$	p = n.s.	5.98 ± 15.31	9.79 ± 21.65	61.23 ± 16.36	$3.447_{(2,16)}$	p = n.s.	/
LVF simple dtc (%)	-40.77 ± 27.92	-76 ± 15.51	$0.932_{(17)}$	p = n.s.	-84.33 ± 19.13	-112.79 ± 28.37	-29.72 ± 21.15	$3.231_{(2,22)}$	p = n.s.	/
LVF complex dtc (%)	-75.74 ± 32.33	-64.06 ± 24.13	$-0.2_{(17)}$	p = n.s.	-69.68 ± 31.63	-116.84 ± 44.73	-32.48 ± 33.81	$1.144_{(2,16)}$	p = n.s.	/

Mean test scores are recorded to 2 decimal places ± SEM. Groups were categorised as pre-HD (total functional capacity (TFC) = 13 and Unified Huntington's Disease total motor score <5) and manifest (all symptomatic stages). The manifest group was further delineated by TFC disease stage (stage 1, TFC =11-13; stage 2, TFC=7-10; stage 3,4, TFC=1-6). All measures in dark blue are recorded as time taken in seconds, where a higher score is indicative of worse performance. All measures in light blue show the number of correct answers given per second where a lower score is indicative of worse performance. Dual task cost is shown in orange and presented as a percentage. A positive percentage is indicative of slower or worse dual task performance relative to baseline, whereas a negative dual task cost is indicative of better performance in the dual task compared to baseline. Significance was set so p < 0.05 and followed by a Bonferroni post-hoc test.
Where TFC= total functional capacity; ANOVA = analysis of variance; TUG = Timed Up and Go; LVF = Letter verbal fluency; bl = baseline; n.s. = not significant; dtc = dual task cost; $^{\$}$ = In the LVF bl simple post-hoc analysis revealed no significant difference between individual TFC disease stages.

There was no difference in dual task cost between disease stage for the TUG and LVF simple or complex (Figure 9b).

Results from a two way repeated measures ANOVA are presented in (Table 9). This revealed that people in stage 3 took significantly longer to perform the TUG in the simple and complex dual task relative to baseline. Performance from no other TUG and LVF measure significantly differed from baseline to the dual task.

Table 9: TUG and LVF two way repeated measures ANOVA

	Dual task item	Baseline vs dual simple	Stages	Baseline vs dual complex	Stages
TUG and LVF	TUG	$F_{(2,22)}$=6.596, p < 0.01	Stage 3: p < 0.001	$F_{(2,14)}$=0.574, p < 0.05	Stage 3: p < 0.001
	LVF	$F_{(2,22)}$=3.124, p = n.s.	/	$F_{(2,14)}$=1.598, p = n.s.	/

This table presents results from a two way repeated measures ANOVA. If this returned as significant (where p < 0.05), a Bonferroni post-hoc was used to identify which disease stage this was relevant to. This is presented in the 'Stages' column. The results from this table and those presented in Table 8 revealed that Stage 3 performed the TUG significantly slower in the dual task conditions compared to baseline. Performance in the LVF did not significantly differ in the dual task compared to baseline (n.s.). Significance was met so p < 0.05.
TUG = Timed up and go, LVF = letter verbal fluency.

2.2.3.4 Step and Stroop

For clarity, a reference of the Step and Stroop test items are presented in Table 10.

Table 10: Step and assessment procedure

	Test order	Dual task item	Brief procedure
Step and Stroop	1	Step baseline	Step onto and off an aerobic step
	2	Stroop baseline simple	Colour words were presented in their coloured ink. For example, pink was printed in the colour pink
	3	Stroop baseline complex	Colour words were presented in a different coloured ink. For example, pink was printed in the colour grey
	4	Step and Stroop simple	Step whilst performing the Stroop simple
	5	Step and Stroop complex	Step whilst performing the Stroop complex

Measures reported in the results includes: number of steps performed in items 1, 4 and 5; The number of correct answers were recorded in items 2-5.

Step and Stroop performance scores and statistical outcomes between Group and TFC disease stage are presented in Table 11.

For the stepping tasks, pre-HD participants stepped significantly more than the Manifest group for the baseline, and simple and complex dual tasks. For the Stroop tasks, the pre-HD group gave significantly more correct answers than manifest for all simple and complex baseline and dual tasks.

When participant data were grouped based on TFC stage, stage 1 participants stepped significantly more than stage 2, stage 3 and stage 4,5 for the baseline, dual simple and dual complex (Figure 9h). For the Stroop simple baseline and dual tasks, participants gave significantly less correct Stroop responses with increasing disease stage (Figure 9i). The Stroop simple baseline better distinguished disease stage compared to the baseline complex. The Stroop complex dual task better distinguished disease stage compared to the Stroop complex baseline.

For dual task cost, stage 3 presented greater dual task costs in the Stroop simple compared to stage 1. There was no difference for any other *Step and Stroop* dual task item between disease stage.

Table 11: Step and Stroop performance scores

Step and Stroop	Pre-HD	Manifest	t-test results		TFC stage				ANOVA results		Pairwise compairsons
			t value (df)	p value	Stage 1	Stage 2	Stage 3	Stage 4,5	F value (df, error)	p value	
Step bl	40.13 ± 1.98	28.72 ± 1.99	$2.252_{(56)}$	p < 0.05	40.83 ± 2. 16	28.78 ± 2.76	20.72 ± 2.44	14 ± 5.97	$15.668_{(3,54)}$	p < 0.001	s1 vs s2 p<0.01; s1 vs s3, s4,5 p<0.001
Step dual simple	36.5 ± 2.9	26.98 ± 1.89	$2.018_{(52)}$	p < 0.05	37.43 ± 2.01	26.29 ± 2.58	17.56 ± 2.41	23 ± 9.65	$13.768_{(3,51)}$	p < 0.001	s1 vs s2 p<0.01; s1 vs s3,4 p<0.001
Step dual complex	35.88 ± 2.72	25.65 ± 2.21	$2.064_{(43)}$	p < 0.05	36 ± 2.11	24 ± 3.13	15.68 ± 2.74	/	$18.013_{(2,42)}$	p < 0.001	s1 vs s2 p<0.01; s1 vs s3,4 p<0.001
Stroop simple bl	79.63 ± 4.14	56 ± 2.72	$3.153_{(63)}$	p < 0.01	77.54 ± 2.96	58.25 ± 3.61	41.32 ±3.08	42.33 ± 8.35	$25.469_{(3,61)}$	p < 0.001	s1 vs s2, s3, s4,5 p<0.001; s2 vs s3 p<0.01
Stroop dual simple	73.13 ± 3.58	48.43 ± 3.23	$3.112_{(52)}$	p < 0.01	69.22 ± 3.19	49.5 ± 4.09	32.35 ± 3.83	12 ± 15.31	$20.984_{(3,50)}$	p < 0.001	s1 vs s3 p<0.001; s1 vs s2, s4,5 p<0.01; s2 vs s3 p<0.05
Stroop complex bl	51 ± 1.92	27.41 ± 1.88	$5.109_{(52)}$	p < 0.001	41.46 ± 2.19	28.67 ± 3.1	18.53 ± 2.6	15 ± 10.73	$16.188_{(3,50)}$	p < 0.001	s1 vs s2 p<0.01; s1 vs s3 p<0.001
Stroop dual complex	51.5 ± 1.68	29.38 ± 2.52	$4.01_{(43)}$	p < 0.001	44.91 ± 2.38	28.9 ± 3.53	17.08 ± 3.1	/	$26.342_{(2,42)}$	p < 0.001	s1 vs s2 p<0.01; s1 vs s3 p<0.001; s2 vs s3 p<0.05
Step simple dtc (%)	9.6 ± 3.76	7.5 ± 4.19	$5.109_{(52)}$	p = n.s.	7.14 ± 5.57	0.21 ± 7.14	15.4 ± 6.52	-1.31 ± 26.88	$0.857_{(3,51)}$	p = n.s.	/
Step complex dtc (%)	11.11 ± 3.37	21.63 ± 4.15	$5.109_{(52)}$	p = n.s.	12.14 ± 4.85	28.62 ± 7.19	25.83 ± 6.31	/	$2.459_{(2,42)}$	p = n.s.	/
Stroop simple dtc (%)	7.98 ± 1.54	21.16 ± 3.19	$5.109_{(52)}$	p = n.s.	10.94 ± 3.63	12.64 ± 4.65	32.94 ± 4.22	70 ± 17.39	$8.78_{(3,51)}$	p < 0.001	s1 vs s3 p<0.001; s1 vs s4,5 p<0.01; s2 vs s3 and s4,5 p<0.05
Stroop complex dtc (%)	-1.31 ± 2.42	2.3 ± 4.35	$5.109_{(52)}$	p = n.s.	-4.11 ± 4.84	-4.66 ± 7.18	16.3 ± 6.3	/	$3.797_{(2,42)}$	p < 0.05	s1 vs s3 p<0.05

Mean test scores are recorded to 2 decimal places ± SEM. Groups were categorised as pre-HD (total functional capacity (TFC) = 13 and Unified Huntington's Disease total motor score <5) and manifest (all symptomatic stages). The manifest group was further delineated by TFC disease stage (stage 1, TFC =11-13; stage 2, TFC=7-10; stage 3, TFC=3-6; stage 4,5, TFC=0-2). All measures in dark blue are recorded as number of steps performed in 45 seconds, where a lower score is indicative of worse performance. All measures in light blue show the number of correct answers given in 45 seconds where a lower score is indicative of worse performance. Dual task cost is shown in orange and presented as a percentage where a lower score is indicative of better performance. A positive percentage is indicative of slower or worse dual task performance relative to baseline, whereas a negative dual task cost is indicative of better performance in the dual task compared to baseline. No participants in stage 4,5 were recruited in the complex dual task. Significance was set so p < 0.05 and followed by a Bonferroni post-hoc test.
Where TFC= total functional capacity; ANOVA = analysis of variance; bl = baseline; n.s. = not significant; s = stage; dtc = dual task cost.

Results from a two way repeated measures ANOVA are presented in Table 12. This revealed that stepping and Stroop performance did not significantly differ in the dual task compared to baseline.

Table 12: Step and Stroop two way repeated measures ANOVA

	Dual task item	Baseline vs dual simple	Stages	Baseline vs dual complex	Stages
Step and Stroop	Step	$F_{(3,50)}$=0.873, p = n.s.	/	$F_{(2,42)}$=0.734, p = n.s.	/
	Stroop	$F_{(2,50)}$=2.488, p = n.s.	/	$F_{(2,42)}$=1.878, p = n.s.	/

This table presents results from a two way repeated measures ANOVA. This revealed that performance in the Step and Stroop did not significantly differ in the dual task relative to baseline. Where n.s. = not significant.

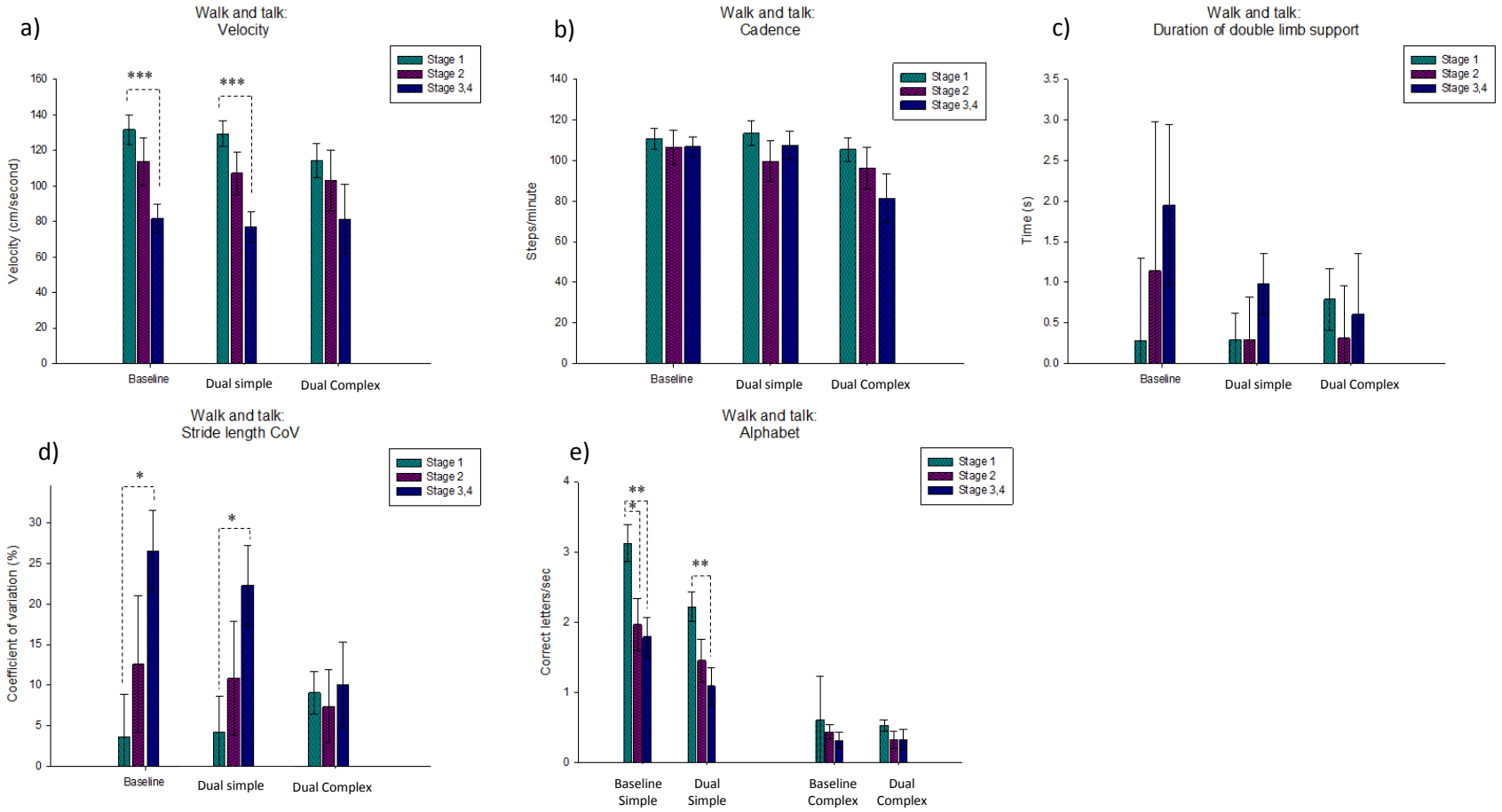
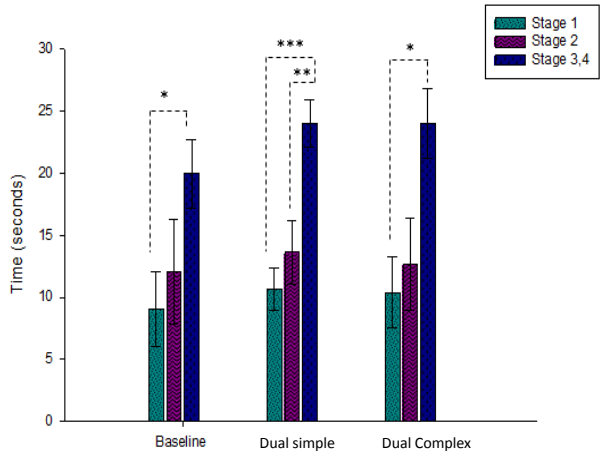
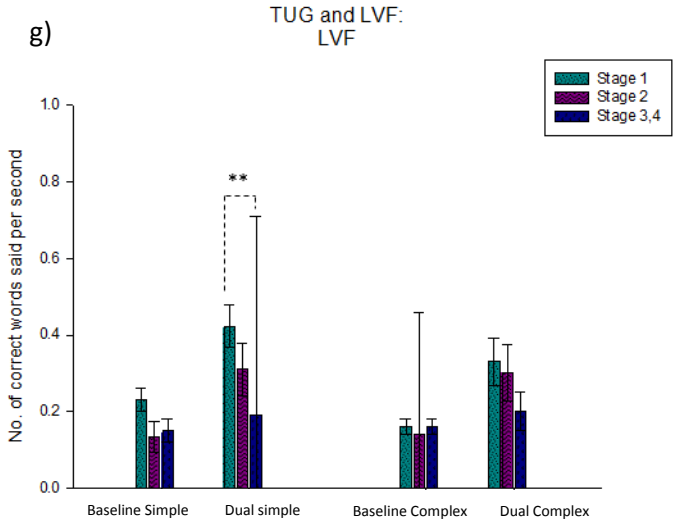


Figure 9 continues on page 833.

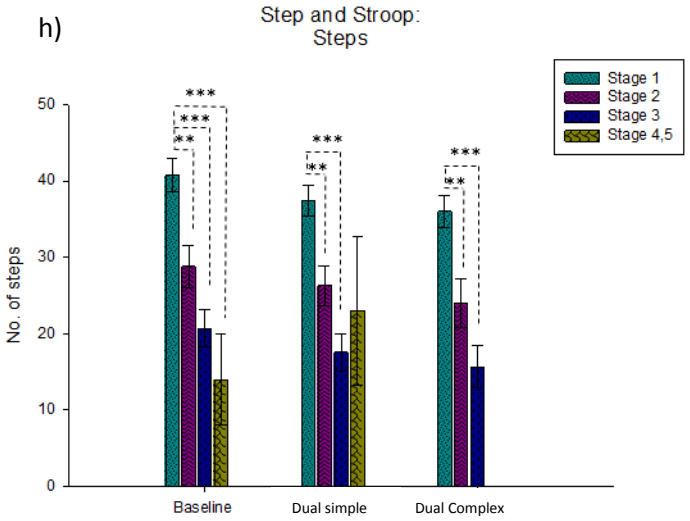
f)


TUG and LVF:
TUG
Stage 1
Stage 2
Stage 3,4
Time (seconds)
0
5
10
15
20
25
30
Baseline
Dual simple
Dual Complex

g)


TUG and LVF:
LVF
Stage 1
Stage 2
Stage 3,4
No. of correct words said per second
0.0
0.2
0.4
0.6
0.8
1.0
Baseline Simple
Dual simple
Baseline Complex
Dual Complex

h)


Step and Stroop:
Steps
Stage 1
Stage 2
Stage 3
Stage 4,5
No. of steps
0
10
20
30
40
50
Baseline
Dual simple
Dual Complex

i)

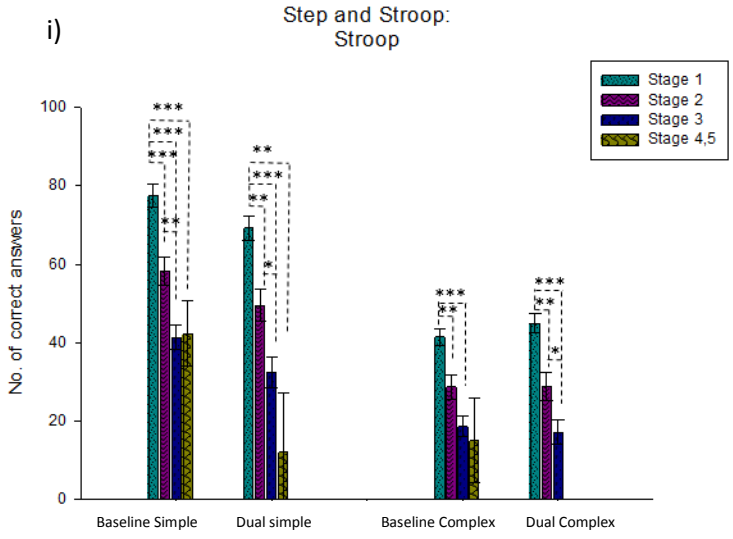

Step and Stroop:
Stroop
Stage 1
Stage 2
Stage 3
Stage 4,5
No. of correct answers
0
20
40
60
80
100
Baseline Simple
Dual simple
Baseline Complex
Dual Complex

*Figure 9: Bar graphs represent the mean performance across each dual task assessment, with standard error of the mean error bars. Figures a-e show the velocity (metres/second), cadence (steps/minute), duration of double limb support (seconds), stride length CoV (%) and the alphabet score (correct letters per second) for the Walk and talk. A greater measure for velocity, cadence and alphabet scores are indicative of better performance, whereas a lower measure is indicative of better performance for duration of double limb support and stride length CoV. Figures f and g show the time taken to perform the Timed up and go (TUG; f) and the letter verbal fluency scores (LVF; g). The TUG is measured in seconds, where a lower time is indicative of better performance and the LVF is measured as number of correct words said per second, where a greater score equates to better performance. Figures h and i show the number of steps and correct answers given for the Step and Stroop, where a greater score is indicative of better performance. Values show averages ± SEM. Where * $p<0.05$, ** $p<0.01$, *** $p<0.001$.*

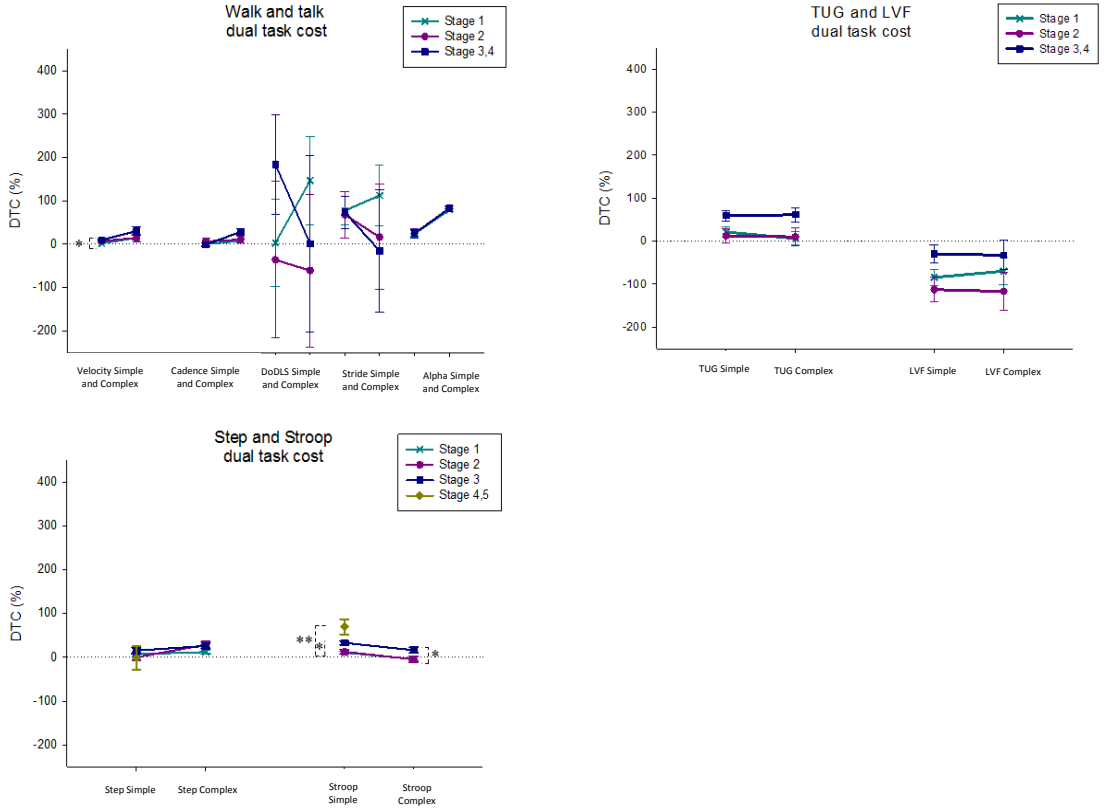


*Figure 10: Dual task cost (Chapter 2, Part 1): Graphs represent the mean dual task cost (dtc) percentage for each TFC disease stage in each dual task assessment, across the simple and complex tasks. Figure a presents the dtc for the Walk and talk and revealed that the dtc for velocity was significantly more in stage 3,4 compared to stage 1. All other parameters were widely variable. Figure b presents the dtc from the TUG and LVF which showed no significant differences between groups. Figure c shows the dtc from the Step and Stroop, which shows stage 3 and stage 4,5 had significantly greater costs that stage 1 and stage 2 in the Stroop simple. Stage 3 also had significantly greater costs compared to stage 1 in the Stroop complex. Where *p<0.05, **p<0.01.*
DTC = dual task cost; DoDLS = duration of double limb support; Alpha = alphabet; TUG = Timed up and go; LVF = letter verbal fluency.

2.2.4 Discussion

In this study, motor-cognitive dual tasks were selected and developed. This led to three lower limb assessments that were tested in people with HD. Dual tasks included the *Walk and talk*, the *TUG and LVF*, and the *Step and Stroop*. As the *Step and Stroop* distinguished between disease stage more than the other dual tasks, this was evaluated in a larger cohort of people with HD.

The Step and Stroop simple dual task best distinguished between disease stage

As part of the *Step and Stroop*, performance in the Stroop baseline and dual tasks distinguished primarily between stage 1 and all other disease stages. In the Stroop simple differences between disease stage were marginally greater than those in the Stroop complex. This finding is in line with previous studies that found that as HD progressed, the greatest changes were in less cognitively demanding tasks, like the Stroop colour and word naming (Stroop simple) compared to the word-colour interference (Stroop complex) (Snowden *et al.*, 2001; Ho *et al.*, 2003). One explanation for this could be because the basal ganglia, specifically the dorsolateral striatum, is implicated in automating behaviour (Ashby, Turner and Horvitz, 2010; Kim and Hikosaka, 2015). Thus, as the same four colour words are repeatedly read in the Stroop simple, this could lead to responses being automated (Flaudias and Llorca, 2014). However, as this circuitry progressively degenerates in HD, the ability to automate tasks may become increasingly difficult (Thompson *et al.*, 2010), meaning more attention is required and less words are recited. Alternatively, speech impairment is another common symptom in people with manifest HD (Rusz *et al.*, 2014; Skodda *et al.*, 2014). As this includes problems with speech rate, it could be that as HD progresses, the ability to promptly respond to verbal tasks, such as the Stroop, becomes increasingly impaired, leading to fewer words recited.

The stepping item from the *Step and Stroop* revealed that on average, the stage 1 group stepped significantly more than stages 2, 3 and 4,5. One explanation for this is that people with HD have problems initiating movement, leading to slower response times (Delval *et al.*, 2007; Goldberg *et al.*, 2010). This is supported by a previous study found that people with manifest HD had problems with self-initiating movement prior to a walking, leading to a slower speed during the first few walking steps, compared to controls (Delval *et al.*, 2007). Anatomically, self-triggered movements, which are required during the stepping task in the Step and Stroop, involve activation of the supplementary motor area and the dorsolateral prefrontal cortex (Jahanshahi *et al.*, 1995). As these regions innervate the basal ganglia (reviewed in: Lanciego et al., 2012; Nambu et al., 2002), the progressive loss of GABAergic neurons which forms the basis of this circuitry (Storey and Beal, 1993), may lead to greater attention and activation of more indirect, compensatory networks required to initiate stepping. Other explanations for a slower stepping rate includes postural disturbances due to increased movement abnormalities leading to imbalance (Delval *et al.*, 2007), or as people with HD become progressively less mobile (Roos, 2010), they may simply tire quicker.

The TUG and LVF dual task

Although the *TUG and LVF* are used as individual tests in the HD population, they have never been combined and tested as a dual task in HD. In this study, the LVF baseline simple was the only LVF item that revealed a significant difference between pre-HD and manifest participants. Surprisingly, when LVF performance was compared across disease stage, this revealed that people in stage 3,4 gave significantly more correct answers than those in stage 1. This was largely influenced by a stage 4 participant who performed particularly well in the LVF, and admitted that she liked to read in her spare time. Although the LVF task has proven sensitive in people with HD in the past (Rosser and Hodges, 1994; Jardim Azambuja *et al.*, 2007), future studies should consider controlling for lifestyle factors such as education and certain hobbies, such as reading. This will allow a greater understanding behind how verbal fluency tasks deteriorate as HD progresses.

As a dual task, the LVF items were also prone to practice effects indicated by the negative dual task costs. As a result, the LVF may not be useful in assessments where a baseline test is required, and then soon repeated, such as in dual tasks.

For the TUG, performance in the *TUG and LVF* dual simple best distinguished between disease stage, over the TUG baseline and TUG dual complex task. This revealed that TUG performance took significantly longer with increasing disease stage. The results in this study suggest that the TUG may be more sensitive when used as part of a dual task rather than a single outcome measure, which is how it is currently used in the observational study, ENROLL-HD. Future research should therefore consider testing different cognitive items that could be combined with the TUG to see how and if this influences TUG performance.

The Walk and Talk dual task

Performance in the Walk and talk was tested in people with HD in a previous study (Fritz *et al.*, 2016), and revealed that the majority of people experienced mutual interference, and that performance in both the walking and the talking task declined in the dual task. As they only measured speed to rate walking performance, in this study, the GaitRite mat was used to measure a range of spatiotemporal walking parameters during dual task performance. Both velocity and stride length CoV significantly differed across disease stage, where stage 1 had a significantly greater velocity and a smaller stride length CoV, compared to stage 3. However, contrasting with the findings from (Rao, Quinn and Marder, 2005) there was no difference between pre-HD and manifest for any walking measure. One explanation for this is some subjects in our study walked off the sensors on the mat, reducing the mean data that could be collected. Furthermore, to conform with the *Walk and talk* performed in HD before (Fritz *et al.*, 2016), each assessment item was for 30 seconds long. Therefore some subjects only completed one length of the GaitRite, limiting the number of steps that could be analysed. To avoid this problem, a previous study standardised the number of steps participants performed to improve the consistency of gait measures (Khalil, 2012). Although this should be considered in future studies that use the

GaitRite, increasing the number of steps or the length of walking time could lead to fatigue, especially in dual task studies where walking would be repeatedly assessed.

Did the dual task have an impact on performance?

In this study, dual task cost was calculated to compare the percentage difference between baseline and dual task performance. When percentage changes were compared across disease stage, this revealed that the Stroop simple best distinguished between disease stage, where stage 3 and stage 4,5 subjects had significantly greater costs compared to stage 1 and stage 2. As there was no significant difference in stepping rate this suggests that participants may have prioritised the motor (stepping) task over the cognitive (Stroop) cognitive tasks when both were performed simultaneously. Interestingly, the opposite pattern was true for the *TUG and LVF*, where TUG performance decreased in the dual condition and performance in the LVF improved. This supports previous findings that suggests dual task interference is dependent upon the motor-cognitive items combined (reviewed in: Carmela et al., 2017), and indicates the importance of validating different dual tasks in a larger group of people with HD in the future.

Part 1 Conclusions

Overall, the results in this study revealed that the *Step and Stroop* assessment items best distinguished different stages of HD compared to the *TUG and LVF* and the *Walk and talk.* In particular the Stroop simple items were most sensitive to disease stage compared to the Stroop complex, suggesting that this may better reflect striatal degeneration than more highly complex cognitive tasks (Snowden *et al.*, 2001). However, a limitation of the *Step and Stroop* dual task was that it required at least two researchers; one to ensure the participant is safe during performance and the other to rate the assessment, which may not be available in all clinical settings.

Taking this study forward, the next step was to correlate dual task performance with HD specific assessments. This analysis is presented in Section 2.3.3.2 of this Chapter.

2.3 Part 2

The dual study in Part 1 was assembled using a combination of existing motor and cognitive tasks, but all involved the lower limb. For reasons described below, it was important to also generate an upper limb task. However, as no tasks existed that could be readily adapted for dual tasking, one had to be created.

2.3.1 Rationale and study design

Motor impairments in HD involves problems with upper limb function, such as problems performing dextrous movements (Klein *et al.*, 2012; Quinn, Busse and Dal Bello-Haas, 2013). Dexterity can be defined as "fine, voluntary movements used to manipulate small objects during a specific task" (Backman, Gibson and Parsons, 1992). There are two main types of dexterity; manual and fine motor. Manual dexterity involves the use of the whole hand, such as grasping the handle bars of a bike, whereas fine motor dexterity involves the manipulation of objects within the hand, such as screwing the lid on a bottle. As such functions form a prominent role in everyday life (Quinn, Busse and Dal Bello-Haas, 2013), it is important objective, ecologically valid outcome measures are available for clinicians and researchers to assess problems with upper limb function in HD. Indeed, there are numerous functional upper limb assessments that have been tested in this population (see Table 13). However, many of these are limited because of the way they are rated, the lack of standardisation and because they are not sensitive to people across all stages of HD. For instance, pegboard tests are well established fine motor tasks which consist of transferring pins into holes on a board (Tiffin and Asher, 1948; Saft *et al.*, 2003; Yancosek and Howell, 2009). A previous study revealed that people with manifest HD transferred pegs significantly slower than the pre-manifest group and healthy controls, but, there was no difference between people with pre-manifest HD and controls (Saft *et al.*, 2003). Other time based functional upper limb tasks include the *10 euro neuro test* which involves turning over ten 1 euro coins (van Vugt *et al.*, 2007). The one study that tested this compared

performance in late stage people with HD and controls and found that people with HD were significantly slower performing the task, and 2 out of 10 could not complete it (van Vugt *et al.*, 2007). The *nut and bolt* test also revealed that performance in people with manifest HD were slower than pre-manifest and controls (Collins *et al.*, 2015). However, as the studies discussed here (Saft *et al.*, 2003; van Vugt *et al.*, 2007; Collins *et al.*, 2015) were tested in people with manifest HD, and symptoms can vary significantly across this group, it is unknown if these would be sensitive across all manifest disease stages. Furthermore, the simplicity of these tests and those included in the *Physical Performance test* (see Table 13) (Reuben and Siu, 1990; Brown *et al.*, 2000) suggests that such simple tests that are rated only using time, may not capture people with different functional abilities, suggesting an accuracy measure is also required.

Another dexterity task includes the *reach to eat*, which involves the subject transferring a small food item from a platform into their mouth (Whishaw *et al.*, 2002; Klein *et al.*, 2011). Using motion analysis, a previous study revealed that people with manifest HD commonly overshot the food target, had problems with wrist rotation, had jerky movement trajectories and impaired temporal sequencing of movements (Klein *et al.*, 2011). However, as this was only tested in 12 people with HD who had very different UHDRS-TMS scores (scores ranged from 19-50 (out of a total of 124)), it is unknown how these deficits differed across people with different disease stages. Furthermore, this test is commonly rated using an ordinal scale, which could lead to subjective scoring. Importantly, the *reach to eat* task was previously translated for pre-clinical use, and revealed that rats with excitotoxic lesions to the lateral striatum had problems with aiming for the food target, pronating and supinating the paw (Whishaw *et al.*, 2007). A similar task used in rodents is the *staircase test*, which involves retrieving small pellets from a descending staircase (Montoya *et al.*, 1991). Similar to the *reach to eat, p*revious studies have shown that excitotoxic lesions to the lateral striatum results in dextrous impairment, which can gradually improve following cell transplantation therapy (Jeyasingham *et al.*, 2001; Döbrössy and Dunnett, 2003; M. D. Döbrössy and Dunnett, 2005). Furthermore, upper motor tasks, such as finger tapping in HD progress over time (Klöppel *et al.*, 2009; Klöppel *et al.*, 2015), and neuroimaging

studies show that such tasks involves striatal function (Scahill *et al.*, 2013; Tabrizi *et al.*, 2013). Together, this suggests that a speed based, quantitative, dextrous assessment could be useful to assess upper limb function following an intervention, such as transplantation in HD. Furthermore, to capture the different stages in HD, including different levels of difficulty may help prise apart different disease stages.

Table 13: Functional upper limb tasks used for HD patients

Test	Procedure	Main scoring method
Pegboard tests (Tiffin and Asher, 1948)	Subjects are timed to transfer a set number of pegs into appropriate holes, or asked to transfer as many pegs as possible in a given time. This is performed twice (once for the left and right hand).	Time taken
10 Euro Neuro test (van Vugt *et al.*, 2007)	The subject is timed to turn over ten 1-euro coins	Time taken
Nut and bolt test (Collins *et al.*, 2015)	The subject uses one hand to screw on three different sized nuts onto bolts.	Time taken
Reach to eat (Whishaw *et al.*, 2002; Klein *et al.*, 2011)	Using one hand, the subject is asked to reach for a small food item (Cheerio™ or Haribo Gummy Bear™) positioned on a pedestal in front of them. This is performed twice (once for the left and right hand).	The test is typically video recorded to qualitatively rate movement performance via a movement element rating scale
Modified Physical performance test (Brown *et al.*, 2000): Pick up a penny Lift a book	The subject is asked to pick up a penny from the floor placed 12 inches in front of their feet. The subject is asked to lift a book and put it on a shelf (above shoulder height, starting position hands at side and standing in front of a table).	Ordinal scoring method where: ≤ 2 sec = 4; 2.1-4 sec = 3; 4.1- 6 sec = 2; > 6 sec = 1; unable = 0

Physical performance test (Reuben and Siu, 1990):		
Write a sentence	Subjects are asked to write: Whales live in a blue ocean.	Ordinal scoring method where: ≤ 10 sec = 4; 10.5-15 sec = 3; 15.5 – 20 sec = 2; >20 sec = 1; unable = 0.
Simulated eating	Subjects are asked to transfer five kidney beans into a tin can using a teaspoon.	

A table presenting previously used functional upper limb assessments in HD. A general limitation of these is that they are either rated using time taken alone, with no accuracy measure, or they are rated on an ordinal scale. In addition, no assessment presented in this table looked at performance in different stages of manifest HD.

2.3.1.1 The development of the Clinch token transfer test

The aim of this study was to develop an upper limb dual task assessment that could be used as a quantitative, performance based functional outcome measure for people with all stages of HD. As a result, the Moneybox test was developed. This name later changed to the Clinch token transfer test (C3t). This section describes the development work that led to this assessment.

The C3t was originally inspired by one of the items from the *Physical performance test*: *Picking up a penny* (Reuben and Siu, 1990). This task requires dexterity as well as postural stability to maintain balance when standing and bending (Blanchet *et al.*, 2014), meaning that it does not directly target upper limb function. To study the *Picking up a penny* item further, *a* small data mining study was carried out using data collected by Busse and colleagues (Busse *et al.*, 2014). This included data from 64 people with all stages of HD who performed a range of different outcome measures. As part of the *Physical Performance test,* the scores from assessment items are usually summed and the total score is then presented as a measure of functional mobility. However, it is unknown if individual item scores, such as *Picking up a penny*, are a useful individual outcome measure, and if they distinguish between different HD

stages. To investigate this, a Kruskall-Wallis H test was performed using the data from the Busse *et al* study (Busse *et al.,* 2014) and revealed that there was no significant difference between people with pre-HD and manifest HD [X^2 (1) = 1.161, p=n.s.], and no significant difference when subject data were grouped by TFC disease stage [X^2 (3) = 1.118, p=n.s.]. Thus, it was concluded that *Picking up a penny* was not demanding enough to tease apart different disease stages in HD. It was therefore decided that a sitting task that focussed on upper limb mobility, which had increasing levels of difficulty could be a useful outcome measure for HD.

To overcome some of the weaknesses associated with the functional upper limb assessments identified in Table 13, a list of criteria were assembled to help form the design and development of a new functional upper limb assessment (Table 14). This resulted in the *Clinch token transfer test* (C3t). This assessment consists of 3 main assessment items that involves transferring British coins between hands into a moneybox, in order of coin size (Baseline simple), coin value, without (Baseline complex) and whilst reciting the alphabet (Dual task). The information presented in Table 14 presents the criteria which aided the development of the C3t.

Table 14: A description of criteria used to develop the C3t

Criteria	Description	C3t
Limited to upper limb function	People with manifest HD fall regularly (Busse, Wiles and Rosser, 2009) and often require a wheelchair during the more advanced stages of disease. Therefore, an assessment restricted to upper limb function would allow wheelchair users and people with severe lower limb functional problems to be 'functionally' assessed.	To isolate motor function to the upper limbs, the C3t is performed whilst sitting. In addition, the C3t was designed so both hands are required to perform the same functions: grasp the coins, transfer and release, the same number of times. Therefore, the C3t effectively measures bilateral function.
A dual task with increasing levels of complexity	Dual and multi-tasking is a common daily activity. People with HD have difficulty performing two tasks simultaneously and appear to have more problems performing motor-cognitive dual tasks compared to motor-motor dual tasks (Delval, Krystkowiak, Delliaux, Dujardin, *et al.*, 2008). Easy and hard assessment items accommodate people with a range of functional abilities (Stout, Glikmann-Johnston and Andrews, 2016). This makes the test applicable to people that manifest a broad range of disease symptoms, such as HD.	The C3t includes three levels of difficulty; the Baseline simple, Baseline complex and dual task. The aim was to develop C3t items simple enough that subjects were willing to attempt the items to their full capacity, ensuring maximum participation. For instance, word fluency and adding/subtracting based secondary tasks can result in a negative response before the test has begun if for instance the subject is not confident with mental arithmetic. Reciting the alphabet was selected for the dual task to increase task difficulty. It was hypothesized that as this is a fairly simple task, it would be more mentally approachable than for instance a secondary task that could be confounded by education or job type, such as adding or subtracting. It was hypothesized this would minimize the negative impact such tasks may have on performance.

Subjects should only continue with the complex assessment items if they pass set criteria in the easier tasks	People in the more advanced stages of disease may struggle to complete more complex tasks due to limited attentional resources (Delval, Krystkowiak, Delliaux, Dujardin, *et al.*, 2008; Vaportzis, Georgiou-Karistianis, Churchyard and Stout, 2015). A hierarchy of items with increasing levels of difficulty, and set criteria for each level could minimize the risk of floor and ceiling effects if the test is too easy or hard for people in different disease stages.	Set criteria for each C3t item included completing the test item in a certain time and committing a limited number of errors. Errors consisted of, transferring the coins in the wrong order, dropping a coin or failing to transfer the coin between hands. Subjects that failed to meet criteria did not continue to the next C3t stage.
The assessment is sensitive to functions that involve the degenerating neuroanatomy in HD	HD is primarily caused by death of medium spiny neurons in the striatum and breakdown of the basal ganglia and cortical projections (Tabrizi *et al.*, 2009; Lanciego, Luquin and Obeso, 2012; Novak *et al.*, 2015). Therefore an assessment, that targets behavioural functions that involve these regions, may correlate with disease progression, meaning the assessment is sensitive to all stages of HD.	C3t items were developed to target: ***Dexterity:*** Previous pre-clinical research revealed the lateral striatum is required for fine motor tasks suggesting a task that incorporated dexterity may sensitively capture deficits caused by striatal degeneration in people with HD (Döbrössy and Dunnett, 2003). To account for this, dexterity was required to pick up and accurately release different sized coins into a defined target (the moneybox slot). ***Repeated motor transitions*:** Rhythmic, repeated motor transitions leads to a change in neuronal firing patterns in the dorsolateral striatum (Ashby, Turner and Horvitz, 2010), and may relate to new skill learning (Turner and Desmurget, 2010). The C3t was designed to take advantage of this, as it requires the subject to repeatedly transfer eight coins as quickly as possible.

		Oculomotor function (Harting and Updyke, 2005)***:*** It was hypothesized optimal C3t performance would require occulo-motor function to rapidly saccade the eyes to the next coin target. ***Attention*** (De Diego-Balaguer *et al.*, 2008)***:*** The increasing levels of difficulty in the C3t were designed to demand increasing levels of attention. Subjects were required to remember to transfer coins between hands and in a given order. In the dual task, attentional capacity is challenged again as subjects are required attend to the C3t rules whilst simultaneously reciting the alphabet as quickly as possible. ***Alphabet recitation:*** Previous studies suggest that tasks with low cognitive demands were more sensitive than those with high cognitive demands (Snowden *et al.*, 2001; Thompson *et al.*, 2010). In addition, pre-clinical research suggests that the dorsolateral striatum is involved in performing fixed, automatic behaviours (Yin, Knowlton and Balleine, 2004). Reciting the alphabet is a fairly simple task that is regularly recited from a young age. For many, by early adulthood, it means that this recitation would pose little attentional demand as the memory is retrieved and automatically recited (Ashby, Turner and Horvitz, 2010; Turner and Desmurget, 2010). It was hypothesized reciting the alphabet would load extra stress on the fronto-striatal circuitry making the dual task even more challenging for people with striatal dysfunction.

Straight forward to train and administer	It is important that outcome measures selected are easy and uncomplicated to set up, whilst also performed in a reasonable time frame to minimize the burden on subjects.	The C3t takes between 5-10 minutes to perform. Due to the set criteria developed for each C3t item, the length of the C3t assessment is dependent on functional ability. In addition, as the C3t is used to measure bilateral function, it only needs to be performed once.
Compact	Clinic space is often limited. Also outcome measures may need to be transported to different clinical locations, therefore it is important assessments are compact and lightweight.	The C3t was designed so construction involved few and small test components. This meant the deconstructed C3t is compact and requires little room for storage.
Quantitatively scored	Outcome measures that are quantitatively reduces subjectivity and also allows for more sensitive change over time (Hobart, Freeman and Thompson, 2000).	The C3t is rated using time as a primary measure. Performance accuracy is also recorded and combined with time taken to produce a C3t total score.

The table presents a list of the criteria which led to the development of the Clinch token transfer test (C3t).

2.3.1.2 C3t test procedure

The C3t consists of three assessment items: 1) C3t Baseline Simple; 2) C3t Baseline Complex; 3) C3t Dual task (Figure 11). The description for each assessment item are explained below.

1. C3t Baseline Simple: The participant was asked to transfer British coins (£2, £1, 50p, 20, 10, 5p, 2p, 1p) into a moneybox in **size** order, starting with the biggest coin (£2) to the smallest coin (5p). Coins were already presented to the participant in size order, with the £2 coin positioned furthest away from them and the 5p coin closest to them (Figure 11). This baseline test was required to see if participants could follow a simple set of task rules, and also pick up coins of varying sizes and release them accurately into a standardised moneybox slot.

2. C3t Baseline Complex: British coins were presented in the same order as the Baseline Simple. This assessment item was designed to add cognitive load. The participant was asked to transfer British coins into a moneybox starting with the highest **value** coin, to the lowest.

3. C3t Dual task: British coins were positioned in the same order as the Baseline tasks. In this assessment item, the participant performed the same test as the Baseline Complex, whilst also reciting the alphabet. This was designed to add a dual element to the assessment. The participant was required to perform two tasks simultaneously which required different overall outcomes; to transfer coins into a moneybox (motor element) and to continuously recite the alphabet (cognitive element).

To ensure that poor performance in the Baseline Complex and the C3t Dual task was because of task complexity and not because the participant could not count backwards, or could not recite the alphabet, the participant was asked to perform a

Value Baseline and an Alphabet Baseline task. This was carried out whilst sitting, allowing full attentional capacity to be directed to these tasks alone. For the Value Baseline, the participant was presented with eight values (200, 100, 50, 20, 10, 5, 2, 1) printed on laminated card and asked to recite the highest value number in order to the lowest value number. For the Alphabet baseline, participants were asked to recite the alphabet once, as quickly as possible. Both the Value baseline and the Alphabet baseline were performed before the C3t Baseline Complex and the C3t dual task. If the participant 'passed' these tasks they proceeded to the C3t Baseline Complex and the C3t Dual task.

Figure 11: The Clinch token transfer test (Version 1): British coins were positioned on a non-slip mat and presented in size order (largest to smallest). The participant was asked to transfer coins in order of size, to the non-dominant hand and into a moneybox (Baseline simple). For the Baseline complex, and the dual task, the coins were presented in the same order as the Baseline simple but they were instead asked to transfer the coins in order of value, without and whilst reciting the alphabet (Baseline complex and dual task respectively).

2.3.1.3 Rating the C3t

The primary measure used to assess performance in the C3t was time taken. Time taken was selected rather than an ordinal scoring method to avoid observer bias. This would also allow a more precise method to measure change in disease symptoms. Consistency in time measures were established by asking participants to start with their hands placed on their legs. The time started when the participant was told to "Go" and the time was stopped as soon as the last coin was released from the participant's fingers into the moneybox. Although a set of written instructions were read to participants prior to the test performance, it was soon clear that some participants would forget to transfer coins between their hands, resulting in a faster performance time. Another common problem was transferring coins in an order which contrasted those instructed. Therefore, a list of *errors* were developed, which were recorded by the researcher if the participant failed to follow the task rules. Any errors committed affected performance accuracy (Equation 1). It was also important that any coins dropped out of reach were also recorded. If a coin was dropped out of the test board then the participant was told to leave it and continue with the next coin. To incorporate test accuracy and the time taken to perform each C3t assessment item, an equation was developed to generate a C3t total score (Equation 2).

Performance in the Alphabet Baseline task was recorded using time taken and the number of correct letters of the alphabet recited. From this, the number of correct letters of the alphabet said per second was calculated (Equation 3). This method was chosen as it meant both time and accuracy were combined to form one score (Alphabet rate). In the C3t Dual task the participant was asked to continuously recite the alphabet until they released the last coin into the moneybox. Calculating the alphabet rate meant that alphabet performance could be compared from the baseline and dual task.

Table 15: C3t errors

Error Type	Error meaning
Transfer error	The subject fails to transfer tokens between hands.
Value error	The subject transfers the tokens in an incorrect size or value order.
Dropping a token	The token falls or rolls outside the test box.

If the subject committed an error presented in this table then this was recorded and acknowledged in the performance accuracy score (Equation 1).

Equation 1: C3t Performance accuracy

$$\left(\frac{(16 - \text{The number of errors made})}{16}\right) * 100 = \text{Accuracy (\%)}$$

Equation 1: Whilst the subject performed the C3t, the total number of transfer and rule errors committed were recorded (refer to Table 15). The C3t required participants to transfer 8 coins into the moneybox. This meant the participant could potentially make 8 transfer errors and 8 rule errors (totalling 16 possible errors). During the C3t performance, any errors committed were recorded. These were summed, subtracted from 16, divided by 16 and multiplied by 100 to give percentage accuracy.

Equation 2: C3t total score

$$\left(\frac{(8 - \text{The number of coins dropped out of reach})}{\text{Time taken (s)}}\right) * \text{Accuracy (\%)} = \text{C3t total}$$

Equation 2: Before the test began, the participant was instructed that if a coin dropped or rolled off the test set up, then to leave it and to continue with the next coin. If a coin did drop out of reach, this was recorded by the researcher. At the end of the assessment, the number of coins dropped out of reach were summed. As the C3t setup involved 8 coins, any coins dropped out of reach were subtracted from 8. This was divided by the time taken to complete the C3t assessment item and multiplied by the accuracy calculated from Equation 1. Essentially this calculation equated to: the average time taken to transfer each coin, multiplied by the percentage accuracy (the number of errors made).

Equation 3: Alphabet rate

$$\frac{\text{Number of correct letters of the alphabet recited}}{\text{Time taken (s)}} = \text{Correct letters said per second}$$

Equation 3: The number of correct letters of the alphabet recited during the alphabet baseline and dual task were recorded. This was divided by either by the time taken to complete the Alphabet Baseline or by the time taken to complete the C3t Dual task to give the alphabet rate.

2.3.2 Methods

Please refer to the Methods in Part 1 (Section 2.2.2) for information on the design, setting, participants, additional participant information and statistics on this study.

2.3.2.1 Dual task procedures

A brief description of the C3t is described in Section 1.3.1.1. A performance threshold was set for each assessment item so participants had to 'pass' set criteria during the Baseline tasks to move onto the complex items. A full description of the testing procedures can be located in Appendix 2. The manual developed for the C3t used in this Chapter is presented in Appendix 3.

2.3.2.2 Rating participant performance

Performance was rated using the time taken, performance accuracy, C3t total score and Alphabet rate and dual task cost, described on page 102.

2.3.3 Results

Demographic data from participants that attempted each dual task assessment are presented in Table 16. Twenty-nine people with HD attempted the C3t from which nineteen completed the full assessment.

People in stages 3 and 4 were combined given the limited number of people recruited in stage 4 (n=1). No people in stage 5 were recruited. Final TFC disease stage groups were stage 1, stage 2, stage 3,4.

The data in Table 16 revealed that stage 1 subjects were significantly younger than stage 3,4. There was no significant difference in the number of years in education or body mass index for any group.

Table 16: Part 2: C3t Demographic data

		Pre-HD	Manifest	Group differences	TFC disease stage			Group differences
					1	2	3,4	
C3t baseline simple	**n (male:female)**	n=3 (3:0)	n=26 (15:11)		n=9 (7:2)	n=5 (2:3)	n=15 (9:6)	
	Age	39.67 (9.87)	54.88 (14.95)	t (27) = -1.717, p = n.s.	40.44 (9.89)	52.8 (19.29)	61.2 (10.8)	F (2,26) = 8.076, p < 0.01 (s1 vs s3)
	TMS	0.33 (0.58)	51.62 (24.41)	t (27) = -3.536, p < 0.001	13.67 (13.41)	41.6 (7.09)	67.47 (17.5)	F (2,26) = 35.993, p < 0.001 (all s.d)
	TFC	13 (0)	6.65 (3.86)	t (27) = 2.804, p < 0.01	12.67 (0.71)	8.4 (1.34)	3.73 (0.96)	F (2,26) = 245.675, p < 0.001 (all s.d)
	Functional scale	25.00	16.36 (6.73)	t (26) = 2.188, p < 0.05	24.56 (0.88)	21.8 (0.44)	11 (3.37)	F (2,25) = 91.172, p < 0.001 (s3 vs s1 and s2)
	Independence scale	100.00	72.08 (17.93)	t (25) = 2.65, p < 0.05	97.22 (6.67)	80 (0)	59.64 (9.9)	F (2,24) = 57.8, p < 0.001 (all)
	CAG disease burden score	235.5 (24.56)	413.58 (84.85)	t (26) = 3.563, p < 0.01	301.56 (79.41)	405.2 (70.96)	440.43 (71.39)	F (2,25) = 11.154, p < 0.001 (s1 vs s3)
	Years of education	12.5 (0.71)	12.875 (3.91)	t (16) = -0.132, p = n.s.	11.67 (1.63)	14 (5.66)	13.3 (4.37)	F (2,15) = 0.451, p = n.s.
	BMI	30.93 (9.48)	26.12 (5.4)	t (23) = 1.332, p = n.s	29.23 (6.4)	24.81 (6.07)	25.8 (5.56)	F (2,22) = 1.119, p = n.s.

The table presents the mean values (Standard Deviation) for those recruited in the C3t baseline simple task. Groups were categorised as pre-HD (total functional capacity (TFC) = 13, Unified Huntington's Disease total motor score (TMS) <5), and manifest (all symptomatic stages). The manifest group was further delineated by TFC disease stage (stage 1, TFC =11-13; stage 2, TFC=7-10; stage 3,4, TFC=1-6. A t-test was used to compare groups differences between pre-HD with manifest subjects, where the t (degrees of freedom) = t value. An ANOVA was used to compare TFC group differences, where F (error, degrees of freedom)=F value. A Bonferroni post-hoc was used if significance was met, where p < 0.05. Demographic data was missing from a maximum of 11 participants. C3t = Clinch token transfer test; TFC = Total Functional Capacity; TMS= total motor score; BMI = body mass index.

2.3.3.1 Testing the C3t in people with HD

For clarity, a reference of the C3t test items are presented in Table 17.

Table 17: C3t assessment procedure

	Test order	Dual task item	Brief procedure
C3t	1	C3t baseline simple	Transfer coins in order of size
	2	Value baseline	Counting backwards from values presented
	3	Alphabet baseline	Recite the alphabet
	4	C3t baseline complex	Transfer coins in order of value
	5	C3t dual	Transfer coins in order of value and recite the alphabet

Measures reported in the results includes: time taken for items 1, 2, 4, 5; Total score, which combines time and any errors, for items 1, 4, 5; Correct letters of the alphabet recited per second for items 3 and 5. Where C3t = Clinch token transfer test.

C3t performance scores and statistical outcomes between Group and TFC disease stage are presented in Table 18.

Pre-HD subjects achieved significantly greater total scores compared to the manifest group, indicative of faster performance and fewer errors in the baseline simple, complex and dual task. There was no significant difference between Pre-HD and manifest subjects for the time taken to perform the C3t for the baseline simple, baseline complex, dual, value baseline or alphabet recited in the baseline or dual task.

When subject data was grouped based on TFC disease stage, performance progressively and significantly slowed with HD progression (Figure 12a). The same pattern of results were true for C3t total score (Figure 12b). For the value baseline, there was an overall significant effect, however following a Bonferroni post-hoc no significant differences were met between groups at any TFC disease stage. Performance results in the alphabet tests revealed that stage 1 subjects performed

significantly better than stage 3, but no differences between any other disease stage for the alphabet were detected (Figure 12c).

Table 18: C3t performance scores

C3t	Pre-HD	Manifest	t-test results		TFC stage			ANOVA results		Pairwise compairsons
			t value (df)	p value	Stage 1	Stage 2	Stage 3,4	F value (df, error)	p value	
Time bl simple (s)	15.16 ± 1.8	35.41 ± 4.05	$-1.667_{(27)}$	p = n.s.	17.12 ± 5.14	22.27 ± 6.89	46.71 ± 3.98	$11.92_{(2,26)}$	p < 0.001	s1 vs s3,4 p<0.001; s2 vs s3,4 p<0.05
Time bl complex (s)	15.53 ± 1.99	33.56 ± 4.6	$-1.485_{(21)}$	p = n.s.	17.3 ± 5.02	26.18 ± 6.74	47.91 ± 5.02	$9.64_{(2,20)}$	p < 0.001	s1 vs s3,4 p<0.001
Time bl dual (s)	16.15 ± 1.68	30.75 ± 2.91	$-2.05_{(18)}$	p = n.s.	18.86 ± 2.77	32.31 ± 3.71	39.98 ± 3.39	$12.33_{(2,17)}$	p < 0.001	s1 vs s2 p<0.05; s1 vs s3,4 p<0.001
Total bl simple	54.14 ± 5.78	28.95 ± 3.43	$2.423_{(27)}$	p < 0.05	50.8 ± 3.83	36.86 ± 5.13	18.25 ± 2.96	$23.28_{(2,26)}$	p < 0.001	s2 vs s1 and s3,4 p<0.001
Total bl complex	53.1 ± 6.26	29.04 ± 3.21	$2.76_{(21)}$	p < 0.05	47.57 ± 3.07	30.09 ± 4.12	17.95 ± 30.7	$23.48_{(2,20)}$	p < 0.001	s1 vs s2 p<0.01; s1 vs s3,4 p<0.001
Total bl dual	50.64 ± 5.39	28.11 ± 3.06	$2.93_{(18)}$	p < 0.01	43.58 ± 3.18	24.28 ± 4.27	19.36 ± 3.9	$13.49_{(2,17)}$	p < 0.001	s1 vs s2 p<0.05; s1 vs s3,4 p<0.001
Value time bl (s)	5.98 ± 1.15	10.77 ± 1.65	$-1.098_{(21)}$	p = n.s.	6.53 ± 2.24	7.77 ± 2.84	14.23 ± 2.01	$3.73_{(2,20)}$	p < 0.05[$]	n.s.
Alphabet bl (letters/s)	2.54 ± 0.24	1.75 ± 0.25	$0.609_{(22)}$	p = n.s.	2.63 ± 0.37	2.02 ± 0.49	1.29 ± 0.28	$4.25_{(2,26)}$	p < 0.05	s1 vs s3,4 p<0.05
Alphabet dual (letters/s)	1.61 ± 0.47	1.24 ± 0.2	$0.73_{(18)}$	p = n.s.	1.86 ± 0.22	0.98 ± 0.29	0.71 ± 0.26	$4.25_{(2,17)}$	p < 0.01	s1 vs s3,4 p<0.05
time bl cost (%)	2.12 ± 2.13	11.51 ± 3.63	$-0.978_{(21)}$	p = n.s.	1.98 ± 4.86	14.52 ± 6.52	16.23 ± 4.86	$2.42_{(2,20)}$	p = n.s.	/
time dtc (%)	6.1 ± 4.89	17.84 ± 4.03	$-1.132_{(21)}$	p = n.s.	9.47 ± 5.02	27.64 ± 6.74	16.35 ± 6.15	$2.336_{(2,17)}$	p = n.s.	/
total bl cost (%)	2.12 ± 2.13	15.12 ± 4.35	$-1.176_{(21)}$	p = n.s.	3.17 ± 5.78	16.4 ± 7.75	22.04 ± 5.78	$2.761_{(2,20)}$	p = n.s.	/
total dtc (%)	6.1 ± 4.89	23.59 ± 4.37	$-1.621_{(21)}$	p = n.s.	11.4 ± 5.32	34.55 ± 7.13	24.01 ± 6.51	$3.54_{(2,17)}$	p = n.s. (0.052)	/
alphabet dtc (%)	76.3 ± 35.77	184.12 ± 54.78	$-0.805_{(21)}$	p = n.s.	50.85 ± 62.21	200.21 ± 83.46	316.71 ± 76.19	$3.752_{(2,17)}$	p < 0.05	s1 vs s3,4 p<0.05

The mean test scores are recorded to 2 decimal places ± SEM. Groups were categorised as pre-HD (total functional capacity (TFC) = 13 and Unified Huntington's Disease total motor score <5) and manifest (all symptomatic stages). The manifest group was further delineated by TFC disease stage (stage 1, TFC =11-13; stage 2, TFC=7-10; stage 3,4, TFC=1-6). All measures in dark blue are recorded in seconds, where a greater score is indicative of worse performance. All measures in light blue show scores where a lower score is indicative of worse performance. Total score has no units and combines time taken with number of errors made. The alphabet is assessed as the number of correct letters of the alphabet recited per second. Dual task cost is shown in orange and presented as a percentage. A positive percentage is indicative of slower or worse dual task performance relative to baseline, whereas a negative dual task cost is indicative of better performance in the dual task compared to baseline. Significance was set so p < 0.05 and followed by a Bonferroni post-hoc test.
Where TFC= total functional capacity; ANOVA = analysis of variance; bl = baseline; n.s. = not significant; s = stage; dtc = dual task cost; [$] = A difference reported but following a post-hoc this need not reach significance between any particular group.

When dual task cost was calculated, stage 3,4 subjects were significantly slower and less accurate in the dual task condition of the alphabet task compared to stage 1 (Figure 12d). There was no difference between any other disease stages for any other C3t item.

Results from a two way repeated measures ANOVA are presented in Table 19. This revealed that people in stages 2 and 3,4 took significantly longer to perform C3t dual task compared to the Baseline simple. People in Stage 3,4 also took significantly longer to perform the Baseline complex compared to the simple. There was no significant difference in the alphabet performance in the dual task, or the C3t total score in the Baseline complex or dual task.

Table 19: C3t two way repeated measures ANOVA

	Dual task item	Baseline simple vs Baseline complex	Stages	Baseline simple vs dual task	Stages
C3t	Time	$F_{(2,20)}$=4.677, p < 0.05	Stage 3: p < 0.001	$F_{(2,17)}$=3.634, p < 0.05	Stage 2: p < 0.001 and Stage 3: p < 0.01
	Total	$F_{(2,20)}$=0.498, p = n.s.	/	$F_{(2,17)}$=1.202, p = n.s.	/
	Alphabet	no test	/	$F_{(2,16)}$=0.244, p = n.s.	/

This table presents the results from a two way repeated measures ANOVA. If this returned as significant (where p < 0.05), a Bonferroni post-hoc was used to identify which disease stage this was relevant to. This is presented in the 'Stages' column. The results from this table and those presented in Table 18 revealed that Stage 2 and stage 3 performed the C3t significantly slower in the dual task compared to Baseline simple. Stage 3 were also significantly slower in the Baseline complex compared to the Baseline simple. C3t total and the alphabet performance did not significantly differ (n.s.) in the Baseline complex or dual task, when compared to the Baseline simple. C3t = Clinch token transfer test.

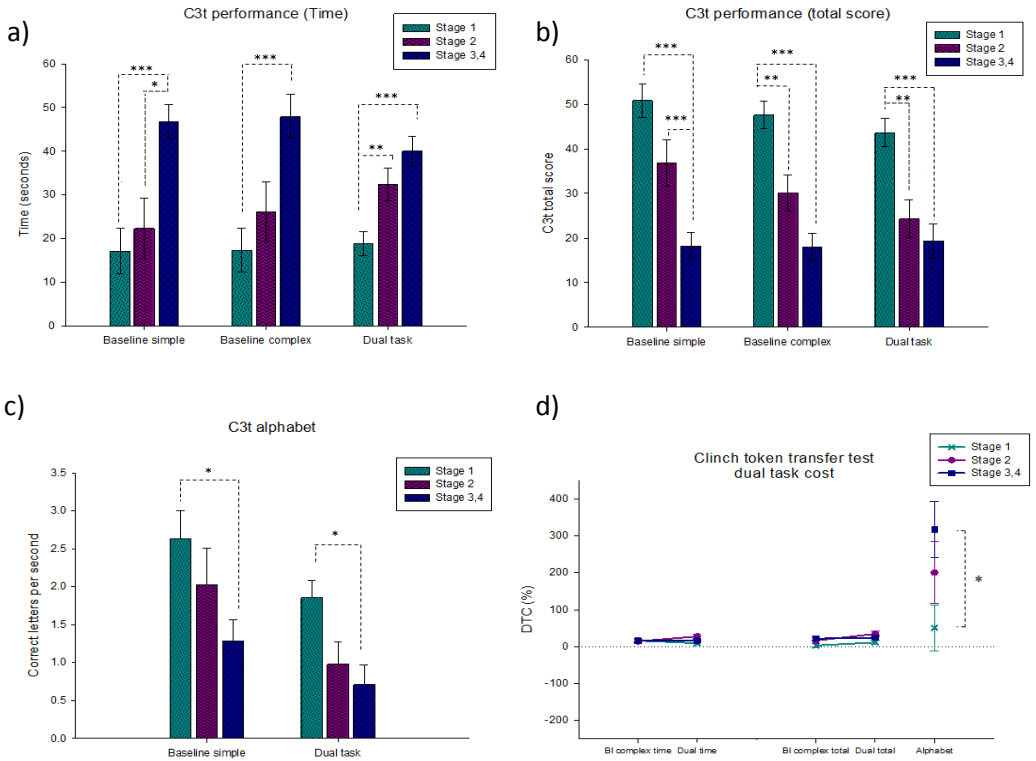


*Figure 12: C3t Version 1 bar graphs. Bar graphs represent the mean C3t performance with SEM error bars. Figure a-c present C3t performance across the Baseline simple, complex and dual task. Figure a shows time taken (seconds), where a lower score is indicative of better performance. Figure b shows C3t total score (no unit), where a higher score is indicative of better performance. Figure c shows alphabet scores (letters/second), where a higher score is indicative of better performance. Figure d presents the dual task cost (%), where a higher percentage is indicative of greater costs in the complex and dual tasks relative to baseline. Where * p<0.05, ** p<0.01, *** p<0.001.*

2.3.3.2 How does the C3t and the lower limb dual task costs compare with HD specific measures?

Correlation results are presented in Table 20.

Performance in the C3t and the *Step and Stroop* items correlated with UHDRS assessments more so than the *TUG and LVF* and *Walk and Talk*. The C3t total simple and complex baseline tasks correlated with the UHDRS TFC, TMS, FAS and independence scores more than any other dual tasks. All C3t items, except for the value and alphabet tasks strongly correlated with the CAG score, SF-12 mental summary, PBA apathy and executive function. C3t baseline simple and complex time and total scores also correlated with the SF-12 physical summary. The LVF hard (baseline and dual), cadence, duration of double limb support, stride length CoV (dual hard) and alphabet hard dual task correlated with the least amount of HD specific and quality of life measures. There was no correlation between any hard baseline or dual item and the UHDRS or quality of life measures for the *Walk and talk* dual task. Cadence and duration of double limb support baseline and dual measures also correlated with few UHDRS and quality of life scores.

Table 20: Correlation between dual task items and HD specific and functional questionnaires

		UHDRS TMS	UHDRS TFC	UHDRS TFC stage	UHDRS FAS	UHDRS Independence scale	UHDRS symbol digit correct	UHDRS LVF	UHDRS Stroop colour naming	UHDRS Stroop word reading	CAG score	SF-12 physical summary	SF-12 mental summary	PBA apathy	PBA executive function
C3t	C3t Time bl simple	.706**	-.717**	.717**	-.759**	-.768**	-.656**	-.636**	-.756**	-.630**	.566**	-.469	-.679**	.669**	.692**
	C3t Time bl complex	.607**	-.720**	.807**	-.603**	-.685**	-.625**	-.613**	-.716**	-.515*	.425*	-.639*	-.661*	.760**	.642**
	C3t Time dt	.627**	-.787**	.762**	-.598**	-.678**	-.697**	-.592**	-.828**	-.650**	.261	-.477	-.602*	.599**	.330
	C3t total bl simple	-.799**	.829**	-.803**	.791**	.827**	.779**	.740**	.807**	.702**	-.645**	.494	.571*	-.648**	-.608**
	C3t total bl complex	-.765**	.835**	-.853**	.711**	.778**	.777**	.753**	.833**	.710**	-.600**	.612*	.674*	-.666**	-.533*
	C3t total dt	-.706**	.768**	-.752**	.583**	.689**	.751**	.606**	.758**	.668**	-.447*	.505	.634*	-.565**	-.361
	Value bl	.583**	-.558**	.528**	-.631**	-.634**	-.543**	-.505*	-.603**	-.573**	.403	-.082	-.219	.532*	.615**
	Alphabet bl	-.399	.554**	-.553**	.454*	.530*	.431*	.542**	.449*	.404	-.446*	.142	.283	-.362	-.407*
	Alphabet dt	-.419	.632**	-.636**	.438	.506*	.474*	.413	.638**	.490*	-.301	.529	.518	-.395	-.189
TUG and LVF	TUG bl	.479**	-.496**	.461**	-.549**	-.531**	-.487**	-.417*	-.609**	-.513**	.349	-.709**	-.225	.424*	.445*
	TUG easy dt	.657**	-.756**	.796**	-.713**	-.733**	-.663**	-.586**	-.776**	-.614**	.455*	-.643**	-.157	.653**	.536**
	TUG hard dt	.513*	-.685**	.753**	-.578*	-.655**	-.561*	-.419	-.669**	-.418	.320	-.803**	-.566	.750**	.556*
	LVF easy bl	-.562**	.428*	-.330	.384	.411*	.601**	.481*	.518**	.544**	-.401*	.082	.036	-.184	-.074
	LVF easy dt	-.630**	.623**	-.561**	.617**	.588**	.612**	.607**	.718**	.709**	-.276	.231	-.005	-.412*	-.454*
	LVF hard bl	-.079	-.078	.098	.037	-.065	.131	.064	.144	.241	.052	.117	.259	.112	.341
	LVF hard dt	-.278	.355	-.384	.231	.213	.376	.286	.644**	.236	.000	.844**	.530	-.439	-.006
Step and Stroop	Step bl	-.637**	.690**	-.667**	.618**	.631**	.647**	.655**	.726**	.666**	-.566**	.714**	.099	-.371**	-.350*
	Step easy dt	-.646**	.680**	-.652**	.619**	.622**	.672**	.663**	.791**	.729**	-.538**	.659**	.182	-.342*	-.423**
	Step hard dt	-.592**	.670**	-.677**	.529**	.518**	.643**	.617**	.845**	.686**	-.445**	.574**	.051	-.207	-.273
	Stroop easy bl	-.707**	.755**	-.707**	.686**	.736**	.756**	.710**	.878**	.831**	-.497**	.465**	.160	-.394**	-.380**
	Stroop easy dt	-.691**	.749**	-.746**	.694**	.694**	.737**	.679**	.847**	.795**	-.552**	.606**	.372*	-.397**	-.497**
	Stroop hard bl	-.695**	.745**	-.697**	.640**	.730**	.785**	.560**	.810**	.743**	-.532**	.415*	.071	-.346*	-.250
	Stroop hard dt	-.648**	.759**	-.744**	.627**	.635**	.740**	.638**	.839**	.733**	-.487**	.507**	.178	-.461**	-.339*

		UHDRS TMS	UHDRS TFC	UHDRS TFC stage	UHDRS FAS	UHDRS Independence scale	UHDRS symbol digit correct	UHDRS LVF	UHDRS Stroop colour naming	UHDRS Stroop word reading	CAG score	SF-12 physical summary	SF-12 mental summary	PBA apathy	PBA executive function
Walk and talk	Velocity bl	-.569**	.676**	-.645**	.627**	.638**	.679**	.540**	.664**	.611**	-.462*	.677**	.290	-.513**	-.459**
	Velocity easy dt	-.621**	.731**	-.707**	.608**	.640**	.674**	.581**	.736**	.609**	-.429*	.752**	.334	-.557**	-.518**
	Velocity hard dt	-.335	.438	-.352	.348	.375	.576**	.232	.617**	.453	-.265	.356	-.061	-.311	-.120
	Cadence bl	-.109	.235	-.241	.146	.216	.278	.211	.087	.186	-.044	.246	-.141	-.224	-.205
	Cadence easy dt	-.020	.151	-.201	-.005	.035	.112	.188	.172	.097	.011	.433	-.052	-.250	-.300
	Cadence hard dt	-.327	.477*	-.417	.370	.394	.429	.267	.590*	.403	-.235	.435	-.212	-.141	-.072
	Duration of double limb support bl	.279	-.241	.200	-.396*	-.343	-.288	-.186	-.266	-.302	.403*	-.383	-.185	.100	.095
	Duration of double limb support easy dt	.352	-.301	.241	-.346	-.387	-.230	-.239	-.217	-.269	.270	-.549*	-.335	.031	.033
	Duration of double limb support hard dt	.049	-.022	-.101	-.005	-.171	-.243	.171	-.132	-.035	-.024	.000	.371	-.120	-.056
	Stride length CoV bl	.528**	-.517**	.471**	-.621**	-.540**	-.466**	-.391*	-.393*	-.513**	.576**	-.506*	-.209	.217	.340
	Stride length CoV easy dt	.485**	-.510**	.454*	-.470*	-.523**	-.366	-.295	-.409*	-.340	.347	-.409	-.212	.191	.196
	Stride length CoV hard dt	.111	-.120	.010	-.089	-.098	-.340	.063	-.232	-.134	.075	.097	.305	.320	.095
	Alphabet easy bl	-.482**	.567**	-.566**	.465*	.530**	.392*	.570**	.458*	.398*	-.545**	.427	-.146	-.371	-.320
	Alphabet easy dt	-.481*	.590**	-.621**	.520*	.524*	.406	.467*	.426	.451*	-.231	.570	.395	-.388	-.373
	Alphabet hard bl	-.514*	.529*	-.502*	.464*	.521*	.538*	.608**	.648**	.603**	-.609**	.265	-.416	-.295	-.294
	Alphabet hard dt	-.404	.414	-.364	.238	.375	.538*	.264	.711**	.446	-.249	.188	-.406	-.105	-.018

p<0.05 (light pink);p<0.01 (dark pink). Unified Huntington's disease rating scale (UHDRS); Total motor score (TMS); Total functional capacity (TFC); Functional assessment score (FAS); Letter verbal fluency (LVF); Short-form 12 (SF-12); Performance behaviour assessment (PBA); Clinch token transfer test (C3t); baseline (bl); dual task (dt); Timed Up and Go (TUG); Coefficient variation (CoV).*

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2.3.4 Discussion

The C3t was developed to supply clinicians and researchers with a functional upper limb assessment specific to the symptoms that manifest in HD. The C3t assessment consisted of five primary items, where performance in three were of primary interest: the C3t Baseline simple, Baseline complex and the dual task. Together, the C3t was designed so it was quick to perform and easy to set up. It also used a quantitative rating method to score performance to improve reliability and test sensitivity. Performance was not only sensitive to disease stage but it also significantly correlated with all the UHDRS measures, equally to the *Step and Stroop* items and more than the *TUG and LVF,* and the *Walk and talk* dual tasks used in Part 1.

The primary finding in this study was that people in the more advanced disease stages took significantly longer to perform the C3t, compared to stage 1 and stage 2 in the Baseline simple task. Importantly performance in the C3t dual task revealed that people in stage 1 performed significantly faster than stage 2. This was evident in the C3t Baseline complex and the dual task when differences between C3t total score were analysed, rather than just time taken alone. This suggests that the C3t could be a useful outcome measure for clinical trials, as developing therapeutics typically aim to target people in the early stages of HD (Kumar *et al.*, 2015). One explanation for this performance deterioration is motor impairment due degeneration of basal ganglia circuitry. This has been shown to affect dextrous movements when grasping small food items in people with HD (Klein *et al.*, 2011). Furthermore, the lateral striatum is implicated in such movements, as rats with unilateral quinolinic acid lesions to this region are impaired in a grasping and dexterity task when using their paw contralateral to the lesion, whilst maintaining performance on the ipsilateral side (Montoya *et al.*, 1991; Döbrössy and Dunnett, 2003, 2004). Importantly, previous studies have shown that performance in such grasping and dexterity tasks gradually improves following cell replacement therapy to the lesioned striatum (Döbrössy and Dunnett, 2003; M. D. Döbrössy and Dunnett, 2005). This suggests that the C3t could be a useful outcome measure to quantify changes following therapies that specifically target the striatum, such as cell replacement therapies. Additional explanations behind performance deterioration in the advancing

disease stages is acknowledged in Chapter 3, which describes the development and validation of a new standardised C3t.

The results in this study also revealed that task complexity had an impact on C3t performance. For instance, people in stage 3,4 performed both the C3t Baseline complex and the dual task significantly slower than the Baseline simple. Whereas, people in stage 2 performed the C3t significantly slower in just the dual task, and performance in stage 1 did not significantly differ between any assessment item. This suggests that adding task complexity is a useful way to tease apart performance differences in different disease stages in HD (Vaportzis *et al.*, 2014; Stout, Glikmann-Johnston and Andrews, 2016). The results also revealed that there was no significant difference in alphabet rate from baseline performance to the dual task, suggesting that people with HD might prioritise cognitive tasks when performing two tasks simultaneously. This could have negative implications when performing complex upper limb tasks at home. For example, using sharp utensils in the kitchen whilst talking, could lead to an increased risk of injury, if more attentional resources are allocated to the cognitive (talking) task. A larger sample size is required to see if these findings are consistent.

Findings from this study also revealed that performance in the C3t and the *Step and Stroop* dual tasks significantly correlated with HD specific measures including the UHDRS motor, cognitive, behavioural and functional questionnaires, as well as the SF-12 and PBA measures, more than the *TUG and LVF* and the *Walk and talk*. Furthermore, C3t performance based on time and accuracy (C3t total) correlated better with all UHDRS measures and teased apart disease stage more so than time taken alone. In addition, the dual task assessment items in the Step and Stroop and C3t significantly correlated with the CAG disease burden score (Penney *et al.*, 1997), suggesting that assessment items from both dual tasks were sensitive to disease progression in HD.

Although the C3t could provide clinicians and researchers with a functional upper limb outcome measure for people with HD, there are a number of limitations that could restrict its use across other clinical sites. For example, the current C3t setup uses British coins. Although, the results in this study were sensitive to disease stage, the same result

might not apply to clinics where the British currency is less familiar. This could lead to longer performance times and less people 'passing' to the more complex C3t items. In addition, the current setup is not standardised and so the set up may vary in consistency, which could lead to more unreliable results.

2.4 Conclusions and future work

The findings in this Chapter revealed that dual task interference and prioritisation strategies may differ in HD depending on the dual task carried out. Where some prioritised the motor task over the cognitive (*Step and Stroop),* whereas the opposite relationship was true for the *TUG and LVF.* Furthermore, mutual interference was more evident in the *Walk and talk* and the *Clinch token transfer test (C3t).*

The dual tasks selected and developed in Part 1 revealed that the *Step and Stroop* best distinguished between disease stage, more so than the *TUG and LVF* and the *Walk and Talk*. As these were being developed, it became evident that current upper limb functional assessments for people with HD are limited. This led to a second study that ran in parallel, in which a new upper limb dual task assessment was developed (Part 2) and was called the Clinch token transfer test (C3t). This consists of three main assessment items which were designed to test bilateral dexterity with increasing task difficulty. Performance in this task revealed that people with HD were significantly slower performing the C3t as HD progressed, and with increasing task difficulty. Further analysis revealed that both the *Step and Stroop* and the C3t significantly correlated with HD specific measures and did so more than the *TUG and LVF*, and the *Walk and Talk*.

This study is the first to examine differences in dual task performance across different stages of HD. This led to a limitation of the studies consisting of relatively small group sizes. Further validation will therefore require larger group sizes. Another limitation were potential practice effects. As the assessment items in each dual task were performed more than once, and in a short period of time, this may have led to results improving or stabilizing (Reeves *et al.*, 2007). One way to truly assess dual task interference in the future would be to counterbalance the baseline and dual task assessment items, or leave a longer period between the baseline and the dual task. As a priority in this study was to ensure subjects could perform the baseline task before proceeding to the dual task, counterbalancing was not an option.

The findings in this study provide evidence that the C3t and the *Step and Stroop* are suitable upper limb and lower limb functional outcome measures for people with all stages of HD. In addition, performance in the C3t was able to tease apart people in early disease stages (stage 1 and stage 2). Distinguishing performance differences within the early stages of HD is of particular interest as this group is most likely to be amendable to neuroprotective treatment (Reetz, Werner and Schiefer, 2015). Therefore, the C3t may be suitable for inclusion in assessment batteries for novel interventions, such as cell transplantation. Prior to this, it is important that some of the design issues associated with the C3t are overcome, such as minimizing cultural bias associated with British coins, and standardising the C3t setup. Therefore, the study in Chapter 3 was developed to overcome some of these fundamental issues, as well as validating the new C3t design in a larger cohort of people with HD.

Chapter 3

Validating a new functional dexterity assessment for people with Huntington's disease

Chapter Summary

The findings in Chapter 2 revealed that the Clinch token transfer test (C3t) could be a useful functional, upper limb assessment for people with HD. However, given that the C3t setup was not standardised, this meant that using the C3t in other clinical sites would lack consistency and decrease the reliability of this assessment. Therefore, the aim of this Chapter was to standardise the new C3t design (C3t version 2; Part 1), evaluate and optimise the C3t v2 in people gene positive with HD (Part 2), and validate this a large group of people with HD and healthy controls (Part 3).

3.1 Introduction

Selecting appropriate outcome measures plays an essential role to assess change in symptoms over time, assist with disease diagnosis or determine the effectiveness of a treatment (Williamson *et al.*, 2012; Iansek and Morris, 2013). However, the usefulness of an outcome hinges on the population being tested and the question being asked. To establish this, selecting outcome measures that have known clinometric properties is desirable (Iansek and Morris, 2013) (See Chapter 1, Section 1.3.1). These are used to assess the quality of an assessment, which includes important questions that should be considered, such as cost and length of time to perform, as well as important properties such as validity and reliability (Kimberlin and Winterstein, 2008) (refer to Chapter 1,Table 1).

Validity is the extent to which an assessment measures what it is intended to measure (Kimberlin and Winterstein, 2008). An assessment that is valid suggests that; when it is used for a specific purpose, such as in a certain population, the outcomes are free from error. Different types of validity exist to measure the extent of these possible errors, and these can be divided into three categories: internal, external and test validity. Furthermore, each of these form subcategories and are described in Chapter 1 (Figure 6). Another important factor is to measure how reliable, or how consistent, the outcomes from an assessment are (Portney and Watkins, 2009). For example, when developing a new assessment it is important to ensure that the rater is not subject to bias and remains consistent scoring an assessment when there are no change in symptoms. Reliability is also important to measure the chance of practice effects, which can be a problem if a patient repeatedly performs an assessment and becomes over familiar with the assessment procedure (Reeves *et al.*, 2007). As a result, improvements are not reflective of a change in symptoms but instead a gradual improvement in the general test procedure. Therefore measuring test-retest reliability in a population where no treatment is being assessed to compare assessment outcomes when no symptoms are expected to have changed is important (Kimberlin and Winterstein, 2008).

In Chapter 2, a new upper limb, functional assessment was developed to overcome the lack of upper limb functional assessments available for people with HD. This was called the Clinch Token Transfer Test (C3t), and involved 3 primary assessment items, which involved; transferring British coins into a moneybox slot in order of size (baseline simple) and value, without and whilst reciting the alphabet (baseline complex and dual task). This was developed so each item performed was more complex than the last. This was used as a way to tease apart people with different levels of functional ability and therefore, different stages in HD. Furthermore, to reduce the chances of floor and ceiling effects, each assessment item consisted of set criteria that the participant had to meet before continuing to the next complex assessment item. To reduce observer bias, C3t performance was rated using a specially developed C3t total score, which was calculated by combining both time taken to perform the C3t and performance accuracy. The findings in Chapter 2**,** suggest that the C3t could have potential to distinguish between different disease stages in HD. Furthermore, C3t performance scores significantly correlated with the UHDRS assessments. To overcome some of the constraints associated with C3t in Chapter 2, the aim of the study in this Chapter was to develop and validate a new standardised version of the C3t (C3t v2) in a larger cohort of people with HD, and to assess how these scores differ, compared to a healthy control group.

The data in this Chapter developed iteratively and formed three stages:

i. Part 1: Standardising the C3t
ii. Part 2: Testing and Optimising the C3t v2a
iii. Part 3: Validating the C3t v2b

Part 2 and Part 3 contain methods and results. These were conducted as part of one protocol. The methodology is therefore only described in detail in Part 2 and referred to where necessary in Part 3. A short summary is presented after Part 2 and a general discussion is presented at the end of this Chapter.

3.2 Part 1: Standardising the C3t

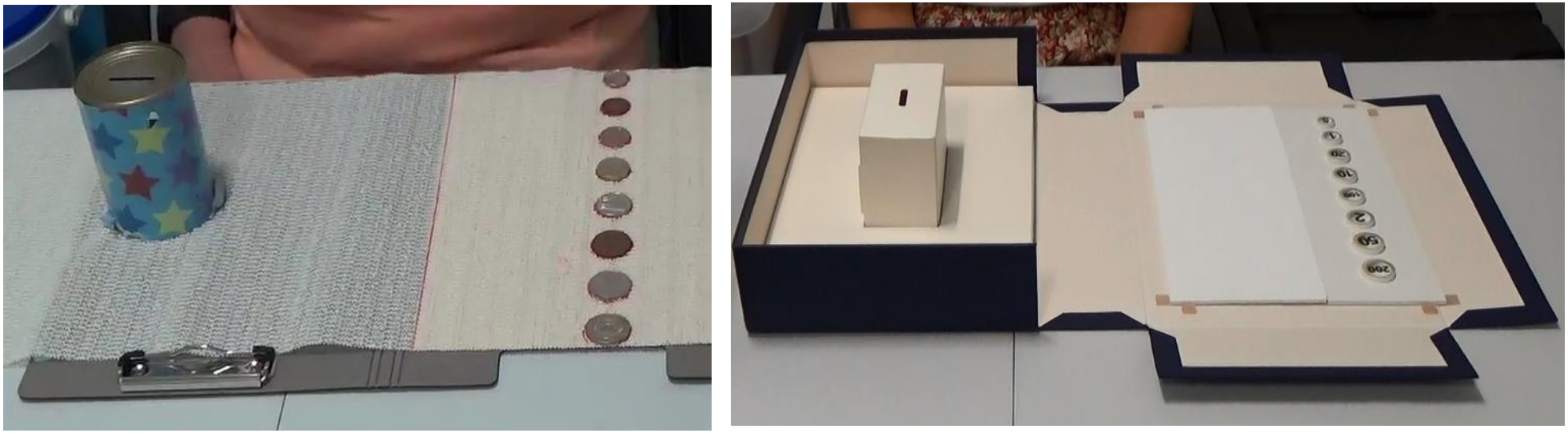
The results in Chapter 2 revealed that C3t performance could distinguish between different stages of HD, and that results significantly correlated with scores from the UHDRS. Although the original C3t test setup provided proof of concept, it was limited due to its unstandardised setup, meaning as a clinical outcome measure, the setup was not easily reproducible if it was used across different clinical sites. For instance, the moneybox used in Chapter 2 consisted of a moneybox slot carved in the base of a tin can. Although this moneybox was not ideal, it was purposely chosen so, if the C3t was used across other clinical sites, a moneybox with the same dimensions could be easily accessed and purchased or easily made. Another limitation was the use of British coins. This meant the assessment was biased towards those that were largely familiar with this currency. Not only could this influence performance accuracy and the time to perform the C3t, but it also limited the number of countries the C3t could be used in. Following the achievement of Strategic Development Funding (Cardiff University), a standardised version of the C3t was developed (C3t version (v)2). From this the aim was to meet criteria according to the clinometric table presented in Chapter 1 (Table 1). The key criteria included:

i. The dimensions and general setup of the C3t v2 were the same as the original C3t
ii. The setup was standardised
iii. Compact when not in use
iv. Robust and cost effective
v. Easy to use

To achieve the criteria set, the following changes were made:

i. The dimensions and general set up the same as the original C3t

For the C3t v2, new tokens and a new moneybox were designed and manufactured (Figure 13). A primary aim was for the C3t v2 to closely resemble the old assessment. This was to ensure that the results achieved in Chapter 2 remained as sensitive (if not more so) than the old version. This meant that the height of the moneybox, the size of the moneybox slot, the size of the tokens and the distance between the position of the tokens and the moneybox slot, remained the same between the two assessment versions. In addition, the values printed on the new tokens were 200, 100, 50, 20, 10, 5, 2, 1, which were the same as those presented in the Value baseline of the original C3t.



C3t v1 (before)

C3t v2 (after)

Figure 13: The C3t original set up (C3t v1) and the standardised set up (C3t v2). The dimensions of each test component remained the same between the unstandardised and standardised C3t.

ii. Standardised set up:

To ensure the C3t set up remained consistent each time it was used, it was important that the test components were always positioned in a fixed location. This was achieved in a number of ways. To guarantee the distance between the tokens and the moneybox slot was fixed, shallow grooves were carved into a 'money tray' which was positioned within a tiled frame on the test board (Figure 14). The grooves in the money tray also meant that the tokens were less likely to slip out of position if they were knocked during testing. One important consideration was to ensure that the grooves on the money tray did not make it harder for the participant to retrieve the tokens, which may have affected the performance results. Therefore, different token groove depths were trialled to prevent this from being a problem. For the moneybox, a space within a platform was designed which ensured the moneybox was positioned in a fixed location (Figure 15). As the moneybox fitted inside the platform, rather than on top of it, this also provided stability, meaning if it was knocked during testing it did not fall over or out of place. Another important change in the C3t v2 was to manufacture tokens in place of British coins. This was to ensure the C3t could be used across sites outside of the UK to minimise bias.

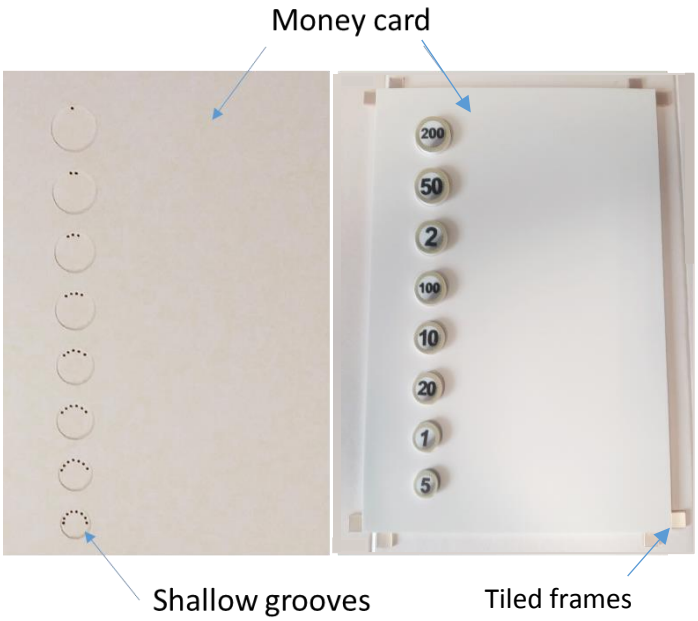


Figure 14: The money card without and with the tokens in position. Shallow grooves were carved into the money card meant that the position of each token was always fixed in the same location. In addition, tiled frames fixed the money card into position, meaning that the distance between the tokens and the moneybox always remained the same.

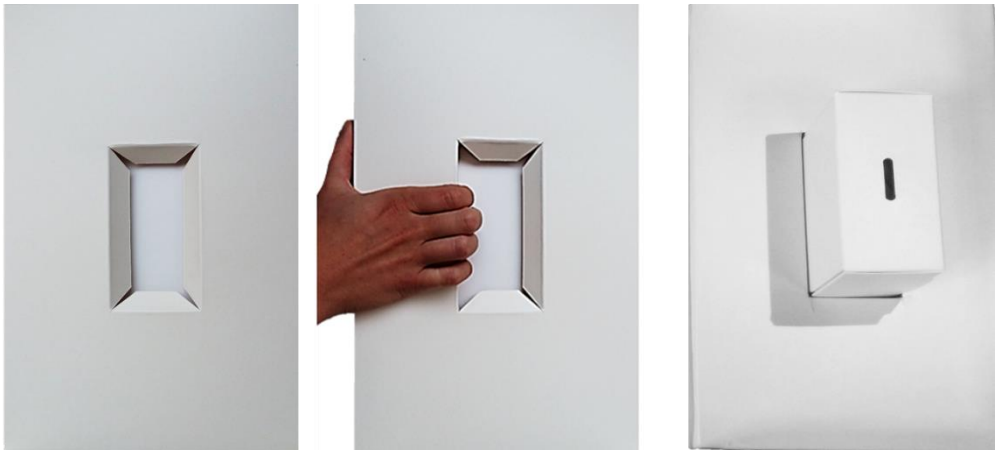


Figure 15: The moneybox fitted in a set location within a moneybox platform. As the moneybox fitted inside the platform, this meant that the platform walls ensured the moneybox remained in position if it was knocked during the testing procedure.

iii. Compact when not in use

The design of the C3t v2 specifically considered storage and transportation requirements, as all test components were housed within the C3t box to enhance the clinical utility (Figure 16).

Figure 16: The C3t v2 is compact and could easily be folded away whilst storing all of the assessment components when it was not in use.

iv. Robust and cost effective

It was important that the materials used to make the test components were strong to minimise breakage with repeated use and therefore maximising its longevity. However, the number of materials that could be used were limited as it was also important that the C3t components were cost effective. As a result, the C3t v2 was made of a mix of dense cardboard and plastic.

v. Easy to use:

An important component of the C3t v2 was the design of the outer box. This needed to be large enough to house all of the assessment components, but also compact so it could be easily stored when it was not in use. As a result, it was important to minimise the number of assessment components so the outer box did not have to house numerous parts. For example, when the C3t v2 was folded away, the tokens were housed inside the moneybox. This was designed so the base of the moneybox opened to access the tokens (Figure 17). In addition, when the C3t v2 was in use, the outer box opened and formed part of the testing board. To ensure the walls of the outer test box did not obstruct the subject from picking up the tokens, the outer walls on the one side of the test box folded down, meaning the subject could easily access the tokens (Figure 18).

To aid the efficiency of the C3t setup, a dot reference was printed on the side of each token which matched the number of dots printed in one of the grooves on the money card. This meant that the researcher could easily locate the placement of each token (Figure 14).

The C3t v2 was also designed so it considered left and right handed people. Therefore, before performing the C3t, participants were asked whether they were left or right handed. For right handed people, the moneybox was positioned on the right side of the test box (Figure 18). If the subject was left handed, the test board was rotated 180 degrees. A different money card was also used to ensure the tokens were still positioned from largest to smallest and maintained the same distance from the moneybox as the right handed setup.

Figure 17: The moneybox was designed so the base could easily open to retrieve the tokens during testing, whilst also providing storage for the tokens when the C3t was not in use.

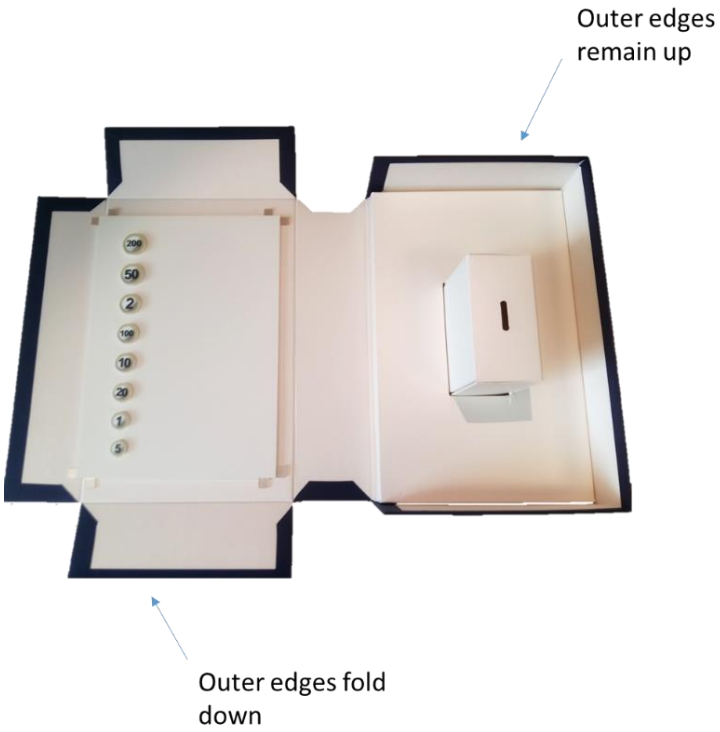


Figure 18: C3t v2 complete setup: The C3t set up. The edges of the outer box folded down on the side of the tokens. This meant that the edges did not obstruct the participant from accessing the tokens when performing the C3t. These edges only folded down on the side of the tokens, but remained fixed upright on the side of the moneybox. This allowed the C3t to fold, forming a rigid box when it was not in use.

3.3 Part 2: Testing and optimising the C3t v2

The aim of this section was to test the design of the new C3t v2 in people gene positive with HD.

3.3.1 Methods

3.3.1.1 Design

This research was a cross sectional, observational study.

3.3.1.2 Setting

People with HD were recruited from the South Wales HD research and management clinic. Recruitment began in September 2015 and continued to February 2016.

Ethical approval was obtained from South East Wales Research Ethics Committee (REC reference: 14/WA/1195). Many patients attending the HD clinic were already enrolled in the ENROLL-HD study (04//WSE05/89), and so their disease progression had been followed for a number of years. One of the optional components within the ENROLL-HD study was permission by participants to be contacted about other additional and affiliated HD research projects. In consenting to be enrolled in the ENROLL-HD study, participants also gave their permission for their unidentifiable data to be accessed by researchers conducting other HD related research.

Potential participants were approached at the beginning of clinic and if interested were given an information sheet on the study. Participants were then given as much time as needed to decide if they wanted to take part and were later approached to discuss the study and asked if they would like to participate. If the

participant was happy to proceed, they were asked to sign a consent form prior to participation. Potential participants also had the option to take part in the study at a future clinic visit.

3.3.1.3 Participants

The aim of this study was to recruit was to recruit all people gene positive for HD.

Inclusion criteria for people with HD

1) Confirmed to carry the HD gene through genetic testing
2) Aged 18 years or above
3) Enrolled in the ENROLL-HD study

Exclusion criteria for all participants

1) Inability to provide informed consent
2) Any comorbid condition that had the potential to confound the results of the study

3.3.1.4 Procedure

The C3t procedure is presented in Table 21 (Figure 19). In each C3t item, there were set criteria developed that the participant had to meet before proceeding to the next, more complex item. The full assessment setup, procedure and instructions can be located in Appendix 13.

Table 21: C3t V2 assessment items and procedure

Test order	C3t item	Procedure
1	C3t baseline simple	The subject was asked to transfer tokens in order of size as quickly as possible.
2	Value baseline	Whilst sitting the subject is presented with 8 values (50, 5, 10, 100, 200, 20, 2, 1) printed on a laminated card placed in front of them. The subject is asked to say aloud the highest value working their way in order to the lowest value.
3	Alphabet baseline	Whilst sitting the subject was asked to recite the alphabet as quickly as possible.
4	C3t baseline complex	The same procedure as (1) but tokens presented had values printed on them. The subject was asked to transfer tokens in value order starting with the highest value token to the lowest.
5	C3t dual task	The subject was asked to simultaneously and continuously recite the alphabet as quickly as possible whilst performing the same C3t procedure as (4). Tokens were presented in a different order to prevent practise effects.

The C3t assessment items were always performed in the order presented here. C3t = Clinch token transfer test.

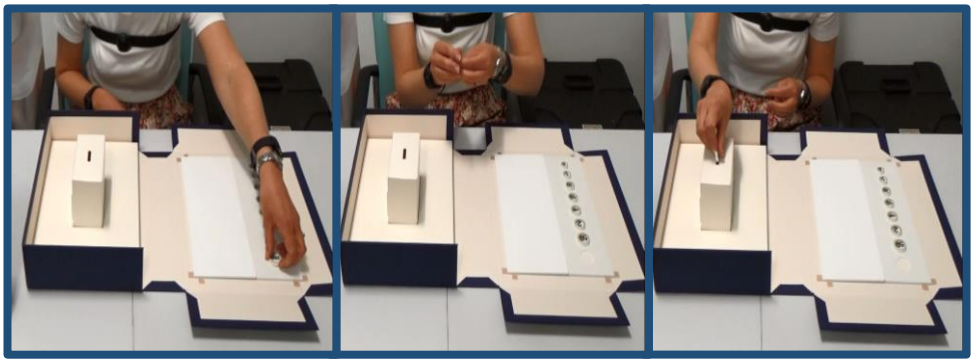


Figure 19: C3t procedure. This involved picking up tokens of different size, transferring them between hands and putting them into a moneybox.

3.3.1.5 Rating C3t performance

All participants recruited were video recorded whilst performing the C3t. The rating procedure for the C3t remained the same as those described in Chapter 2 (Section 2.3.1.3). Briefly, the time taken to complete the C3t baseline simple, complex and dual task was recorded. Participants were asked to start with their hands placed on their legs. As soon as they were instructed to "Go," the stopwatch started, and

stopped as soon as the last token was released into the moneybox. If an error was committed (presented in Table 22) then this was recorded and contributed to the C3t total score calculation. The C3t total score took the time taken to perform the C3t, the number of errors committed and any dropped tokens into account, resulting in a unit-less score which tend to fall in between 0-100 (refer to Chapter 2, Section 2.3.1.3, Equation 2: C3t total score). To measure the alphabet performance, the rate of correct letters said per second were calculated in the alphabet baseline and the C3t dual task.

Table 22: C3t v2 error meanings

Error Type	Error meaning
Transfer error	The subject fails to transfer tokens between hands.
Value error	The subject transfers the tokens in an incorrect size or value order.
Dropping a token	The token falls or rolls outside the test box.

Any errors committed during the C3t performance were recorded and were recognised when calculated in the C3t total score (Section 2.3.1.3).

3.3.1.6 Additional data collected

As part of the ENROLL-HD assessment, participants had already supplied demographic data, current medications, and were assessed on the UHDRS assessments, including the UHDRS-TMS, UHDRS-TFC, UHDRS Cognitive score, UHDRS Functional Assessment scale, UHDRS independence scale, Short Form-12 (SF-12) and Problem Behaviour Assessment (PBA). This data was collected routinely as part of the participant's Registry/ENROLL-HD assessment.

3.3.1.7 Statistical Analysis

SPSS version 20 (PASW) (IBM Corporation, USA) was used to evaluate the results of the study.

Demographic data, UHDRS scores and C3t performance (C3t time taken and C3t total) were evaluated using the mean and standard error of the mean (SEM).

A two way repeated measures analysis of variance (ANOVA) was used to see if participant performance significantly differed with increasing task difficulty. Between subject factors included TFC stage (TFC scores: 11–13, stage 1 (earliest symptomatic stage); 7–10, stage 2; 3–6, stage 3; 1–2, stage 4; and score of 0 is stage 5 (most advanced stage). Within-subject factors included C3t time (Time), C3t total score (C3t Total) and C3t item (C3t baseline simple, C3t baseline complex, C3t dual task). If the sphericity assumption was not met ($P<0.05$), this was corrected using the Greenhouse-Geisser test.

A Bonferroni post hoc test was used for all tests if results were deemed statistically significant ($p<0.05$).

3.3.2 Results

3.3.2.1 Participants

Demographic information for the participants recruited in the C3t baseline are presented in Table 23. Twenty-one people consented to take part in the study from which fourteen people completed the C3t assessment. The number of people in each group that completed each item of the C3t are presented in Appendix 1. People in stage 3 and 4 were combined to form one group (stage 3,4) as only one stage 4 subject was recruited. There was no significant difference between CAG disease burden score, years of education or body mass index. Stage 1 were significantly younger than stage 2 and stage 3.

Table 23: C3t v2b Participant demographics

		Pre-HD	Manifest	Group differences	TFC disease stage			Group differences
					1	2	3,4	
C3t baseline simple	**n (male:female)**	3 (1:2)	18 (12:6)		9 (3:6)	7 (7:0)	5 (3:2)	
	Age	33.67 (0.88)	51.25 (3.48)	t (21) = -1.917, p = n.s.	36.78 (4.22)	57.67 (4.22)	55.2 (5.66)	F (2,20) = 6.91, p < 0.01 (s1 vs s2 and s3)
	TMS	2.33 (0.33)	37.15 (5.48)	t (21) = -2.414, p < 0.05	9.33 (5.96)	45.11 (5.96)	52 (8)	F (2,20) = 12.77, p < 0.001 (s1 vs s2 and s3)
	TFC	13 (0)	8.5 (0.77)	t (21) = 2.211, p < 0.05	12.67 (0.41)	8.22 (0.41)	4 (0.55)	F (2,20) = 83.28, p < 0.001 (All stages)
	Functional scale	25 (0)	37.15 (5.48)	t (21) = 1.869, p = n.s.	24.44 (0.98)	19.56 (0.98)	13.2 (1.32)	F (2,20) = 23.70, p < 0.001 (All stages)
	Independence scale	100 (0)	80.79 (3.51)	t (20) = 2.13, p < 0.05	97.78 (2.94)	77.78 (2.94)	63.75 (4.41)	F (2,19) = 23.69, p < 0.001 (All stages)
	CAG disease burden score	219.5 (42.11)	369.03 (19.45)	t (19) = -2.94, p < 0.01	300.94 (30.03)	394.63 (31.85)	358.88 (45.04)	F (2,18) = 2.33, p = n.s.
	Years of education	13.33 (2.33)	11.68 (0.68)	t (20) = 0.855, p = n.s.	12.33 (1.07)	11.56 (1.07)	11.75 (1.61)	F (2,19) = 0.137, p = n.s.
	BMI	31.9 (2.79)	29.31 (1.7)	t (20) = 0.579, p = n.s.	30.44 (2.61)	29.73 (2.46)	28.32 (3.3)	F (2,19) = 0.127, p = n.s.

The table presents the mean demographics with the standard error of the means. Groups were categorised by pre-HD (TMS < 5) and manifest HD (TMS > 5). The HD groups were further categorised to form 3 groups which included stage 1, TFC =11-13; stage 2, TFC=7-10; stage 3,4 TFC=1-6). A t-test was performed to identify group differences between pre-HD and manifest subjects. To identify group differences between controls and TFC disease stage, a one way ANOVA was performed with Group as a between subject factor and demographics as a within subject factor. If this met significance then a Bonferroni post-hoc was performed. Significance was met when p < 0.05. Where C3t = Clinch token transfer test; TMS = total motor score; TFC = total functional capacity; MBI = body mass index; n.s. = not significant.

3.3.2.2 Testing the C3t v2

Half way through this study, C3t v2 test results were analysed to see if task complexity had an impact on C3t performance scores (See Table 24). This revealed people in stage 3,4 performed significantly slower in the Baseline complex relative to the Baseline Simple, but no difference in C3t total score. There was no difference in time or C3t total score between the Baseline Simple and Baseline complex for Stage 1 or Stage 2. There was also no difference in time taken or C3t total score in the Dual task relative to Baseline complex for any disease stage.

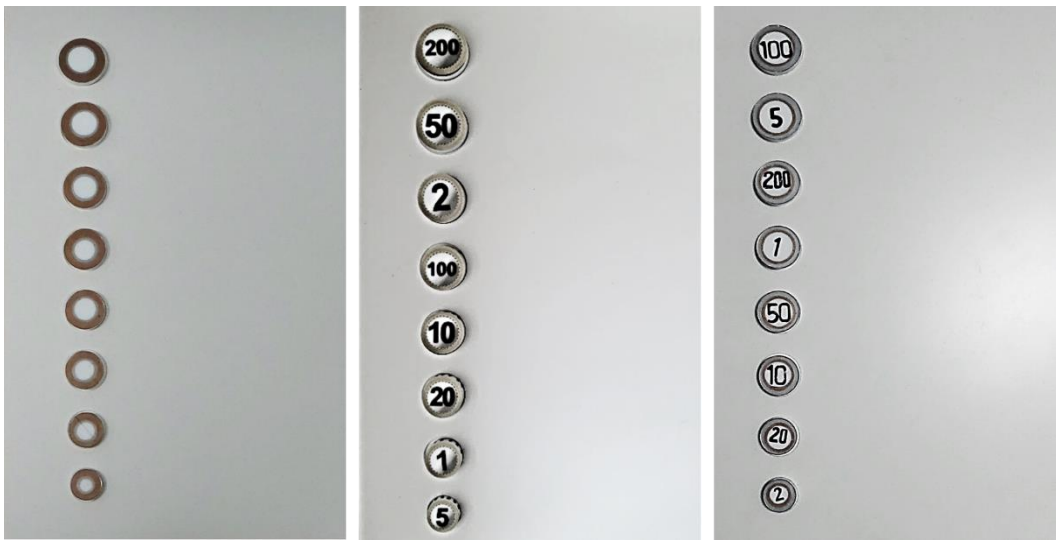
Table 24: C3t v2 performance scores

C3t item	TFC stage		
	1	2	3,4
C3t Baseline Simple time	20.09 ± 4.35	28.7 ± 4.61	49.25 ± 5.83
C3t Baseline Complex time	20.6 ± 6.51	25.26 ± 7.3	57.77 ± 13.63
C3t Dual task time	21.43 ± 1.95	24.42 ± 2.39	44.68 ± 5.85
C3t Baseline Simple total	42.19 ± 3.5	31.12 ± 3.71	18.34 ± 4.69
C3t Baseline Complex total	41.45 ± 3.1	33.15 ± 3.51	13.46 ± 5.36
C3t Dual task total	39.97 ± 3.6	33.91 ± 4.4	17.91 ± 10.79
Baseline Simple vs Baseline Complex time	$F_{(2,16)} = 7.8$, $p<0.01$; Stage 3 $p<0.01$		
Baseline Simple vs Dual task time	$F_{(2,13)} = 0.78$, p = n.s.		
Baseline Simple vs Baseline Complex total	$F_{(2,16)} = 0.641$, p = n.s.		
Baseline Simple vs Dual task time	$F_{(2,13)} = 0.972$, p = n.s.		

C3t = Clinch token transfer test. The C3t time was measured in seconds (light blue), where a lower score was indicative of better performance. The C3t total had no unit (dark blue), but a greater score was indicative of better performance.

3.3.2.3 Optimising the C3t v2

As test difficulty did not have a significant effect on C3t performance for Stage 1 and Stage 2, this suggests that participants were not finding the Baseline Complex or Dual task items any more difficult than the Baseline Simple. One explanation for this may be that participants became over familiar with the order that the tokens were presented in, and so instead of getting slower or less accurate with increasing task difficulty, they were stabilising. To reduce the chances of practice effects, different tokens were designed for the Baseline Simple and the Dual task. In this optimised version (C3t v2b), in the Baseline simple, no values were printed on the tokens (Figure 20). In the Baseline Complex and the Dual task, the same values were used but they were presented in a different order on the money card.



C3t Baseline Simple **C3t Baseline Complex** **C3t Dual task**

Figure 20: C3t v2 money cards: The new token design for the C3t v2b. Tokens for the C3t Baseline Simple had no values printed on them. The C3t Baseline Complex and Dual task had the same values printed but were presented in a different order to each other.

3.4 Part 3: Validating the C3t v2b

The aim of this study was to validate the C3t v2b in healthy controls and people gene positive with HD.

3.4.1 Methods

The methods followed in this study were part of the same protocol as those for Part 2 (Section 3.3.1). Sections that followed the same procedure as those described earlier are referred to where necessary.

3.4.1.1 Design

This research was a cross sectional, observational study. A subset of participants recruited were given the option to return 6 ± 2 weeks to redo the assessment.

3.4.1.2 Setting

People with HD were recruited from the Cardiff HD research and management clinic. Recruitment began in February 2016 and continued to January 2017. The remaining settings were the same as Part 2 (Section 3.3.1.2).

3.4.1.3 Participants

The aim of this study was to recruit was to recruit healthy controls and all people gene positive for HD. The inclusion and exclusion criteria for people with HD was the same as Part 2 (Section 3.3.1.3).

Inclusion criteria for healthy control participants

1) Cannot be at risk for HD
2) Aged 18 years or above

Exclusion criteria for all participants

1) Inability to provide informed consent
2) Any comorbid condition that had the potential to confound the results of the study

3.4.1.4 Dual task procedures

The C3t procedure was the same as Part 2 (Section 3.3.1.4).

3.4.1.5 Rating performance

This was the same as Part 2 (Section 3.3.1.5). In addition, to calculate the change in performance in the Baseline Complex and the Dual task relative to baseline, the dual task cost (DTC) was calculated and expressed as percentage change. This was calculated in the same way as described in Chapter 2 (Section 2.2.2.5). This value could be positive or negative. For example, if the participant performed the C3t faster in the baseline simple relative to the dual task, then the DTC would be positive. If the participant performed the C3t faster in the dual task, compared to the baseline simple, then the DTC would be negative. DTC was calculated using the following equation:

$$\left(\frac{\text{Dual task} - \text{Baseline task}}{\text{Baseline task}} \right) \times 100 = \text{Dual task cost}$$

3.4.1.6 Additional data collected

This was the same as Part 2 (Section 3.3.1.6). The functional component of the *Late-Life Functional Disability Instrument* (LL-FDI) was used also collected in this study (Haley *et al.*, 2002). This is a 32 item questionnaire that focuses on functional day to day activities such as using kitchen utensils to prepare meals, and getting into and out of a car and was used to see how the C3t correlated with self-reported performance in tasks related to daily living. This was rated on an ordinal scale ranging from no difficulty (=0) to cannot do (=5) (See Section 1.3.2.5 for more information on the LL-FDI). A subset of participants also performed the Step and Stroop, described in Chapter 2. A brief description of the Step and Stroop is presented in Table 25.

Table 25: Step and Stroop procedure

	Test order	Dual task item	Brief procedure
Step and Stroop	1	Step baseline	Step onto and off an aerobic step
	2	Stroop baseline easy	Colour words are presented in their coloured ink. For example, pink is printed in the colour pink
	3	Stroop baseline hard	Colour words are presented in a different coloured ink. For example, pink is printed in the colour grey
	4	Step and Stroop easy	Step whilst perfroming the Stroop easy
	5	Step and Stroop hard	Step whlst performing the Stroop hard

The number of steps performed in 45 seconds were recorded for assessment items 1, 4 and 5. The number of correct answers given in 45 seconds was recorded for assessment items 2-5.

3.4.1.7 Statistical Analysis

SPSS version 20 (PASW) (IBM Corporation, USA) was used to evaluate the results of the study.

Demographic data and UHDRS scores were evaluated using the mean and standard error of the mean (SEM).

The mean performance scores were plotted with the standard error of the means (SEM). Group comparisons were made using analysis of variance (ANOVA). Independent subject factors included Group (control, asymptomatic (pre-HD) or Manifest participants) or TFC stage (TFC scores = 13 and UHDRS-TMS < 5, pre-HD; 11–13, stage 1 (earliest symptomatic stage); 7–10, stage 2; 3–6, stage 3; 1–2, stage 4; and score of 0 is stage 5 (most advanced stage), or healthy controls). Dependent subject factors included C3t time (Time), and C3t total score (Total), Value time (Value time) and number of correct letters said per second (Alphabet rate).

A two way repeated measures ANOVA was used to evaluate task difficulty in the C3t Baseline Complex and C3t Dual task compared relative to the C3t Baseline Simple performance, between TFC stage. The C3t item (baseline simple, complex or dual task) and TFC group were used as factors. If the sphericity assumption was not met ($P<0.05$), this was corrected using the Greenhouse-Geisser test.

Receiver operating characteristic (ROC) curves used to evaluate the specificity and sensitivity of each C3t item between healthy controls and HD, and pre-HD and Manifest HD. The area under the curve (AUC) was used to interpret this result, where an outcome close to 1 was indicative of maximal specificity and sensitivity, whereas a result closer to 0.5 was no better than chance (Lalkhen and McCluskey, 2008).

External validity: A Pearson's correlation coefficient was used to reveal any associations between the C3t variables and the ENROLL-HD data collected.

Test validity: Predictive validity was calculated using a multiple linear regression, where UHDRS TMS, TFC and functional scores were used as dependent variables and all C3t items as independent variables. Construct validity was tested using a multiple stepwise linear regression model, using the C3t time and total score as dependent variables and UHDRS-TMS sub-items as independent variables. A Pearson correlation was performed to identify the relationship between items from the Step and Stroop and the C3t.

Test-retest reliability: An intraclass correlation was performed using C3t performance scores from their first and second visit.

A Bonferroni post hoc test was used for all tests if results were deemed statistically significant ($p < 0.05$).

3.4.2 Results

The results in this section were presented in the following order:

1. Participants
2. Validating the C3t v2b
    a. External validity
        i. Is the C3t specific to people with HD?
        ii. Is the C3t sensitive to disease stage?
    b. Test validity
        i. Concurrent validity
        ii. Predictive validity
        iii. Construct validity
    c. How reliable is the C3t v2b?

3.4.2.1 Participants

Demographic information for the participants recruited in the C3t baseline is presented in Table 26. Eighty-four people consented to take part in the study from which twenty-one were healthy controls and sixty-two people were gene positive for HD. From these, twenty controls and fifty-four people gene positive with HD completed the C3t assessment. The number of people in each group that completed each item of the C3t are presented in Appendix 1. People in stage 4 and 5 were combined to form one group (stage 4,5) as only two stage 5 subjects were recruited. There was no significant difference between TFC groups for years of education. Subjects that were pre-HD were significantly younger than the stage 2 group. CAG disease burden score was significantly greater in stage 2 and stage 3 compared to pre-HD. Stage 2 also had a significantly greater body mass index than stage 3.

Table 26: C3t v2b Participant demographics

		Controls	pre-HD	Manifest	Group differences	TFC disease stage					Group differences
						pre-HD	1	2	3	4,5	
C3t v2b	**n (male:female)**	21 (9:12)	8 (6:2)	54 (32:22)	/	8 (6:2)	23 (14:9)	17 (8:9)	11 (7:4)	3 (1:0)	/
	Age	45.52 (2.69)	37.75 (2.27)	51.21 (1.63)	t (62) = -3.049, p < 0.01	37.75 (4.35)	48.44 (2.57)	56.83 (2.91)	50.55 (3.72)	2.75.75 (6.17)	F(5,79) = 3.285, p < 0.01; pre-hd vs s2
	TMS	/	0.38 (0.18)	35.35 (2.87)	t (60) = -4.663, p <0.001	0.37 (4.72)	21.18 (2.85)	34.17 (3.15)	54.82 (4.03)	75 (7.72)	F(4,57) = 30.53, p < 0.001; All stages except for s3 vs s4,5
	TFC	/	12.63 (0.18)	8.87 (0.5)	t (60) = 2.889, p < 0.01	12.63 (0.368)	12.09 (0.22)	8.94 (0.25)	4.63 (0.31)	0.33 (0.6)	F(4,57) = 171.034, p < 0.001; All stages except for pre-hd and s1
	Functional scale	/	24.88 (0.13)	20.04 (0.83)	t (58) = 2.273, p < 0.05	24.88 (0.84)	24.5 (0.51)	20.39 (0.56)	13.44 (0.79)	5 (1.37)	F(4,55) = 74.44, p < 0.001; All stages except for pre-hd and s1
	Independence scale	/	99.38 (0.63)	81.3 (1.95)	t (56) = 3.681, p < 0.001	99.38 (2.72)	91.91 (1.68)	79.44 (1.82)	67.22 (2.57)	50 (5.45)	F(4,53) = 35.465, p < 0.001; All stages except for pre-hd and s1, and s3 vs s4,5
	CAG disease burden score	/	260.71 (26.17)	400.29 (13.81)	t (60) = -3.491, p < 0.001	260.71 (37.7)	373.39 (21.27)	411.78 (23.51)	424.73 (30.08)	329.38 (30.08)	F(4,57) = 3.749, p < 0.01; pre-hd vs s2 and s3
	Years of education	/	12 (0.71)	11.64 (0.38)	t (59) = 0.352, p = n.s.	12 (0.97)	11.81 (0.6)	11.72 (0.65)	11.36 (0.83)	11 (1.59)	F(4,56) = 0.122, p = n.s.
	BMI	/	27.49 (1.14)	27.37 (1.2)	t (56) = 0.035, p = n.s.	27.49 (3.15)	29.27 (1.72)	29.78 (1.82)	20.62 (2.32)	24.95 (4.45)	F (4,52) = 2.42, p < 0.05; s2 vs s3

The table presents the mean demographics with the standard error of the means. Groups were categorised by healthy controls, pre-HD (TMS < 5) and manifest (TMS > 5). The HD groups were further categorised to form 5 groups which included pre-HD (TMS > 5 and TFC = 13), stage 1, TFC =11-13 and TMS > 5; stage 2, TFC=7-10; stage 3, TFC=3-6; stage 4,5 =0-2). A t-test was performed to identify group differences between pre-HD and manifest subjects. To identify group differences between controls and TFC disease stage, a one way ANOVA was performed with Group as a between subject factor and demographics as a within subject factor. If this met significance a Bonferroni post-hoc was performed. Significance was met where p < 0.05.
Where c3t v2b = Clinch token transfer test version 2b; TMS = total motor score; TFC = total functional capacity; MBI = body mass index; n.s. = not significant.

3.4.2.2 Validating the C3t v2b

3.4.2.2.1 External validity

i. Is the C3t specific to people with HD?

The purpose of this section was to see if C3t performance could distinguish between healthy controls and people with HD. Participants were grouped based on their UHDRS-TFC and UHDRS-TMS scores and formed either the Control, pre-HD or Manifest HD Group.

The mean, minimum and maximum values for C3t performance (time and C3t total) scores for Group (Controls, Pre-HD, Manifest) is presented in Table 27.

A univariate ANOVA was used where both time taken and C3t total score were used as factors. Manifest participants performed the C3t significantly slower and achieved a significantly lower C3t total score than Control and Pre-HD participants for the baseline simple, complex and dual task (Controls vs Manifest, All $p<0.001$; Pre-HD vs Manifest: Time, All $p<0.05$; Total, All $p<0.001$; Table 27, Figure 21). There was no between Controls vs Pre-HD participants for any C3t item, and no gender differences for any measure ($p>0.05$). The results suggest that both time and C3t total score accurately measure performance differences between people with manifest HD compared to healthy controls and Pre-HD participants.

For the value and alphabet baseline tests manifest participants performed the value baseline significantly slower and recited significantly less correct letters of the alphabet per second than controls (Table 27) (Simple effects: Value and Alpha baseline: Control vs Manifest $p<0.05$). There was no performance difference between Pre-HD vs Manifest, or Pre-HD vs Control participants for the value and alphabet baseline tests ($p>0.05$).

Table 27: C3t v2b performance scores in controls, asymptomatic and manifest HD

C3t scores	Control				Pre-HD				Manifest				ANOVA results	
	Mean	SEM	Min	Max	Mean	SEM	Min	Max	Mean	SEM	Min	Max	F value $_{(df, error)}$	p value
Time Baseline Simple	14.09	2.92	8.04	43.48	14.22	4.72	9.6	21.7	27.85	1.82	10.54	87.48	9.95 $_{(2, 80)}$	p < 0.001
Time Baseline Complex	13.73	3.12	10.92	17.64	15.43	4.93	12.26	19.8	33.59	1.97	12.07	81.51	17.4 $_{(2, 75)}$	p < 0.001
Time Baseline Dual task	14.08	3.27	11.93	18.16	15.37	5.16	11.02	22.77	33.45	2.15	14.38	99.04	14.75 $_{(2, 71)}$	p < 0.001
Total Baseline Simple	62.42	3.01	18.4	99.5	59.4	4.88	36.87	83.33	34.97	1.88	9.14	75.9	35.18 $_{(2,80)}$	p < 0.001
Total Baseline Complex	59.27	2.46	45.35	73.26	51.48	3.89	40.4	61.17	28.49	1.56	8.59	62.14	61.86 $_{(2, 75)}$	p < 0.001
Total Baseline Dual task	55.86	2.41	38.1	67.06	54.6	3.81	35.13	72.6	28.2	1.59	7.57	55.63	55.86 $_{(2, 71)}$	p < 0.001
Value time Baseline	5.48	1.34	4	8.16	5.41	1.89	3.08	9.81	9.99	0.73	3.28	45.36	5.96 $_{(2, 74)}$	p < 0.01
Alphabet correct/sec Baseline	4.41	0.33	2.4	8.36	3.39	0.55	2	4.92	2.36	0.21	0.49	9.52	14.29 $_{(2, 83)}$	p < 0.001
Alphabet correct/sec Dual task	3.05	0.26	1.19	5.11	2.09	0.39	0.5	4.36	1.46	0.12	0.31	3.21	24.53 $_{(2, 71)}$	p<0.001

The mean C3t performance results with the standard error of the mean, minimum and maximum results in controls, pre-HD and manifest HD are presented. Time and Value time were measured in seconds (light blue), and the alphabet was measured using the number of letters recited per second (yellow). The total scores had no unit (dark blue). A low C3t time and value time, and a high total score and alphabet (correct/sec) score were indicative of better performance. C3t = Clinch token transfer test; SEM = standard error of the mean; Min = Minimum; Max = Maximum; Analysis of variance = ANOVA; Baseline = bl.

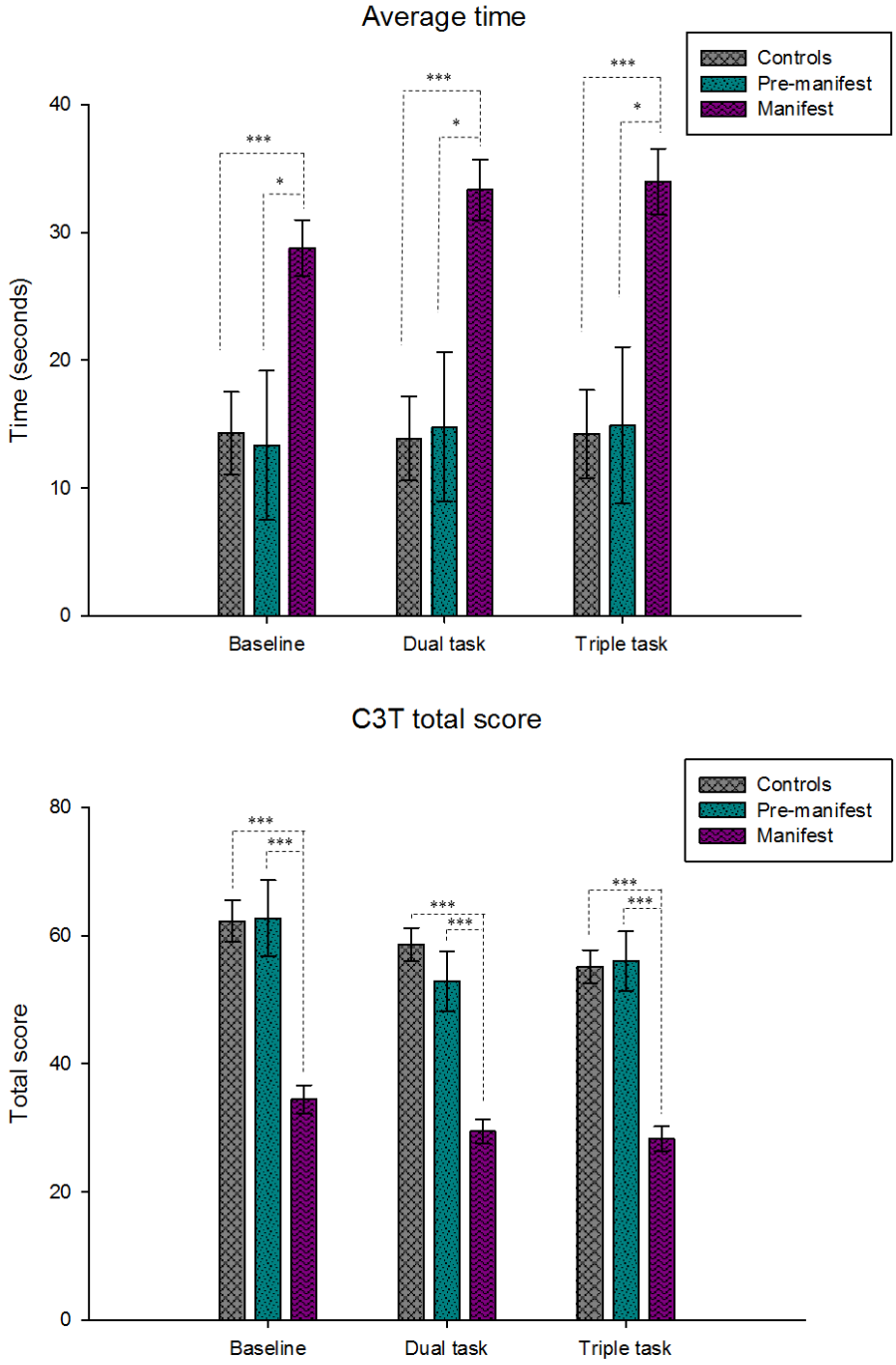


*Figure 21: C3t v2b pre-HD vs manifest bar graphs: C3t performance in healthy controls, pre-manifest and manifest HD. Time taken (Fig a) and C3t total score (Fig b) were used as primary measures where total score was more sensitive to differences between pre-manifest and manifest participants than time taken alone. Values show averages ± SEM. Where * p<0.05, ** p<0.01, *** p<0.001.*

Receiver operating characteristic (ROC) curves were used to determine the sensitivity and specificity of C3t performance scores (time taken and C3t total score) between pre-HD vs Manifest participants, and HD vs Controls in the baseline simple, complex and dual tasks. An AUC outcome of 1 is indicative of 100% sensitivity and specificity.

The AUC values from the ROC curve analysis are presented in Table 28. This revealed that all AUCs were greater than 0.85 indicating excellent specificity between Controls vs HD and sensitivity between Pre-HD and Manifest participants (Lalkhen and McCluskey, 2008).

Table 28: AUC values based on C3t performance

C3t Performance variable(s)		Controls vs HD	Pre-HD vs Man
Time	Baseline simple	.868	.900
	Baseline complex	.905	.914
	Dual task	.889	.939
Total	Baseline simple	.868	.868
	Baseline complex	.923	.923
	Dual task	.870	.870

An AUC value equal to one defines a score with absolute specificity and sensitivity. AUC = Area under the curve, C3t = Clinch token transfer test, HD = Huntington's disease, pre-HD = asymptomatic, Man = Manifest.

ii. Is the C3t sensitive to disease stage in HD?

The results thus far revealed the C3t was specific to people with HD. The purpose of this section was to see if C3t performance was sensitive to disease stage. Six disease stage groups were formed (Controls, pre-HD, Stage 1, Stage 2, Stage 3, Stage 4,5), which were based on their UHDRS-TFC and TMS scores.

The mean, SEM and 95% confidence values for C3t performance (time and C3t total) are presented in Table 29. A univariate ANOVA revealed that Pre-HD participants

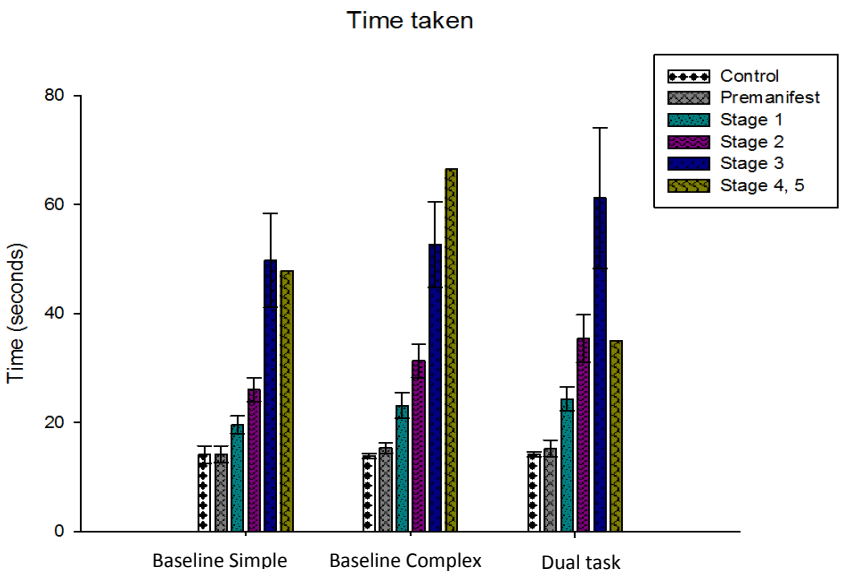
performed the C3t significantly faster (Figure 22) and achieved significantly greater C3t total scores (Figure 23) than those in the later stages of disease for the Baseline Simple, Complex and Dual task. Differences between disease stage were more obvious using the C3t total score as appose to time taken alone, indicating that people with more advanced HD were less accurate and performed the C3t slower than those in the earlier stages of disease. There were no significant Gender differences for any of the measures [Maximum: baseline simple, $F_{(1,60)}$= 0.638, n.s; baseline complex, $F_{(1,54)}$= 0.391, n.s; dual task, $F_{(1,51)}$= 0.839, n.s].

A univariate ANOVA was performed using Value baseline, the Alphabet accuracy (baseline and dual task) and TFC stage as factors. Healthy controls and people in the earliest stages of HD performed the value baseline and alphabet baseline significantly faster than people with more advanced stages of disease did [Value time: $F_{(5,57)}$= 13.54, p< 0.001; Alphabet rate: $F_{(5,60)}$= 5.24, p< 0.001]. Post hoc analysis revealed these differences were between all stages (p<0.05) except for Controls vs Pre-HD, Controls vs Stage 1, Pre-HD vs Stage 1 and Stage 2 vs Stage 3 (p>0.05). People in all TFC stages significantly differed from one another for the Alphabet test (All p<0.05), except between Pre-HD and Stage 4, 5. However, this Group consisted of just one subject.

Table 29: C3t v2b performance scores grouped by TFC disease stage

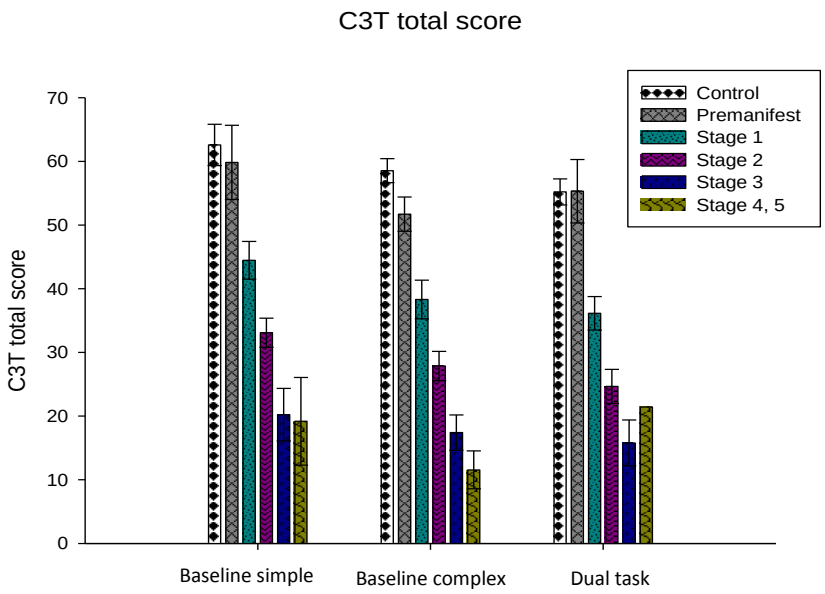
	Group	Time taken (seconds)	95% confidence differece (upper-lower bound)	C3t total (no unit)	95% confidence differece (upper-lower bound)
C3t Baseline Simple	Control	14.34 ± 2.41	9.54 - 19.14	62 ± 2.73	56.55 - 67.45
	pre-HD	13.33 ± 4.46	4.45 - 22.21	62.69 ± 5.06	52.61 - 72.78
	Stage 1	20.59 ± 2.33	15.95 - 25.24	42.2 ± 2.65	36.92 - 47.47
	Stage 2	23.8 ± 2.65	18.51 - 29.08	35.18 ± 3.01	29.18 - 41.19
	Stage 3	46.62 ± 3.42	39.8 - 53.44	20.4 ± 3.89	12.65 - 28.14
	Stage 4,5	37.85 ± 6.3	25.29 - 50.41	27.77 ± 7.16	13.5 - 42.04
	ANOVA: F value and p value	$F_{(5,72)}$=14.25; p<0.001		$F_{(5,72)}$=21.44; p<0.001	
C3t Baseline Complex	Control	13.72 ± 2.39	8.95 - 18.49	59.32 ± 2.11	55.1 - 63.54
	pre-HD	14.78 ± 4.34	6.12 - 23.44	52.88 ± 3.84	45.22 - 60.55
	Stage 1	24.15 ± 2.39	19.38 - 28.91	36.51 ± 2.11	32.29 - 40.73
	Stage 2	30.4 ± 2.68	25.05 - 35.74	22.51 ± 2.37	22.51 - 31.97
	Stage 3	51.51 ± 3.67	44.19 - 58.83	17.05 ± 3.25	10.54 - 23.53
	Stage 4,5	54.64 ± 6.14	42.39 - 66.88	16.37 ± 5.43	5.53 - 27.21
	ANOVA: F value and p value	$F_{(5,67)}$=20.78; p<0.001		$F_{(5,67)}$=38.45; p<0.001	
C3t Dual task	Control	14.14 ± 2.13	9.89 - 18.39	55.67 ± 2.12	51.44 - 59.9
	pre-HD	14.89 ± 3.86	7.17 - 22.61	56.03 ± 3.84	48.35 - 63.71
	Stage 1	25.01 ± 2.13	20.76 - 29.26	34.75 ± 2.12	30.53 - 38.98
	Stage 2	28.97 ± 2.45	24.08 - 33.87	27.32 ± 6.66	22.45 - 32.19
	Stage 3	65.58 ± 3.86	57.86 - 73.3	13.58 ± 3.84	5.9 - 21.26
	Stage 4,5	32.44 ± 6.69	19.07 - 45.81	22.43 ± 6.66	9.12 - 35.73
	ANOVA: F value and p value	$F_{(5,63)}$=29.21; p<0.001		$F_{(5,68)}$=31.38; p<0.001	

The table presents the mean time taken and C3t total score ± SEM, and the 95% confidence difference range for each group across the Baseline Simple, Complex and Dual task. Data is presented to 2 decimal places. Groups were categorised by Controls and TFC disease stage (pre-HD, TFC = 13 and TMS< 5; stage 1, TFC =11-13 and TMS > 5; stage 2, TFC=7-11; stage 3, TFC=3-6; stage 4,5 =0-2). Time taken was measured in seconds and C3t total had no unit. A lower time and a greater C3t total score were indicative of better performance. Group comparisons are highlighted in yellow. pre-HD = asymptomatic; ANOVA = analysis of variance.



	Baseline Simple	Baseline Complex	Dual task
Control	pre-HD Stage 1 Stage 2 Stage 3 *** Stage 4,5 *	pre-HD Stage 1 *** Stage 2 *** Stage 3 *** Stage 4,5 ***	pre-HD Stage 1 ** Stage 2 *** Stage 3 *** Stage 4,5
pre-HD	Controls Stage 1 Stage 2 Stage 3 *** Stage 4,5 *	Controls Stage 1 *** Stage 2 *** Stage 3 *** Stage 4,5 **	Controls Stage 1 *** Stage 2 * Stage 3 *** Stage 4,5
Stage 1	Controls pre-HD Stage 2 Stage 3 *** Stage 4,5	Controls *** pre-HD *** Stage 2 Stage 3 *** Stage 4,5	Controls *** pre-HD Stage 2 Stage 3 *** Stage 4,5
Stage 2	Controls pre-HD Stage 1 Stage 3 *** Stage 4,5	Controls *** pre-HD *** Stage 1 Stage 3 $^{\$}$ Stage 4,5	Controls *** pre-HD * Stage 1 Stage 3 *** Stage 4,5
Stage 3	Controls *** pre-HD *** Stage 1 *** Stage 2 *** Stage 4,5	Controls *** pre-HD *** Stage 1 *** Stage 2 $^{\$}$ Stage 4,5	Controls *** pre-HD *** Stage 1 *** Stage 2 *** Stage 4,5 ***
Stage 4,5	Controls * pre-HD * Stage 1 Stage 2 Stage 3	Controls *** pre-HD *** Stage 1 Stage 2 Stage 3	Controls pre-HD Stage 1 Stage 2 Stage 3 ***

*Figure 22: C3t v2b time bar graph across TFC disease stage. The mean time taken to perform the C3t ± SEM based on participants grouped by healthy controls and TFC stage of disease, where time was measured in seconds. Participants in the later stages of disease took on average significantly longer to perform each item of the C3t compared to those in the earlier stages. A shorter time was indicative of better performance. Less participants passed the Baseline Complex and Dual task in the more advanced disease stages leading to greater variability. See Figure 24 for the percentage of people that attempted but failed these assessment items. The data in the table presents significant differences between disease stage, where $^{\$}$ p<0.056; * p < 0.05; ** p < 0.01; *** p < 0.001.*

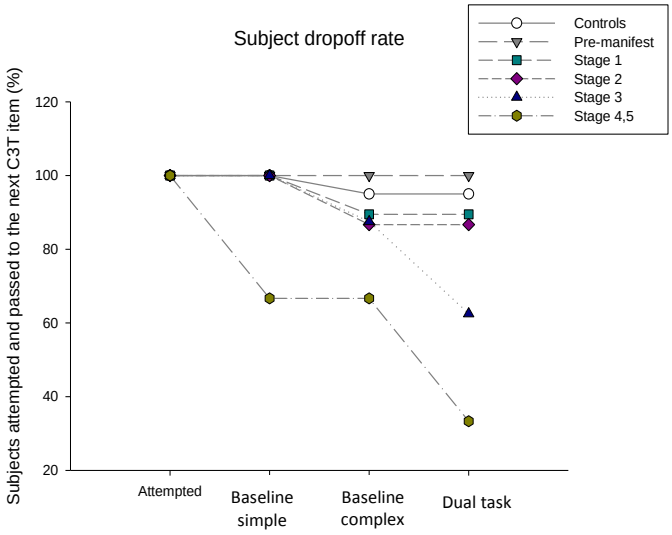


	Baseline Simple	Baseline Complex	Dual task
Control	pre-HD Stage 1 ** Stage 2 *** Stage 3 *** Stage 4,5 **	pre-HD Stage 1 *** Stage 2 *** Stage 3 *** Stage 4,5 ***	pre-HD Stage 1 *** Stage 2 *** Stage 3 *** Stage 4,5 *
pre-HD	Controls Stage 1 ** Stage 2 *** Stage 3 *** Stage 4,5 **	Controls Stage 1 ** Stage 2 *** Stage 3 *** Stage 4,5 ***	Controls Stage 1 *** Stage 2 *** Stage 3 *** Stage 4,5 ***
Stage 1	Controls *** pre-HD *** Stage 2 Stage 3 *** Stage 4,5	Controls *** pre-HD * Stage 2 Stage 3 *** Stage 4,5 *	Controls *** pre-HD *** Stage 2 Stage 3 *** Stage 4,5
Stage 2	Controls *** pre-HD *** Stage 1 Stage 3 $ Stage 4,5	Controls *** pre-HD *** Stage 1 Stage 3 Stage 4,5	Controls *** pre-HD *** Stage 1 Stage 3 $ Stage 4,5
Stage 3	Controls *** pre-HD *** Stage 1 *** Stage 2 $ Stage 4,5	Controls *** pre-HD *** Stage 1 *** Stage 2 Stage 4,5	Controls *** pre-HD *** Stage 1 *** Stage 2 $ Stage 4,5
Stage 4,5	Controls *** pre-HD ** Stage 1 Stage 2 Stage 3	Controls *** pre-HD *** Stage 1 * Stage 2 Stage 3	Controls *** pre-HD *** Stage 1 Stage 2 Stage 3

*Figure 23 C3tv2b total score-bar graphs across TFC disease stage. The mean C3t total scores achieved ± SEM based on participants grouped by healthy controls and TFC stage of disease. Participants in the later stages of disease took significantly longer and were less accurate to perform each item of the C3t compared to those in the earlier stages indicated by a lower total score. This measure had no unit. A greater total score was indicative of better performance. Less participants passed the Baseline Complex and Dual task in the more advanced disease stages leading to greater variability. See Figure 24 for the percentage of people that attempted but failed these assessment items. The data in the table presents differences between disease stage, where $ p<0.056; * p < 0.05; ** p < 0.01; *** p < 0.001.*

3.4.2.2.2 C3t performance deteriorates with increased task difficulty in people with advanced HD

As the C3t items increased in difficulty, more people in the later disease stages failed to meet the set criteria to continue to the complex and dual task items (subject drop-off). This resulted in smaller group sizes in the baseline complex and dual task, and reduced the Stage 4,5 group to a third in the dual task (Figure 24). In comparison, 95% and 100% of the Control and Pre-HD participants completed the whole C3t assessment.



	Participants that passed each C3t item (%)			
	Attempted	Baseline simple	Baseline complex	Dual task
Control	100 (n=21)	100 (n=21)	95 (n=20)	95 (n=20)
Pre-HD	100 (n=8)	100 (n=8)	100 (n=8)	100 (n=8)
Stage 1	100 (n=23)	100 (n=23)	89.47 (n=21)	89.47 (n=21)
Stage 2	100 (n=18)	100 (n=18)	86.67 (n=17)	86.67 (n=16)
Stage 3	100 (n=11)	100 (n=11)	87.5 (n=10)	62.5 (n=8)
Stage 4, 5	100 (n=3)	66.67 (n=2)	66.67 (n=2)	33.33 (n=1)

Figure 24: C3t v2b subject drop off graph. The percentage of participants, grouped by TFC disease stage that passed the set criteria developed for the C3t Baseline Simple, Complex and dual task (See Appendix 1). The figure and the table presents the percentage of participants that were recruited and attempted the C3t (attempted). The plots in the Baseline Simple, Complex and Dual tasks presents the percentage of people that passed each of these items. Set criteria were developed for each C3t item (Appendix 2) and, if the participant met this criteria, they passed to the next, more complex, C3t item.

To see if C3t complexity had an impact on performance, a two way repeated measures ANOVA was performed using TFC stage as an independent subject factor, and C3t time and C3t total as between subject factors. This revealed that people in stage 1, stage 2, stage 3 and stage 4,5 took significantly longer to perform the C3t items with increased task complexity [TFC stage x C3t item: baseline simple vs baseline complex, $F_{(5,72)}$= 4.65, p<0.001; baseline simple vs dual task, $F_{(5,68)}$= 7.68, p<0.001; baseline complex vs dual task, $F_{(5,68)}$= 14.27, p<0.001] (Table 30). Performance did not significantly differ in the control and pre-HD groups as items increased in difficulty.

Table 30: C3t v2b Performance differences with task complexity.

	Group	C3t Baseline Complex	C3t Dual task
C3t Baseline Simple	Control	p = n.s.	p = n.s.
	pre-HD	p = n.s.	p = n.s.
	Stage 1	*	**
	Stage 2	***	**
	Stage 3	*	***
	Stage 4,5	***	p = n.s.
C3t Baseline Complex	Control	/	p = n.s.
	pre-HD	/	p = n.s.
	Stage 1	/	p = n.s.
	Stage 2	/	p = n.s.
	Stage 3	/	***
	Stage 4,5	/	**

The data presented shows Bonferroni post-hoc outcomes to see if the complexity of the C3t item had an impact on performance. All groups except for pre-HD and controls performed significantly worse with increased task complexity (refer to Table 29 and for mean values, which are plotted in Figure 22 and Figure 23). *Where * p < 0.05; ** p < 0.01; *** p < 0.001.*

There was no significant difference in C3t total score with increasing task complexity [TFC stage x C3t item: baseline simple vs baseline complex, $F_{(5,72)}$= 0.63, p=n.s.; baseline simple vs dual task, $F_{(5,68)}$= 0.26, p=n.s.; baseline complex vs dual task, $F_{(5,68)}$= 0.42, p=n.s.]. There was also no significant difference in the alphabet performance between the baseline and dual task [baseline vs dual task, $F_{(5,67)}$= 2, p=n.s.].

3.4.2.3 Test validity

3.4.2.3.1 Concurrent validity

Concurrent validity is a form of criterion validity and is used to understand the extent a measure relates to other gold standard assessments.

A Pearson correlation was used to evaluate if there was a relationship between C3t items and the UHDRS assessments (UHDRS-TMS, TFC, functional assessment score, independence scale and cognitive measures (verbal fluency, symbol digit and the Stroop task), CAG disease burden score, quality of life summaries from the SF-12, the apathy and executive function score from the Problem behaviour assessment and scores from the *Late-Life Functional Disability Instrument*).

Correlation results are presented in Table 31. All C3t performance variables significantly correlated with UHDRS measures except between the alphabet in the C3t Dual task and the verbal fluency and Stroop colour naming. The C3t items significantly correlated with the current performance based functional measures used for ENROLL-HD (Timed up and go, and sit to stand), and the *Late-Life Functional Disability Instrument*. C3t items did not correlate with the SF-12 mental summary or the apathy and executive function scores from the *Problem behaviour assessment*.

Table 31: The correlation between performance in each C3tv2b assessment item and UHDRS, PBA and SF-12 measures

	UHDRS TMS	TFC	TFC stage	FAS	Independance scale	UHDRS SD correct	UHDRS VF correct	UHDRS Stroop colour-name correct	UHDRS Stroop word-reading correct	CAG disease burden score	TUG	Sit to Stand	PBA apathy	PBA executive function	SF-12 physical summary	SF-12 mental summary	LL-FDI total	LLFDI-upper extremity	LLFDI-lower extremity
C3t Time Basline Simple	.775**	-.627**	.621**	-.673**	-.580**	-.565**	-.466**	-.591**	-.589**	.379**	.953**	-.653**	.100	-.070	-.396*	.112	-.423**	-.460**	-.437**
C3t Time Basline Complex	.812**	-.718**	.719**	-.702**	-.651**	-.619**	-.480**	-.608**	-.633**	.411**	.915**	-.700**	.183	.093	-.351*	.039	-.439**	-.448**	-.471**
C3t Time Dual task	.732**	-.678**	.632**	-.678**	-.635**	-.559**	-.391**	-.563**	-.587**	.237	.940**	-.702**	.174	.113	-.399*	-.284	-.517**	-.537**	-.537**
C3t Total Basline Simple	-.756**	.633**	-.618**	.584**	.574**	.716**	.468**	.631**	.609**	-.489**	-.667**	.501**	-.183	-.041	.355*	.109	.507**	.482**	.468**
C3t Total Basline Complex	-.791**	.682**	-.678**	.616**	.639**	.719**	.432**	.599**	.582**	-.487**	-.649**	.560**	-.242	-.147	.345*	.098	.475**	.413**	.441**
C3t Total Dual task	-.777**	.655**	-.604**	.586**	.674**	.741**	.428**	.647**	.633**	-.377**	-.637**	.570**	-.294*	-.155	.326	.257	.488**	.438**	.461**
Value Baseline	.674**	-.631**	.639**	-.701**	-.666**	-.546**	-.432**	-.581**	-.609**	.302*	.704**	-.561**	.392**	.156	-.447**	.081	-.433**	-.513**	-.456**
Alphabet Baseline	-.439**	.475**	-.367**	.462**	.421**	.421**	.348**	.415**	.400**	-.185	-.197	.535**	-.089	-.254*	.393*	.266	.422**	.453**	.462**
Alphabet Dual task	-.405**	.419**	-.388**	.312*	.411**	.428**	.113	.267	.316*	-.202	-.338	.418*	-.365**	-.123	.356*	.280	.567**	.444**	.497**
C3t Time Baseline simple vs Complex cost	.107	-.182	.228	-.079	-.150	-.154	.004	-.052	-.085	.085	-.409*	.187	.100	.356**	-.023	-.070	.040	.100	.063
C3t Total Baseline simple vs Complex cost	.043	-.157	.193	-.039	-.124	-.030	.086	.058	.014	.003	-.447*	.177	.131	.277*	-.045	-.027	.065	.132	.098
C3t Time Dual task cost	.189	-.264	.243	-.192	-.307*	-.188	-.026	-.176	-.255	-.120	-.134	-.028	.320*	.429**	-.411*	-.396*	.000	-.054	-.020
C3t Total Dual task cost	.211	-.307*	.264	-.197	-.348*	-.220	-.043	-.181	-.255	-.089	.016	-.209	.350*	.310*	-.319	-.288	-.006	-.060	-.034
Alphabet Dual task cost	-.021	-.046	.014	-.069	-.109	-.132	.055	.069	-.030	.119	-.063	-.025	.278*	-.009	-.197	-.288	-.206	-.063	-.110

**p<0.05 (light pink); **p<0.01 (dark pink). UHDRS = Unified Huntington's disease rating scale; TMS = Total motor score; TFC = Total functional capacity; LVF = Letter verbal fluency; PBA = Performance behaviour assessment; SF-12 = Short-form 12; LL-FDI = Late life functional disability questionnaire.*

3.4.2.3.2 Predictive validity

Predictive validity is another form of criterion validity and is used to measure how well an instrument predicts the scores of another related assessment.

A multiple linear regression revealed that all C3t items significantly predicted the UHDRS-TMS, UHDRS-TFC and UHDRS-functional score (Table 32).

Table 32: C3t items significantly predict the UHDRS measures

		UHDRS-TMS			UHDRS-TFC			UHDRS Functional score		
		R value	F value	p value	R value	F value	F value	R value	F value	p value
C3t Time	Baseline Simple	0.78	F(1,58)= 89.56	p<0.001	0.63	F(1,58)= 38.25	p<0.001	0.59	F(1,56)= 30.02	p<0.001
	Baseline Complex	0.81	F(1,54)= 104.84	p<0.001	0.72	F(1,54)= 57.47	p<0.001	0.61	F(1,52)= 31.44	p<0.001
	Dual task	0.73	F(1,50)= 58.28	p<0.001	0.68	F(1,50)= 42.7	p<0.001	0.58	F(1,48)= 24.57	p<0.001
C3t Total	Baseline Simple	0.76	F(1,58)= 79.01	p<0.001	0.64	F(1,58)= 39.23	p<0.001	0.68	F(1,56)= 48.7	p<0.001
	Baseline Complex	0.79	F(1,54)= 89.84	p<0.001	0.68	F(1,54)= 47.05	p<0.001	0.7	F(1,52)= 51.13	p<0.001
	Dual task	0.78	F(1,50)= 75.69	p<0.001	0.65	F(1,50)= 37.45	p<0.001	0.68	F(1,48)= 41.62	p<0.001

C3t time was measured in seconds (light blue) and the C3t total had no unit (dark blue). The UHDRS assessments were rated on an ordinal scale by a neurologist at South Wales HD clinic. The data in the table revealed all C3t items (C3t baseline simple, complex and dual task) significantly predict the UHDRS-TMS, TFC and functional score. Where UHDRS = Unified Huntington's disease rating scale, TMS = total motor score, TFC = total functional capacity, C3t = Clinch token transfer test.

To see which UHDRS-TMS sub items best predicated the C3t time and total, a stepwise, linear regression was performed. This revealed that dystonia, pronation and supination of the hand best predicted the C3t Time (Table 33). The UHDRS-TMS sub-items that best predicted the C3t total score included pronation and supination of the hand, chorea and bradykinesia.

Table 33: UHDRS-TMS sub items that significantly predict C3t v2b item scores

C3t item	UHDRS-TMS constructs	R	R squared	Adjusted R squared	p
C3t Time Baseline Simple	Dystonia total	0.817	0.667	0.655	<0.001
C3t Time Baseline Complex	Pronate, supinate/ dystonia	0.912	0.831	0.817	<0.001
C3t Time Dual task	Pronate, supinate/ dystonia	0.872	0.76	0.737	<0.001
C3t Total Baseline Simple	Pronate, supinate/ chorea total	0.846	0.716	0.695	<0.001
C3t Total Baseline Complex	Pronate, supinate, bradykinesia	0.884	0.782	0.765	<0.001
C3t Total Dual task	Pronate, supinate, bradykinesia	0.882	0.778	0.757	<0.001

C3t time was measured in seconds (light blue) and the C3t total had no unit (dark blue). UHDRS-TMS items were rated on an ordinal scale by a neurologist at South Wales HD clinic. The data in the table presents the UHDRS-TMS items including total dystonia and total chorea score, which both consisted of the summed scores for the trunk, and left and right upper and lower extremities (all rated from 0 (normal) to 4 (marked/prolonged)). For the pronate/supinate item, patients had to repeatedly rotate their wrist so their hand faced upwards and downwards (rated from 0 (normal) to 4 cannot perform). Bradykinesia was measured ranging from 0 (normal) to 4 (marked with long delays).

3.4.2.3.3 Construct validity

Construct validity is used to ensure the assessment used measures the constructs it intends to measure.

As both the *Step and Stroop* and the C3t were designed to measure a similar construct (dual task performance), a Pearson correlation was performed to see how well the C3t items related to scores in the Step and Stroop (Table 34). This revealed that all C3t and the Step and Stroop assessment items significantly correlated with each other ($p<0.001$).

Table 34: C3t v2b construct validity

	Step Baseline	Step Simple Dual task	Step Complex Dual task	Stroop Baseline Simple	Stroop Dual task Simple	Stroop Baseline Complex	Stroop Dual task Complex
C3t time Baseline Simple	-.778**	-.791**	-.744**	-.806**	-.815**	-.712**	-.809**
	20	19	19	22	19	22	19
C3t time Baseline Complex	-.770**	-.796**	-.782**	-.880**	-.834**	-.758**	-.833**
	20	19	19	22	19	22	19
C3t time Dual task	-.725**	-.744**	-.749**	-.847**	-.777**	-.692**	-.739**
	17	16	16	19	16	19	16
C3t total Baseline Simple	.911**	.908**	.872**	.941**	.919**	.878**	.923**
	20	19	19	22	19	22	19
C3t total Baseline Complex	.879**	.860**	.849**	.927**	.928**	.885**	.928**
	20	19	19	22	19	22	19
C3t total Dual task	.841**	.866**	.883**	.940**	.862**	.810**	.883**
	17	16	16	19	16	19	16
Alphabet Baseline	.850**	.848**	.810**	.796**	.831**	.752**	.833**
	20	19	19	22	19	22	19
Alphabet Dual task	.795**	.891**	.856**	.790**	.835**	.677**	.806**
	17	16	16	19	16	19	16

A subset of subjects performed both the C3t v2b and the Step and Stroop. All C3t and Step and Stroop items significantly correlated, where p<0.001 (pink). The number of people that performed each item is presented below the correlation. C3t time was measured in seconds, C3t total had no unit, and the alphabet was measured using the number of correct letters of the alphabet recited per second. For the Stroop, the number of steps performed and the number of correct answers in the Stroop were recorded. C3t = Clinch token transfer test.

3.4.2.4 How reliable is the C3t?

One form of reliability is test-retest reliability. This is used to measure the stability of an assessment when no change in subject performance is expected and is usually carried out at two different time points to test the strength or association between the two scores.

To evaluate C3t test-retest reliability, a subset of participants were asked to return to redo the C3t 6 ±2 weeks after their first visit. An intra-class correlation was used and the results are presented in Table 35. This revealed C3t time and C3t total scores for the C3t baseline simple test were most reliable producing an intra-class correlation of 0.973 (n=10). The total score takes number of errors into consideration and as a result produced scores with greater variability and overall lower intra-class correlations, ranging from 0.944 (C3t baseline: n=10) to 0.388 (C3t dual hard: n=7).

Table 35: C3t v2b test-retest reliability

	ICC (95% confidence)
C3t Time Baseline Simple	0.973 (0.898-0.993)
C3t Time Baseline Complex	0.625 (-0.921 - 0.934)
C3t Time Dual task	0.825 (0.106 - 0.969)
C3t Total Baseline Simple	0.944 (0.789 - 0.986)
C3t Total Baseline Complex	0.636 (-0.864 - 0.936)
C3t Total Dual task	0.388 (-2.132 - 0.893)

An ICC of 1 is indicative of 100% reliability. Intraclass correlation coefficient (ICC); Clinch token transfer test (C3t); baseline (bl).

3.5 Discussion

The aim of this study was to design a standardised version of the C3t and validate it in people gene positive with HD. To achieve these aims, this Chapter consisted of three Parts which developed iteratively. In Part 1, the C3t was designed with five main criteria in mind; i) to ensure, the original C3t and the C3t v2 were as similar as possible in terms of dimensions and set up; ii) the set up was standardised; iii) it was compact and could be easily stored when not in use; iv) the materials used were robust but also cost effective; v) it was easy to use. These criteria were made based on the clinometric table presented in Chapter 1 (Table 1). The results in Part 2 revealed that, although the C3t was sensitive to disease stage, the increasing C3t complexity levels did not impact the C3t performance scores. As this may have been due to the familiarity of the tokens, the C3t was optimised, to include different tokens for the C3t Baseline Simple, Baseline Complex and the Dual task. In Part 3 the optimised C3t (C3t v2b) was validated in people with all stages of HD and a healthy control group, and revealed that performance distinguished between people gene positive with HD and healthy controls, and was sensitive to disease stage. Performance significantly worsened with increasing task difficulty. It also significantly correlated with the UHDRS and quality of life and function measures (SF-12, Problem Behaviour assessment, Late life functional disability instrument), and was capable of predicting the UHDRS-TMS scores. All C3t dual task items significantly correlated with items from the Step and Stroop suggesting they measured similar baseline and dual task constructs. In addition, test-retest reliability revealed time taken to perform the C3t was a reliable measure.

C3t performance can distinguish between healthy controls and people with HD

The C3t v2b was tested in healthy controls and people with pre-HD and manifest HD. Manifest participants performed the C3t significantly slower and achieved a lower total score relative to the healthy control and pre-HD groups. ROC analysis revealed the C3t could distinguish between people gene positive with HD and healthy controls, as well as people with pre-HD and manifest HD. However, there was no difference in C3t

performance between healthy controls and pre-HD. As people with pre-HD will inevitably develop HD symptoms, it is more important that outcome measures for HD distinguish between different disease stages to track disease progression. On the other hand, as the pre-HD group was small (n=8), it may be that larger group sizes were required to reveal any performance differences between these groups.

C3t performance can distinguish between stages of disease in HD

As well as dividing participant's scores into pre-HD and manifest groups, to identify different stages of HD, participants were also grouped based on their UHDRS TFC score (stage 1 (early) to stage 5 (advanced)). A limitation of this scoring is that stage 1 typically consists of a mixed group of pre-HD and early manifest which could result in greater performance variability. Therefore, to dissect stage 1 further, people with a TFC score between 11 and 13, and a TMS score greater than 5 were classed as manifest stage 1, and people with a TFC store equal to 13 and a TMS score less than 5 were classed as pre-HD. The mean total scores revealed the C3t was sensitive to all stages of disease, except Controls vs pre-HD participants. Importantly, performance in the C3t was sensitive enough to distinguish between people with pre-HD and stage 1. Overall, C3t performance speed and total score deteriorated in a stepwise manner, where controls and pre-HD performed the C3t quickest with a greater total score, and stage 4,5 performed the C3t slowest. People with more advanced stages of HD performed the C3t baseline simple, complex and dual tasks slower and produced a lower total score compared to those in the earlier disease stages. An explanation for this could be that, because the basal ganglia is involved in motor planning, motor initiation and motor accuracy) (Turner and Desmurget, 2010; Dudman and Krakauer, 2016), as this circuitry degenerates in people with HD, this leads to progressively slower and less accurate performance (Despard *et al.*, 2015).

The C3t assessment items increase in task complexity

The C3t was designed to increase in task complexity so it was applicable to people with different functional ability. In Part 2, the C3t v2a was tested for the first time in twenty four people with HD. In this version, the C3t items were performed using the same tokens for the Baseline Simple, Complex and dual task. Although we expected performance to deteriorate as the items became more difficult, performance differences in stage 1 and stage 2 were marginal. One explanation for this was practice effects due to the familiarity of the token values. Therefore, token values were changed, and in the C3t v2b, all people in all TFC stages took significantly longer to perform the baseline complex and/or dual task relative to the baseline simple. Contrasting this, healthy controls and people with pre-HD maintained performance across the C3t Baseline Simple, Complex and dual task and did not perform any slower or produce a lower test score as the tests increased in complexity. These results are supported by a previous study that found healthy controls and people with pre-HD had the ability to gradually improve a motor skill when it was repeatedly performed, whereas people with manifest HD were impaired in motor skill learning (Shabbott *et al.*, 2013). In this study, they tested motor skill learning and motor execution in a reaching based task (Shabbott *et al.*, 2013), where participants used their finger to direct a cursor to a target. The target was reflected from a computer screen onto a mirror, where only the mirror was visible to the participant. The findings revealed that people with HD were slower and less accurate directing the cursor to the target and produced more variable trajectories over repeated sessions. In comparison, people with pre-HD and healthy controls improved, gradually becoming quicker whilst remaining accurate over sessions (Shabbott *et al.*, 2013). One reason for this could be that people with pre-HD and controls have the ability to automate movement, allowing the allocation of more attentional resources on task accuracy compared to people with manifest HD that do not (Thompson *et al.*, 2010). As the basal ganglia is implicated in motor skill learning and automating responses (reviewed in: Turner and Desmurget, 2010; Dudman and Krakauer, 2016), this ability may gradually deteriorate as the circuitry progressively degenerates in HD.

C3t performance significantly differed between Stage 1 and pre-HD and controls. Stage 1 performed the C3t significantly slower and produced a lower C3t score. To our knowledge, there is no other functional upper limb assessment available that can distinguish the subtle differences between pre-HD and Stage 1. Furthermore, this was only evident in the more complex C3t items (Baseline Complex and Dual task), but not in the Baseline Simple, which supports that, different levels of complexity within assessments are important to capture all disease stages. The C3t was unable to distinguish differences between Stage 1 and Stage 2. Therefore, a more sensitive measure may be required to tease apart these disease stages. One option is to use accelerometers to quantify movement signatures, such as acceleration and deceleration, which cannot be captured using the current C3t rating method. Such methods have been applied in people with HD which found that accelerometers were a sensitive method to quantify gait and balance between people with pre-manifest and manifest HD (Dalton, Khalil, Busse, Rosser and Deursen, 2013). Furthermore, accelerometers have also been used in people with Parkinson's disease to quantify tremor severity (Patel *et al.*, 2012), and were recently worn by a small group of people with HD whilst performing the C3t (Bennasar *et al.*, 2016). The latter study revealed accelerometers could distinguish between manifest and pre-manifest HD groups. However, due to the small sample size, differences in movement parameters between disease stage were not tested, and as such, recruiting more people with HD is a current aim for future research. This is discussed in further detail in the General discussion (Chapter 5). The findings in this study also revealed that performance in the Stage 4,5 group significantly differed consistently from controls and pre-HD, but no other stage. An explanation for this could be the small sample size in this group (maximum n=3) which reduced to n=1 in the dual task. Thus, more people from Stage 4 and 5 are required to better represent C3t performance in these disease stages.

Performance in the C3t significantly correlates with the UHDRS assessments, quality of life, and function related outcome measures

Performance scores (time taken and C3t total) in the C3t items (Baseline simple, complex and dual task) significantly correlated with all UHDRS measures, evidencing strong concurrent validity. This was also true for the SF-12 physical summaries, the function component of the *Late-Life Functional Disability Instrument*, the *Timed up and Go* and the *Sit to stand*, which are all measures used to assess performance in daily functional tasks. Importantly, the C3t correlated with CAG disease burden score, which again suggests that the C3t performance is capable of tracking disease stage in HD. This score is especially useful for people with pre-HD, given that these groups can be heterogeneous, with some people closer to disease onset than others (Klöppel *et al.*, 2015). However, a larger pre-HD group is required to identify if C3t performance is sensitive enough to tracking this, given the pre-HD group size in this study was n=8. The C3t did not correlate with the apathy score from the *Problem behaviour assessment.* As there is no gold standard apathy outcome measure, other apathy assessments, such as the *Apathy Evaluation scale* (Clarke *et al.*, 2011)*,* could be used to prove or disprove these findings. In addition, the C3t did not correlate with the executive function summary from the *Problem behaviour assessment.* However, as the C3t items significantly correlated with the *Symbol digit test, the Stroop tasks* and the *Letter verbal fluency*, which are both measures of executive function (Craufurd and Snowden, 2002), this suggests that the ordinal scale used to rate patient responses in the *Problem behaviour assessment,* may not be an accurate executive function measure.

Predictive Validity

The C3t items (C3t Baseline simple, complex and dual task) significantly predicted the UHDRS-TMS, TFC and functional score, suggesting C3t performance accurately predicts motor and functional symptoms associated with HD.

Further analysis revealed the UHDRS-TMS sub-items that predicted C3t performance included: dystonia, chorea, bradykinesia and pronate/supinate hand. Dystonia and chorea are involuntary movements common in people during the early to mid-stages of HD, which manifest due to degeneration of the indirect pathway (Cepeda *et al.*, 2007). Theoretically, involuntary movements, such as chorea and dystonia could push the hand away from its intended trajectory during performance, increasing the time to complete the C3t. This study also revealed that the bradykinesia sub-item predicted C3t performance. Bradykinesia is a symptom that tends to manifest in the advanced stages of HD, and results from degeneration of the direct pathway (Drago *et al.*, 1998). This is required to initiate movement and is sometimes referred as the 'Go' pathway (Braunlich and Seger, 2013; Nelson and Kreitzer, 2014). Thus, increased bradykinesia will ultimately make synchronous upper limb movements required for the C3t more physically demanding and inefficient. This could result in an increased time to perform the C3t compared to people with pre-HD and those in the early stages. Furthermore, pronation and supination sub item scores also significantly predicated C3t performance. Pronation and supination are particularly important for grasping and release based tasks (Klein *et al.*, 2011, 2012). Typically, when someone grasps an item, the hand will pronate above the target and prepare the first and second digits as the hand draws closer to the target, followed by a grasp and supination (Klein *et al.*, 2011, 2012). A previous study revealed that people with HD had abnormal pronation and supination as well as mistiming when preparing to grasp an object (Klein *et al.*, 2011). This could affect the speed and accuracy of picking up tokens and manipulating the token within the hand during the C3t. Surprisingly, the oculomotor TMS sub-item was not a predictor of C3t performance. Previous studies suggests oculomotor function is required during reaching and grasping tasks (Whishaw *et al.*, 2002; Klein *et al.*, 2011, 2012). An explanation for this could be that the UHDRS-TMS sub-item is not sensitive enough to identify subtle changes in oculomotor function. Such changes have however been identified in people with pre-HD using computerized oculomotor assessments (Tabrizi *et al.*, 2009). Therefore, objective tools that can quantify subtle changes in oculomotor function are required to confirm the findings reported in this study.

Construct Validity

Measuring construct validity is challenging. This is because an assessment such as the C3t is multidimensional, meaning it consists of multiple potential constructs (Kimberlin and Winterstein, 2008). Given that the C3t significantly correlated with UHDRS and functional measures, this suggests that the C3t assessment captures, motor, cognitive and function (using the LL-FDI and the UHDRS functional assessment score) constructs (Westen and Rosenthal, 2003). Furthermore, given the C3t and the Step and Stroop items significantly correlate, this suggests they to measure similar constructs, and could relate to the motor, cognitive and functional constructs they both possess.

C3t reliability

Time taken to perform the C3t resulted in high test-retest reliability for the Baseline Simple, Complex and Dual task, but was lower for the C3t total score. An explanation for this could be that participants that committed an error one week, may have remembered the C3t instructions on their return visit. As a result, less, or no errors were committed, leading to a change in C3t total score, but not in the C3t time. Another consideration is that it may not be surprising that performance differs in functional outcome measures, such as the C3t, from one week to the next. People with HD are known to have 'good and bad days' in terms of symptom severity and general function (Sparbel *et al.*, 2008). As a result, performance in a multi-dimensional outcome measure, such as the C3t, could differ from one week to the next, resulting in greater performance variability. As test-retest reliability was assessed in a small sample size (n=10), retesting reliability in a larger group of people with HD is required to confirm these hypotheses.

Study limitations

One of the main caveats in this study were the small TFC group sizes, particularly pre-HD and Stage 4,5. In addition, as the C3t increased in difficulty, more people with advanced HD, mainly Stages 3 and Stage 4,5 were unable to meet the set criteria to pass

the complex C3t items. This resulted in more people in these groups 'failing,' meaning they could not proceed to the next (more complex) assessment item. Therefore group sizes got smaller for the more complex assessment items, and reduced the Stage 4,5 group to one person in the dual task (Figure 24). As previously mentioned, the small sample size used to assess C3t test-retest reliability may have also increased the amount of variability in the results test-retest results. Therefore, a greater sample size is required to truly identify differences between TFC disease stage and to calculate test-retest reliability with greater experimental power.

Another study constraint was that no other upper limb outcome measure, such as the pegboard test was used to compare performance with the C3t. Therefore, there is no way of knowing if the C3t is more sensitive than current upper limb assessments on the market. However, although it is important to test this in the future, as all C3t items significantly correlated with the upper limb score of the *Late-Life Functional Disability Instrument* (described in Chapter 1, Section 1.3.2.5), this suggests that C3t performance is related to daily tasks that require upper limb function. Furthermore, all C3t scores also correlated with lower limb score of the *Late-Life Functional Disability Instrument*, suggesting that the C3t could be used as an assessment that relates to overall body function in people with HD.

The C3t takes between 5-10 minutes to perform. Although this means that assessment results can be obtained quickly, and the patient burden is reduced, a limitation is that this may have led to practice effects in the Baseline complex and dual task, leading to faster, more accurate (indicated by the C3t total) performance. For instance, the dual task requires the participant to transfer tokens in value order, whilst simultaneously reciting the alphabet. However, the same values are presented here as in the value baseline and the C3t baseline complex. Thus, due to the familiarity of the values, this could mean less cognitive effort is required in the dual task. To overcome this, a study is underway to redesign the C3t. This includes developing new tokens with different values for the baseline complex and dual task (Appendix 4) and is discussed in more detail in the General discussion (Chapter 5).

Conclusions: The C3t as a functional outcome measure

The C3t is a novel dual-task assessment that has potential to provide sensitive feedback regarding upper limb function in HD. Importantly, performance differences were evident in early disease stages (pre-HD vs Stage 1), which are the stages commonly targeted for clinical trials to test the effectiveness of new treatments (Glorioso *et al.*, 2015; Kumar *et al.*, 2015). Furthermore, for treatments where a specific brain region is targeted, such as cell replacement therapy in HD; it is important that outcome measures which involve basal ganglia function (i.e. the circuitry being 'fixed'), and that are sensitive to HD symptoms, are available to assess . In addition, sensitive, objective scoring methods that are used in the C3t, as appose to ordinal rating scales, are crucial for interventions such as cell transplantation, where symptom changes are gradual and can be subtle (Wijeyekoon and Barker, 2011).

As an assessment, the C3t requires minimal researcher training and is inexpensive to produce. It is also small and compact, and can easily be stored, which avoids the common problem of restricted space in clinical settings. Furthermore as the C3t is independent of both language and culture barriers, this makes it an attractive outcome measure for clinical trials globally.

Chapter 4

Identification of the neural correlates underlying successful performance of a rodent analogue of the Stroop task

Chapter Summary

The Stroop task is a widely used task measuring attention and conflict resolution, which shows sensitivity across a range of diseases, including Huntington's disease (HD). A rodent analogue of the Stroop task, the 'Response-Conflict task', allows for systematic investigation of the neural systems underlying performance in this test.

The aim of this study was to determine whether neural regions relevant to HD are utilised during conflict resolution. To achieve this, the expression patterns of the immediate early genes (IEG) Zif268 and C-fos were analysed throughout cortical and basal ganglia subregions of the rodent brain.

4.1 Introduction

In Chapters 2 and 3, the rationale for developing dual task assessments developed was because previous studies suggest that people with HD are impaired when required to perform multiple tasks simultaneously (see Chapter 1, Table 2). Furthermore, one explanation for this could be because of striatal dysfunction (see Chapter 1, Section 1.4.3.1, page 41). In people, neuroimaging techniques such as fMRI, can be used to quantify the neural regions activated in behavioural tasks. However, this becomes increasingly difficult in people with HD due to increased involuntary movement, which can cause artefacts in the image (Andersson *et al.*, 2017). Furthermore, fMRI cannot be used pre-clinically. Understanding the neural correlates that underlie preclinical outcome measures is crucial to assess the effectiveness of phase I clinical trials. Therefore, in this study, it was of interest to take the Stroop task utilized in Chapter 2, and which is currently used in people with HD, to identify which neural regions were employed in a rodent analogue.

The Stroop task is a bi-conditional discrimination task which requires a subject to select task relevant information whilst ignoring task irrelevant stimuli (Bullard *et al.*, 2012). This task is commonly used to test executive functions such as attention and conflict resolution. In the classic Stroop task, subjects are required to either read colour-words, written in their coloured ink (e.g. PINK in the colour pink), or say the coloured ink of the word which do not correspond (e.g. PINK in the colour yellow) (Bullard *et al.*, 2012). This is one of the few cognitive assessments reported to measure cognitive decline in HD, where performance gradually deteriorates as the disease progresses (Barker *et al.*, 2013) and directly correlates with striatal degeneration (Sanchez-Pernaute *et al.*, 2000). Neuroimaging studies have highlighted several regions of the brain activated for successful performance of the Stroop task, including the striatum (caudate and the putamen), dorsolateral prefrontal cortex (DLPFC) and anterior cingulate cortex (ACC) (Egner and Hirsch, 2005; Harrison *et al.*, 2005; Ali *et al.*, 2010).

A rodent response conflict task (rRCT) was previously developed to target the same neural processes as those underlying performance in the human Stroop task (Haddon and Killcross, 2005). This operant based task tests a rodent's ability to follow task-specific rules by suppressing task irrelevant stimuli. This involves the presentation of two audio (A1 or A2) and two visual stimuli (V1 or V2), which are each associated with either a left or right lever. The type of stimuli presented (audio or visual) is dependent upon the one of two contexts the animal is placed in (C1 or C2). After intensive training, animals are tested under a congruent or incongruent condition. In the congruent scenario, both the auditory and visual stimuli correspond to the same lever press. In the incongruent condition, the audio-visual stimuli presented are associated with different levers. The animal is required to disambiguate the audio-visual stimuli by attending to the context it is placed in (the 'rule'), thereby suppressing the tendency to respond to the opposing lever.

Previous lesion based studies suggest that involvement of the medial prefrontal cortex (mPFC) is required for successful performance in the rRCT. This is understood to be most analogous to primate DLPFC, and can be sub divided into three sections; the ACC, prelimbic cortex (PrL), infralimbic cortex (IL) and precentral cortices. Specifically, rats with PrL, IL and ACC lesions all performed worse in a rodent response conflict task either through ablation of goal directed behaviour, lack of inhibitory control or lack of cognitive control (Haddon and Killcross, 2005, 2011; Marquis, Killcross and Haddon, 2007; Dwyer *et al.*, 2010; Oualian and Gisquet-Verrier, 2010). Additional lesion based studies revealed that lesions to the retrosplenial cortex (RSC), impaired performance in the incongruent trials of the rRCT (Nelson *et al.*, 2014), whereas no performance differences were seen in animals with hippocampal lesions (Haddon and Killcross, 2007). Surprisingly, no study has yet investigated if subregions of the striatum are involved in rRCTs, which given its involvement in the human Stroop task (Ali *et al.*, 2010) is of particular relevance seeing as the focal disease in this thesis is HD.

Understanding which regions of the brain are activated during specific tasks can be assessed using fMRI in humans. As this is not possible in rodents, an alternative strategy is the use of immediate early gene (IEG) markers. These are a class of response genes that are rapidly transcribed upon learning and memory, and encode for a variety of different transcription factors (reviewed in: Minatohara, Akiyoshi and Okuno, 2015). In turn IEGs regulate the expression of "late" genes responsible for producing long-term cellular modifications (Sheng and Greenberg, 1990). Given stimulation does not require an initial round of protein synthesis this facilitates a rapid cellular response lasting from seconds to minutes (Pérez-Cadahía, Drobic and Davie, 2011). As a result, IEGs can be used as an effective molecular marker of primary cellular responses, which can be quantified in brain regions of interest to measure neural activity implicated in learning and memory (Herdegen and Leah, 1998).

The main aim of this study was to identify neural regions activated by or recruited during the performance of the rRCT using IEG expression. Although the striatum is understood to be involved in the human Stroop task (Ali *et al.*, 2010), no previous studies have researched the involvement of the striatum in the rRCT. Therefore, as well as analysing IEG expression in a variety of cortical and temporal lobe structures, a particular interest was to see if subregions of the striatum were implicated in successful performance in the rRCT.

4.2 Methods

4.2.1 Experimental design

In this study, the aim was to compare IEG activity in selected areas of the brain between two groups of rats that underwent behavioural rRCT operant training (rRCT control (congruent) and rRCT incongruent) and Cage Controls. The behavioural procedure for the rRCT controls and rRCT incongruent groups are described below. Cage Controls received no training and were perfused at the end of the study. The recipes used for the immunohistochemistry can be located in Appendix 5.

4.2.2 Subjects

Twenty-four female Lister-hooded rats (Harlan Olac, UK) were included in this experiment, 16 of which underwent operant testing and 8 remained as cage controls. Rats were housed in groups of 4 and maintained on a rat chow diet at 85-90% of their original *ab libitum* weights, each fed 12g per day. Cage controls were handled regularly and given food *ad libitum* for a total of 6 weeks before being perfused. Holding rooms were maintained in a temperature ($21^{+/-}2^{\circ}$) and humidity ($55^{+/-}10\%$) controlled room at a 14:10-h light/dark cycle.

4.2.3 Apparatus

Behavioural training took place in a bank of 12 skinner operant boxes (Campden Instruments, Loughborough, UK), controlled by the Cambridge Cognition Control BNC software (Campden instruments, Version 1.23), as described (Dunnett and White, 2006). Each operant box was fixed with two retractable levers fitted either side of a magazine where rats retrieved 45mg of sucrose pellet rewards dispensed following a correct response. Lights were fitted above each lever, the magazine and a 3W house light on the ceiling of the operant chamber. Six operant boxes were fitted with a laminated checked context with the smell of cumin, which was mixed with the

sawdust in the tray below the floor, and 6 were fitted with a laminated stripy context with the smell of cinnamon.

4.2.4 Behavioural testing

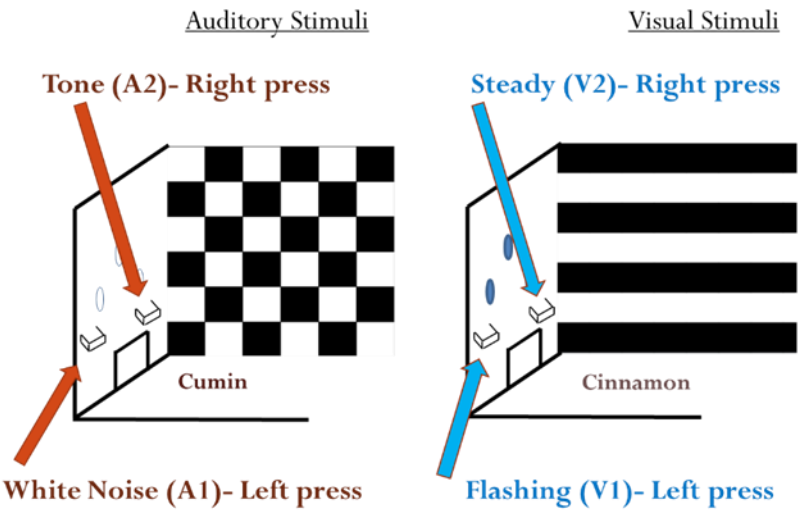
Behavioural training took place between 9am and 17.00 from Monday to Friday with one training session taking place in the morning in one context and a training session in the other context in the afternoon.

4.2.4.1 Pre-training

Each animal received one magazine training session and two lever press-training sessions in each context.

4.2.4.2 Context/stimuli training

Operant training was based on the study by (Haddon and Killcross, 2005) with minor modifications. Each training session lasted for 48 minutes. The context, for example context 1 (C1; checked context and cumin) was always associated with audio stimuli (A1 or A2), whereas context 2 (C2; stripy context and cinnamon) was always associated with visual stimuli (V1 or V2; Figure 25). Auditory stimuli consisted of either white noise (A1) or tone (A2), and visual stimuli consisted of flashing (V1) or a steady light (V2). Animals were trained to associate each stimulus with either the left or the right lever. Following a correct response, a sucrose pellet reward was dispensed via a lit magazine. Stimulus-response associations were fully counterbalanced across all rats. An example of the stimuli presented during a training session is presented in Figure 25. Each training session (in either context 1 or 2) consisted of 24 pseudorandom stimulus presentations, 12 of each (either A1 and A2 or V1 and V2). Stimulus presentations lasted for 60 seconds with 60 seconds inter-stimulus interval. Rats were trained to learn stimulus-response associations for 23 days until they performed to 80% accuracy.



	Context			
	Check + cumin		Stripe + cinnamon	
	LP1	LP2	LP1	LP2
Pre-training	A1	A2	V1	V2
rRCT Control	A1V1	A2V2	A1V1	A2V2
rRCT Incongruent	A1V2	A2V1	V1A2	V2A1

Figure 25: Operant setup of the rRCT. This figure presents a schematic of the operant set up for the rRCT during training. This illustrates the two different contexts rats were placed in, which were either associated with two different types of auditory (A1 and A2) or two different types of visual stimuli (V1 and V2), and each corresponded with a correct left or right lever press response. Briefly, the rRCT control task consisted of pressing the lever associated with the 'congruent' audio-visual stimuli. The rRCT incongruent was where audio-visual stimuli were incongruent. Therefore, rats had to attend to the context or the 'rule' to decipher the correct lever press response.
Where: rRCT = rodent response conflict task; LP1 and LP2 = Left and right lever press; LP2 = Lever press right; A1 and A2 = auditory stimuli 1 and 2; V1 and V2 = visual stimuli 1 and 2.

4.2.4.3 Extinction tests

All animals received 8 testing sessions (x4 rRCT control tests and x4 rRCT incongruent tests), each test lasting for 25 minutes and was run in extinction (i.e. in the absence of sucrose rewards). The extinction tests were used to ensure that the reinforcement of a reward was not guiding the rat to the correct response. The extinction test is used to probe an 'extinction burst'; an short increased response in an attempt to achieve the desired reward (Lattal and Lattal, 2012). Animals then received two days of training (as described in Section 4.2.4.2, context/stimuli pre-training) before the next extinction test day.

For the control test, animals were placed in one of the two contexts and presented with simultaneous audio-visual stimuli followed by the exposure of the left and right levers. In this condition, both stimuli corresponded to the same lever. In the incongruent test, animals were exposed to audio-visual stimuli, which corresponded to opposing levers. To respond correctly, the rat had to utilize the context ('rule') and determine which context-stimulus association had been previously learnt. Responding according to the previously learnt context-stimulus association meant that a 'correct' lever press was one that was associated with the stimulus that had previously been paired with the context.

To ensure that IEG expression was only activated based on the rRCT, rats were put into a darkened room for 90 minutes before and after each training session.

4.2.4.4 Final extinction test

Based on performance scores from the previous testing sessions, groups were biased so those with highest performance accuracy were put into the rRCT incongruent group and those remaining were put into the rRCT control group. This allowed us to study IEG expression in animals that could perform the incongruent rRCT to a high level of accuracy. Tissue was harvested 90 minutes after the central point in the testing

session, which coincided with peak *C-fos* activation. All rats were placed into a darkened room before and after the test to minimize any environmental stimuli that could have activated IEG expression.

4.2.5 Perfusion (performed by Dr M.J. Lelos)

Rats were terminally anaesthetized with sodium pentobarbital (Euthatal, Merial, Woking, UK) and sacrificed by transcardial perfusion with 0.01M phosphate buffered saline (PBS). Each rat received 250ml of 4% paraformaldehyde (PFA) over 5 minutes. Rats were decapitated and brains removed and stored in 4% PFA for 4 hours before transferred into a 25% sucrose solution in PBS at room temperature.

4.2.6 Sectioning

Brain tissue was cut coronally into 40µm thick sections on a freeze microtome. Sections were stored in a 1 in 12 series at -20°C in 48 well plates in ethylene-glycol-based cryoprotectant.

4.2.7 Immunohistochemistry

A 1:12 series of free floating sections were used for each immunohistochemical assay. *Zif268* staining was performed by Jessica Griffiths, an undergraduate student. Endogenous peroxidases were quenched by incubating sections in 10% methanol, 10% hydrogen peroxide and 80% distilled water. Sections were blocked with 3% normal serum and then incubated in the primary antibody *Zif268* (1: 1000; Egr-1 (C-19) Santa Cruz Biotechnology, Santa Cruz, CA, USA) and 1% normal serum in room temperature overnight. The same procedure was performed using the *C-fos* rabbit polyclonal antibody (1:5000; Ab-5, Synaptic Systems GmBH, Germany, cat. 226003/4), except for no blocking stage was implemented prior to primary antibody incubation. The following day sections were incubated with a biotinylated secondary antibody with 1% normal serum for 3 hours. The sections were

immersed in avidin-biotinylated enzyme complex (Vector Laboratories, Peterborough, UK) with 1% normal serum for 2 hours and stained with 3,3'-Diaminobenzidine (DAB) until light brown. Sections were mounted onto gelatinized glass slides and left to air dry overnight. The sections were dehydrated in ascending concentration of 70%, 95% and 100% of alcohols and then immersed in xylene. Slides were cover slipped using di-n-butyl phthalate in xylene (DPX) mounting medium and air-dried.

Sections were visualized using a brightfield Leica DMRB microscope at x5 objective and captured using an Olympus DP70 camera on Leica Application Suite Imaging software. Some images were captured and stitched together using Windows Live Photo Gallery.

4.2.8 Image J analysis

All images were analysed blind. IEG expression was quantified using Image J software (Version 1.51, National Institutes of Health, USA). To quantify the number of immunoreactive nuclei, an individual threshold was set for each region of interest and remained consistent for all animals. A watershed was applied and the number of immunoreactive positive nuclei were counted in set area and averaged across both hemispheres. The number of immunoreactive positive (IR) nuclei per $10\mu m^2$ were calculated using the equation:

$$100 * \frac{\text{(Average number of IR positive nuclei)}}{\text{Average area}} = \text{Average IR nuclei} / 10\mu m^2$$

4.2.9 Regions of interest

Cytoarchitectonic subfields of the prefrontal cortex (prelimbic cortex, infralimbic cortex and anterior cingulate cortex), striatum (dorsolateral, dorsomedial and ventral), hippocampus (CA1, CA2, CA3 and dentate gyrus), retrosplenial cortex

(dysgranular (RSCa) and granular (RSCb)) and the auditory cortex were identified in all sections using nomenclature based on (Paxinos and Watson, 2007) (Figure 26). immunoreactive positive counts were also made in the auditory cortex which was used as an internal control for IEG staining specifically between rRCT groups (rRCT incongruent and rRCT control) and Cage controls.

The neuroanatomical coordinates used when counting were between 4.7mm to 2.70mm from bregma for all prefrontal cortical regions; 1.60mm to -1.40mm from bregma for all striatal regions; -2.30mm to -4.30mm from bregma for all hippocampal subregions; -1.60mm to -4.52mm from bregma for the retrosplenial cortex; -3.14mm to -4.42mm from bregma for the auditory cortex.

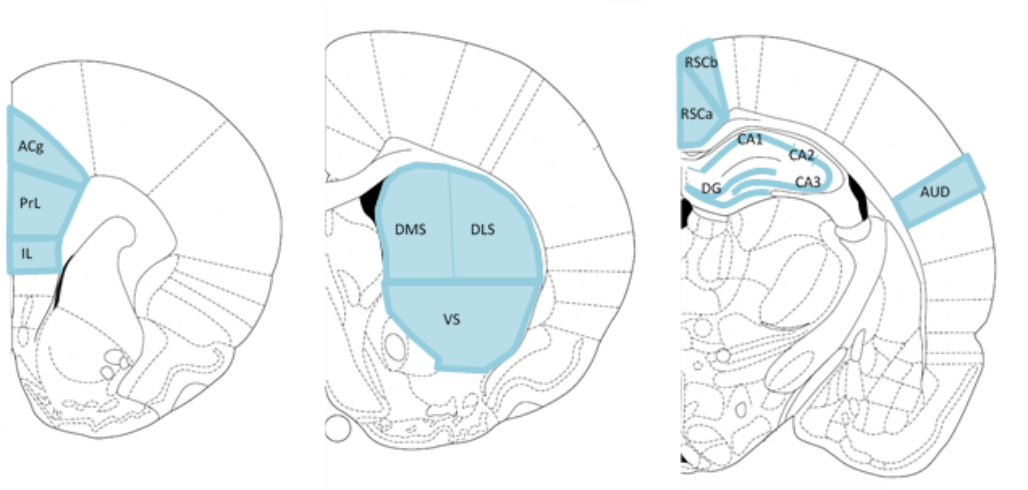


Figure 26: IEG brain regions of interest: Coronal sections representing brain regions of interest taken from Paxinos and Watson 1997: ACC*, anterior cingulate cortex,* ACC*; AUD, auditory cortex; DG, dentate gyrus; DLS, dorsolateral striatum; DMS, dorsomedial striatum; IL, infralimbic cortex; PrL, prelimbic cortex; RSC, retrosplenial cortex; VS, ventral striatum.*

4.2.10 Statistical Analysis

SPSS version 20 (PASW) (IBM Corporation, USA) was used to analyse the data. For all behavioural training data, performance during the first 10 seconds of stimuli presentation was used (Haddon and Killcross, 2006). In the final extinction test, this was taken only from the first 12 minutes of the final test, coinciding with peak *C-fos* activation (Chaudhuri, Matsubara and Cynader, 1995; Kaminska, Kaczmarek and Chaudhuri, 1996). The percentage accuracy and the average number of correct vs incorrect lever presses were presented in graphs with the standard error of the mean (SEM). Differences between the numbers of correct vs incorrect responses during the final test were calculated using a paired t-test.

For the IEG counts, the mean number of immunoreactive positive nuclei per μm^2 were presented in graphs with the SEM. A one way ANOVA was used with a between-subjects variable of 'Group' (Cage Control, rRCT control or rRCT incongruent) and using the brain region of interest as the dependent variable.

A Pearson correlation was used to correlate IEG expression with performance accuracy during the first 10 to 60 seconds of stimuli presentation in the first half of the final task.

All ANOVAs and t-tests performed used $p< 0.05$ to reject the null hypothesis.

4.3 Results

4.3.1 Subjects

Due to illness, one rat was perfused before the end of the experiment. An additional two rats were removed from analysis due to an inability to learn the behavioural task and inconsistent staining. All remaining rats in the Cage Control (n=8), rRCT control (n=7) and rRCT incongruent group (n=6) completed the experiment.

4.3.2 Behavioural results

4.3.2.1 Previous training and testing

All rats performed the training sessions to a high level of accuracy, successfully responding to the correct lever following stimulus presentation, performing greater than 85 ± 2% accuracy.

The final rRCT control and incongruent groups were biased, based on performance from the eight previous extinction tests (x4 control and x4 incongruent). The best performers formed the rRCT incongruent group and achieved on average 59.08 ± 2.35% correct responses in the previous rRCT incongruent extinction tests. Rats that formed the final rRCT control group achieved on average 51.03 ± 3.34% correct responses in the previous rRCT control extinction tests.

4.3.2.2 Final test

The first six minutes of the final test were used for analysis. Rats in the rRCT incongruent group achieved significantly more correct than incorrect lever responses, achieving on average 71.96 ± 4.8% accuracy [$t_{(5)}$=4.39, p<0.01; Figure 27]. The rRCT control group achieved on average 51.34 ± 4.85% correct responses [$t_{(6)}$=0.121, p= n.s].

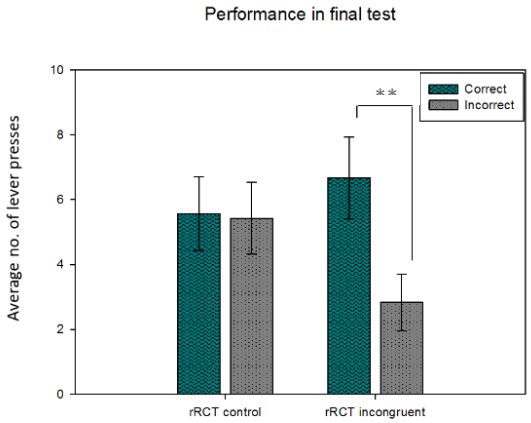


*Figure 27 The Mean number of correct and incorrect lever presses in the final rRCT. Bars showing the SEM. Where * p<0.05.*

4.3.3 IEG results

4.3.3.1 Zif268 results

Representative images of *Zif268* staining are presented in Figure 28.

Striatum. Immunoreactive staining in the DLS and DMS were no different between any of the groups [Group: DLS, $F_{2,18} = 2.27$, p = n.s.; DMS, $F_{2,18} = 3.201$, p = n.s.; Figure 29a and 29b]. However, a trend was seen in the VS which was driven by a downregulation of *Zif268* in the rRCT control groups relative to Cage Controls [Group: $F_{2,18} = 3.463$, P = 0.053; rRCT control vs Cage Controls p = 0.086; Figure 29c].

Prefrontal cortex. Group differences in *Zif268* counts were identified in subregions of the PFC. In the ACC, there was a significant upregulation of *Zif268* between Cage Controls and rRCT controls [Group: $F_{2,17} = 4.569$, $p < 0.05$; Cage Controls vs rRCT controls <0.05; d]. In the IL cortex, there was an upregulation of *Zif268* in the rRCT incongruent group compared to Cage Controls, and a trend towards greater *Zif268* expression between rRCT incongruent and rRCT controls [Group: $F_{2,13} = 6.195$, $p < 0.05$;

rRCT incongruent vs Cage Controls p<0.05; rRCT incongruent vs rRCT controls p = 0.051; Figure 29f]. No difference in IEG expression was seen between groups in the PrL [Group: $F_{2,14}$ = 0.094, n.s.; Figure 29e].

Hippocampus. In the CA1 there was a significant upregulation of *Zif268* in the rRCT controls compared to Cage Controls [Group: CA1, $F_{2,18}$ = 4.335, p< 0.05; rRCT controls vs Cage controls p<0.05; Figure 29g]. In the CA3 there was also a significant upregulation between both rRCT groups and Cage controls [Group: $F_{2,18}$ = 5.109, p< 0.05; rRCT incongruent and rRCT controls vs Cage Controls p<0.05, Figure 29h]. There was a trend toward significance in the DG driven by an upregulation in the rRCT incongruent group compared to Cage Controls [Group: $F_{2,18}$ = 3.487, p = 0.052; rRCT incongruent vs Cage Controls p=0.095;Figure 29j], No differences were seen between Groups in the CA2 [Group: $F_{2,18}$ = 1.393, n.s. Figure 29k].

Retrosplenial cortex. IR positive counts in the RSCb revealed a significant upregulation of *Zif268* expression in rRCT incongruent group relative to Cage Controls [Group: $F_{2,18}$ = 4.734, p<0.05; rRCT incongruent vs Cage Controls p<0.05;Figure 29 l]. There was a main effect of Group in the RSCa, however no individual comparisons met significance Figure 29k). Initial differences seemed to be driven by an upregulation in *Zif268* in the rRCT incongruent group relative to Cage controls [Group: RSCa, $F_{2,18}$ = 3.667, P<0.05; rRCT incongruent vs Cage Control p = 0.071].

Auditory cortex. There was a significant upregulation in *Zif268* expression in the rRCT incongruent and rRCT contols relative to Cage Controls, but no significant difference between rRCT controls and rRCT incongruent [Group: $F_{2,17}$ = 6.453, P<0.01; rRCT incongruent and rRCT congruent vs Cage controls p<0.05; Figure 29m].

***Zif268* staining**

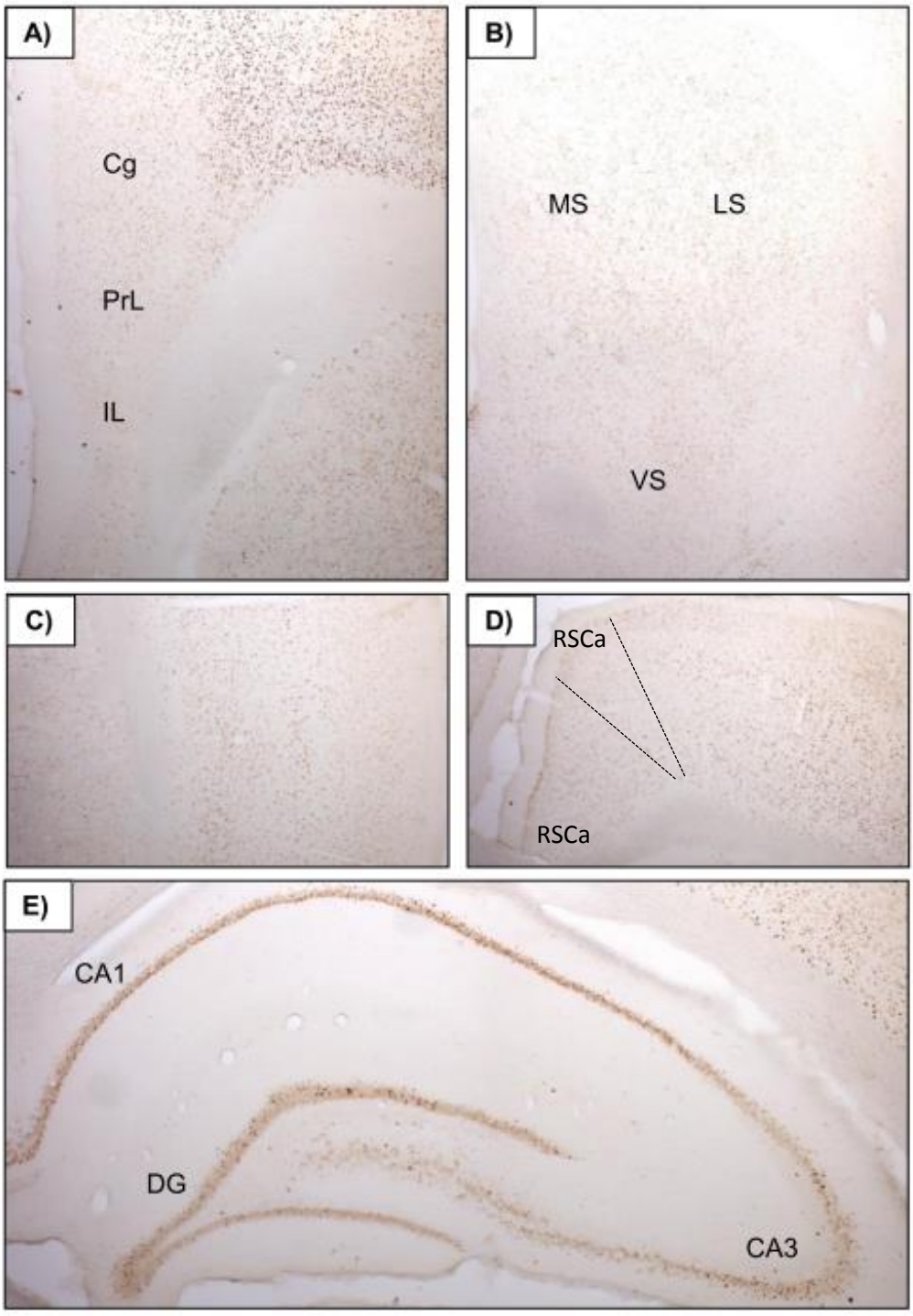


Figure 28: Representative images of Zif268 staining in subregions of the prefrontal cortex (a), striatum (b), auditory cortex (c), Retrosplenial cortex (d) and hippocampus (e). Where ACC=anterior cingulate cortex, PrL=prelimbic cortex, IL=infralimbic cortex, MS =medial striatum, LS=lateral striatum, RSC = Retrosplenial cortex, VS=ventral striatum, DG=dentate gyrus. Images presented here were captured by Jessica Griffiths. Images were taken at x1.25 magnification.

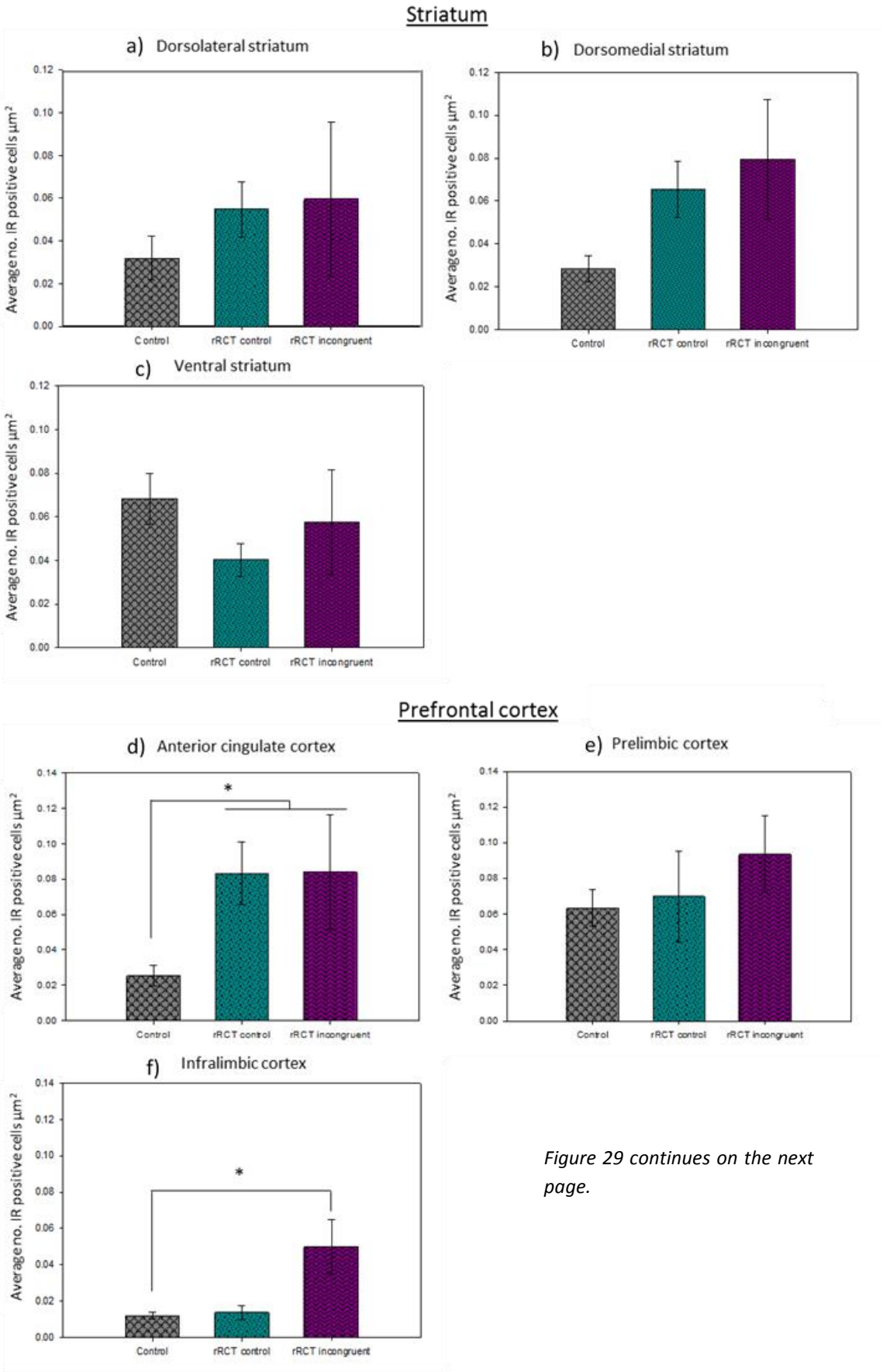


Figure 29 continues on the next page.

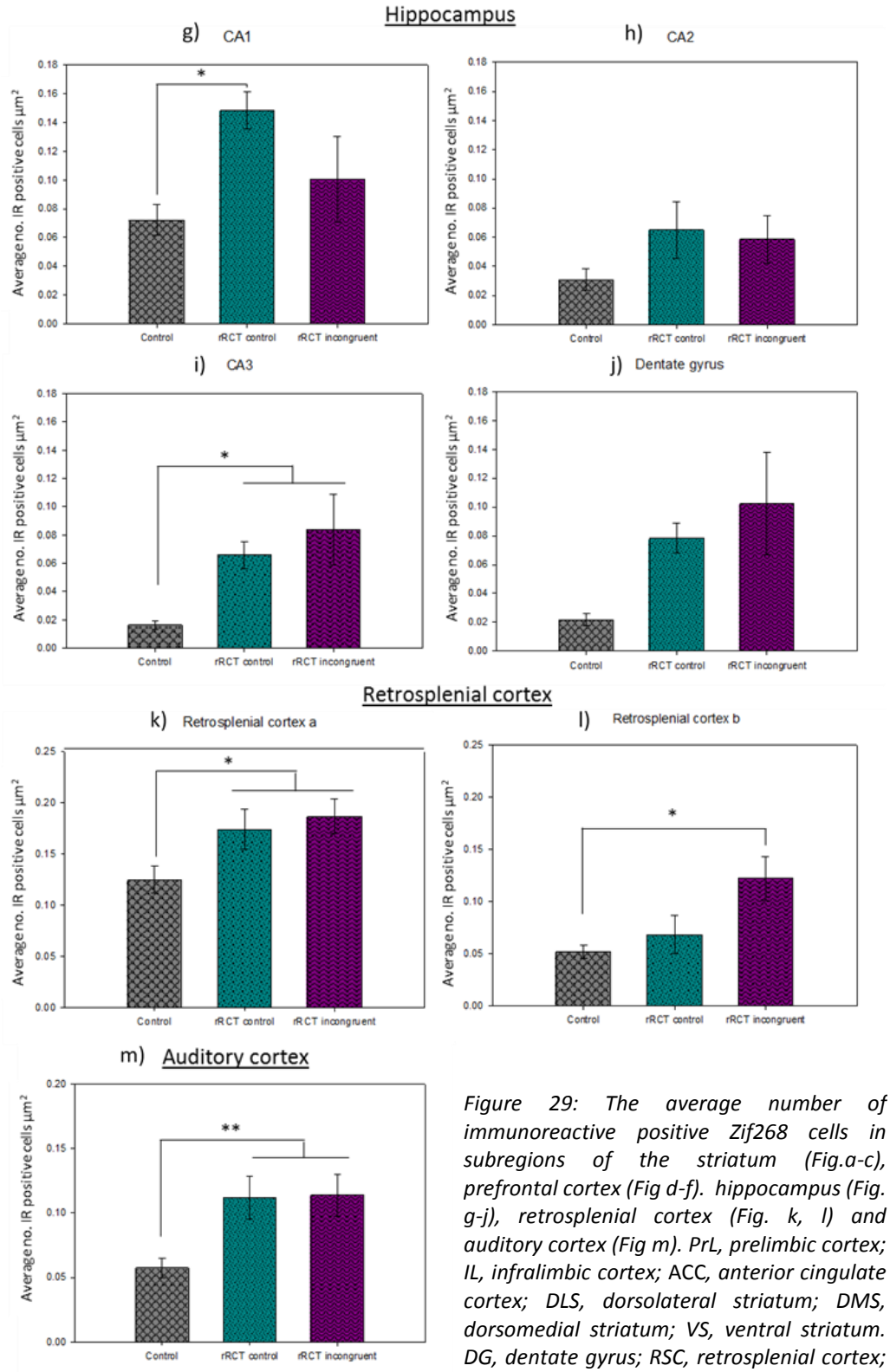


Figure 29: The average number of immunoreactive positive Zif268 cells in subregions of the striatum (Fig.a-c), prefrontal cortex (Fig d-f). hippocampus (Fig. g-j), retrosplenial cortex (Fig. k, l) and auditory cortex (Fig m). PrL, prelimbic cortex; IL, infralimbic cortex; ACC*, anterior cingulate cortex; DLS, dorsolateral striatum; DMS, dorsomedial striatum; VS, ventral striatum. DG, dentate gyrus; RSC, retrosplenial cortex; Aud, auditory cortex. Error bars show ± standard error of the mean. *P<0.05, **P<0.01.*

4.3.3.2 *C-fos* results

Representative images of *C-fos* staining in the Retrosplenial cortex and the auditory cortex are presented in Figure 30.

The RSCb was the only region where *C-fos* expression differed across group, revealing a significant upregulation in the rRCT incongruent group relative to Cage Controls. [Group: $F_{2, 18}$ =4.826, P< 0.05; rRCT incongruent vs Cage Controls, p<0.05;Figure 31a].

There was a trend toward significance in the PrL, driven by an upregulation in the rRCT controls relative to Cage Controls, but a downregulation in the rRCT incongruent group relative to the Cage Control group [Group: PrL, $F_{2,14}$ =3.511, p= 0.058].

There were no significant differences in *C-fos* expression in any of the following regions of interest: PFC [Group: IL, $F_{2,14}$ =1.108, p = n.s.; ACC, $F_{2,18}$ =1.218, p= n.s.], striatum [Group: DLS, $F_{2,18}$ =2.451, p = n.s.; DMS, $F_{2,18}$ =2.005, p = n.s.; VS, $F_{2,18}$ =1.228, p = n.s.], hippocampus [Group: CA1, $F_{2,17}$ =1.553, p= n.s.; CA2, $F_{2,17}$ =1.066, p= n.s.; CA3, $F_{2,16}$ =0.26, p= n.s.; DG, $F_{2,17}$ =0.129, p = n.s.) and RSCa [Group: $F_{2,18}$ =1.063, p=n.s.].

In the Auditory cortex, there were no significant differences in *C-fos* expression between any Group [Group: $F_{2,16}$ = 0.76, p = n.s.; Figure 31b]. As IEG counts were taken from the Auditory cortex as a control region (refer to Section 4.2.9), given that there were no differences in *C-fos* expression between cage controls and rRCT groups that undertook operant training, this made us question the *C-fos* staining patterns for the brain regions recorded.

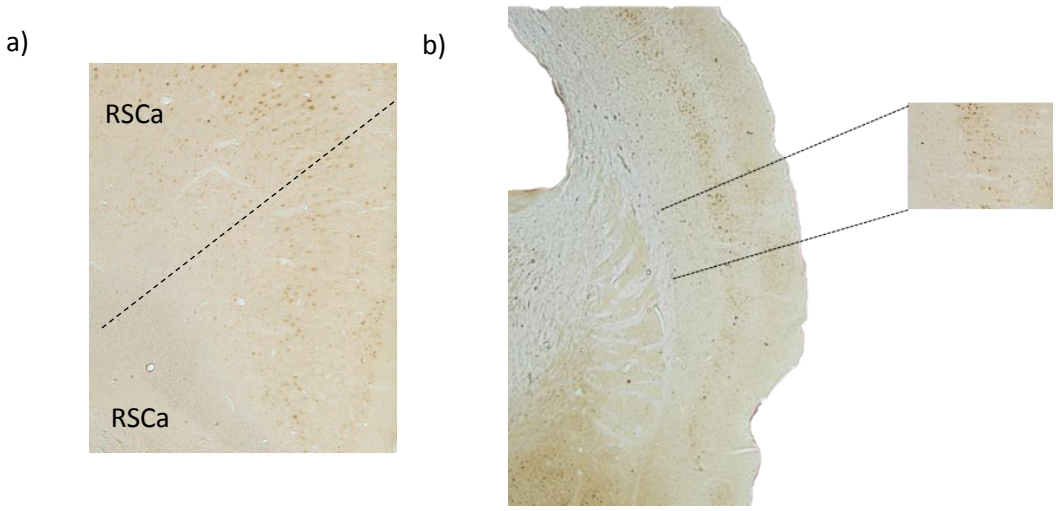


Figure 30: Representative C-fos images in the retrosplenial cortex and the auditory cortex of a rat that undertook operant training. Figure a) was taken at x5 magnification and figure b) was was taken at x1.25 magnification, and the magnified image was taken at x5. Where RSC = retrosplenial cortex (agranular (a) or granular (b)).

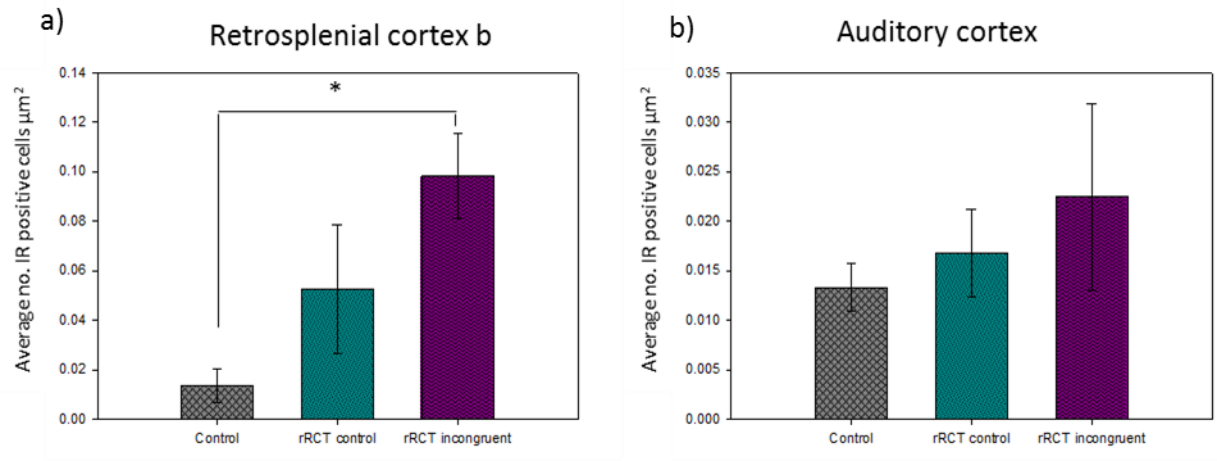


Figure 31: The average number of immunoreactive C-fos positive nuclei counted in the Retrosplenial cortex b (a) auditory cortex (b) in the Cage Control, rRCT control and rRCT incongruent groups. This revealed no significant differences between any of the groups (P>0.05). Bars show standard error of the mean.

4.3.3.3 The relationship between behavioural performance and IEG expression

Correlation results between *Zif268* expression and performance accuracy throughout the entire stimuli presentation are presented in Table 37.

The *C-fos* counts significantly, positively correlated with performance accuracy from the rRCT incongruent group in both the RSCa [r=0.879, n=6, p<0.05] and RSCb [r=0.8596, n=6, p<0.05], but for no other region for any Group (p>0.05).

Table 37: The correlation between performance accuracy and Zif268 expression sub-regions of the brain

Performance accuracy		Striatum			Prefrontal cortex			Hippocampus				Retrosplenial cortex	
		DLS	DMS	VS	ACC	PrL	IL	CA1	CA2	CA3	DG	RSCa	RSCb
First 10 seconds	rRCT Incongruent	0.823*	-0.28	.959**	0.44	-0.47	-0.60	0.40	0.34	0.37	0.26	0.23	0.33
		6	6	6	6	4	4	6	6	6	6	6	6
	rRCT control	-.463	-0.43823	-.025	-0.721	.254	-.206	.527	-.343	.031	.404	.307	.699
		7	7	7	7	5	4	7	7	7	7	7	7
First 20 seconds	rRCT Incongruent	0.35	-.891*	0.66	-0.31	-.972*	0.21	0.27	0.10	0.10	0.17	-0.34	-0.39
		6	6	6	6	4	4	6	6	6	6	6	6
	rRCT control	-.650	-.891**	-.270	-.803*	-.129	.861	.446	-.064	-.364	.020	.519	.381
		7	7	7	7	5	4	7	7	7	7	7	7
First 30 seconds	rRCT Incongruent	0.08	-.920**	0.24	-0.33	-0.68	-0.13	-0.07	-0.36	-0.19	-0.18	-0.72	-0.19
		6	6	6	6	4	4	6	6	6	6	6	6
	rRCT control	-.372	-.793*	.013	-0.70799	-.197	.852	.591	.072	-.056	.279	.756*	.541
		7	7	7	7	5	4	7	7	7	7	7	7
First 40 seconds	rRCT Incongruent	-0.04	-.878*	0.37	-0.40	-.966*	0.19	-0.01	-0.21	-0.11	-0.02	-0.24	-0.24
		6	6	6	6	4	4	6	6	6	6	6	6
	rRCT control	-.400	-.781*	-.155	-0.521	-.284	.985*	.364	.014	-.086	.128	.712	.542
		7	7	7	7	5	4	7	7	7	7	7	7
First 50 seconds	rRCT Incongruent	-0.15	-0.68	-0.11	-0.35	-0.68	0.80	0.27	0.07	0.17	0.30	-0.28	-0.66
		6	6	6	6	4	4	6	6	6	6	6	6
	rRCT control	-.050	-0.443	.064	-0.379	.214	.730	.574	.021	-.001	.587	.878**	.486
		7	7	7	7	5	4	7	7	7	7	7	7
First 60 seconds	rRCT Incongruent	-0.28	-0.63	-0.06	-0.58	-0.81	0.84	0.17	0.07	0.03	0.23	-0.20	-0.76
		6	6	6	6	4	4	6	6	6	6	6	6
	rRCT control	-.120	-.496	.109	-.525	.272	.616	.689	-.045	.059	0.68	.905**	.641
		7	7	7	7	5	4	7	7	7	7	7	7

*DLS = dorsolateral striatum; DMS = dorsomedial striatum; VS= ventral striatum; ACC=anterior cingulate cortex; PrL=prelimbic cortex; IL=infralimbic cortex; RSC=Retrosplenial cortex. Where * p<0.05, ** p<0.01 (hall significant values highlighted in beige).*

4.4 Discussion

The aim of the study described in this Chapter was to identify the neural regions recruited during response conflict processing, using the operant rRCT. More specifically, given that people with HD are impaired in RCTs such as the Stroop task, a particular interest was to identify whether the striatum was activated during performance in the rRCT. Immediate early genes, *C-fos* and *Zif268* were used to measure neural recruitment in numerous cortical and midbrain structures. The results revealed significant changes in *Zif268* activity in the prefrontal cortex (IL and ACC), hippocampus (CA1, CA3, DG), retrosplenial cortex (RSCa and RSCb) and the auditory cortex between Cage Controls, rRCT controls and/or rRCT incongruent groups. *Zif268* expression in the dorsomedial striatum consistently correlated with performance accuracy throughout the duration of stimulus presentation in both the rRCT control and rRCT incongruent groups. *C-fos* expression significantly differed between groups in the RSCb. However, there were no changes in *C-fos* expression in any other brain region, including auditory cortex, which was used as an internal control. This is explained in further detail in the discussion below.

Behavioural performance in the rRCT extinction test

During the final extinction test, rats in the rRCT incongruent group were required to disambiguate audio-visual stimuli based on the context in which they were placed in. Rats in the rRCT incongruent group achieved significantly more correct than incorrect responses, supporting previous studies that rats can successfully identify and respond to conflicting information (Haddon and Killcross, 2005, 2011; J. Haddon and Killcross, 2006; Marquis, Killcross and Haddon, 2007). Groups formed for this final test were sorted in such a way as to ensure optimal performance in the incongruent tests, since this was the neural process that we were interested in investigating. As a result, the RCT control group contained rats that had learnt the associations less well and therefore only achieved 51% accuracy. However, as these rats were exposed to auditory stimuli, visual stimuli, odours, handling, contexts and produced motor

movements - they formed a critical control group to account for the effect of these external and internal stimuli on IEG expression.

IEG expression

The neuronal IEGs *C-fos* and *Zif268* both increase and decrease downstream gene expression and are used regularly as markers for general neural activity and neuronal activation during learning or memory retrieval (Martinez, Calvo-Torrent and Herbert, 2002; Rygh *et al.*, 2006). This study utilised changes in IEG expression as a means of identifying which brain regions were recruited during performance in the rRCT. The IEGs used in this study peak at different time points, with *C-fos* peaking between 90-120 minutes after stimulus onset contrasting with *Zif268* which peaks between 30-60 minutes and reduces to basal levels within 24 hours (Chaudhuri, Matsubara and Cynader, 1995; Kaminska, Kaczmarek and Chaudhuri, 1996; Davis, Bozon and Laroche, 2003). In our study, cage control animals were not exposed to any sound stimuli. As a result, it was expected a significant difference in *Zif268* expression in the auditory cortex of cage controls, relative to the auditory cortex in animals that underwent operant testing. This region was therefore used as an IEG internal control and, when using *Zif268* this confirmed a significant upregulation in the rRCT groups compared to cage controls. However, no differences were observed between Cage Controls and rRCT groups when *C-fos* were analysed. In addition, *C-fos* staining appeared patchy and inconsistent, which may have been due to notorious sensitivity of the *C-fos* antibody and perhaps subtle differences in tissue perfusion and fixation. Therefore, it is acknowledged that there is a fundamental caveat associated with the data derived from the *C-fos* staining in this study.

Zif268 expression in the PFC, hippocampus and retrosplenial cortex

In this study, changes in IEG expression in subregions of the PFC in rRCT groups were observed. There was a significant upregulation of *Zif268* positive nuclei in the IL cortex in the rRCT incongruent group relative to Cage Controls, but no difference between rRCT controls and Cage Controls. This suggests that the upregulation in the

rRCT incongruent group may have been required for conflict resolution. This contrasts with findings from a previous finding that used the rRCT and revealed that temporary inactivation of the IL did not affect the accuracy of performance in the incongruent test (Marquis, Killcross and Haddon, 2007). Other studies had revealed different roles for the IL cortex including behavioural flexibility (Oualian and Gisquet-Verrier, 2010) and extinction learning (Barker, Taylor and Chandler, 2014), whilst others have shown it is fundamental for fixed behaviour such as habits (Marquis, Killcross and Haddon, 2007). In our study, as rats had experienced the extinction test numerous times, the IL may have been recruited for extinction learning or perhaps the transition from goal directed to a more habitual response, leading to an increase in *Zif268* expression.

The results in this study also showed an upregulation of *Zif268* in the ACC in both rRCT groups relative to Cage Controls, suggesting that this may have been recruited due to the exposure of stimuli and/or motor responses rather than conflict resolution per se. This finding is somewhat surprising as numerous studies suggest that the ACC is involved when evaluating conflict scenarios (Pardo *et al.*, 1990; Braver *et al.*, 2001; De Pisapia and Braver, 2006; Haddon and Killcross, 2006). However, this region has also been implicated in the evaluation of action-outcomes and may serve an explanation to the *Zif268* upregulation in both rRCT groups (Botvinick, Cohen and Carter, 2004).

Counts recorded from the hippocampus revealed significant changes in *Zif268* activation in the CA1 and CA3 subregions between Cage Controls and the rRCT groups. This supports previous findings that suggest the CA1 and CA3 subregions of the hippocampus are involved in associative learning, specifically pairing a stimulus to a context (Izuma, Saito and Sadato, 2010), but not necessarily for conflict resolution (Haddon and Killcross, 2007).

Changes in *Zif268* expression in the RSC were identified between Cage Controls and the rRCT groups. Interestingly, there was a *Zif268* upregulation in the rRCT incongruent group relative to Cage Controls, but no difference in the rRCT control group. This supports a previous finding that suggest the RSC is involved in conflict

resolution (Nelson *et al.*, 2014). They found rats had no problem learning context-dependent rules but did have difficulty in selecting task relevant stimuli (Nelson *et al.*, 2014). However, they did not look at differences between the RSCa and RSCb subregions. The RSCb is more extensively connected with cortical and subcortical regions than RSCa (Hindley *et al.*, 2014), suggesting this region may be more involved in resolving conflict and why a general upregulation was seen in the RSCa but not specific to the rRCT incongruent group.

Is the striatum necessary for conflict resolution?

Huntington's disease provides a unique model for studying striatal involvement in the Stroop task, where a previous study revealed striatal degeneration correlated with performance accuracy in the incongruent Stroop task (Sanchez-Pernaute *et al.*, 2000; Ali *et al.*, 2010). As striatal involvement has not yet been tested in the rRCT, analysing the potential involvement of this region during a conflicting scenario was of particular interest in this study. The results revealed a significant positive correlation between *Zif268* expression in all subregions of the striatum at various time points during stimulus presentation of the final test. Conversely, no changes were observed in general *Zif268* expression counts between any of the Groups.

Zif268 expression in the DMS correlated with performance accuracy more than any other subregion in both the rRCT control and rRCT incongruent group in the first 20-40 seconds of stimuli presentation. The DMS is analogous to the human caudate and anterior putamen, and has previously been implicated in decision making and in particular goal directed behaviour (Yin *et al.*, 2005; MacDonald *et al.*, 2014). As decision making behaviours are more likely to occur at the beginning of stimuli presentation, this may explain why the final 20 seconds of stimulus presentation did not significantly correlate with performance accuracy. With regards to goal directed learning, in this particular task the animal learns to respond to a stimulus, which is reinforced with a sucrose. During extinction tests, no reward is given (Haddon and Killcross, 2005; Haddon and Killcross, 2006; Marquis, Killcross and Haddon, 2007). This

is carried out in an attempt to achieve an 'extinction burst', which is where the rat's behaviour temporarily increases in an attempt to achieve the desired food reward (reviewed in: Lattal, 2012). However, over time, as no food reward is given, this leads to extinction learning, and a decrease in the goal-directed response as the rat learns no reward is given (reviewed in: Todd, Vurbic and Bouton, 2014). In this study, as there was no significant relationship between Zif268 expression and performance accuracy during the final 20 seconds of stimulus presentation, this may have been a result of initial goal-directed behaviour, which gradually decreased with increased extinction learning.

The DLS and VS of the rRCT incongruent group also significantly correlated with performance accuracy during the first 10 seconds of stimulus presentation. As the DLS is implicated in stimulus response associations (Stalnaker *et al.*, 2010), which form the basis of habit formation after excessive goal-directed learning (Yin, Knowlton and Balleine, 2004; Balleine, Liljeholm and Ostlund, 2009), this suggests initial responses to stimuli presentation may have been driven by repeated exposure to the incongruent stimuli during previous extinction tasks. Interestingly, a significant correlation between *Zif268* expression and performance accuracy was also seen in the VS in the first 10 seconds of stimulus presentation. As the VS is activated under rewarding experiences (Burton, Nakamura and Roesch, 2015), this suggests responses may have been driven by the expectation of a food reward.

In HD as the disease progresses the greatest changes appear to be in the less cognitively demanding tasks, specifically in the time taken to recite colour names and words read, i.e. the congruent task (Snowden *et al.*, 2001; Ho *et al.*, 2003). An explanation for this could be that information processing speed and recitation of automatic over-learned sequences deteriorates as the disease progresses, which is thought to involve a striatal contribution (reviewed in: Saling and Phillips, 2007). Therefore, in this study, the striatum may be more important for the rRCT control task than the incongruent rRCT and could explain why no changes in *Zif268* expression were observed in the rRCT incongruent group using the immunoreactive counts alone. Furthermore, as the rRCT control group consisted of animals that had learned the rRCT

tasks less well, this would also explain why no *Zif268* changes were observed in this group either.

Experimental caveats

A main limitation to this study was the small sample size used. Larger group sizes in this study would have increased the experimental power.

In this study, there was reason to believe extinction learning may have driven some of the *Zif268* expression changes in the rRCT groups. Certain methodological differences between this study and the existing literature might explain this discrepancy. In this study, rats were exposed to nine extinction tests (compared to 2 in the original rRCT study (Haddon and Killcross, 2005), interspersed with behaviour training. This experimental procedure was designed as previous attempts to carry out this study resulted in rats unable to perform the rRCT incongruent tests. To reduce this risk, the rRCT was optimised by utilizing contexts that were more obvious, and repeated extinction tests to ensure rats were able to perform the incongruent rRCT and therefore obtain the optimal rRCT incongruent group.

In this study, IEGs were used to understand the neural processes that may have been activated during response conflict. However, using this method does not definitively determine the precise role of that brain region. As a result, this study should be treated as the first of a series of studies, which would include lesion and inactivation experiments to fully determine what each brain region does in this task. As previously mentioned, a limitation to this study was the poor *C-fos* staining resulting in inconclusive results for this IEG. Although the *C-fos* immuno-histological staining was optimised prior to this study, *C-fos* is a notoriously temperamental antibody and may therefore explain the inconsistent staining observed.

Future work and Conclusions

To conclude, this study revealed that rats could successfully respond to conflicting stimuli in the incongruent rRCT. This allowed comparison of *Zif268* differences between Cage Controls, rRCT controls and rRCT incongruent groups in numerous brain regions, including sub-regions of the PFC, striatum, hippocampus and RSC. Interestingly, *Zif268* expression correlated with performance accuracy in all sub-regions of the striatum. However, whether this was due to genuine recruitment required either for conflict resolution or for the rRCT control task, or if this was a result of extinction learning is inconclusive. Therefore, additional studies are required to determine the precise role striatal involvement in the rRCT.

As mentioned previously, future work could include using larger sample sizes in a similar study for greater experimental power and sensitivity to smaller effects. Furthermore, additional optimisation of the *C-fos* antibody is of interest given the inconclusive results using this IEG. It should be noted, other IEGs such as *Arc* are also activated and important for learning and memory (reviewed in: Minatohara, Akiyoshi and Okuno, 2015) and could therefore be used as an additional IEG marker in a repeated study.

Future work could also include further analysis of IEG results through structural equation modelling. This statistical technique allows the analysis and testing of observed IEG activity using a computational model of anatomically connected brain regions. This would allow us to test and compare interdependences between and within networks of neural activity as shown by other groups (Lelos and Good, 2012; Kinnavane, Albasser and Aggleton, 2015). In our case, this would allow a more in depth analysis of the neural networks recruited for successful performance of the rRCT, whilst informing us of other interconnected brain regions that have been implicated in the human Stroop task, such as the parietal cortex, cerebellum and the thalamus (Coulthard, Nachev and Husain, 2008; Ide and Li, 2011; Becerril and Barch, 2013).

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Chapter 5

General Discussion

Discussion Summary

In this thesis I have described the development and evaluation of novel assessments that could be used to sensitively track neurodegeneration in people with HD and in the future, potentially a preclinical model of HD. In Chapters 2 and 3, dual task assessments were developed to specifically recruit the basal ganglia circuity. In Chapter 2, this revealed that the Step and Stroop and the Clinch token transfer test (C3t) significantly distinguished between disease stage in HD, more so than other dual task assessments that were tested. To take the C3t findings further, in Chapter 3, the C3t design was standardised and validated in people gene positive for HD and in healthy controls. This revealed that the C3t had high external and test validity, and most importantly that it distinguished between the earliest stages of HD, suggesting that this could be an informative functional outcome measure for future HD clinical trials. As the Stroop task is a common cognitive assessment used in people with HD, in Chapter 4, the aim was to understand the neurobiology associated with a rodent analogue of the Stroop task. This revealed that striatal activation significantly correlated with the Stroop performance from rats in the incongruent group, and the control (congruent) group. Therefore, this could be the first in a series of studies to understand if the striatum is involved during response conflict.

5.1 Thesis summary

The aim of the work in this thesis was to develop functional outcome measures that are sensitive to the deficits seen in HD. This is crucial to accurately track disease progression, and to assess the efficacy of developing therapeutics, such as cell transplantation. If we are going to develop any new treatments, we need to show a benefit from the cellular level (graft integration) to the clinic and importantly, if this improves the standards of daily living. In addition, with regards to cell transplantation, we also need to start thinking about what functions we might expect to improve if we are reconnecting the cortico-basal ganglia-thalamo circuitry. However, current outcome measures with high ecological validity and that are sensitive to HD are limited and do not allow such sensitive assessment.

One improvement that we might expect to see is the ability to perform two or more tasks simultaneously. Dual tasking is one example of a function consistently performed in daily life, and therefore may be a useful outcome measure to assess functional performance in people with HD. In addition, previous studies suggest that dividing attention between two or more tasks may involve the fronto-striatal circuitry for optimal performance (Yogev-Seligmann, Hausdorff and Giladi, 2012; Yildiz and Beste, 2015). As this is a primary site of degeneration in people with HD, it is not surprising that HD patients have difficulty performing two tasks simultaneously (See Chapter 1, Table 2). Furthermore, in some cases, this may require prioritising attention when two potential outcomes are presented. An important aspect of decision making is to select an appropriate action in the face of conflicting information (response conflict), and this is required to successfully perform the Stroop task.

The findings in this thesis support that people with HD are deficient when performing two tasks simultaneously, although the extent to which performance deteriorates is not consistent, where in some tasks the motor task is prioritised and in other tasks the cognitive task is prioritised. Therefore attention does not seem to be

allocated definitively toward the motor or the cognitive element of the dual task and instead it depends on the motor-cognitive tasks combined. The C3t involved automating aspects of behaviour for optimal performance and, from all of the dual tasks tested, this was the most sensitive to disease stage. This suggests that the basal ganglia circuitry may be recruited during this assessment due to the clear stepwise deterioration of performance with increasing disease stage. Furthermore, the findings in Chapter 4 suggest that the striatum could be implicated during response conflict in a rodent analogue of the Stroop task suggesting that the striatum may be involved in selecting and prioritising one task over another when two outcomes are presented. Understanding the neural correlates that are required for such tasks could help explain why people with HD have difficulty performing some common daily tasks, such as allocating attention when two outcomes are presented.

In Chapter 2, Part 1, three lower limb dual tasks were selected and developed for testing in HD. This included, the *Walk and talk*, which had been used in a previous HD study (Fritz *et al.*, 2016); the *TUG and LVF*, which are two single tasks that are commonly used in HD, and were previously used as a dual task in the elderly (van Iersel, 2007), but never in HD; and the *Step and Stroop* which was newly designed for this study. Whilst planning Part 1, it become evident that the dual tasks selected thus far all focussed on lower limb function. As no current upper limb tasks could be adapted for dual task testing, a new upper limb dual task assessment was designed and developed for this study. This novel assessment was originally named the Moneybox test, but was later changed and referred to throughout this thesis as the Clinch token transfer test (C3t). As part of the assessment development, it was important to include functions that tapped into the degenerating neurocircuitry in HD. Furthermore, to ensure that these dual tasks were sensitive to people with different levels of functional ability, it was important that they included different levels of complexity.

The results in Chapter 2 revealed that different motor-cognitive dual tasks elicit different levels of interference in people with HD. The *Step and Stroop* and the C3t best

distinguished between disease stage in HD and significantly correlated with UHDRS scores and quality of life measures, more than the *Walk and talk*, and the *TUG and LVF*.

In Chapter 2, it became evident that there was a need to standardise the C3t. Thus, the aim of this chapter was to design and develop the C3t version 2 and then validate this in a large cohort of people gene positive with HD. When the new design was tested in people with HD (Chapter 3, Part 2), it was clear that performance was not changing with increased task complexity, suggesting that there were practice effects. Therefore, the C3t was optimised, which involved re-positioning the tokens in the Baseline complex and dual task. In Part 3, this was validated in people with all stages of HD and in healthy controls, and revealed that C3t performance was significantly slower and less accurate in people with manifest HD compared to healthy controls. In addition, performance declined in a stepwise pattern between each disease stage, where people with pre-HD performed the C3t fastest and achieved the best C3t total score, whereas people in stage 4,5 performed the C3t most slowly and achieved the lowest C3t total score. The design changes made in Part 2 also meant that participants performed less well with increasing task difficulty, although this performance decline was significant only for the mid to advanced disease stages (stage 3 and stage 4,5). Overall, the results in Chapter 3 revealed that the C3t had both external and test validity. Time taken to perform the C3t was also reliable in the few people recruited to redo the assessment. Whilst there was more variability in the Baseline complex and dual task using the C3t total score, this may have mimicked the fluctuating disease symptoms experienced by HD patients, which may become more apparent with increased task difficulty. A greater sample size is required to confirm this hypothesis.

As well as developing sensitive outcome measures for people with HD, it is just as important that such assessments are available in pre-clinical models of HD. As the Stroop task is regularly used in clinic, in Chapter 4, a rodent analogue of the Stroop task was tested in a group of healthy rats. To understand the neural correlates associated with this task, immediate early gene expression was used as a marker in the striatal and cortical brain regions. The findings revealed that *Zif268* expression in the striatum significantly

correlated with performance in both the Stroop control (congruent) and Stroop incongruent task. Therefore, this could provide the first of a series of experiments to understand the extent the striatum is involved for optimal performance in this task.

5.2 Methodological considerations

Symptoms associated with HD can significantly vary from one individual to the next (Ross *et al.*, 2014), where some people experience greater motor abnormalities whilst maintaining cognitive function, whereas others show the reverse. This makes categorising people into disease stage challenging. Two common methods to categorise disease stage is to divide people into pre-manifest and manifest groups, or categorising people based on their UHDRS-TFC score. However, there are well known limitations using both of these methods (see Chapter 1, Section 1.1.7). In Chapters 2 and 3, HD performance was compared using both methods. This resulted in small group sizes particularly when subjects were grouped by TFC disease stage. In addition, group sizes got smaller in the more advanced disease stages as they failed to meet the criteria set to proceed to the complex dual task items, which reduced the experimental power. However, as the CAG disease burden score significantly correlated with the *Step and Stroop*, and the C3t total score this suggests that these assessments accurately tracked disease progression in HD.

It is important to acknowledge that the participants in the dual task studies may not have reflected the whole HD population. It is well known that people with HD are apathetic and less motivated than healthy controls (van Duijn *et al.*, 2014). Therefore, it is possible that results generated from certain studies are biased towards people who are more motivated and perhaps less affected by behavioural changes, resulting in the neuropathological profile for this group not being accurately captured. As people with all disease profiles attend South Wales HD clinic, I attempted to overcome this problem by approaching people whilst they were at clinic to ask if they were interested in taking part in the study. As the study did not take an excessive amount of time (between 15-45 minutes), all participants approached in Chapters 2 and 3 agreed to take part in the study

whilst they were at clinic. This meant that no additional journey for the study was required, thus reducing the impact of apathy on recruitment.

In Chapters 2 and 3, as part of the study design, it was important that participants were able to pass the baseline criteria before proceeding to the dual task. This meant that the test order remained consistent across all participants, and it ensured participants could perform the baseline task before continuing to the more complex dual task. However, this may have resulted in practice effects, leading to less dual task interference. This was more apparent in certain tasks, such as the LVF, but not in the C3t v2b. In the LVF, subjects performed better in the dual task condition than at baseline, whereas in the C3t v2b, practice effects that were evident in previous C3t versions were eliminated following optimisation of the assessment setup and procedure. Therefore, future studies that are interested only in calculating dual task interference should consider counterbalancing dual task items.

5.3 Future directions

5.3.1 Potential techniques to measure neural activity during dual task performance

The rationale for selecting and developing the dual tasks in Chapters 2 and 3 was because they were understood to target the brain regions affected in HD. One way to clarify this would be to use neuroimaging, which would be a feasible future study. Functional neuroimaging techniques, such as fMRI, are sensitive to motion artefact meaning that involuntary movements that increase in HD could result in distorted images (Zhang *et al.*, 2014). Although some techniques are now available to reduce the impact of movements on image quality, it would nevertheless reduce the range of disease stage assessed. Furthermore, assessing the direct neural correlates implicated during the *Step and Stroop*, and the C3t cannot be performed in a scanner. One option is to use nuclear neuroimaging techniques such as near-infrared spectroscopy (NIRS) and transcranial magnetic stimulation (Bakker *et al.*, 2007). Functional NIRS (fNIRS) is in some ways

comparable to fMRI in that it can recognise changes in oxygenated and deoxygenated haemoglobin (Gramigna *et al.*, 2017). Thus, when a brain region is required for a particular function, oxygenated blood is directed to that region to support those functional processes (Cutini and Brigadoi, 2014; Gramigna *et al.*, 2017). However, this technique is limited because it is unable to measure activity more than a few centimetres from the surface of the skull (Figure 33) (Gramigna *et al.*, 2017), meaning that basal ganglia activity is not accessible using this measure. Another option is to use transcranial magnetic stimulation. Similar to fNIRS, this also detects cortical activity (Deng, Lisanby and Peterchev, 2014). However, this measure differs from fNIRS in that, computational models can be used to identify the ongoing subcortical circuitry involved in various tasks and can provide a link between behaviour and cortical regions activated (Geeter, Dupré and Crevecoeur, 2016). Such techniques could be used during performance in the *Step and Stroop* and the C3t to further understand the neurocircuitry activated during these assessments.

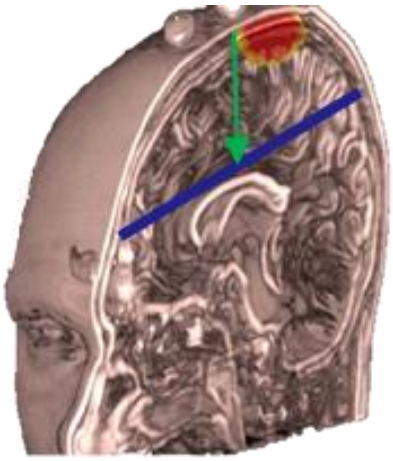


Figure 33: A brain reconstruction adapted from (Gramigna et al., 2017) of the penetration depth achieved using fNIRS. This reaches just a few centimetres below the surface of the skull. Although the caudate and putamen is not clear in this image, the green arrow and purple line give an indication of the estimated depth required to measure functional activity in the basal ganglia and more specifically the caudate-putamen.

5.3.2 The C3t: What next?

As well as using the techniques previously described to understand the regions activated and the circuitry involved when performing the C3t, there are a number of avenues being actively investigated to optimise the C3t development further. It is the intention that the C3t will, in the future, be considered a standard outcome measure for use in clinical settings and in clinical trials to measure upper limb function in people with HD. The standardisation of the C3t was fundamental to optimise the assessment sensitivity and setup (see Chapter 3, Part 1). Whilst this could not have been achieved without funding from a School of Healthcare Sciences Research Development award (Cardiff University), the limited funding allocated for the C3t development minimised the materials that could be used to develop the C3t v2. As a result, the C3t v2 is prone to breakage with repeated usage. To overcome this, funding from the MRC Confidence in Concept, as well as funding from Wales Brain Repair and Intracranial Neurotherapeutics (BRAIN) unit, and the REPAIR-HD FP7 European consortium, has allowed the development of a new, robust C3t design (Figure 34). The aim was to design and develop the new C3t using strong materials that could be cleaned easily, whilst also ensuring that it was compact. The design of this new C3t is described further in Appendix 4. As some of the test procedures have changed compared to those described in Chapters 2 and 3, the next step involves recruiting people with HD to validate the new design.

Figure 34: C3t v3 new design. A prototype of the new C3t. The new design is described in detail in Appendix 4. This work was funded by REPAIR-HD, Wales Brain Repair and Intracranial Neurotherapeutics (BRAIN) and the MRC Confidence in Concept.

The neurological basis behind successful performance of the C3t is intact basal ganglia circuitry relaying to the motor cortex for the grasping and dexterity component (Kim and Hikosaka, 2015) and also the frontal cortical regions to plan (Glover, Wall and Smith, 2012), coordinate (Serrien, Burgunder and Wiesendanger, 2001) and attend to the cognitive components (Vaportzis, Georgiou-Karistianis, Churchyard and Stout, 2015). Therefore, the C3t could also be sensitive to other populations that have basal ganglia dysfunction, such as Parkinson's disease (Rochester *et al.*, 2014; Rolinski *et al.*, 2015; Weingarten *et al.*, 2015) and subtypes of epilepsy (Panayiotopoulos, Obeid and Tahan, 1994; Vollmar *et al.*, 2011; Wandschneider *et al.*, 2014). Funding from an MRC Confidence in Concept application, means that the new C3t design (mentioned in the paragraph above; Appendix 4) will soon be evaluated in people with Parkinson's and juvenile myoclonic epilepsy to evaluate its sensitivity in populations other than HD.

C3t performance is currently rated using time and number of errors committed. Although this method sensitively distinguished subtle differences between people with Pre-HD and Stage 1, it was not able to detect differences between healthy controls and Pre-HD (Chapter 2, Part 3). Explanations for this include (i) the small sample size in the pre-HD group, reducing the experimental power; and (ii) it may have been that the

majority of pre-HD people recruited were far away from disease onset (Klöppel *et al.*, 2015). As this latter group was heterogeneous some people might have been closer to disease onset than others, leading to greater variability in performance.

One method that could increase the sensitivity of the C3t is to use objective instruments, such as accelerometers. These can be worn on the body to quantify movement parameters, and were recently tested using the C3t (Bennasar et al., 2016). In this study, triaxial accelerometers were worn on each wrist and the chest whilst people with HD and healthy controls performed the C3t. Signal processing and pattern recognition methods were applied and identified specific movement signatures associated with the HD group compared to controls. In a separate study, motion analysis techniques were used to validate the accelerometers (Jones et al., 2016: Unpublished data). This technique involved light reflective sensors positioned on certain joints of each arm, to capture a number of movement parameters, and revealed that the accelerometers accurately quantified movement parameters during C3t performance (Figure 36). Additional work in collaboration with Cardiff School of Engineering also includes validating the accelerometers worn during the C3t in a larger cohort of people with HD. A previous study revealed that different levels of variance could be identified in the movement paths taken by people with manifest HD, relative to pre-HD and controls (Bennasar et al., 2017. Unpublished data). Thus, future work involves i) the continued recruitment of people with HD to perform the C3t whilst wearing accelerometers ii) to identify if involuntary movement is affected with increased cognitive load, and iii) to optimise the algorithms developed for the C3t to interpret these into clinically relevant and understandable outcomes.

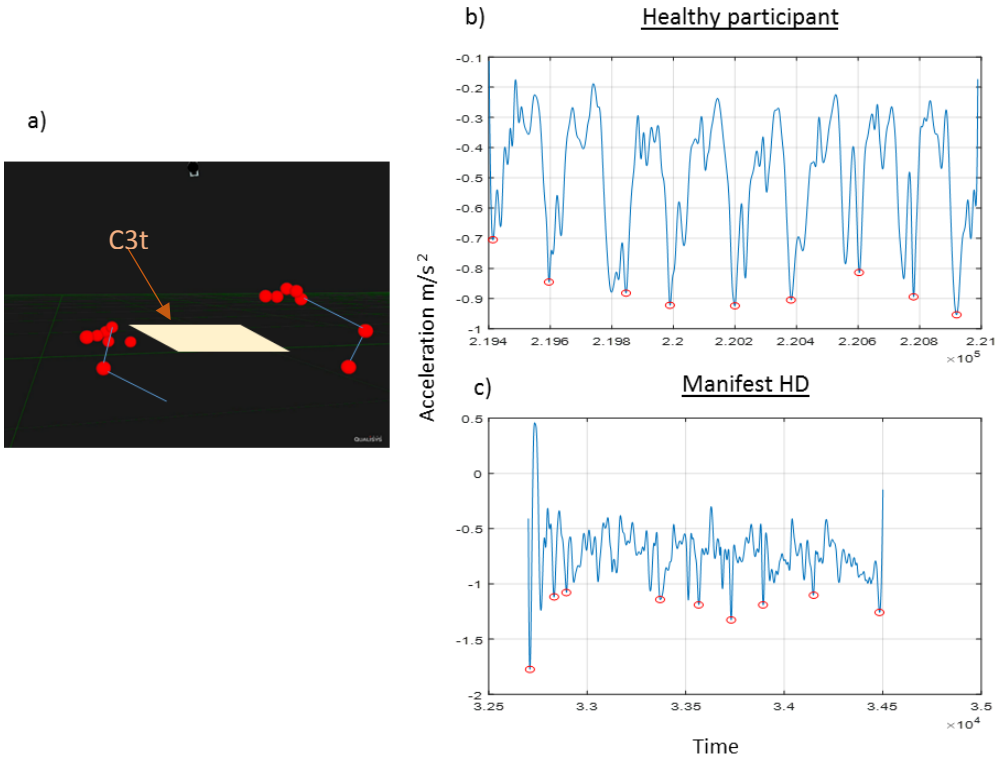
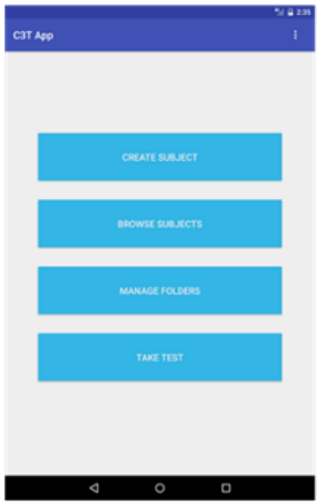


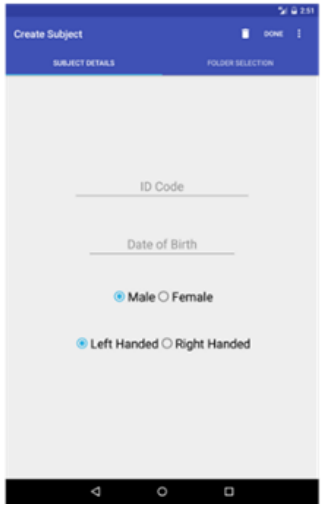
Figure 36: C3t accelerometers: Outcomes using objective instruments to quantify C3t performance. The graphs presented were produced in collaboration with Jones et al, 2017 (unpublished data) (a) and Bennasar et al, 2017 (unpublished data) (b, c). Figure a, shows an image captured using motion analysis. The reflective markers (red dots) were positioned on the accelerometers on the wrist, the elbow and upper arm and quantified movement parameters to validate the accelerometer outputs. Figures b and c shows the amount of chaos quantified when performing the C3t. Each cycle (picking up the token > transferring the token > token in the moneybox) is identified as the periods in between the red dots on the graph. In Figure c, the cycles are distinct, whereas the cycles in the participant with manifest HD are variable and unclear. The y axis presents all negative values in b) due to the direction the axis with respect to gravity. This work was funded by REPAIR-HD and MRC Confidence in Concept funding.

As previously suggested, accelerometers worn during the C3t and the new C3t design could provide an informative measure of functional dexterity for clinicians and researchers to assess C3t performance in people with HD. However, a caveat of the accelerometers currently used is the amount of raw data produced, meaning that data output cannot be easily interpreted. To overcome this, funding from the Wellcome Trust Institutional Strategic Support Fund was awarded to develop a C3t Android App in collaboration with Cardiff University, School of Engineering (Figure 37). This is currently under construction, but, upon completion, the aim is to incorporate the specific algorithms developed for the accelerometers (described in the paragraph above) with the App. The App will also be made available to rate the C3t without the accelerometers, which will be used to calculate the time taken and the C3t total score. A future consideration is to integrate radio-frequency identification tags into the tokens to generate a more sensitive breakdown of C3t performance, which does not require the cost of accelerometers. This will reduce the labour intensive recording of C3t performance by the rater. Ultimately, the App will provide instantaneous, informative feedback to the clinician or researcher, which in the future can be adapted for using accelerometers and develop it so it is specific for other disease populations.

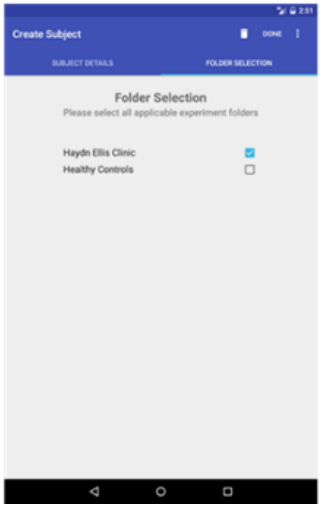
a)


C3T App
CREATE SUBJECT
BROWSE SUBJECTS
MANAGE FOLDERS
TAKE TEST

b)


Create Subject
DONE
SUBJECT DETAILS
FOLDER SELECTION
ID Code
Date of Birth
Male
Female
Left Handed
Right Handed

c)


Create Subject
DONE
SUBJECT DETAILS
FOLDER SELECTION
Folder Selection
Please select all applicable experiment folders
Haydn Ellis Clinic
Healthy Controls

d)


Alphabet Baseline
VALUE BASELINE SIMPLE
VALUE BASELINE COMPLEX
ALPHABET BASELINE
TASK BASELINE SIMPLE
TASK BASELINE COMPLEX
DUAL TASK
ADD SENSOR DATA
SAVE & FINISH

e)


Task Baseline Simple
VIEW RESULTS
SAVE & FINISH
00:00
START
Rule Errors
0
Transfer Errors
0
Dropped Coins
0

f)

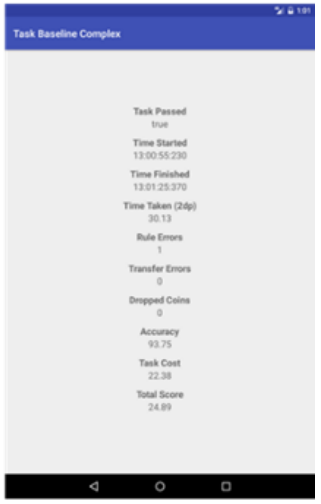

Task Baseline Complex
Task Passed
true
Time Started
13:00:55:230
Time Finished
13:01:25:370
Time Taken (2dp)
30.13
Rule Errors
1
Transfer Errors
0
Dropped Coins
0
Accuracy
93.75
Task Cost
22.38
Total Score
24.89

Figure 37: C3t App; Screenshot examples of the C3t App, developed by Woodgate et al., 2017 (Unpublished data). To begin the clinician/researcher is given 4 options: create a subject; browse subjects that have previously performed the C3t; manage subjects from previous studies; take the test (Figure a). In Figure b, the subjects information is added, and saved in a research folder (Figure c). The researcher selects which C3t item they would like the subject to perform (Figure d) and presented with a rating page specific to that C3t item (Figure e). Once the subject has completed the assessment they are instantly presented with C3t outputs. The completed App will also provide the accelerometer outputs. This work was funded by REPAIR-HD and ISSF Wellcome trust funding.

5.3.3 Can dual task training be used as a rehabilitation strategy post transplantation?

A primary outcome in Chapters 2 and 3 were the development and evaluation of dual task performance in people with HD. Although the idea was that these could be useful outcome measures, there is evidence to suggest that dual task training could be used for rehabilitation purposes for people for neurological conditions such as stroke (Yang *et al.*, 2007; Plummer *et al.*, 2014), dementia (Schwenk *et al.*, 2010) and Parkinson's disease (Fernandes *et al.*, 2015; Sahu and Sri Vastava, 2015). Although specific dual task training has not yet been tested in HD, this may be a promising avenue to investigate in terms of rehabilitative training post transplantation. As discussed in Chapter 1 (Section 1.2.2), there is a wealth of preclinical evidence to suggest that striatal specific training post transplantation is required for optimal graft integration and graft function. However, this has been has been largely neglected in clinic. Therefore, future studies could be used to investigate the effects of dual task training as a potential rehabilitation strategy post transplantation. Alternatively, dual task outcome measures could be used to validate alternative and newly developed rehabilitation strategies for use post transplantation. Alongside *learning to use the transplant*, additional factors such as the dosage (amount of rehabilitative training), type of training, and importantly when to begin training the graft must also be considered (Clinch *et al.*, 2017).

Can cell transplantation improve automated behaviour?

One explanation of why people with HD are impaired when performing during dual tasks is an inability to automate performance in one or both behaviours (Ashby, Turner and Horvitz, 2010; Thompson *et al.*, 2010). It is well understood that habitual (automatic) behaviour involves the dorsolateral striatum (Yin, Knowlton and Balleine, 2004; Ashby, Turner and Horvitz, 2010; Manuscript, 2013). Therefore, automating behaviour that is required for performance in some dual tasks could be an outcome that improves following cell transplantation. However, this has not yet been tested in clinic or

in previous pre-clinical studies. Thus, future studies could investigate whether habitual learning could be affected by transplants.

5.3.4 Final conclusions

The work presented in this thesis evidences new and previously developed outcome measures that could be used to assess disease symptoms in clinic and in pre-clinical models of HD. It is crucial that the effectiveness of clinical trials in HD are accurately assessed. In clinic, one way of doing this is to use outcome measures that tap into basal ganglia circuitry to sensitively track disease progression and have high ecological validity. Another important consideration is, where possible, to translate pre-clinical and clinical outcome measures. This is important to optimise and develop new treatments and translate findings from the bench to bedside.

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Appendix 1

In Chapters 2 and 3, dual task assessments were developed assessed, which each consisted of five assessment items. Furthermore, set criteria was developed for each assessment item, which the participant had to meet before proceeding to the next, more complex task. This meant that a different number of people performed each assessment item, for each dual task. The data presented in Table A-1 shows the number of people that completed each assessment item of the *Walk and talk, TUG and LVF, and the* C3t v1 (performed in Chapter 2). The number of people that performed each assessment item in the *Step and Stroop* is presented in Table A-2 (performed in Chapter 2). The number of people that performed each assessment item in the C3t v2a is presented in Table A-3 (See Chapter 2, Part 2), and the number of people that performed the assessment items in the C3t v2b is presented in Table A-4 (See Chapter 2, Part 3). All groups were categorised as pre-HD (total functional capacity (TFC) = 13 and Unified Huntington's Disease total motor score < 5) and manifest (all symptomatic stages). The manifest group was further delineated by TFC disease stage (stage 1, TFC =11-13; stage 2, TFC=7-10; stage 3, TFC=3-6; stage 4, TFC=1-2; stage 5, TFC=0.

Table A-1: The sample sizes in the Walk and talk, TUG and LVF and C3t

	Assessment item	Pre-HD	Manifest	TFC disease stage			Total
				1	2	3,4	
Walk and talk	Walk baseline	4	28	13	5	14	32
	Alphabet Simple	3	23	11	4	11	26
	Alphabet Complex	3	23	12	4	10	26
	Walk and talk Simple	3	16	10	3	6	19
	Walk and talk Complex	3	15	10	3	5	18
TUG and LVF	TUG baseline	4	28	12	6	14	32
	LVF Simple	4	23	12	6	9	27
	TUG and LVF Simple	4	21	11	5	9	25
	LVF Complex	4	17	9	4	8	21
	TUG and LVF Complex	3	15	7	4	7	18
C3t v1	C3t baseline v1	3	26	9	5	15	29
	Value baseline v1	3	20	8	5	10	23
	Alphabet baseline v1	3	21	8	5	11	24
	C3t Simple v1	3	20	9	5	9	23
	C3t Complex v1	3	17	9	5	6	20

Performance in the Walk and talk, TUG and LVF and C3t v1 were presented in Chapter 2. Due to the small sample size recruited in stage 4, people in stage 3 and stage 4 were combined to form stage 3,4. The column in orange presents the total number of people with HD recruited for the assessment items. Where Pre-HD = pre-manifest HD; TUG = Timed up and go; LVF = Letter verbal fluency; C3t = Clinch token transfer test.

Table A-2: The sample sizes in the Step and Stroop

	Assessment item	Pre-HD	Manifest	TFC disease stage				Total
				1	2	3	4,5	
Step and Stroop	Step baseline	8	50	23	14	18	3	58
	Stroop Simple	8	57	23	16	19	3	61
	Step and Stroop Simple	8	46	23	14	16	1	54
	Stroop Complex	8	46	23	12	16	1	52
	Step and Stroop Complex	8	37	22	10	13	0	45

Performance in the Step and Stroop was presented in Chapter 2. Due to the small sample size recruited in stage 5, people in stage 4 and stage 5 were combined to form stage 4,5. The column in orange presents the total number of people with HD recruited for the assessment items. Where Pre-HD = pre-manifest HD.

Table A-3: The sample sizes in the C3t v2a

	Assessment item	Pre-HD	Manifest	TFC disease stage			Total
				1	2	3,4	
C3t v2a	C3t baseline v2a	3	18	9	7	5	21
	Value baseline v2a	3	15	9	6	3	18
	Alphabet baseline v2a	3	13	9	6	1	16
	C3t Simple v2a	3	15	9	6	3	18
	C3t Complex v2a	3	13	9	6	1	16

Performance in the C3t v2a was presented in Chapter 3, Part 2. Due to the small sample size recruited in stage 4, people in stage 3 and stage 4 were combined to form stage 3,4. The column in orange presents the total number of people with HD recruited for the assessment items. Where Pre-HD = pre-manifest HD; C3t = Clinch token transfer test.

Table A-4: The sample sizes in the C3t v2b

	Assessment item	Controls	Pre-HD	Manifest	TFC disease stage					Total	
					pre-HD	1	2	3	4,5	Controls	HD
C3t v2b	C3t baseline v2b	21	8	54	8	23	17	11	3	21	83
	Value baseline v2b	16	8	53	8	22	17	11	3	16	77
	Alphabet baseline v2b	20	8	49	8	21	16	9	3	20	77
	C3t Simple v2b	20	8	50	8	21	16	10	3	20	78
	C3t Complex v2b	20	8	46	8	21	15	8	2	20	74

Performance in the C3t v2b was presented in Chapter 3, Part 3. Due to the small sample size recruited in stage 5, people in stage 4 and stage 5 were combined to form stage 4,5. The column in orange presents the total number of healthy controls recruited for the study, and in the second column, the total number of people with HD. Where Pre-HD = pre-manifest HD; C3t = Clinch token transfer test.

Appendix 2

This following dual task procedures, instructions and task rules developed are presented for the *Walk and talk, TUG and LVF* and the *Step and Stroop.* These were performed in Chapter 2. The task rules were developed based on clinical observations alongside Professor Monica Busse that were made before the study in Chapter 2 had begun.

Walk and Talk

Walk baseline

Task:

The participant was asked to walk at their steady pace to the end of the GaitRite, turn around a cone positioned 1m past the end of the GaitRite and walk back. The participant repeated this process for 30 seconds.

Instructions:

"When I say "Go" I want you to walk at your normal steady pace to the end of the mat, around the cone and back, like this (example)*. I will tell you to stop after 30 seconds of walking. Do you have any questions? Ready? Go"*

Rules:

- The subject had to walk to the end of the GaitRite mat to proceed to the dual task.

Baseline: Alphabet simple

Task:

Whilst sitting the participant was asked to recite the alphabet beginning with the letter A. This was timed and the participant was asked to stop when they finished the alphabet.

Instructions:

"I would like you to recite the alphabet beginning with the letter A, pronouncing each letter, as quickly as possible, like this (example*). Do you have any questions? Ready? Go"*

Rules

- The alphabet must complete the alphabet within 60 seconds
- The subject must achieve 75% answers correct to proceed to the walking while taking dual task.
- If a wrong answer was given then an error was recorded and the number of correct answers from then on was counted.

Baseline: Alphabet complex

Task:

Whilst sitting the participant was asked to recite every other letter of the alphabet beginning with the letter A. This was timed and the participant was asked to stop when they finish the alphabet.

Instructions:

"I would like you to recite every other letter of the alphabet beginning with the letter A, pronouncing each letter, and doing this as quickly as possible, like this (example). *Do you have any questions? Ready? Go"*

Rules

The same rules applied as the alphabet simple task but subjects were given 90 seconds to complete the test.

Walking whilst talking dual simple

Task:

Whilst walking along the GaitRite, the participant was asked to recite the alphabet, beginning with the letter A. This was timed and the participant was asked to stop after 30 seconds.

Instructions:

"When I say "Go" I want you to recite the alphabet as quickly as you can beginning with the letter A. As you do this walk along the mat, at your normal walking speed. When you reach the end of the mat, walk around the cone and back. If you reach the end of the alphabet before I stop you, begin again with the letter A. I will stop you after 30 seconds. Do you have any questions? Ready? Go".

Rules:

- The subject had to walk one length of the GaitRite to proceed to the more difficult dual task.
- The subject was encouraged to walk if they stopped for longer than 5 seconds. They were reminded to keep walking once.
- If a wrong answer was given then an error was noted and the number of correct answers from then on was counted.

Walking whilst talking dual complex

Task:

Whilst walking along the GaitRite, the participant was asked to recite every other letter of the alphabet beginning with the letter A. This was timed and the participant was asked to stop after 30 seconds.

Instructions:

"When I say "Go" I want you to recite every other letter of the alphabet as quickly as you can beginning with the letter A. As you do this walk along the mat, at your normal walking speed. When you reach the end of the mat, walk around the cone and back. If you reach the end of the alphabet, begin again pronouncing every other letter of the alphabet beginning with the letter A. I will stop you after 30 seconds. Do you have any questions? Ready? Go"

Rules:

- The same rules as the walking and talking dual simple task.

TUG and LVF dual task

TUG baseline:

Task:

Participants were asked to stand from a hard chair, walk 3m, around a cone and back, finishing by sitting in the chair.

Instruction:

"When I say, I want you to start with your back against the chair and your hands on your lap, stand from the sitting position, walk to and around the cone and back, finishing by sitting in the chair, like this (example). Do you have any questions? Ready? Go"

Rules:

- The subject started by sitting with their backs against the chair and hands on their legs
- The time is stopped as soon as the subject completes the sitting descend and their back is straight.
- If the subject has a walker the subject could do the test but it must be noted.
- If the participant took longer than 45 seconds to complete the test they did not proceed to the TUG and LVF dual task.

Letter verbal fluency baseline simple

Task:

Whilst sitting, the participant was asked to say as many different words they could think of beginning with the letter 'R'. The participant was asked not to repeat themselves and not to include the names of people or places. Each correct word given was recorded as well as any repeated words or names of people or places (errors). The test lasted for 30 seconds.

Instructions:

"I am going to give you a letter of the alphabet and I would like you to tell me as many different words you can think of that start with that letter as quickly as you can. For example if I say S you might say snake, slide or stir. Try to avoid any repetition and do not include the names of people or places. During the test try to sit as still as possible. I will tell you when the test is over. Do you have any questions? Now please give me as many different words beginning with the letter R. Go."

Rules:

- Errors were given if the subject repeated a word already stated or if they named people or places.
- A minimum of 3 answers had to be given to pass

Letter verbal fluency baseline complex

Task:

The same procedure as the Simple version but a more challenging letter was given.

Instructions:

"I am going to give you a letter of the alphabet and I would like you to tell me as many different words you can think of that start with that letter as quickly as you can. For example if I say S you might say snake, slide or stir. Try to avoid any repetition and do not include the names of people or places. During the test try to sit as still as possible. I will tell you when the test is over. Do you have any questions? Now please give me as many different words beginning with the letter N. Go."

Rules:

- The same rules as the LVF simple applied

TUG and LVF dual simple

Task:

The participant was asked to perform the timed up and go whilst performing a letter verbal fluency task

Instructions:

"When I say, I want you to start with your back against the chair and your hands on your lap, stand from the sitting position, walk to and around the cone and back, finishing by sitting in the chair. Whilst doing this I want you to give me as many different words you can think of beginning with a letter. Try not to repeat yourself and do not say the names of any people or places. Do you have any questions?
Please give me as many different words beginning with the letter R. Go"

Rules:

- The subject had to complete the TUG in 1 minute to proceed to the more difficult dual task
- The subject was reminded to continue walking if they stopped for longer than 5 seconds. This could be done once.
- The same rules as the baseline TUG and LVF applied to proceed to the TUG and LVF dual complex.

TUG and LVF dual complex

Task:

The same as the TUG and LVF simple dual task but a harder letter was given for the LVF.

Instructions:

"When I say, I want you to start with your back against the chair and your hands on your lap, stand from the sitting position, walk to and around the cone and back, finishing by sitting in the chair. Whilst doing this I want you to give me as many different

words you can think of beginning with a letter. Try not to repeat yourself and do not say the names of any people or places. Do you have any questions?
Please give me as many different words beginning with the letter N. Go"

Rules:

- The same rules as the TUG and LVF simple applied.

Step and Stroop

Stepping baseline

Task:

The participant was instructed to face forwards with the step in front of them. They were asked to step onto the step, one foot at a time, and then back down, whilst staring straight ahead for 45 seconds.

Instructions:

"When I say "Go" I would like you to step onto the box one foot at a time and then step down as quickly but as safely as you can, ensuring that your whole foot is on the step and staring straight ahead when doing this like this (example)*. If you look down or if you don't put your whole foot on the step I will remind you during the test. I will stop you after 45 seconds. Do you have any questions? Ready? Go"*

Rules

- The subject was asked to look straight ahead when stepping
- The subject was asked to put their whole foot on the step and to avoid running (half a foot placed on the edge of the step to maximise speed). If the subject did this during the test they were instructed to stop running.
- One step was counted when two feet were either on top of the step or on the floor.
- The subject had to complete 4 steps to proceed to the Step and Stroop dual task.

Stroop reading and colour baseline

Task:

Whilst sitting, the participant was asked to name colours (either pink, grey or yellow) from a total of x8 blocks presented on a PowerPoint presentation. They were then asked to read the words of colours (either pink, grey or yellow) from a total of x8 blocks. Subjects were not timed to do this but had to achieve 12 out of 16 correct answers to continue to the next stage of the test.

Instructions

"*When I say "Go" I would like you to name the colours you see in the boxes on the screen, naming them from left to right. You will then see words reading the names of colours, and again I would like you to read the colours from left to right. You will see either yellow, pink or grey for all options shown*. *Do you have any questions? Ready? Go"*

Rules:

- The subject had to achieve 6 out of 8 in both of the tests to proceed to the Stroop tasks.

Stroop Simple baseline

Task:

Whilst sitting, the participant was asked to read the colour names displayed (written in either yellow, pink or grey). The colour words were written in their coloured ink, for example pink was in the colour pink. The participant was given 45 seconds to give as many answers as possible. Subjects were shown 4 words at a time via a PowerPoint presentation and the slides were changed using a USB pen.

Instructions

"*When I say "Go" I would like you to name the words you see in the boxes on the screen, naming them from top to bottom, as quickly as you can, like this* (example). *A new*

colour sequence will appear on the screen when you reach the bottom. You will see either yellow, pink or grey for all options shown. I will stop you after 45 seconds. Do you have any questions? Ready? Go"

Rules:

- The subject had to achieve 75% pass rate to proceed to the Stroop complex and the Step and Stroop simple dual task.
- The number of correct answers and errors were recorded

Stroop complex baseline

Task:

Whilst sitting, the participant was asked to say the coloured ink of the colour-words shown. The ink of the words were incongruent to the colour-names. The participant was given 45 seconds to give as many answers as possible. Subjects were shown 4 words at a time via a PowerPoint presentation and the slides are changed using a USB pen.

Instructions

"*When I say "Go" I am going to show you words on the screen and I would like you to say the coloured ink of the words shown. For example, the correct answer here would be pink* *point to example on screen*. *I will stop timing you after 45 seconds and I want you to work as quickly as you can. You will see either yellow, pink or grey for all options shown. Do you have any questions? Ready? Go"*

Rules:

- The same rules as the Stroop simple applied.

Step and Stroop dual task: simple

Task:

Whilst stepping the participant was asked to read the colour words displayed (written in either yellow, pink or grey). This test was the same as the Stroop congruent baseline test. The participant was given 45 seconds to give as many answers as possible.

Instructions

"*When I say "Go" I would like you to read the words you see in the boxes on the screen, naming them from top to bottom, as quickly as you can, like this* (example). *A new colour sequence will appear on the screen when you reach the bottom. You will see either yellow, pink or grey for all options shown. Whilst doing this I want you to step as quickly but as safely as you can, as you did before. I will stop you after 45 seconds. Do you have any questions? Ready? Go"*

Rules:

- The subject had to perform 2 steps to proceed to the more difficult dual task
- The subject was encouraged to continue stepping if they stopped for longer than 10 seconds. This was only done once.
- The same rules applied as the Stroop baseline tasks.

Step and Stroop dual task complex:

Task:

Whilst stepping, the participant was asked to say the coloured ink of the colour-words shown. The ink of the words are incongruent to the words. The participant is given 45 seconds to give as many answers as possible. Subjects are shown 4 words at a time via a PowerPoint presentation and the slides are changed using a USB pen.

Instructions

"*When I say "Go" I am going to show you words on the screen and I would like you to say the coloured ink of the words shown. For example, the correct answer here would*

be pink *point at the onscreen example*. *I will stop timing you after 45 seconds and I want you to work as quickly as you can. You will see either yellow, pink or grey for all options shown. Whilst doing this I want you to step as quickly but as safely as you can, as you did before. Do you have any questions? Ready? Go"*

Rules:

- The same rules applied as the Stroop Simple dual task

Appendix 3

The Clinch token transfer test manual

In Chapters 2 and 3 a new upper limb dual task assessment was developed and was called the Clinch token transfer test (C3t). This was originally called the Moneybox test. To ensure the testing procedure remained consistent when the C3t was carried out, a manual was developed (Figure A-5).

The Moneybox Test Manual

Authors:

Susanne Clinch, Monica Busse,
Mariah Lelos, Anne Rosser

Contents

What does the Moneybox test measure?

The Moneybox test (MBT) is a compact, newly developed multi-task assessment of bilateral, upper motor function (Figure 1). This relatively simple task is unique as it can be used individually as a single, dual or triple task or as a three test assessment with added difficulty. The MBT is currently being validated in people across all stages of HD, with the aim that this can be used as a sensitive outcome measure for people with HD and potentially other neurological diseases in the future.

Rationale

Loss of upper limb dexterity is a core component of hand function, which can impact upon the completion of daily activities such as eating and getting dressed. Many of these tasks are often carried out whilst doing another task, such talking, which for someone with no neurological disease should be fairly simplistic. However, for people with a neurological disorder, such as Huntington's disease (HD), added cognitive load can result in reduced performance in either one or both tasks being performed. This is not only dangerous under certain conditions but it can also affect quality of life. As there is currently no cure for many of these diseases, ongoing research and clinical trials are relentlessly look for ways to slow the progression of disease and improve quality of life. With this in mind sensitive outcome measures must be designed and utilised to accurately detect any improvement in disease symptoms, such as upper limb and cognitive function, following an intervention.

a) b)

Figure 1: The Moneybox test enclosed in the case when not in use (a) and open ready for testing (b)

Moneybox test contents

All items required for the Moneybox test (MBT) come enclosed in the MBT case (Figure 2). The MBT includes:

- A MBT case: When opened three edges fold down
- Money card: x1 A4 size
  X1 of two parts
- Moneybox platform: Money cards can be stored within this (open the short edge of the platform) when the test is not in use
- Moneybox: the bottom of which opens to retrieve the tokens
- Two sets of 8 tokens of various size, all of which will have values (200,100,50,20,10,5,2,1) printed on at least 1 side. Tokens are double sided. Bronze are used for the baseline task, gold are used for the dual task and silver are used for the triple task.
- Laminated token baseline test

Extra equipment needed

- Stopwatch

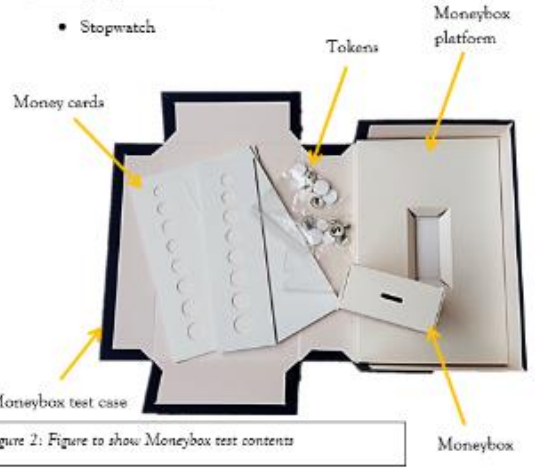


Figure 2: Figure to show Moneybox test contents

Setting up the MBT

When using the MBT:

1. Open the case and fold down the three sides of the case (Figure 3). This will be the money card side.

Figure 3: Opening the Money box case

2. Remove the x2 bags of tokens from the moneybox by gently opening the base of the moneybox (Figure 4). Also, remove the money cards from the moneybox platform by opening the short end of the platform and taking out the three pieces that make up two money cards.

Figure 4: Opening the base of the monevbox

3. Position the platform on the enclosed side of the case (where the sides do not fold down).

4. Put the money box in the space provided in the platform. You may need to fold back the arms in the platform space to allow the moneybox to fit in position (Figure 5).

Figure 5: Preparing space for the moneybox in the platform

5. Position the money card on the opposite, open side of the box, inside the tiled corner frames. The indentations should be decreasing in size, from the top to the bottom of the card. The indentations should also be on the outer length of the money card. If the subject is right handed the test is set up as shown in Figure 6. If the subject is left handed, turn the box 180 degrees, use the money card with two parts and ensure the tokens are positioned on the outer side of the card to the right of the moneybox.

6. Identify which tokens to use. This is dependent on the test carried out (baseline, dual or triple task). For the baseline test the tokens with no values printed are used. Those with a gold rim and values printed are used for the dual task and those with a silver rim and values printed are used for the triple task.

7. Tokens are always positioned from the largest token (top) to smallest (bottom) positioned in the money card indentations. Look to the outer edge of each token and match the number of dots with the number of dots in the indentations of the money card (ranging from 1-8 dots) for the correct token positioning (Figure 7). For example the largest token has 1 dot printed and the smallest token has 8 dots printed. Alternatively refer to page 11 for the token order for the dual and triple tasks.

Figure 6: Set up ready for testing for a right handed subject.

Figure 7: Token set up – matching the dots.

8. Testing is ready to begin (Figure 6).

General test procedure and set up

The subject is asked to sit in a hard backed chair with the MBT positioned in front of them placed on a table. Find out if the subject is right or left handed. If they are right handed the moneybox should be on the right side of the MBT (Figure 6), if they are left handed rotate the box 180 degrees so the moneybox is on the left side of the MBT, using the money card with two parts so the tokens are aligned along the outer edge. The tokens should be positioned so the largest token is furthest from them and the smallest is closest to them.

1. The baseline MBT requires the subject to transfer the tokens in order of size as quickly as they can. The tokens presented do not have any values printed on them for this test.
   Use the bronze tokens that have no value printed on them and set these up matching the number of dots on the outer edge of the token with the number of dots in the indentations of the money card.

2. Whilst sitting the subject is shown 8 values (50, 5, 10, 100, 200, 20, 2, 1) using the laminated MBT token baseline test (enclosed) placed in front of them on a table. The subject is asked to say aloud the highest value in order to the lowest value.

3. The MBT dual task follows a similar process as the MBT baseline but this time the tokens have values printed on them. The subject is asked to transfer the tokens from the highest value to the lowest value as quickly as they can. Use the gold printed tokens and set up as instructed on page 11 or match the dots on the side of the tokens as before. Whilst reading the instructions keep the tokens covered with for example the token baseline card. Reveal the tokens when the subject is told to 'Go.'

4. Whilst sitting the subject is asked to recite the alphabet as quickly as they can. The alphabet can be recited in any language recognised by the researcher.

5. The MBT triple task follows the same procedure as the MBT dual task, but different tokens, using the same values but placed in a different order. The subject is asked to transfer the tokens from the highest value to the lowest value as quickly as they can whilst also continuously reciting the alphabet, in the same language as the alphabet baseline, as quickly as they can. Use the silver tokens and set up as instructed on page 11 or match the dots on the side of the tokens as before. Whilst reading the instructions keep the tokens covered with for example the token baseline card. Reveal the tokens when the subject is told to 'Go.'

Instructions

The subject is asked to sit facing the table. The following instructions are then given for each of the MBT tests:

1. *MBT baseline task:*

When I say "Go," using your non-dominant hand I want you to pick up each token individually, pass it to the hand you write with and put it in the moneybox. I want you to start with the largest token, so the one furthest from you and work your way down to the smallest token which is closest to you. I want you to do this as quickly as possible and I will stop timing you after you have released the last token into the box. If you drop a token outside the test board please leave it, if you drop the token on the test board, pick it up and continue. I would like you to start with your hands on your legs. Do you have any questions? Ready? Go"

2. *Token value test baseline*

"Using the values printed on this card, I want you to say aloud the highest value and work your way down to the lowest value as quickly as you can. I will stop you once you have announced your final value. Do you have any questions? Ready? Go"

3. *MBT dual task*

(Keep the tokens covered whilst reading the instructions below)

"When I say "Go," using your non-dominant hand I want you to pick up each token individually, pass it to the hand you write with and put it in the moneybox. I want you to transfer the tokens in order of value, starting with the highest value to the lowest. I want you to do this as quickly as possible and I will stop timing you after you have released the last token into the box. If you drop a token outside the test board please leave it, if you drop the token on the test board, pick it up and continue. I would like you to start with your hands on your legs. Do you have any questions? Ready? Go" (Reveal the tokens).

4. *Alphabet baseline*

"I would like you to recite the alphabet, pronouncing each letter, as quickly as possible. I will stop the clock once you have said your final letter. Do you have any questions? Ready? Go"

5. *MBT triple task-*

(Keep the tokens covered whilst reading the instructions below)

"When I say "Go," using your non-dominant hand I want you to pick up each token individually, pass it to the hand you write with and put it in the moneybox. I want you to transfer the tokens as quickly as possible in order of value, starting with the highest value to the lowest value. Whilst doing this I want you to recite the alphabet as quickly as you can as you did before. If you finish the alphabet before you finish this transfer task, start reciting the alphabet again. I will stop timing you after you have released the last token into the box. If you drop a token on the floor or outside of the test board please leave it, if you drop the token on the test board, pick it up and continue. I would like you to start with your hands on your legs. Do you have any questions? Ready? Go" (Reveal the tokens).

Rating the MBT

- The MBT is scored using the time taken to complete the MBT. The researcher should start timing as soon as they instruct the subject to "Go" and the subject's hands are moved from their legs. The time is stopped as soon as the last token is released from the subject's fingers into the moneybox.
- If the subject does not transfer the tokens between their hands this is recorded as a **transfer error**.
- If the subject transfers the tokens in the wrong order this is recorded as a **rule error**.
- If the subject drops a token and this falls out of reach, outside of the test board, remind the subject to leave it and continue with the next token. Record this as a **token dropped out of reach** in Equation 2. If the token is dropped out of reach but the subject quickly retrieves it with little test disturbance record this as a **rule error**. Refer to the last point in the FAQ section (page 10) for more information.
- Test accuracy is calculated using Equation 1. This value as well as the time taken is used to calculate a MBT total score (refer to Equation 2).
- For the alphabet baseline and triple task, correct letters said per second are recorded (Equation 3). Equation 4 is then used to calculate percentage triple task cost.

Equation 1 – Accuracy

$$\left(\frac{16 - \text{number of errors made}}{16}\right) * 100 = \text{Accuracy (\%)}$$

Whilst the subject is carrying out the MBT record the total number of transfer and rule errors committed. Subtract any errors made from 16 (8 possible transfer errors + 8 possible rule errors), divide by 16 and multiply by 100.

Equation 2 – MBT total

$$\left(\frac{8 - \text{number of tokens dropped out of reach}}{\text{Time taken (s)}}\right) * \text{Accuracy (\%)} = \text{MBT total}$$

Subtract the number of tokens dropped out of reach (and therefore left) from 8 (8 tokens). Divide this by the time taken to complete the MBT and multiply by the accuracy calculated from Equation 1. Follow the same equation for the baseline, dual and triple task. A higher score is indicative of better performance with a faster time and fewer errors.

Equation 3 – Alphabet letters per second (baseline and triple task)

$$\frac{\text{Number of correct answers}}{\text{Time taken (s)}} = \text{Correct letters said per second}$$

Record the number of letters said correctly for the alphabet baseline and triple task. Divide this by either the time taken to complete the alphabet baseline or by the time taken to complete the MBT triple task.

Equation 4 – Dual task and triple task cost

$$\left(\frac{\text{Baseline test} - \text{dual task or triple task}}{\text{Baseline test}}\right) * 100 = \text{Dual or triple task cost (\%)}$$

Subtract either the dual or triple task value from the baseline value and divide by the same baseline value. Multiply this by 100 to give a percentage cost.Follow this equation using the MBT total values (Equation 2) and also the alphabet letters per second (Equation 3).

Pass/Fail procedure for the MBT assessment

The subject must pass set criteria to pass onto the harder versions of the MBT.

The following pass/fail procedure (Figure 8) should be used when the MBT assessment is carried out. If the subject passes the rules, written in orange, then the subject passes that test and can continue to the next test of the assessment. If the subject does not meet one or more of the rules highlighted, the assessment ends there.

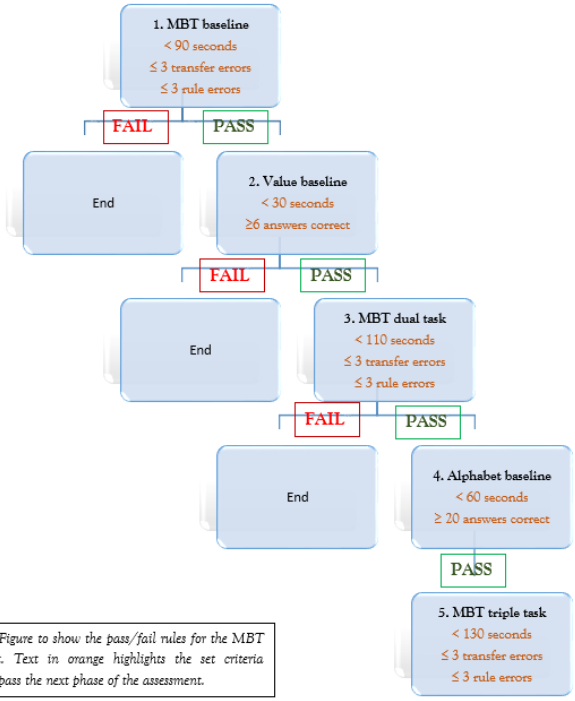


Figure 8: Figure to show the pass/fail rules for the MBT assessment. Text in orange highlights the set criteria needed to pass the next phase of the assessment.

Frequently asked questions (FAQs)

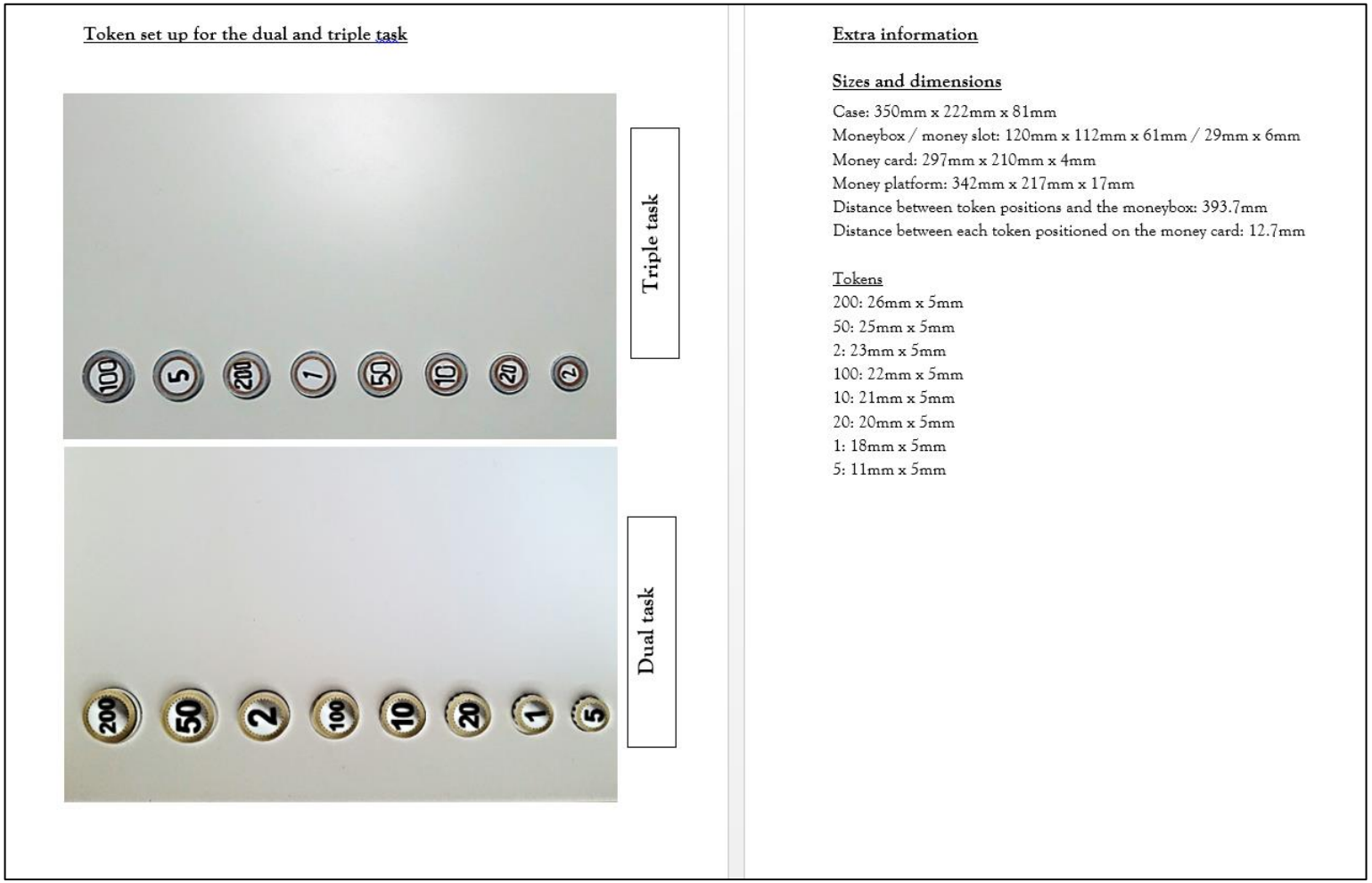
- Why is one of the money cards split in 2 pieces?
  This allows the test to be set up for left handed people, allowing the tokens to be positioned on the outer edge of the card from largest to smallest, on the right of the Moneybox.

- How do I fold away the MBT?
  The MBT is folded by first putting the tokens in the bags provided and storing the token bags in the moneybox. Put the money cards in the moneybox platform, by opening its short edge. Position the platform and the moneybox on the open side of the case where the money card would normally sit when conducting the test. Fold up the 3 sides of the case and fold over the enclosed side of the case ready to put away.

- What if the subject drops the token on the floor?
  If a token is dropped out of reach (outside the test box) or on the floor, remind the subject to leave it and continue with the next token. Record the number of tokens dropped out of reach and use in Equation 2 to calculate the MBT total.

- What if the subject drops a token outside the box but quickly retrieves it?
  If the token is dropped just outside the box or on the subject's lap and the token is immediately retrieved with little disturbance to the test then do not count this as a token fallen out of reach when calculating the MBT total in Equation 2. This should instead be recorded as a rule error, as the subject has not ignored the dropped token as instructed. Ensure the subject still follows the rule of transferring the token between the correct hands if the token is retrieved.

- What if the subject starts transferring the tokens in the wrong order or forgets to transfer the tokens between their hands?
  After this error has been made, you can remind the subject of the rules once by saying for example 'remember to transfer the token between your hands' or 'remember from the highest value to the lowest.'



Token set up for the dual and triple task

Triple task

Dual task

Extra information

Sizes and dimensions

Case: 350mm x 222mm x 81mm
Moneybox / money slot: 120mm x 112mm x 61mm / 29mm x 6mm
Money card: 297mm x 210mm x 4mm
Money platform: 342mm x 217mm x 17mm
Distance between token positions and the moneybox: 393.7mm
Distance between each token positioned on the money card: 12.7mm

Tokens

200: 26mm x 5mm
50: 25mm x 5mm
2: 23mm x 5mm
100: 22mm x 5mm
10: 21mm x 5mm
20: 20mm x 5mm
1: 18mm x 5mm
5: 11mm x 5mm

Figure A-1: The Moneybox test manual developed and used for Chapter 2, Part 2.

Appendix 4

The development of the C3t v3

The standardised C3t v2 was manufactured by MinuteMan Press, Cardiff, and was funded by a Strategic development fund application, from the School of Healthcare, Cardiff University. The development of the C3t v2 was an integral part of the C3t development process as this meant the C3t setup was more reliable. Furthermore, as the British coins were changed to tokens this meant that it was no longer biased to people very familiar with this currency and therefore could be used in other countries. As a result the C3t v2 has been tested in clinical sites in Cardiff, Manchester, Germany and France as part of REPAIR-HD (an FP7 European consortium grant). However, a limitation of the C3t v2 is that the materials it is constructed from cannot be cleaned easily and they are prone to breakage. Therefore, through the achievement of an MRC Confidence in Concept grant, I have worked closely with Thread design, Cardiff, to improve the construction of the C3t and setup. This development process is described below.

To begin, it was important that the designers understood the assessment concept and therefore, the key properties of the C3t that needed developing or redesigning. From this, key features were developed to optimise the assessment procedure for both the participant and for the researcher. For the participant, it was important that the C3t had an approachable design and interface. For the researcher it was important that the following criteria were met:

a. Smooth transition throughout from one C3t item to the next
b. A quick setup and transition between assessment items
c. Easy to set up
d. Consistent (standardised) testing
e. Compartments to ensure components do not go missing
f. Compact

Whilst meeting these criteria was important, a primary aim was that the test procedure did not change from the C3t v2. This was to maintain the promising results developed in Chapter 3 using this test. Therefore the overall dimensions of the assessment remained the same (Figure A-2).

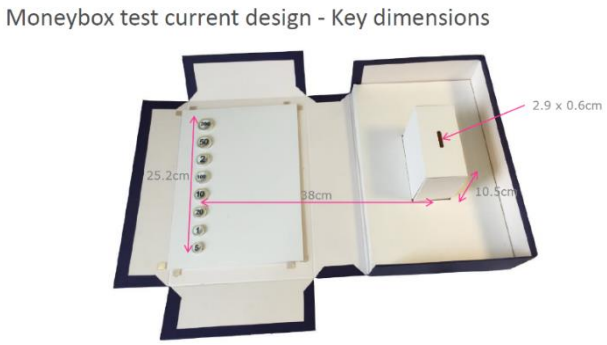
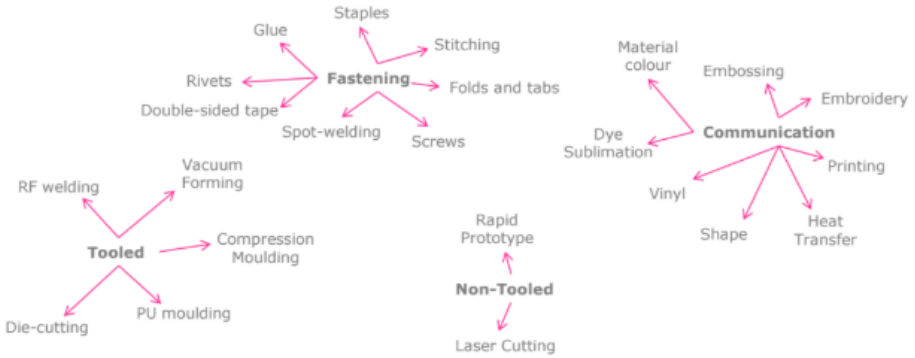


Figure A-2: Dimensions of the C3t v2 were taken. This was done to maintain the consistency of sensitive results achieved using the C3t v2 with the new C3t test design.

An important factor that drove the decision making process for the new C3t development was that it needed to be affordable. Therefore, a target price of £100 per unit was chosen. Using this budget as a benchmark, a list of the physical parts of the C3t that needed to be incorporated and designed was made, and the materials and tools that could be used to construct the new C3t were brain stormed (Figure A-3).



Test components

- Moneybox
- Test platform that could be folded – the C3t would be set up on this
- Tokens – 3 sets (for the Baseline simple, complex and dual task)
- Token trays (x3 so the C3t test setup can be prepared before the participant takes the test
- Value baseline card
- An overall case, which would contain compartments to house the C3t test platform, accelerometers and a tablet (for the C3t App)

Figure A-3: The test components and possible materials and tools that were suggested to construct these.

Design

It was important that the C3t were made of materials that did not ware easily over time and could also be cleaned. The overall aims regarding the design of the C3t were:

- Colour – off white
- Clinical appearance – clean look
- Friendly feel – not intimidating
- Sense of quality and robustness
- Easy maintenance
- Integrated

Design ideas for the C3t

A number of promising designs for the C3t were brainstormed with Thread design. This generated the options presented in Figure A-4. Although these were compact

and integrative, the main limitation with each of these was the way the moneybox was constructed and positioned. The concern with these designs was that people with a movement disorder, such as HD, would use the length or platform of the moneybox to stabilise their wrist when performing the C3t. This could lead to a faster performance time, and reduce the sensitivity of this assessment. The final C3t design is presented in Figure A-5. This design is compact and avoids the moneybox limitation associated with the designs in Figure A-4. The idea is that this design will fit into a larger case that will also support the tablet for the C3t App and accelerometers.

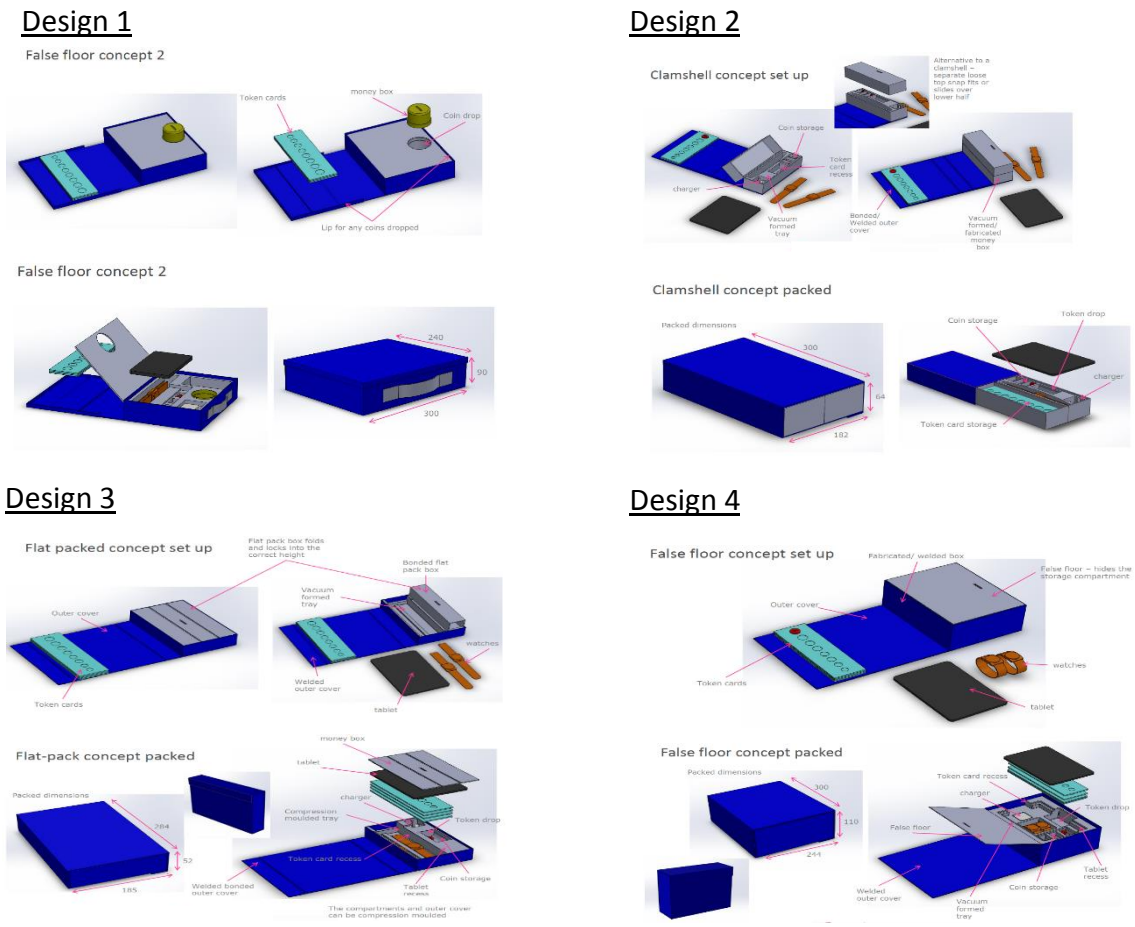
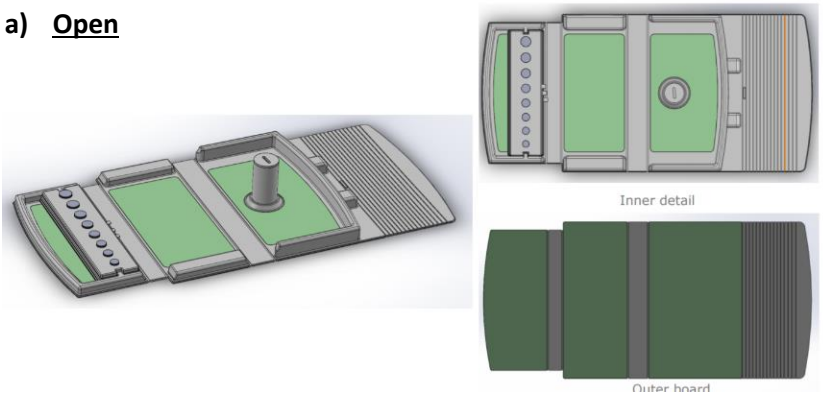
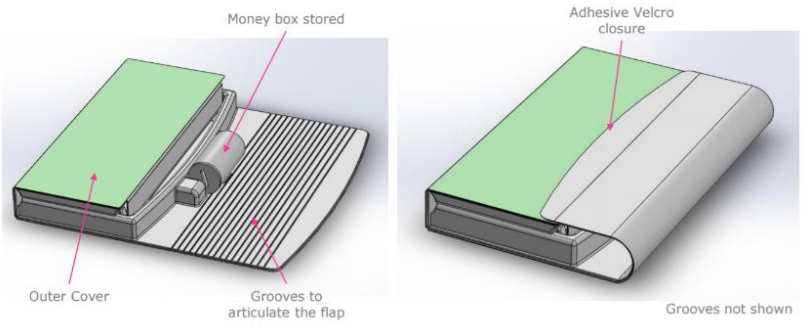


Figure A-4: C3t design ideas. The designs presented were put together by Thread design. These were abandoned because of the construction of the moneybox, which could have provided a platform to stabilize the wrist and essentially make the assessment easier and less sensitive for people with a movement disorder.

a) <u>Open</u>



b) <u>Closed</u>



c) <u>Housed inside the outer assessment box</u>

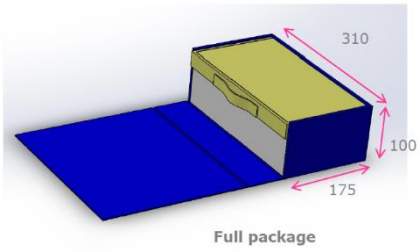


Figure A-5: The figures present different orientations of the final C3t design, both open (a), closed (b) and positioned within the outer assessment box (c) which will also house the accelerometers and tablet for the C3t App.

Tokens

Given that the C3t assessment can be performed in 5 minutes, there is a risk of practice effects as the token values become more familiar to the subject. The tokens used in the C3t v2 present same values for the Baseline complex and the dual task. To prevent potential practice effects, in Chapter 3 (Part 2), the C3t v2 was optimised by keeping the values the same, but changing the order the tokens were presented in for the Baseline complex and the dual task. One of the biggest changes that has been made to the new C3t design is that different token values are used for the Baseline complex and the dual task. As this could mean that the dual task is more difficult, the new C3t will need to be re-validated in people with HD. However, in making this change, it is anticipated that it will improve the sensitivity of the C3t and lead to greater dual task interference between disease stages in HD.

One risk using the C3t in people with a motor deficits is that there is an increased risk that the tokens are accidently knocked during the testing procedure. This was considered in the C3t v2, and to prevent this from happening shallow grooves were made on the money card (token trays), which the tokens were positioned in. In the new C3t, different design ideas were suggested to ensure the tokens were even more secure when positioned in the grooves of the token trays (see Figure A-6). The final design of the new C3t tokens houses a magnet, which is sandwiched inside the token. As the token trays also contain a magnet and shallow grooves (see Figure A-7), this means that the tokens are less likely to knock out of position during testing.

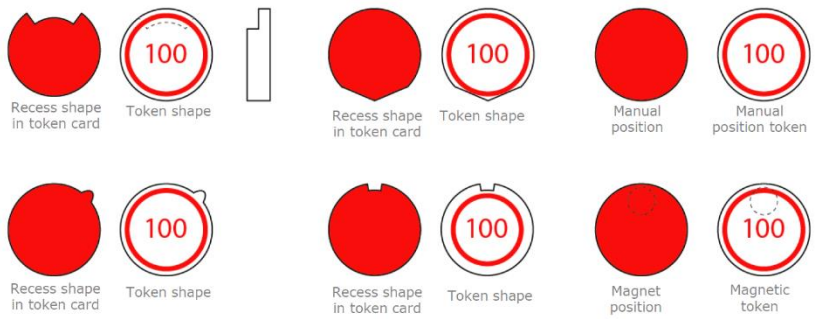


Figure A-6: Different token design ideas to ensure the tokens remained in position during the C3t testing procedure

Token trays

To ensure the new C3t is easy to use, three trays were designed so they stacked on top of one another, meaning that the C3t setup can be prepared before the subject arrives (Figure A-7). In addition, each tray contains grooves which contain markings. The markings match the token required for that location and assessment item.

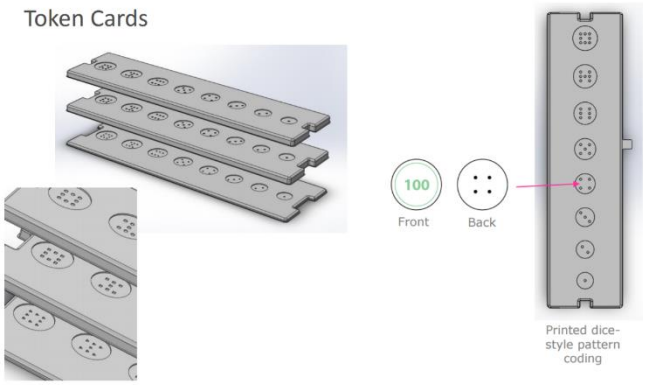


Figure A-7: Token trays are stacked to allow for quick and easy setup for the researcher. In addition, the markings on the tokens match the markings in the groves of the token tray so each token is positioned in the right location for each assessment item.

Moneybox

Another change to the C3t is the moneybox (Figure A-8). Although the original idea was to keep the design the same as the C3t v2, a new, compact moneybox has been designed for the C3t. Importantly, the moneybox slot and the height of the moneybox have remained the same as the C3t v2.

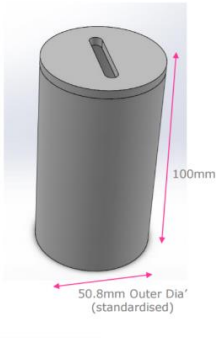


Figure A-8: The moneybox design for the new C3t. The height and moneybox slot have remained the same as the C3t v2.

Logo

The logo designed for the C3t v2 was printed onto a sticker and attached to the outer assessment box. However, a concern was that, over time, a sticker could begin to ware and start peeling off. An alternative option was to imprint the logo onto the outer box. However, due to the tooling restrictions involved in this process, this meant designing a much simpler logo with less detail.

A lot of thought went into the logo design, as I wanted the logo to relate to the C3t procedure or concept in some way. The final C3t orientation was designed so it represented an abstract shape of the caudate and putamen, the brain regions affected in people with HD could be required for successful performance in the C3t. One option was to put the C3t into a brain shaped template, or into a disc shape to represent the token. It was decided that the simplest design was the clearest and so the logo in Figure A-9a was selected. Other logo options that were designed are presented in Figure A-9b.

a)

b)

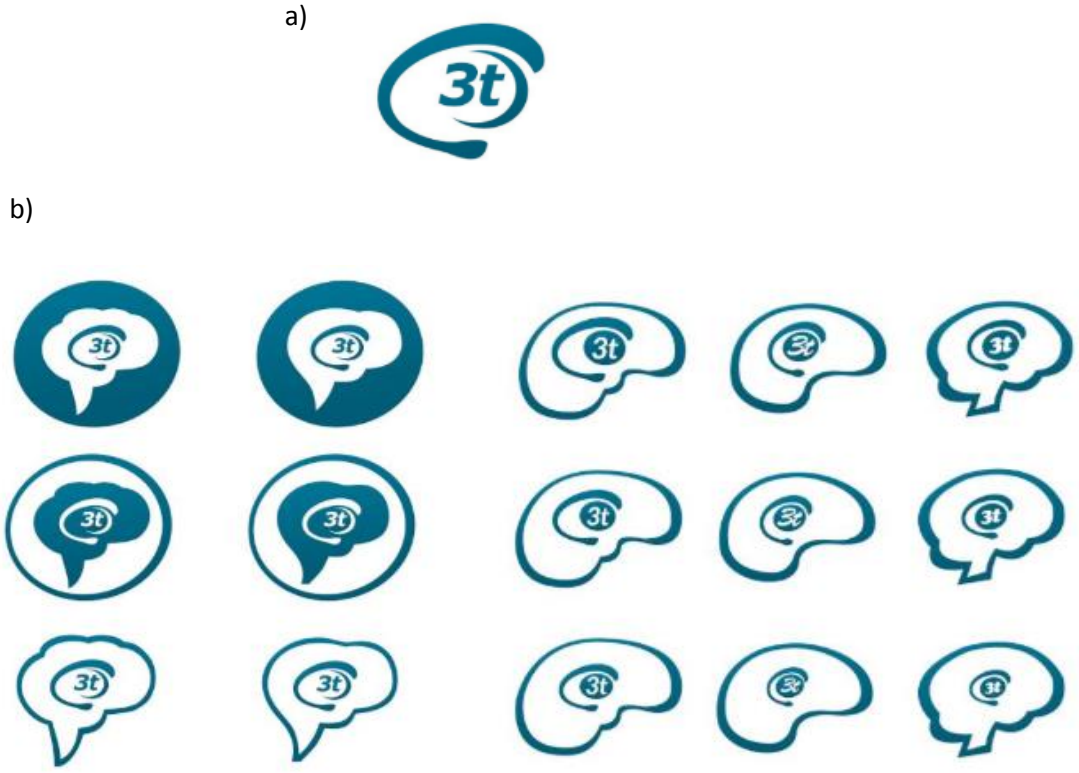


Figure A-9: C3t logo designs. The logo in Figure a) presents the final logo design selected for the new C3t. Figure b) shows the alternative logo options that were eliminated.

Appendix 5

Recipes

The following recipes were used for the histological procedures performed in Chapter 4.

Perfusion and tissue storage

Paraformaldehyde (PFA) solution - stored at 4 degrees Celsius
40g PFA (4%)
1 L Prewash buffer (recipe below)
Heat to dissolve
pH 7.3 (orthophosphoric acid)

Prewash buffer (PBS) – stored at 4 degrees Celsius
18g di-sodium hydrogen phosphate
9g sodium chloride
1L distilled water
pH 7.3 (orthophosphoric acid)

Sucrose (25%)
250g sucrose
1L PBS
pH 7.3

Antifreeze (800ml)
4.36g Di-sodium hydrogen orthophosphate (A)
1.256g Sodium di-hydrogen orthophosphate (B)
Dissolve in 320 ml distilled water (C)
A, B and C dissolved fully and pH 7.3-7.4 then add:
240ml Ethylene Glycol
240ml Glycerol

Histology and Immunohistochemistry

4x Tris buffered saline
96g Trizma base
72g sodium chloride
2L distilled water
pH 7.3-7.4

0.2% Triton X-100 in TBS (TXTBS)
0.5ml Triton X-100
250ml TBS

Tris non-saline (TNS)
6g Trizma base
1L distilled water
pH 7.3-7.4

Endogenous Peroxidase Quench
10ml methanol
10ml hydrogen peroxide (30%)
40ml distilled water

ABC solution
5ul A (DAKO)
5 ul B (DAKO)
1ml 1 % serum in 1 x TBS

Dehydration
70% alcohol (5 minutes)
95% alcohol (5 minutes)
100% alcohol (5 minutes)
Xylene (10 minutes)
Coverslip using DPX