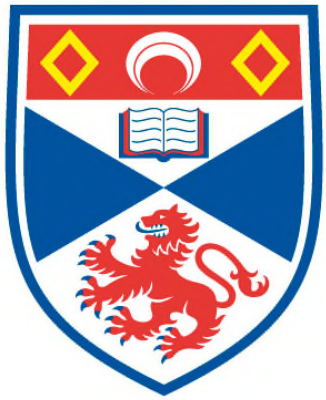
AN EVALUATION OF COGNITIVE DEFICITS IN A RAT-MODEL OF HUNTINGTON'S DISEASE

Ana I. García Aguirre

A Thesis Submitted for the Degree of PhD
at the
University of St Andrews



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University of
St Andrews

This thesis is submitted in partial fulfilment for the degree of
PhD
at the
University of St Andrews

Date of Submission

December 2015

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Abstract

The purpose of this thesis was to develop methodology by which treatments for the cognitive impairments in Huntington's disease (HD) could be tested. As such, the thesis focused mainly on evaluating rats with quinolinic acid (QA) lesions of the striatum, as this manipulation mimics some aspects of the neural damage in Huntington's disease, to try to identify cognitive deficits of HD resulting from cell loss in the striatum.

In the first part (Chapters 3-5), the role of the striatum in implicit memory was investigated. Chapter 3 compared the performance of rats and humans on a reaction time task that evaluated implicit memory by presenting visual stimuli with differing probabilities which change over time. Although rats made higher percentage of incorrect responses and late errors, both groups showed a similar pattern of reaction times. Chapter 4 investigated whether implicit memory (the computation of probabilities to predict the location of a stimulus) was affected by selective blockade of dopaminergic transmission at the D_1 or D_2 receptors by SCH-23390 and raclopride, respectively. Reaction times were slower with SCH-23390 and raclopride, but only SCH-23390 reduced errors to the least probable target location. Chapter 5 used the same task to evaluate implicit memory in rats with QA lesions of the dorsomedial striatum (DMS). Implicit memory was not affected by lesions of the DMS, which suggested that once a task that requires implicit memory has been learned, the DMS was not involved in sustaining the performance of the task. The second part of this thesis (Chapter 6), explored the contribution of the DMS in habit formation. DMS lesioned rats did not show habitual responding, and were not impaired in learning a new goal-directed behaviour. The third part (Chapters 7 and 8), investigated the role of the dorsal striatum in reversal learning, attentional set-formation, and set-shifting. Dorsal striatum lesioned rats were not impaired in reversal learning, but had a diminished shift-cost, which suggested that dorsal striatum lesions disrupted the formation of attentional sets.

These results showed that although QA lesions of the dorsal striatum mimic some aspects of the neural damage in HD, they did not result in the same cognitive deficits observed in patients with HD, at least using the tasks presented in this thesis. However, other animal models of HD could be evaluated using the different tasks presented in this thesis to continue the search of a reliable animal model of HD in which treatments for the disease could be evaluated.

Acknowledgements

I wish I had enough words to express my gratitude to my primary supervisor Prof Verity Brown for giving me the opportunity of studying a PhD at the University of St Andrews. I am also especially grateful to Dr David Tait for his advice and support, for everything he taught me, and for also supervising me. I would like to thank Dr Eric Bowman for his guidance, supervision and contribution to this thesis. Thanks to Dr Hugh Marston and Dr Ines Jentzsch for their advice during our collaboration and to my examiners Dr Jamie Ainge and Dr Jeff Dalley for their comments to improve this thesis. I would also like to thank my undergraduate supervisor and friend Vladimir Orduña for introducing me to the world of animal learning theory several years ago. Thanks to the technical staff of the School of Psychology and Neuroscience Animal House for looking after my animals.

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Chapter 1

General Introduction

Huntington's disease

1.1 Introduction

Huntington's disease (HD) is a hereditary, autosomal dominant neurodegenerative disorder that is progressive and that it is characterised by a triad of motor, cognitive, and psychiatric symptoms (Novak and Tabrizi, 2010; Novak and Tabrizi, 2011; Petersén and Brundin, 2002; Ross and Margolis, 2001; Wagner et al., 2008).

The disease was named after George Huntington (1850-1916), a physician, who in 1872 presented his manuscript, "On chorea", in which he described the choreiform movement disorder, the psychiatric symptoms associated with the disease, the inheritance pattern, and the progressive and fatal course of the disease (Huntington, 1872; reprinted in Huntington, 2003). Although he was not the first to describe the disease (Hayden, 2012, for a review), his description was so concise and accurate that his name became attached to the disease (Berg, 1948; Hayden, 2012; Heathfield, 1973; Rüb et al., 2015).

1.2 Epidemiology

Studies that incorporated genetic and clinical diagnostic standards have shown that the prevalence of HD in western populations is 10.6-13.7 individuals per 100,000 (or 1 in 7,300) are affected (Bates et al., 2015).

In the United Kingdom, the prevalence of HD is 12.3 per 10,000: the London region had the lowest prevalence (5.4 per 100,000); while the North East of England (18.3 per 10,000) and Scotland (16.1 per 10,000) had the highest prevalence (Evans et al., 2013).

1.3 Genetics

HD is caused by an expanded cytosine-adenine-guanine (CAG) trinucleotide repeat in exon 1 of the huntingtin gene (or IT-15, interesting transcript 15), encoding Huntingtin protein (HTT), which is located on chromosome 4 (Gusella et al., 1983; MacDonald et al., 1993). The disease is an autosomal dominant inheritance disease (i.e., there is 50% chance that each child of an affected parent will inherit the abnormal gene). A normal gene contains between 6 to 35 polyglutamine (PolyQ) repeats; however, increased CAG repeats can vary from 36 to 180. Symptoms of HD have not been reported in individuals with fewer than 36 CAG repeats; however, an abnormal gene containing more than 40 repeats will ultimately result in symptoms of HD. Patients with genes containing between 36 to 39 CAG repeats show reduced penetrance (i.e., some would develop HD and others will not; Snell et al., 1993). Since the causative gene mutation was discovered (MacDonald et al., 1993), genetic testing for HD has become available. A blood sample (or other tissues, even autopsy material) can determine the CAG repeat length in the *HTT* gene (Nance et al., 2011). Testing could be performed in three clinical situations: to diagnose if the symptoms observed in a patient are due to HD, to predict if a person with a parent with HD has an expanded CAG and will develop HD, or prenatally to evaluate if the foetus has an expanded *HTT* gene (Rosenblatt et al., 1999; See, 1994).

The mean age of onset of symptoms is between 35 and 45 years of age; but, depending on the severity of the genetic mutation (i.e., number of repeats), there may be juvenile (< 20 years) or late (> 70 years) onset of symptoms. The onset of the disease is inversely proportional to the length of the PolyQ repeats (i.e., the longer the CAG repeat length, the earlier the onset of symptoms; a genetic phenomenon known as “anticipation”). Juvenile HD is commonly seen with 60 or more CAG repeats; in adult onset, the average number of CAG repeats is 44 and in

patients with late onset, the number of CAG repeats varies from 42 to 46. In adult onset, death generally occurs within 15 to 20 years after onset (Novak and Tabrizi, 2011). Patients diagnosed with a repeat expansion that do not exhibit the full clinical syndrome yet are in the prodromal, preclinical, asymptomatic or pre-symptomatic phase of HD (Nance et al., 2011; Paulsen, 2011).

1.4 Neuropathology

Although HD affects many regions of the brain, the most prominent neuropathological characteristic of HD is the selective atrophy and neuronal loss of the striatum (i.e., caudate nucleus and putamen). The earliest and most affected area by the disease is the caudate nucleus, but as the disease progresses there is a dorsal to ventral, anterior to posterior, and medial to lateral direction of striatal degeneration (Vonsattel et al., 2008). Within the striatum, neuron populations are divided into two groups. One group consists of aspiny neurons, which are interneurons whose connections are contained within the striatum. The other group consists of spiny neurons, which are the projection neurons that use gamma-aminobutyric acid (GABA) as their principal neurotransmitter, and are often referred to as GABAergic projection neurons. Spiny neurons constitute 90-95% of striatal neurons (Kita and Kitai, 1988). In patients with HD, almost all types of interneurons in the striatum show evidence of dysfunction. However, the neuropathological hallmark of HD is the premature and progressive degeneration of medium spiny projection neurons, while the large aspiny interneurons in the striatum are relatively preserved (Ferrante et al., 1987; Ferrante et al., 1985; Ferrante et al., 1986).

In addition to the striatum, the cortex also suffers from atrophy and the cortical neuronal degeneration leads to excessive thinning of the cerebral mantle of the entire brain (Hedreen et al., 1991). Similar to the striatum, while the cortical interneurons are preserved, the projection neurons in the cortex are the ones that are more vulnerable to degeneration (Vonsattel et al.,

2008). It also appears that the large cortical neurons are the most affected; most of the neuronal loss is seen in projections to the thalamus, while there is a significant loss in neurons that project to the caudate nucleus and putamen (Ross and Margolis, 2001). Other nuclei such as the globus pallidus (GP), thalamus, hypothalamus, subthalamic nucleus (STN), substantia nigra (SN), and cerebellum also are affected in HD (Vonsattel et al., 2008, for a review). Vonsattel et al. (1985) introduced a neuropathological five-point grading system that stages the extent of neuronal degeneration in the striatum, gliosis, and cell loss as the disease progresses. Depending on the progression of the disease, other common neuronal changes in HD include thinning of the cortical mantle, ventricle enlargements, and decreased brain weights and volume. During the last stages of the disease, with the combined reduction in the striatal volume (60%), and the atrophy of the neocortex (20%), the HD brain may lose nearly 25-30% of its weight (Roze et al., 2011).

In order to understand the mechanisms by which degeneration of neurons in the basal ganglia produce symptoms in HD, the neuronal circuitry of the normal basal ganglia first needs to be examined in more detail.

1.4.1 Organisation of the basal ganglia

The basal ganglia are a group of interconnected subcortical nuclei incorporating the striatum (caudate nucleus and putamen), the GP, which is divided into internal (GPi) and external (GPe) segments, the STN, and the SN, divided into the pars reticulata (SNpr) and the pars compacta (SNpc; Albin et al., 1989).

Albin et al. (1989) proposed a model in which specific types of basal ganglia disorders are associated with changes in the function of striatal projection neurons. In this model, the caudate nucleus and the putamen integrate the *input* compartment, since they receive inputs from

the cerebral cortex, intralaminar thalamic nuclei, and the SNpc. The STN, SNpr, and GPi, integrate the *output* compartment, their target nuclei are in the thalamus, which has excitatory effects on the cortex. The input and the output compartments are integrated in two major pathways: the *direct pathway*, which facilities movement by disinhibition, and the *indirect pathway*, which inhibits movements. In this model, dopaminergic innervations of striatal neurons originate in the SNpc. When dopamine is realised into the striatum, it inhibits the indirect pathway by acting on D_2 receptors and stimulates the direct pathway by acting on D_1 receptors. Figure 1.1 shows the location and circuitry of the basal ganglia.

The direct pathway consists of medium spiny neurons (MSNs) that mostly express D_1 receptors (Gerfen et al., 1990) and substance-P (Haber and Nauta, 1983) that project to the GPi and SNpr. The GPi and the SNpr then project to the thalamus, which then projects to the cortex to initiate movements (Albin et al., 1989). To initiate movements, the cortex sends excitatory (glutamatergic) input to the putamen and caudate nucleus; this process increases the activity of the neurons of the striatum, which are inhibitory (GABAergic). The striatum then sends this increased inhibitory signal to the GPi and the SNpr. The GPi and the SNpr normally send inhibitory (GABAergic) signals to the thalamus; however, since the GPi and the SNpr are briefly suppressed, the thalamus is disinhibited; permitting the thalamus to send excitatory (glutamatergic) signals to the cortex, the activation of the cortex ultimately facilitates movement.

The indirect pathway consists of MSNs that mostly express D_2 receptors (Gerfen et al., 1990) and enkephalin (Haber and Nauta, 1983), and project to the GPe. The GPe projects to the STN, which projects to the GPi or to the SNpr. The GPi and the SNpr then project to the thalamus, which then projects to the cortex to inhibit movements (Albin et al., 1989). To inhibit movements, the cortex sends excitatory (glutamatergic) input to the putamen and caudate

nucleus; this process increases the activity of the neurons of the striatum, which are inhibitory (GABAergic). The striatum then sends this increased inhibitory signal to the GPe and the SNpr. The role of the GPe is to inhibit (via GABA) the STN; however, since the striatum is inhibiting the GPe, the STN is disinhibited. The STN sends increased excitatory (glutamatergic) signals to the GPi and the SNpr. The GPi and the SNpr normally send inhibitory (GABAergic) signals to the thalamus; however, since the GPi and the SNpr are more active, they send an increased inhibitory (GABAergic) signal to the thalamus. Leading to a decrease in the excitatory (glutamatergic) signals that the thalamus sends to the cortex, this effect results in inhibition of movement.

1.4.1.1 The basal ganglia in HD

MSNs of the indirect pathway are the most affected neurons in HD. In early and middle stages of the disease, striatal spiny projection neurons containing enkephalin, which project to the GPe (a reliable marker for the indirect pathway), are more affected than substance-P neurons, that project to the GPi (a reliable marker for the direct pathway). At the most advanced stages of the disease, almost all striatal projections are depleted (Reiner et al., 1988).

Disruption of the afferent pathways in the striatum results in the motor dysfunction observed in HD (Vonsattel et al., 2008). The degeneration of the striatal inhibitory neurons of the indirect pathway leads to a reduced inhibitory output of the GPe upon the subthalamic nucleus (STN). Thus, the STN becomes hypo functional, reducing the inhibitory action of the GPi upon the thalamus, which increases the excitation of the cortex, causing chorea (Albin et al., 1990). Thus, Albin et al. (1990) hypothesised that chorea, which occurs early in the disease, results from preferential dysfunction and loss of striatal MSNs projecting to the GPe (indirect pathway), and that akinesia and dystonia, which occurs later in HD, is a result from the additional

loss of striatal MSNs projecting to the GPi direct pathway. Additionally, it has been suggested that the imbalance within the two GP also impairs voluntary movements (Chesselet and Delfs, 1996; Matsumura et al., 1995). Figure 1.1 shows a summary explanation of the hyperkinetic disorder seen in HD.

It has been reported that loss of basal ganglia volume begins many years prior to diagnosable HD (Aylward et al., 1996). Patients in the prodromal phase present loss of corticostriatal connectivity and striatal atrophy (Tabrizi et al., 2012; Tabrizi et al., 2011). Using magnetic resonance imaging (MRI) scans, Aylward et al. (2004) measured the volumes of the caudate nucleus and the putamen on asymptomatic preclinical subjects with the HD gene expansion (i.e., people that have more than 36 CAG repeats but that had not developed the characteristic motor deficits of the disease) and reported that they had smaller volumes of the basal ganglia than aged matched control subjects. These results suggested that atrophy in the striatum begins long before onset of the disease. An increasing number of studies using positron emission tomography (PET) and functional MRI (fMRI) in prodromal HD carriers have provided evidence that clinical impairments and brain atrophy can be detected prior to a clinical diagnosis (Paulsen, 2009, for a review).

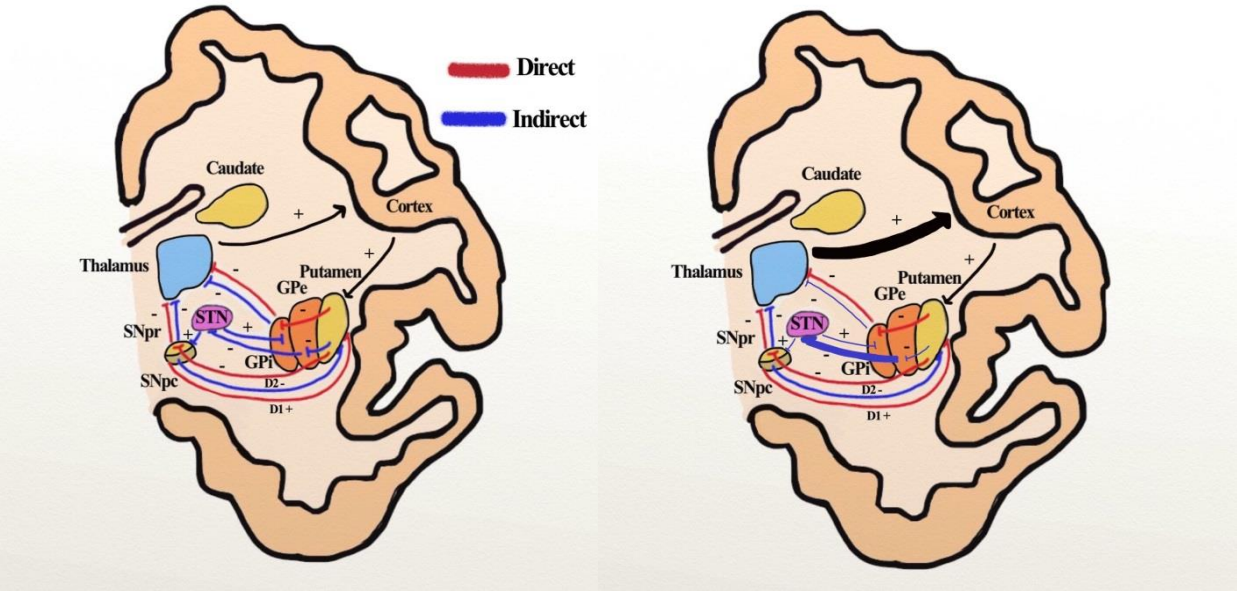

Figure 1.1. *Schematic representation of the direct/indirect pathway in the normal brain (left) and in HD (right). Red lines indicate the direct pathway (facilitates movement); blue lines, the indirect pathway (inhibits movement). The inhibitory connections are marked with "–"; the excitatory with "+".*
Left: basic circuit in the normal brain.
Right: In HD, in the indirect pathway (blue lines) the projection from the putamen (and caudate, not shown) to the GPe is diminished (thinner line). This increases the inhibition from the GPe to the STN (thicker line), which makes the excitatory STN less effective in opposing the action of the direct pathway (thinner line between the STN-Gpi, STN-SNpr, and between GPi-thalamus and SNpr-thalamus). Resulting in an increased activation (thicker arrow) from the thalamus to the cortex, this leads to increased motor activity.
Abbreviations: GPe = external segment of the globus pallidus; GPi = internal segment of the globus pallidus; SNpc = substantia nigra pars compacta; SNpr = substantia nigra pars reticulata; STN = subthalamic nucleus.
(Redrawn from Calabresi, Picconi, Tozzi, Ghiglieri, & Di Filippo, 2014)

1.5 Symptoms

HD is characterised by a triad of progressive clinical symptoms: motor dysfunction, psychiatric disturbances, and cognitive decline. Although the onset of HD is generally defined as the time when motor symptoms become evident (Siesling et al., 1998), it has been reported that mood changes and cognitive deficits appear before the motor symptoms manifest (Aylward,

2007; Aylward et al., 2000; Duff et al., 2007; Paulsen, 2011; Paulsen et al., 2006; Paulsen et al., 2008; Paulsen et al., 2001b).

1.5.1 Motor symptoms.

There are two main categories of motor symptoms: increased involuntary movements (e.g., chorea, dystonia) and impaired ability to sustain voluntary muscular effort (e.g., dysphagia, dysarthria, spasticity, rigidity, bradykinesia). The most common motor manifestation of HD is chorea, which is defined as involuntary, quick irregular movements. The most prominent regions where chorea manifests are the orofacial regions and the distal musculature of hands and feet. Chorea can be an early manifestation of HD (Berardelli et al., 1999), may manifest in individuals at risk of HD (McCusker et al., 2000), and, in late onset of HD, may be the only symptom (Myers et al., 1985). It has been reported that early in the disease, patients may not be aware of the presence of chorea, but as the disease progresses, larger muscle groups are involved which interferes with voluntary movements; the incorporation of chorea into purposeful movements is known as parakinesia (Novak and Tabrizi, 2011).

Dystonia (slower movements caused by increased muscle tone and involuntary contraction of muscles) is also seen in most patients with HD. Different displays of dystonia include: twisting, tilting, turning of the neck (torticollis), involuntary arching of the back (opisthotonos), rotatory dystonic movements of the shoulder, hands, and arching of the feet (Ghosh and Tabrizi, 2015; Novak and Tabrizi, 2011). Cervical dystonia has been proposed as an initial manifestation of HD and is more prominent in juvenile onset of HD (Ashizawa and Jankovic, 1996). Bradykinesia (slowness in execution of movements), clumsiness, and akinesia (slowness of initiation of movements) are also common in patients with HD. Patients with juvenile onset tend to have more bradykinesia, rigidity, dystonia and little or no chorea, while

later onset patients predominantly manifest chorea (Louis et al., 2000). The combination of bradykinesia, dystonia, and chorea, which is observed in many patients with HD, leads to gait disorders, which is a major and disabling feature of HD that leaves patients highly prone to falls. Some of the characteristics of gait dysfunctions that are observed in patients with HD are a decrease in gait velocity and stride length, spontaneous flection of the knees and broad based gait; these symptoms are commonly confused which drunkenness by people unaware of the disease (Koller and Trimble, 1985). Eye movement abnormalities are also present in patients with HD. These manifest through significant abnormalities of saccades, difficulty maintaining fixation and smooth pursuit. Oculomotor abnormalities manifest early in the disease and persist throughout the course of it (Lasker et al., 1987). Severe myoclonus (sudden brief jerkin of groups of muscles) and tics may also be present in patients with HD. Tics can be stereotyped movements such as blinking, nose twitching, head jerking, or transient abnormal postures; they can also cause sounds like sniffs, snorts, grunts, coughs, and sucking through involvement of respiratory and vocal structures and may be severe enough to cause significant speech difficulties (dysarthria), problems with swallowing (dysphagia), and balance, causing frequent falls (Jankovic and Ashizawa, 1995).

1.5.2 Psychiatric and mood symptoms.

Psychiatric and mood symptoms are common in HD and precede motor dysfunctions by many years (Duff et al., 2007). Around 72-98% of HD patients exhibit psychiatric symptoms (Paulsen et al., 2001a; Van Duijn et al., 2007). A review by Van Duijn et al. (2007) showed that the most common psychiatric symptoms that HD gene carriers will experience before establishing the diagnosis and during the course of the disease include: depression, suicide risk, apathy, anxiety, irritability and agitation, obsessive-compulsive behaviours, and psychosis.

While depression is common in HD, it is not clear if it is an intrinsic feature of the disease, if it is a response to being diagnosed with a terminal disease with increase disability, or if it is a combination of both (Wahlin and Byrne, 2012). A survey conducted in 2,835 patients with HD found that 40% presented symptoms of depression and more than 10% had made at least one suicide attempt (Paulsen et al., 2005a). Suicide risk is higher in HD patients (Di Maio et al., 1993; Schoenfeld et al., 1984), patients at risk for HD (Sørensen and Fenger, 1992), and even in people without the disease but with a family history of HD (Robins Wahlin et al., 2000). The suicide rate in HD is 4-6 times higher than in the general population, and it was the cause of death in 7.8% of HD patients in one study (Di Maio et al., 1993). Schoenfeld et al. (1984) noted that half of the suicides occurred in individuals who showed early signs of the illness but who had not been diagnosed, which suggested that suicide risk is greater in early stages of the illness. Further examination by Paulsen et al. (2005a) reported that there are two critical periods for increased risk of suicide in patients with HD: before receiving a formal diagnosis, but when they start showing motor symptoms of HD, and when their independence decreases as a result of the onset of the disease. Novak and Tabrizi (2010) suggested that some of the risk factors for suicide are depression and the idea that it is the rational response to their fear of loss of independence. But in some other cases, patients without depression also attempted suicide, especially if they had no offspring (Lipe et al., 1993).

Apathy, which is characterised by diminish energy and activity, lack of drive, and impaired performance of daily tasks (Van Duijn et al., 2007), can sometimes be confused as a symptom of depression. However, it has been reported that apathy is not correlated with depression (Levy et al., 1998) and that it is a separate clinical symptom in neuropsychiatric disorders (Levy et al., 1998; Marin, 1991). Different studies have reported that apathy is another

symptom observed in patients with HD (Craufurd et al., 2001; Kulisevsky et al., 2001; Paulsen et al., 2001a; Van Duijn et al., 2007), with a prevalence varying between 36-76% depending on the scale used, and it has been reported to increase as the disease progresses (Levy et al., 1998).

Other psychological problems are also apparent in HD patients. For example, anxiety has also been reported in patients with HD (Craufurd et al., 2001; Kulisevsky et al., 2001; Murgod et al., 2001; Paulsen et al., 2005b; Paulsen et al., 2001a; Van Duijn et al., 2007). The highest prevalence of anxiety (61%) has been reported after showing the first motor symptoms of the disease (Murgod et al., 2001). Irritability, a mood disorder characterised by a diminishes control over temper, that may result in verbal or physical altercations (Snaith and Taylor, 1985), has a prevalence of 38-73% in patients with HD (Craufurd et al., 2001; Kulisevsky et al., 2001; Murgod et al., 2001; Paulsen et al., 2001a; Van Duijn et al., 2007). There has also been reported a prevalence of 10-52% of obsessive and compulsive thoughts and behaviours in HD (Anderson et al., 2001; Craufurd et al., 2001; Murgod et al., 2001). The three most common obsessions in HD are related to other people, to the self, and ritualistic behaviours (Novak and Tabrizi, 2010). Psychotic symptoms, such as delusions and hallucinations, are rare in HD, but low prevalence (3-10%) has been reported (Craufurd et al., 2001; Kulisevsky et al., 2001; Murgod et al., 2001; Paulsen et al., 2001a; Van Duijn et al., 2007).

1.5.3 Cognitive symptoms.

It was previously believed that cognitive deficits began only after the motor symptoms were apparent. However, recent studies have reported that by the time diagnostic tests confirms the disease, there is already significant cognitive impairment, and subtle cognitive impairment could be among the earliest manifestations of the disease (Aylward, 2007; Aylward et al., 2000; Hahn-Barma et al., 1998; Paulsen, 2011; Paulsen et al., 2006; Paulsen et al., 2008; Paulsen et al.,

2001b). Additionally, it has been proposed that cognitive changes might be one of the first symptoms of HD (Hahn-Barma et al., 1998), and it has also been reported that cognitive and motor impairments in HD are independent from each other (Rothlind et al., 1993).

1.5.3.1 Cognitive Diagnoses in HD

Even though there are several rating scales to assess features of HD, there is no universal battery that has been used for cognitive assessment of HD. Some HD centres rely on the Unified Huntington Disease Rating Scale (UHDRS; Kremer and Group, 1996), a battery that combines different tests to measure four domains of clinical features of HD: motor function, cognitive function, behavioural abnormalities (psychiatric symptoms), and functional capacity. Motor function is assessed by rating oculomotor function, dysarthria, chorea, dystonia, gait, and postural stability. Higher scores, indicate more severe motor impairments. Cognitive function is evaluated by using a series of tests: the phonetic verbal fluency test, where participants have to say as many words as possible from a category in a given time (Ho et al., 2002); the Symbol Digit Modalities Test, where using a reference key, subjects have 90 seconds to pair specific numbers with given geometric figures (Smith, 2002); and the Stroop Interference Test (Stroop, 1935). In this section of the battery, higher scores, indicate better cognitive performance. Behavioural assessment, which is more related to a psychiatric assessment, is evaluated by rating both the severity and frequency of symptoms related to mood, self-esteem, anxiety, suicidal thoughts, aggressive behaviour, irritable behaviour, obsession, compulsion, delusions, and hallucinations. Higher scores in this section of the battery indicate more severe disturbance. Finally, the functional assessment include the Huntington's Disease Functional Capacity Scale (HDFCS), which assesses a patient's capacity in functional domains including employability, financial tasks, domestic capacities, and self-care skills (Shoulson and Fahn, 1979); the

independence scale and a checklist of common daily tasks. Higher scores on the functional scales indicate better functioning. Nevertheless, although there have been some studies that have found the scale useful for monitoring the progression of motor deficits in patients with HD, the changes observed on the scale during longitudinal follow-up studies have not been reported for all of the patients (Marder et al., 2000; Siesling et al., 1998). In other cases, no change on the motor scores of the UHDRS (except rigidity) was observed during a 3-year follow-up study (Reilmann et al., 2001). Likewise, Pavese et al. (2003) did not find a correlation between the progression of striatal loss of D_2 receptors and the individual changes in UHDRS motor scores in a longitudinal study over 3 years, which suggests that the UHDRS does not reflect progression of striatal reduction of dopaminergic receptors alone. Other limitations of the UHDRS is that the cognitive assessment throughout this rating scale is very limited (Beglinger et al., 2010) and, that it is only designed as a screening tool (Paulsen et al., 2001b). Therefore, other cognitive tests should be conducted to evaluate the specific cognitive mechanisms that are impaired in HD.

Another cognitive screening battery that has been used for cognitive assessment in HD is the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS; Randolph et al., 1998). The battery was developed to identify and characterise abnormal cognitive decline in older adults and to perform neuropsychological screening in younger patients. The battery evaluates five cognitive domains: 1) Immediate Memory (measured through two subtests: list learning and story memory); 2) Visuospatial/Constructional (consisting of two subtests: figure copy and line orientation), 3) Language (structured with the following two subsets: picture naming and semantic fluency), 4) Attention (including two subtests: Digit Span and Coding), and 5) Delay Memory (evaluated through four subtests: list recall, list recognition, story recall, and figure recall. Randolph et al. (1998) reported that HD patients were impaired on the Attention

and the Visuospatial/Constructional subsections. A limitation of this battery is that it has not been extensively explored, as it has only been reported in three published studies (Beglinger et al., 2010; Duff et al., 2010; Randolph et al., 1998) and two of the studies had small sample sizes. Randolph et al. (1998) only tested 20 HD patients and Beglinger et al. (2010) had a sample of 38 patients with HD, which questions the generalisability of the data to other patients with HD. For example, when the sample size was increased to 75 patients, Duff et al. (2010) reported that HD patients were impaired on all the five cognitive domains (on 11 of the 12 subtests), which were different from the results reported from the two other experiments (Beglinger et al., 2010; Randolph et al., 1998). Another limitation is that only Beglinger et al. (2010) retested the patients to evaluate if the task measured the cognitive decline as the disease progressed. After 16 months, when the patients were retested, they showed a decline in performance on the attentional index and on the subtest scores on coding, digit span, list recognition, figure copy, and figure recall (Beglinger et al., 2010). However, further research will be needed to evaluate if the battery continues measuring cognitive decline as the disease progresses. Another limitation is that the battery does not provide information about the other characteristics of the patients (e.g., severity of motor dysfunction and psychiatric symptoms); therefore, it should be used with other batteries that examine other markers of HD to better characterise the sample of HD patients that are being evaluated.

Given the absence of valid batteries for cognitive assessment of HD, new cognitive tasks should be considered. The next section summarises the cognitive impairments that have been reported in patients with HD in an attempt to identify those areas that have the most potential to yield a cognitive biomarker of HD.

1.5.3.2 Cognitive changes in HD

The major feature of the cognitive deficits in HD is a progressive decline of executive functions, defined as the set of processes that serves to optimise performance in complex tasks with many cognitive or behavioural components (Lawrence et al., 1998b). The abilities to plan, organise, show mental flexibility, switch from one way of responding to another (e.g., shifting of attention), and multitask are impaired in patients with HD; furthermore, organisation of time, thoughts, and activities become harder resulting in significant problems in daily functioning and as the disease progresses, patients lose their independence (Ghosh and Tabrizi, 2015). Some of the tests that require executive functions in which patients with HD are impaired are: the Wisconsin Card Sorting Test (Paulsen et al., 1995b) and the computerised Intra-Dimensional/Extra-Dimensional (ID/ED) attentional set-shifting task (Josiassen et al., 1983; Lange et al., 1995), which have been used to evaluate cognitive flexibility and shifting attentional set. Also, HD patients showed impaired planning in the Tower of London test, which requires the ability to plan a sequence of actions to solve the test (Lange et al., 1995). Likewise, patients with HD perform poorly on verbal fluency tasks, which involve generation of words belonging to a specified category or beginning with a specified letter (Monsch et al., 1994).

The first cognitive changes in HD may appear 12-15 years before the clinical (i.e., motor symptoms) onset of the disease (Stout et al., 2011). Paulsen (2011) reported that the earliest cognitive deficit detected in HD patients is *emotional recognition*. Compared to healthy controls, patients at the prodromal stage of HD were not able to identify facial expressions or verbal tones representing fear, anger, or disgust (Snowden et al., 2008). A review of the literature showed that using different tests of emotion recognition varying from facial expressions, vocal

expressions, and short verbal vignettes, recognition of emotions of disgust and anger associated with social disapproval were the most frequently impaired in HD (Calder et al., 2010).

Impaired *timing* (e.g., perception of time and the production of timed output) has also been reported in pre-symptomatic carriers of HD 15 years before the motor diagnosis, as well as in patients with HD (Hinton et al., 2007; Rowe et al., 2010; Zimbelman et al., 2007). These studies are consistent with the animal research showing that interval timing depends on the intact striatum (Buhusi and Meck, 2005, for a review).

Changes in *psychomotor speed* have also been reported as an early sign of cognitive changes in prodromal HD patients. For example, completion of ordinary mental tasks become more tiring and requires more time. Thus, cognitive or motor tasks that require speed are sensitive to the detection and progression of prodromal HD and HD patients (Paulsen, 2011). Some of the tasks that have been used to detect psychomotor slowing in patients with HD are the Stroop, Symbol Digit Modalities Test, and Trail Making tests (Ho et al., 2003). Slower reaction times have also been reported in patients with HD (Jahanshahi et al., 1993; Sprengelmeyer et al., 1995).

Changes in psychomotor speed also affect *communication*, as patients with HD have reported that they required increased effort and concentration to communicate (Hartelius et al., 2010). Although patients with HD have language difficulties, they are not aphasic. They understand words and speak grammatically correct sentences; early in the disease, language difficulties in HD are primarily related to muscle control impairments that make patients unable to articulate and speak clearly. As the disease progresses, the ability to speak is limited, but language comprehension may remain (Paulsen, 2011).

Learning and memory dysfunction is a symptom frequently reported in HD that may precede the onset of motor symptoms (Montoya et al., 2006a; Solomon et al., 2007). Although several studies have reported that patients with HD may have difficulty learning new information and retrieving previously learned information (i.e., explicit memory; Montoya et al., 2006a; Solomon et al., 2007), procedural learning is more likely to be impaired in HD (i.e., implicit memory; Redondo-Verge, 2000; Sprengelmeyer et al., 1995). In comparison to patients with severe amnesia or Alzheimer's disease (who may be able to learn new motor procedures even though they are unable to retrieve previously learned information), older memories are often unaffected in patients with HD, even when they have implicit learning impairments and are unable to learn new motor skills or procedures (Paulsen et al., 1995a). This dissociation between learning a skill and an explicit recall of learning episodes suggested that the distinct cognitive profiles are associated with the different underlying pathologies of the two dementias. In the case of HD, some deficits in implicit learning are associated with the neuronal loss of the striatum (Paulsen et al., 1995a).

Until recently, the implications of the areas affected in HD were poorly understood and it was even believed that neuronal loss in the striatum was only responsible for the motor impairments in HD, while the cognitive symptoms resulted from atrophy in the neocortex. However, a number of studies have shown a relationship between cognitive performance and changes in the striatum, and have shown that the progressive neurodegeneration of the striatum produce both cognitive and motor symptoms in HD (Montoya et al., 2006b). Additionally, selective cortical thinning and semi-independent of striatal atrophy have been correlated with cognitive deficits in HD (Rosas et al., 2005). Furthermore, different lines of research have shown that many of the cognitive changes in HD result from the atrophy and neuronal loss in the

corticostriatal circuit, leading to the hypothesis that executive function depends on circuits of multiple interconnected neural structures that code for specialised subprocesses (Lawrence et al., 1998b).

1.6 Treatment

Even though the first clinical description of HD was reported in 1872, currently, there are no treatments to cure, or even to slow the progression of the disease. The medications available merely ameliorate the motor and psychiatric symptoms (see Rosenblatt et al., 1999 for a detailed handbook of HD treatment options) but do not treat the cognitive decline that is observed in HD. Several studies have reported that cognitive and psychiatric functions are better predictors of impaired functioning than the movement disorder, and they are closely related to HD patients' independence and quality of life (Hamilton et al., 2003; Nehl et al., 2004). For this reason, it is widely accepted that clinical research and drug trials should focus on targeting cognitive treatments of HD. The use of animal models of HD is an essential step in drug-discovery, before the pursuit of a full-scale human trial, for preclinical validation of the therapeutic targets and/or compounds (Yang and Gray, 2011).

1.7 Choosing an animal model for the study of Huntington's disease

Even though clinical studies in patients with HD have shown that cognitive changes precede motor symptoms, mechanistic studies are difficult to perform. In this respect, animal models of HD have become an important tool for mechanistic examination.

Although there is no animal model that simulates all the aspects of HD, models that presents some aspects of the disease are often more valuable in exploring specific domains (e.g., cognitive) without contamination from other impairments of the disorder (e.g., motor). Therefore, partial models are often accepted as appropriate (Willner, 1991) and sometimes

superior. Nevertheless, it is common approach to require that an animal model resembles the clinical disorder in many details as possible, including symptomatic expression, treatment responses, pathophysiology, and, ideally, aetiology. The validity of animal models has been evaluated using three main criteria: face, predictive, and construct validity (Willner, 1986). More specifically, an adequate model of HD should resemble the fundamental motor, psychiatric, and cognitive symptoms, as well as the pathophysiology, found in HD patients (face validity). Should conform to a theoretical rationale, such as the known genetics of HD (construct validity), and allow us to make predictions about HD, based on the performance of the model (predictive validity). From a pharmacological perspective, predictive validity is the ability of an animal model to respond to treatments in the same manner that humans respond to the treatment and vice versa (Willner, 1984). Currently there are no effective treatments available for HD, and thus this criterion cannot be used to validate the existing animal models of HD.

In an attempt to gain a better understanding of the progression of HD and its treatments, various animal models that try to resemble the neuropathology, genetic mutation, and symptoms of HD have been proposed. In general terms, these animal models have been divided into two broad categories: toxin and genetic models.

Before the mutation that causes HD was discovered (MacDonald et al., 1993), the animal models of HD relied on neurotoxic lesions of the striatum (Beal et al., 1986; McGeer and McGeer, 1976; Schwarcz et al., 1984). However, after the discovery of the *HTT* mutation in 1993 (MacDonald et al., 1993), a number of genetic approaches have been used to generate animal models with a similar genetic background to HD. There are several genetic models of HD which vary on the number of CAG repeat expansion, the behavioural phenotype, the level of neuropathology affected, and the time course of the disease (Ferrante, 2009; Menalled, 2005;

Menalled and Chesselet, 2002; Pouladi et al., 2013; Ramaswamy et al., 2007, for a review). In general, the different genetic mouse lines fall into three broad categories: knockout, transgenic, and knock-in models.

The first genetic mouse models were the huntingtin knockout models. However, the homozygous knockout of this gene in mice is embryonic lethal (Duyao et al., 1995; Nasir et al., 1995), which diverges to the late onset of the disease in humans. Therefore, although this early mouse models have demonstrated that huntingtin has an important role in embryonic development, they are not good models of the disease (Menalled, 2005; Menalled and Chesselet, 2002).

The transgenic models are those in which the human mutant huntingtin (*HD*) gene, or a fragment of the gene, is inserted randomly into the mouse genome. Therefore, this mouse model will express the two normal copies of the endogenous mouse huntingtin (*Hdh*) gene and the additional mutant gene inserted (Menalled, 2005; Menalled and Chesselet, 2002). This category includes the R6/1, R6/2 and N171-82Q lines. The main differences between R6/1 and R6/2 lines are the number of CAG repeat expansion. The R6/1 has 116, while the R6/2 has 144-150 CAG repeats. The R6/2 was the first transgenic mouse model and has been the most studied. These mice present body weight loss, diabetes, dystonia with limp clasping, reduced limp performance, tremors and seizures (Carter et al., 1999; Hurlbert et al., 1999; Mangiarini et al., 1996). In addition, they also have an early death at 12-18 weeks and although the brain volume is reduced, the neuronal loss is minimal and delayed in comparison to the behavioural symptoms (Mangiarini et al., 1996; Turmaine et al., 2000). The N171-82Q mice have 82 polyglutamines (Schilling et al., 1999). Between the behavioural changes that are observed, these mice present body weight loss, and motor performance deficits that include loss of coordination, gait

abnormalities, hypokinesia, hind limb clasping behaviour, and muscle weakness (Schilling et al., 1999; Yu et al., 2003). The neuropathology of the model is similar to that observed in humans, as the model presents neuronal loss of the striatum and enlarged ventricles (Yu et al., 2003). However, the model has a short life span which ranges between 130-180 days. In addition, it has been reported that the phenotype of this model is more variable than that of R6/2 mice; therefore, a larger sample of mice are required for drug trials (Ferrante, 2009).

The knock-in mouse models have a CAG repeat expansion inserted into the mouse huntingtin gene. Given that the mutation is genomically correct and under the endogenous Hdh promoter, they have been suggested as the closest genetic models of the human disease (Ferrante, 2009; Menalled, 2005; Menalled and Chesselet, 2002). However, some of the lines do not show behavioural phenotypes that are characteristic of HD (Wheeler et al., 2000; White et al., 1997), making these models inappropriate for investigating treatments for the symptoms observed in HD. Some knock-in models are the chimeric HdhQ lines, which, depending on the model, can contain 48-111 CAG repeats. Other knock-in models with greater CAG repeats are the CAG140 HD mice and the $Hdh^{(CAG)150}$, with 140 and 150 CAG repeats respectively. These models have been suggested for investigating early mechanisms and preclinical biomarkers of HD (Ferrante, 2009; Menalled, 2005; Menalled and Chesselet, 2002).

As these genetic animal models of HD have only recently begun being investigated, they present some disadvantages that should be considered when choosing an animal model for studying HD. For example, the motor impairments that some of the genetic models have are so severe that the animals are unable to control neither their voluntary movements or their involuntary movements, which prevent them from performing certain behavioural tasks (Brooks et al., 2004). Additionally, the long polyglutamine repeats in some of the genetic models have

been suggested to replicate the juvenile form of the disease instead of the adult onset form of HD (Ferrante, 2009). Likewise, another relevant characteristic to consider when designing an experiment is the lifespan of the model, which can limit the duration of the research that could be conducted in these animal models. Hence, most of the research that has been conducted in genetic models has predominantly evaluated motor phenotypes (Brooks and Dunnett, 2009), while the cognitive aspects of the models still remain unknown. The neuropathology of these models should also be considered, as it has been reported that in some genetic modified mice, there is no evidence of neuronal loss in the striatum or other regions (Ferrante, 2009, for a review). Without neuronal cell loss, these animal models are inadequate for investigating treatments for the neuropathology observed in HD; thus they will not be discussed further.

Excitotoxic lesions of the striatum offer an alternative model for the study of HD (Beal et al., 1986; Kim et al., 2011; Schwarcz et al., 1984). Although the excitotoxic models do not mimic the pathogenesis of HD, and thus they are less effective for investigating the development of the disease, as noted above, there are some advantages over the genetic models. For example, excitotoxic lesions are not limited to mouse models. Using rats rather than mice, has the advantage that they tend to live longer, and there is a richer repertoire of behavioural tasks available for the rat, which can help to pinpoint the dysfunction and degeneration of neurons in HD.

In addition, lesions of the striatum are a controlled and rapid method to induce HD neuropathology, as well as cognitive and motor phenotypes, which allows validating tests in which the striatum might be involved. By controlling the "onset" of the disease, comparisons between pre- and post-surgery can be done to understand the cognitive and behavioural phenotypes caused by the neuronal loss in the striatum. This direct comparison cannot be done

in neurodegenerative models, as the disease will not be diagnosed until the first motor symptoms appear, even though cognitive and psychiatric symptoms may precede the motor dysfunctions (Aylward, 2007; Aylward et al., 2000; Duff et al., 2007; Paulsen, 2011; Paulsen et al., 2006; Paulsen et al., 2008; Paulsen et al., 2001b). Besides providing information about the role of the striatum in behaviour, the excitotoxic models have been proposed as "first pass" model systems in order to evaluate therapeutic compounds, cell replacement therapies and other interventions before the use of genetic modified models (Brooks and Dunnett, 2013). Given the strengths and limitations of the currently available animal models of HD, this thesis investigated an excitotoxic model of HD.

1.7.1 Quinolinic acid (QA) lesions of the rat striatum: an animal model of Huntington's disease

Among the most important neurotoxin-based models of HD is the quinolinic acid (QA) lesion of the striatum. As previously mentioned, the most prominent neuropathological feature of HD is selective neuronal degeneration in the striatum, where medium spiny neurons with GABAergic striatal projection neurons are affected early and most severely (Ferrante et al., 1987; Ferrante et al., 1985; Ferrante et al., 1986). QA is an excitatory amino acid acting via N-methyl-D-aspartate (NMDA) receptors (DiFiglia, 1990). Injections of QA in the striatum results in a depletion of GABA, which induces apoptotic cell death of the striatal MSNs while leaving aspiny neurons with somatostatin unaffected. Similarly as in patients with HD, QA lesions also show marked gliosis (Björklund et al., 1986), produce a loss in $GABA_A$ receptors in the striatum and an increase in the GP (Faull et al., 1993), and a decrease of substance-P and enkephalin GABAergic striatal projection neurons (Beal et al., 1986; Ferrante et al., 1993). Therefore, the model resembles the most similar pattern of striatal neuropathology and receptor changes of HD

(Beal et al., 1986; Kim et al., 2011; Wang et al., 2012). QA cannot cross the blood-brain barrier; thus, in order to create the excitotoxic animal model of HD, it needs to be injected directly into the striatum (Foster et al., 1984). In rats (Brasted et al., 1998; Dunnett et al., 2012; Shear et al., 1998), mice (McLin et al., 2006), and non-human primates (Emerich et al., 2006; Ferrante et al., 1993), excitotoxic lesions of the striatum produce anatomical changes that resemble the pathology of HD (face validity).

In addition, striatal lesions produce behavioural changes that, whilst might not be identical to the symptoms observed in HD, are analogous (Brasted et al., 1998). In this sense, the face validity of the model has focused on similarity of impaired behaviours that have been established as behaviours affected in HD. For example, performance in reaction time tasks has been shown to be impaired by QA lesions of the rodent striatum (Brasted et al., 1998; Brooks et al., 2007; Hauber and Schmidt, 1994; Jay and Dunnett, 2007), which are also characteristics of patients with HD (Jahanshahi et al., 1993; Kim et al., 2004; Knopman and Nissen, 1991; Sprengelmeyer et al., 1995).

In order to evaluate the effects of lesions of the striatum in reaction time tasks, rodents have been tested in nine-hole operant chambers. In basic reaction time tasks, the three central holes are used, and the rat is required to sustain a nose poke in the central hole until a brief visual stimulus is presented in one of the two holes (on the left or right side). In order to receive a reward, the rat must withdraw its nose from the central hole (reaction time) and nose poke into the signalled hole (movement time). Using this task, different studies have reported that reaction time, but not movement time (i.e., the initiation but not the execution of a learned response), is affected by lesions of the striatum (Brasted et al., 1997; Brown and Robbins, 1989; Mittleman et al., 1988).

In reaction time tasks, when regular patterns of stimuli appear in random sequences, human subjects normally show progressive improvements in speed and accuracy to the sequences, without being consciously aware of them (Nissen and Bullemer, 1987). It is not clear if this type of implicit learning is impaired in patients with HD. While some studies have found that HD patients showed implicit learning deficits in the serial reaction time task (Kim et al., 2004; Knopman and Nissen, 1991; Willingham and Koroshetz, 1993), several others have failed to replicate these deficits (Brown et al., 2001; Ghilardi et al., 2008; Maki et al., 2000; Schneider et al., 2010). Differences in data analysis may account for some of the discrepancy in results. To evaluate this type of implicit learning, a form of procedural learning, in rodent models of HD, the serial implicit learning task (SILT) was developed (Trueman et al., 2005). Using the operant nine-hole box, the SILT adapted the methodology used in the 5-choice serial reaction time task (5-CSRTT; Robbins, 2002), where rodents have to nose poke into one of the five holes that presented a light stimulus in order to receive a reward. On the SILT, rodents needed to respond to two consecutive light stimuli, presented at two of the five holes, before receiving a reward. The light stimuli at the holes were randomly presented; however, amongst these random two-sequence stimuli presentations, an implicit learning probe trial was introduced. On these trials, an embedded two-sequence combination was presented, where a light stimulus at the second hole was always followed by a light stimulus at the fourth hole. Implicit learning was measured by faster reaction time and greater accuracy to the predictable stimuli (light presented at the second hole followed by the presentation of the light at the fourth hole) and then compared to the performance to the corresponding mirrored unpredictable stimuli (light presented at the fourth hole followed by the presentation of the light on the second hole). Rats with QA lesions of the striatum did not show implicit learning deficits on the SILT (i.e., there was no evidence that rats

utilised the predictable information embedded within the task; Jay and Dunnett, 2007). The same results were observed in mice with QA lesions of the striatum (Trueman et al., 2005), and it was also reported that acquisition of the task was not impaired with QA lesions of the striatum (Brooks et al., 2007). Since the SILT is the only task available to evaluate implicit learning in rodents, and it has been suggested that implicit learning is mediated by the striatum (Kim et al., 2004; Knopman and Nissen, 1991; Knowlton et al., 1996b; Wilkinson and Jahanshahi, 2007), new tasks for rodents that assess implicit learning, and other behavioural impairments after cell loss in the striatum, should be designed.

Despite the numerous animal models of HD available, most research has evaluated the motor impairments of the models, while the characterisation of the cognitive impairments remains poorly investigated. The reduced number of tasks available for evaluating cognitive impairments in animal models of HD, which are caused by cell loss in the striatum, has slowed the progress for testing treatments for the disease. Currently, the only treatments available for HD ameliorate the motor and psychiatric symptoms, but there are no treatments available for the cognitive decline observed in HD. This suggests the necessity to develop and validate new tests of striatal function in which the effectiveness of treatments for HD could be evaluated. Accordingly, the present thesis examines three different tasks that measured implicit learning, habit formation, and attentional set-shifting in rats with QA lesions of the striatum in order to assess the validity of these tasks and to evaluate the effectiveness of potential treatments for HD.

1.8 Behavioural paradigms used in this thesis to evaluate QA lesioned rats

Considering the cognitive deficits that have been previously reported in HD, this thesis uses three tasks to evaluate QA lesioned rats to try to identify cognitive biomarkers of HD caused by cell loss in the striatum. Having valid cognitive tasks is necessary for evaluating the

effectiveness of potential treatments for HD. The remainder of this section summarises the relevant principles, theories, and previous studies of implicit memory tasks, habit formation tasks, and attentional set-shifting tasks.

1.8.1 Implicit memory tasks

As previously mentioned in section 1.5.3.2, older memories are often unaffected in patients with HD; however, they have implicit learning impairments, as they are unable to learn new motor skills or procedures. Some of the deficits in implicit memory have been attributed to the neuronal loss of the striatum and other basal ganglia structures (Paulsen et al., 1995a). The basal ganglia is connected to motor and premotor areas of the frontal lobe, regions that have been implicated in the planning of movements as well as their execution (Alexander et al., 1986). These corticostriatal connections, as the connectivity in the striatum, have suggested that the basal ganglia are not only involved in the motor execution, but also in motor learning (Graybiel et al., 1994). In particular, within the striatum, it has been suggested that motor and cognitive skill learning are mediated by the caudate nucleus. In effect, patients with HD are impaired in learning and retaining certain motor and cognitive skills, which in healthy patients improve through practice and are not expressed verbally, which suggested that the neuronal loss in the striatum in HD impairs implicit memory (Knowlton et al., 1996b). Implicit memory is a descriptive label that refers to the performance that has been influenced by past experiences, without conscious recollection of a learning episode. In this sense, it is assumed that performance on implicit tests reflects unconscious or unaware expression of retention (i.e., although the performance of the task improves through practice, participants cannot verbalise how they solve the task). Other terms that have been used instead of implicit memory are:

memory without awareness, indirect memory, and non-declarative memory (Schacter et al., 1993).

Patients with HD were impaired on a number of implicit memory tasks. For example, patients with HD were impaired in rotor-pursuit perceptual-motor learning, a task that requires tracking a rotatory object with a stylus (Heindel et al., 1988) and impaired in learning to read mirror-reversed text (Martone et al., 1984). In the weight judgement study, where subjects lift a set of weights (heavy or light) and then later judge the heaviness of a new set of weights. Prior experience with the weights affects the weight judgements in healthy controls (e.g., the standard set of weights are perceived heavier if subjects were exposed to the light weights, but the weights are perceived lighter if they were exposed to the heavy weights). However, patients with HD showed impaired adaptation-level effects for weight judgements, as they were not influenced by the prior exposure of the weights (Heindel et al., 1991). Another task in which patients with HD showed impairments is in the prism adaptation task, where participants wear distorting prism goggles that displace the perceived location of objects to the right or left while being required to point to a target. With practice, healthy participants improved their performance on the task, whereas HD patients showed impaired prism adaptation (Paulsen et al., 1993). The results from these studies have suggested that impairments in motor skill leaning are related to neostriatal dysfunction.

In addition, as previously mentioned, some studies have reported that patients with HD have difficulty learning the repeated sequence in a serial reaction time task, as their reaction time is not faster in the embedded sequence in comparison to the random sequence (Heindel et al., 1988; Heindel et al., 1989; Kim et al., 2004; Knopman and Nissen, 1991; Willingham and Koroshetz, 1993). Additionally, functional imaging studies have shown increased metabolic

activity in the striatum when subjects are performing a serial reaction time task, which suggests that the striatum is involved in implicit learning (Kim et al., 2004). Nevertheless, others studies have not reported a performance deficit in patients with HD in learning the embedded sequences in the serial reaction time task (Brown et al., 2001; Ghilardi et al., 2008; Maki et al., 2000; Schneider et al., 2010). The contradictory results on the performance of this task call into question its validity for investigating deficits in implicit memory in patients with HD. These conflicting results should be considered before using this behavioural paradigm as a means of testing the animal models of HD.

Although the findings from the previously described tasks suggest that the striatum and its cortical connections may be critical for implicit memory tasks, it is important to mention that all of these tasks are restricted to motor components (e.g., pursuit rotor learning) or require changes in motor programs in response to perceptual information (e.g., prism adaptation, weight judgements, mirror reading). To investigate if the implicit memory deficits in HD are not only limited to motor learning, Knowlton et al. (1994) investigated implicit memory in the probabilistic classification task, a task that was not restricted to motor components, but that required learning a cognitive skill with practice. Briefly, the probabilistic classification task involves predicting the weather with a set of cards. There are four cues in the task (i.e., cards with geometric shapes), which predict sun or rain 60-85% of the time. Even though the participants report that they feel they are guessing, generally, they learn to choose the more highly associated outcome after 50-100 trials. Knowlton et al. (1996b) reported that patients with HD were impaired in learning this task, which suggested that the basal ganglia is related to incrementally learned cognitive skill tasks that require trial-by-trial practice and that are not conscious (i.e., implicit memory); also, that the striatum is not limited to only motor skill tasks.

However, Gluck et al. (2002) reported that the task could be solved using at least three different strategies. First, a one-cue strategy, which involves responding in the presence of absence of a single cue, regardless of all the other cues. The second approach uses an optimal multiple-cue strategy, which involves responding to each pattern based on the associations of all four cues with each outcome. The third strategy that could be used is a singleton strategy, which requires learning only about the four patterns that have only one cue present and all others absent. Given the variability of strategies to solve the task, it has been argued that different brain regions are involved in solving this task. Thus, the task is not a reliable measure to evaluate cognitive impairments related to neuronal cell loss in the striatum.

The tasks that have been used to evaluate implicit memory in HD (e.g., rotor-pursuit perceptual-motor learning, weight judgement, prism adaptation, mirror reading, weather probabilistic classification task) were designed for humans, and the behaviours that they require cannot be evaluated in rats. Therefore, the first aim of this thesis was to develop a valid task that could be performed by rats and humans to evaluate implicit memory. Because it has been suggested that the striatum is involved in the calculation of probabilities that predict an outcome (Knowlton et al., 1996b), a reaction time task was modified to allow the study of implicit memory by incorporating the computation of probabilities to predict the location of the stimuli. In this reaction time task, the location of the target (spatial probability) changed as a function of the length of the foreperiod (temporal probability). Thus, at shorter foreperiods, targets on the left side were more probable than targets on the right side, but at longer foreperiods, targets on the right side became more probable than the targets on the left side. If implicit learning occurred it was expected that, during the task, the subjects would gradually have faster reaction times on the left side at shorter foreperiods, and have faster reaction times on the right side at

longer foreperiods (the more probable location of the targets), even though they would not have conscious knowledge of the location where the stimuli were more likely to appear.

To summarise, this section has presented a series of implicit memory tasks in which patients with HD were impaired. The results from these studies have suggested that these impairments are related to striatal dysfunction. However, this has not been investigated in animal models of HD, as there are no tasks available for rats. Chapter 3 describes a task developed to evaluate implicit memory (the computation of probabilities to predict the location of a stimulus) in rats and humans.

1.8.1.1 Dopamine D1 and D2 Receptors in HD

As previously mentioned in section *1.4.1.1 the basal ganglia in HD*, it has been suggested that altered dopamine receptors contribute to the pathophysiology of HD. In the direct pathway, the striatum projects to the SNpr and the GPi. In the indirect pathway, the striatum projects to the GPe, then the SNT, and finally to the GPi (Albin et al., 1989). In the striatum, D_1 receptors are mostly expressed by the substance-P neurons of the direct pathway, while D_2 receptors are mostly expressed by the enkephalin neurons of the indirect pathway. Dopamine inhibits the indirect pathway by acting on D_2 receptors and stimulates the direct pathway by acting on D_1 receptors. The direct pathway facilities movement, and the indirect pathway inhibits movements (Ferré et al., 1997). Albin et al. (1989) proposed that the chorea observed in HD results from the selective loss of D_2 receptors enkephalin neurons of the indirect pathway. However, recent results from PET studies argue against the differential loss of D_1 and D_2 receptors in HD. These new studies have observed a parallel reduction of both caudate and putamen D_2 and D_1 receptor binding in pre-symptomatic mutation carriers of HD (Andrews et al., 1999; Weeks et al., 1996) and in HD patients regardless if chorea or rigidity is predominant (Turjanski et al., 1995).

Although these findings have not found a selective loss of striatal dopamine receptor in HD for chorea, Turjanski et al. (1995) found that HD patients with rigidity present an increased loss of striatal D_1 and D_2 receptor compared to those patients without rigidity. In addition, Andrews et al. (1999) found that the annual percentage reduction in D_1 and D_2 binding were not related to each other and proposed that in pre-symptomatic mutation carriers of HD striatal D_2 measures were more sensitive for detecting disease progression. Thus, suggesting that differential loss and dysfunction of the neurons that express these two dopamine receptors may contribute to the different symptoms of the disease.

Correlations between striatal dysfunction and cognitive performance have also been found using functional studies of neurotransmission systems. Different PET studies have found reductions in dopamine binding in the striatum in patients with HD (Backman et al., 1997; Berent et al., 1988; Bohnen et al., 2000; Ginovart et al., 1997).

Changes in cognitive performance induced by reduction of dopamine binding have also been observed in patients with HD. Brandt et al. (1990) showed that HD patients with reduced D_2 receptor binding in the caudate nucleus presented impairments in tasks that required rapid coordination and set alternation. Reductions in D_1 and D_2 receptor density in the striatum in HD patients have also been correlated with the severity of impairments of executive function, perceptual speed, visuospatial skill, verbal fluency, episodic memory, and reasoning (Backman et al., 1997). These correlations between reduced D_1 and D_2 receptor binding and cognitive deficits have also been reported in preclinical carriers of HD (Lawrence et al., 1998b; Lawrence et al., 1998c). The relationship between D_1 and D_2 receptors in the striatum and cognition in HD are an area of inquiry that requires further research.

Previous research has reported that in addition to motor deficits, dopaminergic receptor blockade of the brain dopamine (DA) system also induces cognitive deficits (Amalric et al., 1993; Baunez et al., 1995; Courtière et al., 2003; Domenger and Schwarting, 2006; Mayfield et al., 1993). However, the functional differences between D_1 and D_2 receptors in different behaviours are not clear. In order to evaluate the differences between these two receptors, DA antagonist drugs that selectively block D_1 or D_2 have been used. SCH-23390 is a DA antagonist with high affinity for the D_1 DA receptor (Billard et al., 1984; Hietala et al., 1992). Contrastingly, raclopride is a DA antagonist with a high affinity for the D_2 DA receptor and has no effect on D_1 receptors (Hall et al., 1988; Ögren et al., 1986). Differential role of the D_1 and D_2 DA receptors in the execution of a reaction time motor task have been reported. While D_1 receptor antagonist SCH-23390 did not affect the performance on a reaction time task, D_2 receptor antagonist raclopride increased the number of incorrect responses, which suggests that reaction time could be differentially affected by a selective dopamine receptor blockade (Amalric et al., 1993). Research in HD could benefit if the PET studies also incorporate a cognitive task that could detect differences between the loss of striatal D_1 and D_2 receptors. Incorporating both methodologies could be useful for monitoring the effects of new treatment interventions in HD.

The experiment in Chapter 4 was designed to investigate if the performance on a probabilistic serial reaction time task was differently affected by the dopamine D_1 receptor antagonist SCH-23390 or the D_2 receptor antagonist raclopride. Given that changes in cognitive performance induced by reductions in D_1 and D_2 receptor density in the striatum in HD patients and preclinical carriers of HD have been observed (Backman et al., 1997; Berent et al., 1988; Bohnen et al., 2000; Brandt et al., 1990; Ginovart et al., 1997; Lawrence et al., 1998b), it was hypothesised that selective blockade of dopaminergic transmission at the D_1 or the D_2 receptor

would affect implicit memory (the computation of probabilities to predict the location of a stimulus).

1.8.1.2 QA lesions of the rat striatum in an implicit task

The results presented in section 1.8.1 show that patients with HD are impaired in acquiring new motor skills, which suggests that the striatum is necessary for learning implicit tasks. However, the question of whether the striatum, once a task that requires implicit memory has been learned, is necessary for continuing performance in the task, has yet to be addressed. This will be investigated in Chapter 5 using an animal model of HD with QA lesions of the striatum. If the striatum is necessary for continuing performance in the task once it has been learned, it would be predicted that post-surgery the performance on the Spatiotemporal Target Probability Signal Reaction Time Task would be affected.

1.8.2 Habit formation tasks

In addition to having a general role in the initiation and patterning of different behaviours, the striatum has also been associated with habit formation (Packard and Knowlton, 2002; Yin and Knowlton, 2006).

Goal-directed behaviours (i.e., flexible, deliberate actions) are controlled by their consequences; thus, they require an association of an action and an expected outcome (A-O). When the value of the expected outcome changes, the action should consequently be affected. Conversely, habits (i.e., inflexible, automatic actions) are regulated by antecedent stimuli from the environment and are behaviours that have become routine and predictable. Thus, action selection is controlled through association of stimulus-response (S-R) without associating the

outcome to those actions; therefore, changes in the value of the expected outcome should not affect the behaviour (Adams and Dickinson, 1981).

To evaluate if the behaviour is goal-directed or habitual, the value of the outcome could be decreased (devalued) or increased (inflated). For example, to devaluate the value of a reward, animals are given access to the reward before the task. If the performance of the task is unaffected after the value of the outcome is manipulated, then the behaviour is habitual. Conversely, if the performance is affected after the value of the outcome is manipulated (e.g., the rate of responding decreases after the reward is devalued), then the behaviour is goal-directed. In addition, if degrading the contingency between action-outcome (e.g., rewards are delivered regardless of the behaviour), has no effect on the performance of the task, then the behaviour is habitual and not goal-directed (Dickinson, 1985; Dickinson and Balleine, 1994).

Whereas S-R associations mediating automatic habitual behaviours require implicit memory, it has been suggested that deliberate actions (i.e., goal-directed behaviours) are encoded in declarative memory (Dickinson, 1980; Knowlton et al., 1996a).

As previously mentioned (section 1.8.1), it has been suggested that the basal ganglia mediates some forms of implicit or automatic (habit) learning, and the dorsal striatum, in particular, has been reported as a critical brain structure for implicit tasks that require motor skills (Graybiel, 1995; Salmon and Butters, 1995). In rats, the DMS have been suggested to be involved in A-O learning. To assess the role of the striatum in habit learning, rats were trained to press a lever in order to receive a reward under a variable interval schedule. They then received a devaluation of the reward using conditional taste aversion, which consisted of pairing of the reward with administration of lithium chloride (which makes rats sick). Rats with lesions of the

DMS responded habitually (i.e., the effects of devaluating the outcome did not affect the performance), which suggests that lesions of the DMS do not affect habit learning (Yin et al., 2004). Yin et al. (2005) continued this line of research by investigating the effects of lesions of the DMS in the performance of goal-directed instrumental actions. Using a procedure with two actions and two outcomes under variable ratio schedules, the authors found that lesions of the posterior DMS (pDMS), as well as local inactivation of this area, abolished sensitivity to devaluation and degradation, which suggested that the pDMS was necessary for the acquisition and expression of goal-directed actions.

The role of the dorsal striatum in habits has also been investigated using the place and response learning task. In this task, the rats were always placed in the same starting point, and they were trained to retrieve the reward from a consistent arm in a cross-maze. Probe trials consist of moving the starting point to the opposite side of the maze and allowing them to approach a maze arm. If the rats turned to the opposite side to the one that was learned during training, they were considered place learners. Accordingly, it was suggested that the behaviour was flexible as they were incorporating spatial cues to decide the side to turn. Conversely, if rats continued performing the same response (i.e., continued turning to the same side) as in the training phase, they were considered response learners, which shows that the behaviour was inflexible and response specific. At the beginning, most rats used the place strategy, but after repeated testing, they switched to a response strategy (inflexible and response specific behaviour). Rats with inactivation of the dorsal striatum continued using the place strategy, independently of the number of trials, which suggests that the dorsal striatum was required to switch between goal-directed actions to habitual responding (Packard and Knowlton, 2002; Packard and McGaugh, 1996).

Instead of characterising habitual behaviours as associations between stimulus and response, another approach in the study of habits has defined them as a stereotypic, ritualistic behaviour that consists of sequential movements (Graybiel, 1998). To study the formation of sequential habits, Desrochers et al. (2010) designed a task in which monkeys were presented with a grid of four or nine dots, one of which was randomly baited after a variable delay. Once the monkey captured the baited target by fixating or saccading through the target dot, a reward became available. However, given that the baited dot changed every trial, it was unpredictable where and when the target would become baited. Therefore, no particular response or sequence of responses would produce a reward, and no fixed S-R habit would be required to solve the task. Using this task, Desrochers et al. (2010) found that even though the monkeys were free to move their eyes in any direction to find the target, there was no explicit training to develop a pattern of saccades to scan the grid. Monkeys developed a dot-looking visual habit to scan the target grids without training and without explicit S-R associations. However, even though Desrochers et al. (2010) described the scanning pattern as habitual behaviour, it remains to be investigated if the behaviour is affected by the outcome. As Dickinson (1985) suggested, if the performance of the task is unaffected after the value of the outcome is manipulated, then the behaviour is considered habitual. Chapter 6 implemented a task, adapted from that of Desrochers et al. (2010) in which rats nose poked a row of five holes to find a target hole selected randomly on each trial (no cue would indicate which hole would produce the reward) to evaluate habit formation in rats with QA lesions of the DMS.

Changes in goal value were assessed by using a devaluation of the reward, in which rats had free access to the reinforcement in their home cages before testing and the reward was not delivered during the session. It was hypothesised that if habits were formed, the performance on

the task would not be affected even though the value of the reward was devalued. In addition, to evaluate if lesions of the DMS would impair goal-directed behaviour (i.e., the association of an action and an expected outcome), in the last phase all of the conditions remained the same, but the central hole never produced a reward. Since there were no cues to indicate the change, the rat had to learn through the task that nose poking into the central hole would never produce a reward. Habit responding would consist of continuing responding to the central hole. Thus, it was hypothesised that if the DMS is required to learn goal-directed behaviours, rats with DMS lesions would show habit responding and would continue responding to the central hole regardless of no further reinforcement for that response.

1.8.3 Attentional set-shifting tasks

One of the cognitive deficits in patients with Huntington's disease is a decrement in behavioural flexibility (i.e., the ability to adjust responses according to the specified context and requirements of varying situations). Attentional set-shifting, which is a process of shifting attention from one perceptual dimension to another, is an aspect of behavioural flexibility that has been assessed in humans using the Wisconsin Card Sorting Test (WCST; Berg, 1948). In this test, the participants are given 60 response cards. Each card has one to four identical figures of a single colour. There are four different figures (stars, crosses, triangles, and circles) and four different colours (red, yellow, blue, and green). The participants are presented with four stimulus cards that differ in number, form and colour and they are asked to match the response cards to one of the four stimulus cards. Each card could be sorted according to number, form, or colour of the figures; however, the participants are not told the stimulus dimension to use in order to sort the cards. They only receive feedback when a particular match is right or wrong; therefore, the strategy to sort the cards must be inferred from the feedback provided. After five consecutive

correct responses, the sorting rules are changed and the participants not only need to discover the new sorting rule, but they also need to stop responding to the old rule for the newly sorted cards. The WCST measures the ability to make this shift by analysing the errors, time to complete the task, and the amount of perseveration (i.e., previously rewarded responses that persist when they are no longer beneficial).

Although the prefrontal cortex (PFC) mediates different forms of behavioural flexibility (Chase et al., 2012; Dias et al., 1996a), flexible behaviour is not only supported by this structure but by a larger neural network which includes the prefrontal-basal ganglia circuits. The striatum is one of the brain structures that has been suggested to interact with the PFC to mediate behavioural flexibility (Ragozzino, 2007). Ragozzino et al. (2002b) examined the effects of inactivating the dorsomedial striatum in a task that required rats to switch from a response discrimination to a visual cue discrimination and vice versa. Rats were trained either on a response or on a visual cue discrimination task. On the response discrimination task, regardless of where a white visual cue was placed, rats always had to make a 90° turn to the right to receive cereal reinforcement. Alternatively, rats on the visual cue discrimination always had to enter the visual cued arm, which required making turns to the right or to the left. Inactivation of the DMS by infusing 2% tetracaine, a local anaesthetic, when rats were changed between the conditions, impaired switching from a response to a visual cue discrimination and vice versa. This impaired behavioural flexibility was due to an inability to maintain the new task strategy. Likewise, excitotoxic lesions of the DMS impaired rats in reversing an instrumental spatial discrimination (the previously correct lever became incorrect and the previously incorrect lever became correct). The reversal deficits were present because rats failed to suppress perseverative responding from the previous contingency (Castañé et al., 2010).

Impaired performances on reversal learning after lesions of the dorsomedial striatum have suggested that this region is important for flexible alternation of strategies or for response patterns when environmental conditions change (Castañé et al., 2010; Ragozzino, 2007; Ragozzino et al., 2002b). To support the idea that the striatum plays a critical role in behavioural flexibility, it has been reported that in diseases where the striatum is affected, such as HD, patients manifest flexible behaviour deficits on the WCST. Josiassen et al. (1983) compared the performance of patients with recently diagnosed HD (diagnosed for one year or less) with moderate HD patients (diagnosed for more than a year to eight years) on the WCST. While both groups were able to infer the first sorting rule without difficulty, changing the sorting rule showed that patients with moderate HD had deficits in behavioural flexibility (i.e., they perseverated in using the past sorting rule). Various imaging studies have suggested that the deficits seen in HD patients in the WCST result from changes in striatal function rather than in the frontal cortex (Lawrence et al., 1998b, for a review). For example, using high resolution single photon emission computerised tomography (SPECT), Hasselbalch et al. (1992) showed that blood flow is reduced in the caudate nucleus of patients with HD and that there is a positive linear relationship between blood flow in the caudate nucleus and performance of the WCST and in patients with HD.

Although the WCST examines the transference from perceptual dimensions between stimuli (colour to shape, shape to number, number to colour, etc.) when the sorting rule changes, the task can be solved using different strategies, including matching to sample. Furthermore, given the structure of the task, successful completion of the WCST is not only limited to behavioural flexibility. Other executive processes such as: remembering the goal of the task, the ability to respond to feedback to infer the sorting rule, recognizing that new sorting rules are

required along the task, flexibility to change strategies to find the new sorting rule, maintaining that response and stopping to respond to the previous sorting rules, are required to successfully complete the task. However, deficits in any of these cognitive processes are not accounted in the scoring system, which makes it impossible to identify if the impaired performance on the WCST are limited to deficits in behavioural flexibility. Therefore, attentional set-shifting abilities become difficult to assess.

To address some of these issues, Roberts et al. (1988) modified the task so that the early stages could identify these executive functioning deficits and would prevent the subject to progress to the stages that evaluate set-formation and shifting. The Intra-Dimensional/Extra-Dimensional attentional set-shifting task is a computerised test that displays white lines superimposed onto blue-filled shapes on a touch screen as the two perceptual dimensions. The ID/ED attentional set-shifting task, studies the formation, maintenance and shifting of cognitive sets. In this task, humans and marmosets were required to learn a series of two-choice discriminations in which one stimulus from one of the two dimensions (shape or line) was correct. Feedback of incorrect and correct responses was provided by the computer. The ID/ED attentional set-shifting task consisted of a series of stages. The first stage was the simple discrimination (SD) stage in which subjects had to choose one of two stimuli which are equal of the same dimensions (i.e., either shape or line). For example, from the shape dimension, two different shapes were presented, but only one was the correct stimulus. Subjects completed 60 trials per day for a given stage until they reached a criterion of 90% correct responses in one session. At the following stage, the simple reversal (SR) stage, the contingencies were changed and the previously correct shape stimulus became incorrect and vice versa. The criterion for the reversal stage was 18 correct responses out of the last 20 trials. At the third stage, the compound

discrimination (CD) stage, stimuli from the alternative dimension were introduced. The lines (i.e., irrelevant stimuli) were superimposed over the shapes (i.e., relevant stimuli) to form a compound stimulus. Therefore, there were four compound stimuli: Line-A/Shape-A, Line-A/Shape-B, Line-B/Shape-A, and Line-B/Shape-B. On any one trial, the lines and shapes were intermixed. On some trials, Line-A/Shape-A was combined with Line-B/Shape-B; on others, Line-A/Shape-B was presented with Line-B/Shape-A. In order to respond correctly, subjects had to stay with the previously relevant stimulus (shape) and ignore the irrelevant stimulus (line). Therefore, if Shape-B had been reinforced in the previous stage, it remained reinforced regardless of whether it formed a compound stimulus with Line-A or Line-B. Given that each shape was paired with each line, an irrelevant dimension stimulus only predicted a reward 50% of the time, whereas the relevant dimension stimulus predicted reward 100% of the time. This stage continued until 90% correct responses were made in a session of 60 trials. At the fourth stage, the compound discrimination reversal (CDR) stage, the compound stimulus containing the previously incorrect shape became the correct choice and vice versa. The criterion to move to the next stage was 18 correct responses out of 20. At the fifth stage, the probe test, new exemplars of the irrelevant dimension were introduced (Line-C and Line-D), but the relevant dimension stimuli did not change (Shape-A and Shape-B were used) and the reinforcement contingencies were similarly unaltered. The previously rewarded shape remained reinforced regardless of whether it formed a compound stimulus with Line-C or Line-D. This stage was used only for one session to measure if the subject had learned to ignore the irrelevant dimension in the compound stimuli. In the next stage, subjects were returned to the previous compound stimuli used in the CDR stage. This stage continued until 90% correct responses were made over 60 trials over the course of two consecutive days. For the final stage, subjects received either an

Intra-, or Extra-dimensional shift stage. For these stages, new compound stimuli were introduced which were composed of new shapes and lines. In the ID stage, the relevant dimension was the same from the previous stages (e.g., shape). Thus, subjects had to continue to ignore the line dimension and base their choices solely on the shape's dimension. In the ED shift stage, subjects needed to shift their attention between the different stimuli dimensions. In this stage, the shape's dimension became irrelevant, and the line became the relevant stimuli dimension. It has been suggested that this stage is analogous to the change in category in the WCST (Grant and Berg, 1948), whereby the experimenter introduces an ED shift by changing the sorting rule and the subject has to learn to shift to the new sorting rule (Grant and Berg, 1948). Finally, the last stage was a reversal stage. The group that had an ID stage, received an ID reversal (IDR), whereby the compound stimulus containing the previously incorrect shape became the correct choice and vice versa. For the group that received the ED stage, they received an ED reversal (EDR), whereby the compound stimulus containing the previously incorrect line became the correct choice and vice versa.

The ID/ED attentional set-shifting task showed that the subjects that had to distinguish between stimuli varying along a different dimension (ED) from the previous stage (i.e., lines to shapes or shapes to lines) made more errors than those required to distinguish between stimuli varying along the same dimension (ID) from the previous stage (i.e., shapes to shapes or lines to lines). Suggesting that humans and marmosets were able to learn to attend to the relevant dimension of a stimulus (Roberts et al., 1988). It is important to note that the example used to describe the test was based on shapes being the initial relevant dimension. However, in the test, the conditions are counterbalanced so that half of the subjects receive a line as the initial relevant dimension and the other half receive a shape as the relevant dimension.

The Intra-Extra Dimensional Set Shift (IED) task from the Cambridge Neuropsychological Test Automated Battery (CANTAB; 2002) is a modified version of the task that has been used to measure behavioural flexibility. As in the ID/ED attentional set-shifting task from Roberts et al. (1988), participants are told that there is a rule that they can learn in order to find the correct stimulus each time; however, this rule will change once it is apparent that they have understood the currently correct rule. In this version of the task, subjects are tested in both the ID and the ED shift stages. The test consists of nine stages: SD, SR, C_D (first compound discrimination, where the irrelevant stimuli are introduced and are paired side by side with the relevant stimuli), CD, CDR, ID, IDR, ED, and EDR. After six consecutive correct choices at one stage, the test automatically proceeds to the next stage. If the criterion of six correct choices is not reached within 50 trials, the test is discontinued. Since the task is designed as a series of stages, executive functioning deficits in learning from feedback, maintain a response over time or keeping a future goal in mind, are detected in the early stages and prevent the subject to progress to the next stages. So, any impairment in performance on the ID or ED shift stages, which are the last stages, cannot be attributed to deficits to those cognitive processes. Therefore, the structure of the ID/ED attentional set-shifting task provides a more controlled measure than the WCST for the cognitive processes that are involved to successfully complete the task.

Patients with damage to the striatum arising from HD, exhibit deficits in the ID/ED attentional set-shifting task (Lawrence et al., 1998b). Depending on the progression of the disease, patients with HD had shown different impairments in the ID/ED attentional set-shifting task. Patients in the early stage of HD showed impairments in the ED shift (i.e., they had deficits when the task required them to shift responding from stimuli of one perceptual dimension to

stimuli of another dimension) and they made more perseverative than non-perseverative errors (i.e., they kept on responding to the previous relevant stimuli dimension, when it was no longer relevant). However, they were not impaired in set-formation or reversal learning. Less than 20% of the participants in early stage HD were able to complete all the stages of the task (Lawrence et al., 1996). To investigate the impaired ED shift performance in HD patients, Lawrence et al. (1999b) used two modified versions of the ID/ED attentional set-shifting task. Until the ED stage, the initial stages were the same for both tasks. However, the ED stage for the *perseveration* condition replaced the irrelevant dimension with a new dimension (which became the relevant dimension) and the *learned irrelevance* condition replaced the previously relevant dimension with a new dimension (which was irrelevant for task performance). Perseveration was described as the inability to release attention from relevant perceptual dimension, and learned irrelevance was defined as the inability to reengage attention to the previously irrelevant dimension (Owen et al., 1993). Results showed that early stage HD patients were able to learn that the previously irrelevant dimension became relevant. Accordingly, their set-shifting impairments resulted from perseverative responding (i.e., inability to stop responding) to the stimulus dimension that was relevant in the stages before the ED (Lawrence et al., 1999b). These results are consistent with previous data that shows that HD patients are impaired in shifting sets (Josiassen et al., 1983). However, patients in advanced HD showed reversal impairments (i.e., they continued selecting the previously reinforced stimuli even though it was no longer correct) and also failed to complete the task. Patients in advanced stages of HD cannot complete the reversal stages and rarely reach the ED stage (Lange et al., 1995). It has also been reported that preclinical carriers of the HD mutation, before the onset of any clinical movement disorder, showed specific deficits in the ED stage of the ID/ED attentional set-shifting task. Therefore, the

ID/ED attentional set-shifting task seems to be a tool that is capable of detecting subtle impairments earlier than many other cognitive tasks and before the development of the movement disorders in HD (Lawrence et al., 1998a).

Birrell and Brown (2000) adapted the ID/ED attentional set-shifting task for rodents. Like the human version, the rodent ID/ED attentional set-shifting task consists of multiple stages (simple and compound discrimination, reversals, ID and ED shifts) in which the shifting rules change. Using different digging medium and odour stimuli as the stimulus modalities, rats are presented with a pair of bowls in which only one is baited. Rats need to dig in the bowl that contains the correct stimulus exemplar to retrieve the hidden food reinforcement. In this task, the rats perform a series of seven stages that are analogous to those in the human task.

Mice have also been tested on the rodent ID/ED attentional set-shifting task (Brigman et al., 2005; Colacicco et al., 2002; Garner et al., 2006). However, even though there are several transgenic rodent models of HD (Menalled and Chesselet, 2002, for a review), at present, there is only one study that had tested a knock-in model of HD in the attentional set-shifting task (Brooks et al., 2006). A 24 week old HD homozygous *Hdh*$^{(CAG)150}$ knock-in mouse line was tested in a modified version of the rodent ID/ED task. This version consisted of only five discrimination stages (simple discrimination, compound discrimination, compound reversal, intradimensional acquisition, and extradimensional shift). Each stage was tested on separate days. Although there was no difference between the Wild type (Wt) mice and the knock-in mouse model of HD on the reversal stage, the knock-in line required more trials to complete the ED shift stage. However, this difference might be the result from the decreased number of trials of the Wt mice at the ED stage compared to the ID stage, which suggests that the Wt mice formed no attentional set. Two other characteristics of the mouse line that was used were that they present only mild HD-like

behavioural symptoms and they lack striatal atrophy; therefore, the role of the striatum in the rodent ID/ED task remains unknown.

Given that the ID/ED task has been able to identify cognitive deficits in HD patients even before any motor symptoms manifest (Lawrence et al., 1998a), and since different studies have suggested that the striatum is essential for effective set-shifting (Castañé et al., 2010; Ragozzino et al., 2002b), Chapter 7 evaluates if rats with bilateral quinolinic acid lesion of the dorsomedial striatum present similar cognitive deficits in the attentional set-shifting task as those seen in patients with HD. Based on the performance on the ED/ID task of HD patients (Josiassen et al., 1983; Lange et al., 1995; Lawrence et al., 1998a; Lawrence et al., 1996; Lawrence et al., 1999b), it was hypothesised that rats with QA lesions of the DMS would show impairments in the ED shift (i.e., they would have deficits when the task requires them to shift responding from stimuli of one perceptual dimension to stimuli of another dimension), would make more perseverative than non-perseverative errors (i.e., they would kept on responding to the previous relevant stimuli dimension, when it would no longer be relevant), and would have reversal impairments (i.e., they would continue selecting the previously reinforced stimuli even though it would no longer be correct).

To test if attentional set-formation is impaired in rats with lesions of the DMS Chapter 8 presents a series of behavioural tasks designed to further elucidate set-shifting performance with the possibility of drawing conclusions on set-formation. In addition, differences between DMS and the dorsolateral striatum (DLS) in reversal performance have been reported using an instrumental two-lever spatial discrimination task in rats. While rats with DLS lesions showed no reversals impairments, rats with lesions of the DMS showed a significant impairment in reversal learning (Castañé et al., 2010). Therefore, given that it has been suggested that different

striatal sub-regions may be implicated in different forms of flexible behaviour (Castañé et al., 2010) to assess the specificity of the role of the DMS in set-shifting performance, a group with lesions of the DLS was added.

Thus, Chapter 8 evaluates the contributions of the DMS and the DLS in reversal learning, set-formation, and set-shifting in rats.

1.9 Outline of experimental work in this thesis

This thesis focused mainly on evaluating the behaviour of rats with QA lesions of the striatum, which mimic some aspects of the neural damage in HD, in order to try to identify cognitive deficits of HD caused by cell loss in the striatum.

The experiments described in this thesis may be divided into three parts.

In the first part (Chapters 3-5), the role of the striatum in implicit memory is investigated. Chapter 3 reports on the development of a new task in rats and humans for examining reaction time and computation of probabilities of a location of a target. The task presents lateralised visual stimuli with differing probabilities that change over time. At short foreperiods (i.e., the interval between a warning signal and the stimulus to which the subject was to respond), the stimuli were more likely on the left side but, as time elapsed, presentation of the stimuli on the right side became more likely. It was expected that with practice, reaction times would be faster on the side in which the stimulus was more likely to appear (left side at shorter foreperiods and right side at longer foreperiods). The aim of this experiment was to find a task that could detect deficits in implicit memory (computation of probabilities of a location of a target) in rodents that were transitional to humans, so potential treatments for HD could be tested. Since a loss of striatal D_1 and D_2 receptors in HD has been reported. Chapter 4 uses the same task used in

Chapter 3 in order to investigate if implicit memory (the computation of probabilities to predict the location of a stimulus) is affected by a selective blockade of dopaminergic transmission at the D_1 or D_2 receptors by SCH-23390 and raclopride, respectively. Chapter 5 also uses the task described in Chapter 3 as a means of evaluating the role of the striatum in computation of probabilities of a location of a target in rats with bilateral QA lesions of the dorsomedial striatum (an animal model of HD).

In the second part (Chapter 6), the contribution of the dorsomedial striatum (DMS) in habit formation is explored. Chapter 6 implements a task, adapted from that of Desrochers et al. (2010), in order to evaluate habit formation in rats with QA lesions of the DMS.

In the third part (Chapters 7 and 8), the role of the dorsal striatum in attentional set-shifting is investigated. Chapter 7 tests rats with bilateral QA lesions of the DMS in the attentional set-shifting task in order to determine if the animal model presents similar cognitive deficits in behavioural flexibility as those seen in patients with HD. Chapter 8 presents a series of experiments as a means of investigating the role of the dorsolateral or the dorsomedial striatum in reversal learning, attentional set-formation, and set-shifting in rats with QA lesions.

Chapter 2

General Methods

2.1.1 Animals

The animals used in Chapters 3, 4, and 8 were experimentally-naïve male hooded Lister rats (Charles River, UK Ltd). While the animals used in Chapter 6 were experimentally-näive male hooded Lister rats (Harlan, UK Ltd). Table 2.1 shows the start and finish weights of all the rats used in this thesis. The rats were housed, in groups of up to four, in plastic box cages (50 x 25 x 30 cm) and handled five days a week. After habituation to the conditions of the animal colony, food was restricted to 15-20 g per rat, per day, of standard laboratory rat chow (Special Diet Services, Essex, UK). Body weight was monitored weekly to ensure steady gain. Water was available *ad libitum* in the home cage. Lights were on a 12:12 hr light-dark cycle with lights on at 7 a.m. Testing was conducted during the light phase. All experimental and welfare practices described herein complied with the Animal (Scientific Procedures) Act 1986, and were carried out under the authority of project licenses approved by the United Kingdom Home Office and the University of St Andrews Animal Welfare and Ethics Committee.

Table 2.1.
Start and finish weights of all the rats used in this thesis

Chapter	Number of rats	Start weights (range)	End weights (range)
Chapters 3, 5 & 7	24	276 – 307 g	473 – 599 g
Chapter 4	8	289 – 315 g	447 – 520 g
Chapter 6	16	256 – 321 g	407 – 572 g
Chapter 8	36	288 – 330 g	417 – 580 g

2.1.2 Surgery

The surgical protocol was based on that previously described by Castañé et al. (2010). Anaesthesia was induced by inhalation of 5% isoflurane (Abbott Laboratories Ltd) in oxygen, and was maintained between 1-2% throughout the surgery at a flow rate of 2L/min delivered by a nosecone fitted on the incisor bar of the stereotaxic frame. Once anaesthetised, the rats were administered a 0.05ml injection (SC) of the anti-inflammatory carprofen (Carprieve, Norbrook Laboratories LTD, Newry, N. Ireland, UK), and then secured in a stereotaxic frame (David Kopf Instruments, Tujunga, CA, USA). Rats were fitted with atraumatic ear bars and with the incisor tooth bar set at -3.3 mm relative to the interaural line for a level skull position. A midline incision was made along the scalp, and skin and tissues were retracted. Once the skull surface was exposed, small holes in the skull were made above the appropriate stereotaxic coordinates using a dental drill.

Bilateral lesions of the DMS or DLS were made by infusions (0.125µl per site; 1 min 40 sec infusion time per site) of 0.09M quinolinic acid (Sigma Aldrich) buffered to pH 7.3-7.4 in 0.1M phosphate-buffered saline (PBS) using a 0.5µl Hamilton syringe with a 30-gauge round-tipped needle. Stereotaxic coordinates for DMS were: AP +1.2, ML ±2.0, DV -4.5 and -5.5; and AP +0.2, DV ±2.0, DV -4.5 and -5.5. Stereotaxic coordinates for DLS were: AP +0.7, ML ±3.6, DV -5.5 and -6.0; and AP -0.3, DV ±3.6, DV -6.0 and -6.5. The needle was left *in situ* for a further 1 min 40 sec after infusion, before being slowly moved to the next DV coordinate, or withdrawn. Coordinates were taken from the skull surface using the bregma as the point of origin. Coordinates for all the surgery are presented in Table 2.2 (based on Castañé et al., 2010)

Surgery for control rats was identical to that described above, with the exception that the infusions were 0.01M PBS. After surgery, animals were individually housed; body weight, food intake, wound condition, and general health was monitored.

Table 2.2.
Injection parameters for dorsomedial striatum lesions.

Lesion area	Injections per site	Excitotoxin	Coordinates			Injection		Diffusion
			AP	L	DV	Vol (µl)	Time (min:s)	Time (min:s)
DMS	1	0.09 M Quinolinic acid	+0.2	±2.0	-5.5	0.125	1:40	1:40
					-4.5	0.125	1:40	1:40
			+1.2	±2.0	-5.5	0.125	1:40	1:40
					-4.5	0.125	1:40	1:40
DLS	1	0.09 M Quinolinic acid	-0.3	±3.6	-6.5	0.125	1:40	1:40
					-6.0	0.125	1:40	1:40
			+0.7	±3.6	-6.0	0.125	1:40	1:40
					-5.5	0.125	1:40	1:40

Note. Abbreviations: DMS, dorsomedial striatum; DLS, dorsolateral striatum; AP anteroposterior; L, lateral from midline; DV, dorsoventral. DV coordinates were taken from the skull surface (table adapted from Castañé et al., 2010).

2.1.3 Histology

Once the behavioural testing was completed, rats were anaesthetised with a lethal dose of 0.8 ml Dolethal (intraperitoneal, IP; Univet, Bicester, UK) and perfused transcardially with 4% paraformaldehyde in 0.1M phosphate buffer (PB; disodium hydrogen orthophosphate and sodium dihydrogen orthophosphate in distilled water). Brains were removed and stored overnight at 4°C

in a 20% sucrose solution, then washed three times in distilled water and allowed to dry. Brains were placed in individual wells and surrounded with egg yolk, before being placed in a 40% formaldehyde bath for five days to allow the tissue to fix. Brains were then cut to 50μm sections on a microtome (Jung Histoslide 2000, Reichert-Jung, Cambridge Instruments GmbH) into 0.1M PBS (0.9%). Brain sections were double-stained for neuronal nuclei (NeuN) and with cresyl violet to visualise cell nuclei and cytoarchitecture in order to map lesion extent.

For NeuN, sections were washed 5 times for three minutes in 0.1M PBS, then placed on a stirrer for 1 hour in blocking solution (0.1M PBS, 20% normal goat serum, 0.1% triton). Sections were washed, as previously in 0.1M PBS, then incubated in anti-NeuN (1:4000; Chemicon International, Temecula, CA, USA) in antibody diluting solution (ADS; 0.1M PBS, 1% normal goat serum, 0.1% triton) on a stirrer for 1 night. Subsequently sections were washed in 0.1M PBS as before, then incubated in a stirrer in vector IgG solution (anti-mouse IgG at 5μl/ml ADS; Vector Laboratories Ltd, Peterborough, UK) for 1 hour. After being washed in 0.1M PBS again, sections were incubated in a stirrer in Vectastain ABC complex (Vector Laboratories Ltd, Peterborough, UK; reagents A and B at 10μl/ml ADS) for a further hour. Sections were then washed in 0.1M PBS again and finally immersed in Sigma Fast 3.3-Diaminobenzidine tablets (DAB; Sigma Chemical Company, St Louis, MO, USA) for approximately 10 minutes, with the time being determined by visual inspection of the tissue. The tissue was removed during the point at which background staining was minimal but neurons were clearly visible. Sections were washed again in 0.1M PBS and then mounted on treated glass slides.

Sections were then de-fatted with xylene, and re-hydrated with ethanol, then 50% ethanol solution, then water. Sections were immersed in cresyl violet solution (cresyl fast violet acetate

dissolved in distilled water and glacial acetic acid, pH adjusted to 3.5 with sodium acetate) for 2 minutes then washed in running water for 5 minutes. Sections were subsequently dehydrated in 50% ethanol solution, ethanol and finally xylene before being cover slipped with DPX mountant (BDH Laboratory Supplies, Poole, UK).

Lesions were verified by light microscope examination of areas and cell damage was noted by lack of neuronal staining. The extent of lesions was mapped onto standardised sections of the rat brain using a stereotaxic atlas by Paxinos and Watson (2007).

2.1.4 Data analyses

Statistical analyses. All analyses were conducted in IBM SPSS (v 21, SPSS Inc., Chicago, IL). The criterion for significance (alpha level) was $p < .05$ in all cases. Sidak's-corrected pairwise comparisons were calculated, which is less conservative than Bonferroni's correction, and there is no loss of power associated with Bonferroni corrected values; it controls the family wise error only when the comparisons are independent (Field, 2013).

Effect size was computed to gain an index of the strength of the contribution of the manipulated variable. A partial eta^2 (ηp^2) statistic was used to determine the variance accounted for by an effect and that effect plus its associated error variance: $\eta^2_{partial} = \frac{SS_{effect}}{SS_{effect}+SS_{error}}$, where SS_{effect} was the sum of squares associated with each effect in the model and SS_{error} was the sum of squares for the error term associated with that effect.

Chapter 3

Spatiotemporal Target Probability Signal Reaction Time Task: Performance of Rats and Humans

Rats and humans were tested in the Spatiotemporal Target Probability Signal Reaction Time Task: a reaction time task that presented visual stimuli with differing probabilities which changed over time. At shorter foreperiods, the stimuli were more likely to appear on the left side, but at longer foreperiods, the stimuli were more likely to appear on the right side. Having a task that detects deficits in the computation of probabilities of the location of stimuli in rats, on which humans can also be tested, will allow further investigation of animal models of HD to evaluate potential treatments for the disease.

Do rats and humans have faster reaction times to stimuli presented in a more probable location, even when the location changes as a function of time-in-trial?

3.1 Introduction

Prediction of future events is a pervasive function of the brain. However, in order to make accurate predictions, time-based patterns need to be recognised, stored and recalled (Hawkins et al., 2009). Most predictions are based on the implicit learning that occurs when information is acquired from an environment of complex stimuli, without conscious access either to what was learned or to the fact that the learning occurred (Janacsek and Nemeth, 2012). Implicit learning has been defined as the knowledge that is acquired with practice but without awareness of the process or the product of the acquisition (Reber et al., 1991). It has been suggested that a great variety of everyday situations require the retrieval of previous implicit learning. For example, operations of appliance, computer applications, and even playing an instrument require implicit memory (Romano et al., 2010). Some social situations that require the prediction of emotions, or prediction of others' behaviour based on previous verbal and nonverbal social communication have also been considered to require implicit memory (Janacsek and Nemeth, 2012). Therefore, it has been suggested that implicit memory is not only related to motor skills, but also to cognitive and social skills (Kaufman et al., 2010), which are important aspects across life.

The basal ganglia have been reported as a critical brain structure for implicit learning (Paulsen et al., 1995a); in effect, deficits in implicit learning have been observed in diseases in which there is neuronal loss of the striatum, such as HD (Knowlton et al., 1996b). While the performance of HD patients is normal on implicit memory tasks that involve lexical, semantic and pictorial priming, their performance is impaired on implicit tasks that require the generation and modification of central motor programs to guide behaviour (Butters et al., 1994). For example, some of the implicit learning tasks in which HD patients have shown impairments

include rotor-pursuit perceptual-motor learning, weight judgement, prism adaptation, mirror reading and weather probabilistic classification task (see section 1.8.1 from the General Introduction). However, as previously mentioned, these tasks were designed for humans, and the behaviours required to perform the tasks cannot be tested in animals. Thus, implicit learning in animal models of HD has been restricted. This suggests the necessity to develop and validate new tests of striatal function in which the effectiveness of treatments for HD could be evaluated.

Therefore, the aim of this experiment was to develop a valid task that measured implicit memory which could be performed by both rats and humans. Because it has been suggested that the striatum is involved in the calculation of probabilities that predict an outcome (Knowlton et al., 1996b), a reaction time task was modified to allow the study of implicit memory by incorporating the computation of probabilities to predict the location of the stimuli. In this reaction time task, the location of the target (spatial probability) changed as a function of the length of the foreperiod (temporal probability). Thus, at shorter foreperiods, targets on the left side were more probable than targets on the right side, but at longer foreperiods, targets on the right side became more probable than the targets on the left side. If implicit learning occurred it was expected that, during the task, subjects would gradually have faster reaction times on the left side at shorter foreperiods, and have faster reaction times on the right side at longer foreperiods (the more probable location of the targets), even though they would not have conscious knowledge of the location where the stimuli were more likely to appear.

3.2 Method

3.2.1 Subjects

Twenty four experimentally-naïve male hooded Lister rats (Charles River, UK Ltd) with a mean *ad libitum* weight of 290 g (range = 276 - 307 g) at the beginning of the experiment. The

rats were housed in pairs in plastic box cages (see the General Methods sections in Chapter 2 for more details). Housing and husbandry conditions were described in the General Methods in Chapter 2.

In addition, 35 human adults (11 males, 24 females; with an age range of 18-40 years) students at the University of St Andrews participated in the study for a fee of £5. All participants had normal or corrected-to-normal vision and were recruited through the University of St Andrews SONA experiment participation scheme. The study was approved by the University of St Andrews University Teaching and Research Ethics Committee (UTREC; see Appendix).

3.2.2 Apparatus

For rats. All phases of the experiment were conducted in a set of four nine-hole operant chambers (Paul Fray Ltd, Cambridge, UK; see left image of Figure 3.1). Each chamber was enclosed in a ventilated, sound-attenuating cubicle. An extractor fan provided a constant low-level of background noise and a continuous airflow. The floor of the chamber was comprised of a stainless steel grid. The rear wall of each chamber was concave and had a horizontal array of nine holes; however, only the three central holes were used for this study (holes 4, 5 and 6 from left to right) and the other three holes located on the left (holes 1, 2 and 3) and on the right (holes 7, 8 and 9) of the three central holes were blocked with metal covers. Visual stimuli were presented by the illumination of a white bulb placed at the rear of each hole. Each hole contained a vertical photocell beam located at the front of each hole to detect nose entries. In the opposite wall, a 5.1 cm × 5.1 cm pellet hopper with a hinged panel was located 2.5 cm above the floor, and this received, according to the schedule, 45-mg TestDiet precision food pellets (Richmond, IN, USA) from a magazine pellet dispenser. The panel occluding the hopper was connected to a micro-switch that indicated its opening and contained a 28-volt cue light. A 3-W yellow house

light was located on the ceiling of the chamber. Auditory stimuli were produced from a tone generator, connected to a loudspeaker, located in the centre of the ceiling of the chamber. The presentation of stimuli and the collection of data were controlled by computers with a custom built interface, programmed in a version of BBC Basic (SPIDER; Paul Fray Ltd, Cambridge, UK). The temporal resolution of the instrumental set-up was 0.1 sec.

For humans. One of the nine-hole operant chambers was modified by removing the front wall, sterilising the apparatus, and adding a button outside the box to initiate the trials (see right image of Figure 3.1).

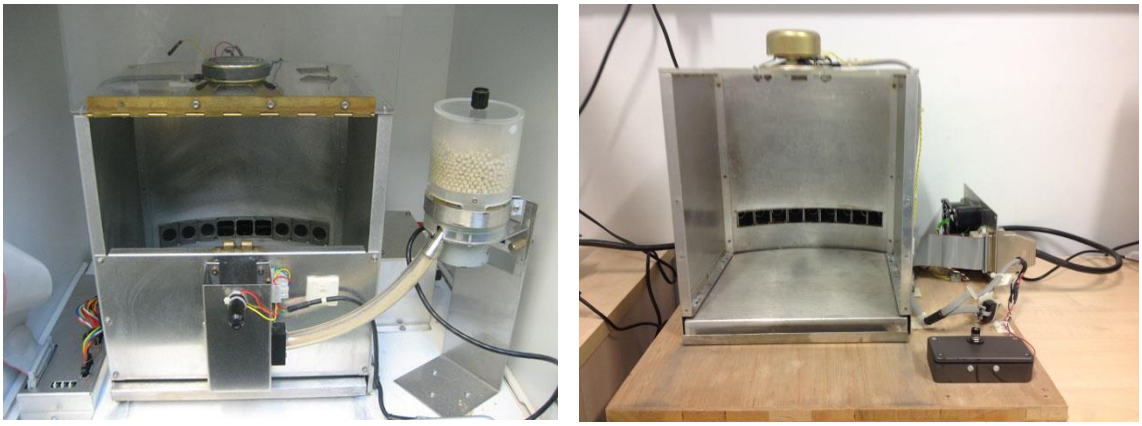

Figure 3.1. *Nine-hole operant chamber.*

Left: Operant box used for rats. *Right: Modified operant box for humans.*

3.2.3 Procedure

3.2.3.1 Behavioural protocol for rats.

Pre-training. In the initial session, rats were habituated to the operant boxes during one 30 min session, in which the house light was turned on and there were about 60 pellets available in the pellet hopper. Habituation was completed when the rat had eaten all the pellets in the designated time. During the next sessions, a pellet was dispensed every time the rat pushed the

hinged panel of the hopper. When rats earned 100 reinforcers in a 30 min session within this procedure, which typically required five sessions, the pre-training phase finished.

Instrumental conditioning. During the subsequent sessions, the animals were trained to poke their nose into the illuminated central hole to receive food. The trial began with the house light and the hopper light on. Once the rat pushed the panel of the hopper, the light of the hopper was turned off and the light inside the central hole was illuminated to serve as a signal of trial commencement. The hole light remained lit until the rat poked its nose; once this occurred, the light was turned off and the rat was required to sustain the nose poke in the hole until a tone was presented. The duration of the tone was 100 ms and was delivered from the speaker located in the ceiling of the chamber. The duration of time between the nose poke and the tone onset was gradually increased across sessions, according to the individual rat's performance (rats needed to complete 120 correct responses within 30 min session), from 100 to 600 ms. If the animal held the nose poke until the onset of the tone a single pellet reinforcer was delivered and a *correct response* was counted; however, if the animal withdrew its nose from the hole before the tone was turned on, no pellet was delivered, and a time-out interval was initiated, wherein the house light was turned off for 1 sec, and an *anticipatory error* was registered. To initiate a new trial the rat needed to press the pellet panel. Sessions finished when the rats received 120 reinforcers in total. Once the animals had a low rate of anticipatory errors (fewer than 20 per session) in the 600 ms foreperiod and completed at least 120 trials within the 30 min session, they progressed to a simple two choice discrimination task in the next session. This phase was carried out over 14 training sessions.

Simple two choice discrimination task. During the next sessions, all the rats were trained in a reaction time task to respond to lateralised visual stimuli to receive food. Each trial was

initiated once the rat pushed the panel of the hopper. The beginning of a trial was indicated by the illumination of the central hole which remained lit until the rat made a nose poke. In this phase, the rat was required to sustain the nose poke for a variable, unpredictable, foreperiod delay of 200, 300, 400, 500 or 600 ms. The end of the foreperiod was indicated by the onset of a continuous tone and a target light stimulus on either the right or the left hole (50% to each side through a complete session). The tone was turned off once the animal removed its nose from the central hole, but the target light remained on until the rat responded by making a nose poke into one of the side holes. The duration of the target light was initially 5 sec, but it was gradually reduced to 3, then 2, and finally 1 sec during the following sessions. If the animal removed its nose from the central hole after the onset of the tone, and the target light, and poked its nose into the illuminated side opening while the light was on, a food pellet was delivered and a *correct response* was counted; however, if there was no response on either of the side holes within 2 sec, a *late error* was recorded. An *incorrect response* was registered when the animal removed its nose from the central hole after the onset of the target stimuli but poked its nose into the side opening that was not illuminated. If the rat failed to hold the nose poke in the central hole for the duration of the foreperiod, an *anticipatory error* was counted. Late, incorrect and anticipatory errors initiated a time-out interval where no pellet was delivered and, after the rat pushed the hopper panel, the same trial was restarted. The session was terminated after either 120 correct responses or after 40 min. A stable baseline of about 80% correct responses was achieved in 14 training sessions, after which they progressed to the next phase.

Spatiotemporal Target Probability Signal Reaction Time Task. The final behavioural task differed from the previous one in terms of the probability of the target location cues. During the early foreperiods, the stimuli were more likely to appear on the left, but as time elapsed,

presentation of the stimuli became more likely to appear on the right. A complete schematic representation of the Spatiotemporal Target Probability Signal Reaction Time Task is shown in Figure 3.2.

In each session, there were 24 trials for each of the five different foreperiods (200, 300, 400, 500 or 600 ms), which were pseudo-randomly distributed in a 45 min session (120 trials in total).

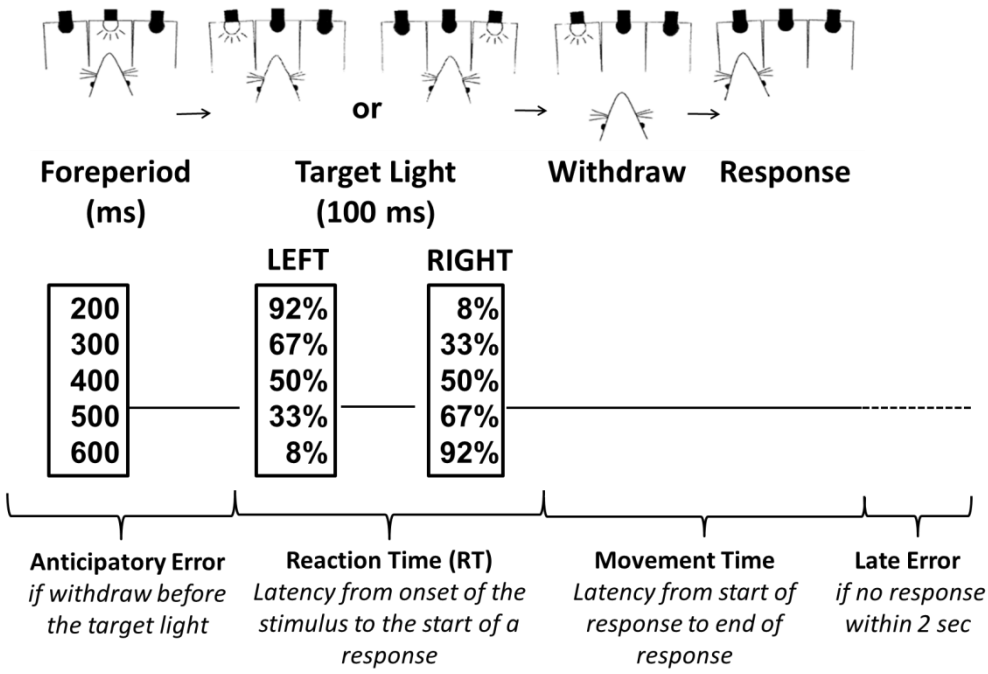

Figure 3.2. *Schematic representation of the Spatiotemporal Target Probability Signal Reaction Time Task. Foreperiods could have durations of 200, 300, 400, 500 or 600 ms and were followed by a light stimulus of 100 ms on either left or right side. At the early foreperiods, the stimuli were more likely on the left side; as time elapsed, presentation of the stimuli became more likely on the right.*

Training in this condition continued until the rats were able to complete 120 correct trials and had fewer than 25% incorrect responses within a 45 minutes session, for 5 consecutive days.

Data was collected for another 10 additional days (1200 trials) and were used for the analysis. On average, this phase required 140 sessions.

3.2.3.2 Behavioural protocol for humans.

Participants sat in front of the apparatus and were instructed to press the button outside the box to initiate the trials. This would turn off the light on top of the box and would turn on the light on the central hole. Participants were instructed to insert their index finger into the middle hole when it was lit, wait until a light on either the right or the left adjacent holes appeared and to move their finger as fast as possible to the hole where the light appeared. They were informed that, when an incorrect response was made, a time-out interval, during which time no lights would be on, would initiate for 1 sec. No further information about the structure of the task was given to the participants. After completing five trials correctly, the experiment would begin. Trials were initiated by poking a finger into the central hole. After a foreperiod of 200, 300, 400, 500 or 600 ms a target light on the right or the left (left more likely at short foreperiods and right more likely at longer foreperiods) was turned on. Subjects were required to respond to the location of the light. A new trial was initiated by pressing the button outside the box for humans. The data were collected in one session that consisted of 1000 trials.

Two factors could influence the performance of the task: location (spatial probability of the target), and time (foreperiod). The relative contribution of each of these two factors to the overall performance of the task is shown in Table 3.1 to illustrate the information available at any given point in time during the task. *A priori temporal probability* is the likelihood of the stimuli appearing at the different foreperiods. *Conditional temporal probability* shows that, as time elapses, the likelihood of a target to appear increases. For example, the probability of occurrence of the signal is 20% before 200 ms (as there are 5 foreperiods in which the stimulus could

appear), 25% before 300 ms (as there are now only 4 foreperiods left in which the stimulus could appear), 33% before 400 ms (with only 3 foreperiods left), 50% before 500 ms (as there are now only 2 foreperiods left in which the stimulus could appear), and 100% before 600 ms (since the stimuli did not appear in any of the other foreperiods, it must appear at the last foreperiod). *A priori spatial probability* shows the probability of the light appearing in each side at any given foreperiod; at short foreperiods, stimuli were more likely on the left side, but as time elapsed, presentation of the stimuli on the right became more likely. *Conditional spatiotemporal probability* shows the likelihood of target occurrence as determined by both *a priori* spatial probability and conditional temporal probability (i.e., is the product of multiplying *a priori* spatial probability by conditional temporal probability and dividing it by 100). As time elapses the probability of a target occurrence increases, and targets on the right side become more likely to appear.

Table 3.1.
Relative probabilities of the tone and the target lights

Foreperiod (ms)	200	200	400	500	600
A Priori Temporal Probability (tone)	20%	20%	20%	20%	20%
Conditional Temporal Probability (tone)	20%	25%	33%	50%	100%
A Priory Spatial Probability (target light)					
Left	92%	67%	50%	33%	8%
Right	8%	33%	50%	67%	92%
Conditional Spatiotemporal Probability (target light)					
Left	18%	17%	17%	17%	8%
Right	2%	8%	17%	33%	92%
Rats					
Number of trials per session (target light)	24	24	24	24	24
Left	22	16	12	8	2
Right	2	8	12	16	22
Humans					
Number of trials per session (target light)	200	200	200	200	200
Left	184	134	100	66	16
Right	16	66	100	134	184

Note. *A priori* temporal probability is the likelihood of the stimuli appearing at the different foreperiods. Conditional temporal probability shows that as time elapses, the likelihood of a target to appear increases. *A priori* spatial probability shows the probability of the light to appear in each side at any given foreperiod; at short foreperiods, stimuli were more likely on the left side, but as time elapsed, presentation of the stimuli on the right became more likely. Conditional spatiotemporal probability shows the likelihood of target occurrence as determined by both *a priori* spatial probability and conditional temporal probability (i.e., is the product of multiplying *a priori* spatial probability by conditional temporal probability and dividing it by 100).

3.2.4 Data Analysis

Reaction time (RT). Latency from the onset of the stimuli (tone and side hole light) to the start of the response (withdrawal of the nose/finger from the central hole).

Movement time (MT). Latency from the start of the response (withdrawal of the nose/finger from the central hole) to the end of the response (nose/finger poke in either side hole).

Percentage of incorrect responses. Removal of the nose/finger from the central hole after the onset of the target stimuli, followed by a response into the side opening that was not illuminated. This was computed by dividing the number of incorrect responses by the number of incorrect responses and the number of correct responses, and multiplying by 100.

Percentage of late errors. No response on any side hole within 2 sec after successful removal of the nose/finger from the central hole when the target stimuli were onset. This was computed by dividing the number of late errors by the total number of correct, incorrect and late trials, and multiplying by 100.

Percentage of anticipatory errors. Withdrawal of the nose/finger poke from the central hole before the onset of the target stimuli. This was computed by dividing the number of anticipatory errors by the total number of trials (i.e., including correct, incorrect and late errors) and multiplying by 100. Anticipatory errors were not influenced by the spatial location of the side stimuli because the nose/finger poke was withdrawn from the central hole before the onset of the side target stimuli; therefore, they were analysed without including the variable side.

The results were expressed as means across the multiple sessions for each subject for each variable. For reaction time both mean and mode were recorded; however, previous research in our laboratory has reported that the modal reaction time is a more accurate measure for the

central tendency of the reaction time distribution for this task, because the mean reaction time is more sensitive to extreme values at the high ends of the probability distribution which contained fewer trials (Farovik, 2007; O'Neill, 2005).

To show the cost/benefit of varying the spatial probability, the reaction time on the left side was subtracted from the reaction time on the right side for each foreperiod.

Statistical analyses. All analyses were conducted using IBM SPSS (v 21, SPSS Inc., Chicago, IL). The criterion for significance (alpha level) was $p < .05$ in all cases.

Given the difference in motor responses required to perform the task between humans and rats (i.e., having to move a finger to respond for humans vs. having to move the entire body to respond for rats) the reaction times would not be equivalent for comparison. Therefore, the data for each group were analysed separately but the pattern of performance of the task was compared.

A two factor, 2 x 5 within-subjects analysis of variance (ANOVA) with the variables of Side (Within-subjects factor with 2 levels: Left vs. Right) and Foreperiod (Within-subjects factor with 5 levels: 200, 300, 400, 500 or 600) was conducted to evaluate if there were significant differences between the two groups on each of the dependent measures. Sidak's-corrected pairwise comparisons for significant effects or interactions were performed. When data violated the assumption of sphericity, Huynh-Feldt corrections were reported.

3.3 Results

Reaction time (RT). Figure 3.3 shows the modal reaction time, across foreperiods, for humans and rats. The upper row shows the modal RT for right and left sides across foreperiods for humans (left column) and rats (right column). The lower row-left column shows the same data from the upper row subtracting the RT from the left side minus the right side to illustrate the

effect of conditional spatiotemporal probability (i.e., the probability of the stimulus on the right was lower at early foreperiods; but at later foreperiods, the probability of the stimulus on the right was greater). The negative values on the graph at shorter foreperiods indicate that reaction times on the left side were faster than on the right side (at shorter foreperiods, the stimuli were more probable on the left). Likewise, the positive values at longer foreperiods indicate that reaction times on the right side were faster than on the left side (at longer foreperiods, the right side stimuli became more probable). The lower row-right column shows the RT of the average of the two sides to illustrate the effect of foreperiod.

As foreperiod increased, reaction times were faster for both humans (main effect of Foreperiod: $F(4, 136) = 5.68$, $p = .004$, $\eta_p^2 = .14$) and for rats (main effect of Foreperiod: $F(4, 92) = 193.30$, $p < .001$, $\eta_p^2 = .89$). Faster RTs by foreperiod was greatest for targets on the right side for humans (Side x Foreperiod interaction: $F(4, 136) = 5.66$, $p = .002$, $\eta_p^2 = .14$) and for rats (Side x Foreperiod interaction: $F(4, 92) = 6.26$, $p = .01$, $\eta_p^2 = .21$). Although there was a trend, in both groups, showing faster RTs to the left side at shorter foreperiods and faster RTs to the right side at longer foreperiods (see Figure 3.3). Sidak's-corrected pairwise comparisons indicated that, for humans, RT to the right side was faster than to the left side at the longest foreperiod (FP 600; $p = .01$) and for rats, RT on the right side was faster than on the left side at longer foreperiods (FP 500; $p = .01$ and 600; $p = .01$). This showed that at the longest foreperiods both species had faster reaction times to the right side (i.e., the side where the target was more likely to appear).

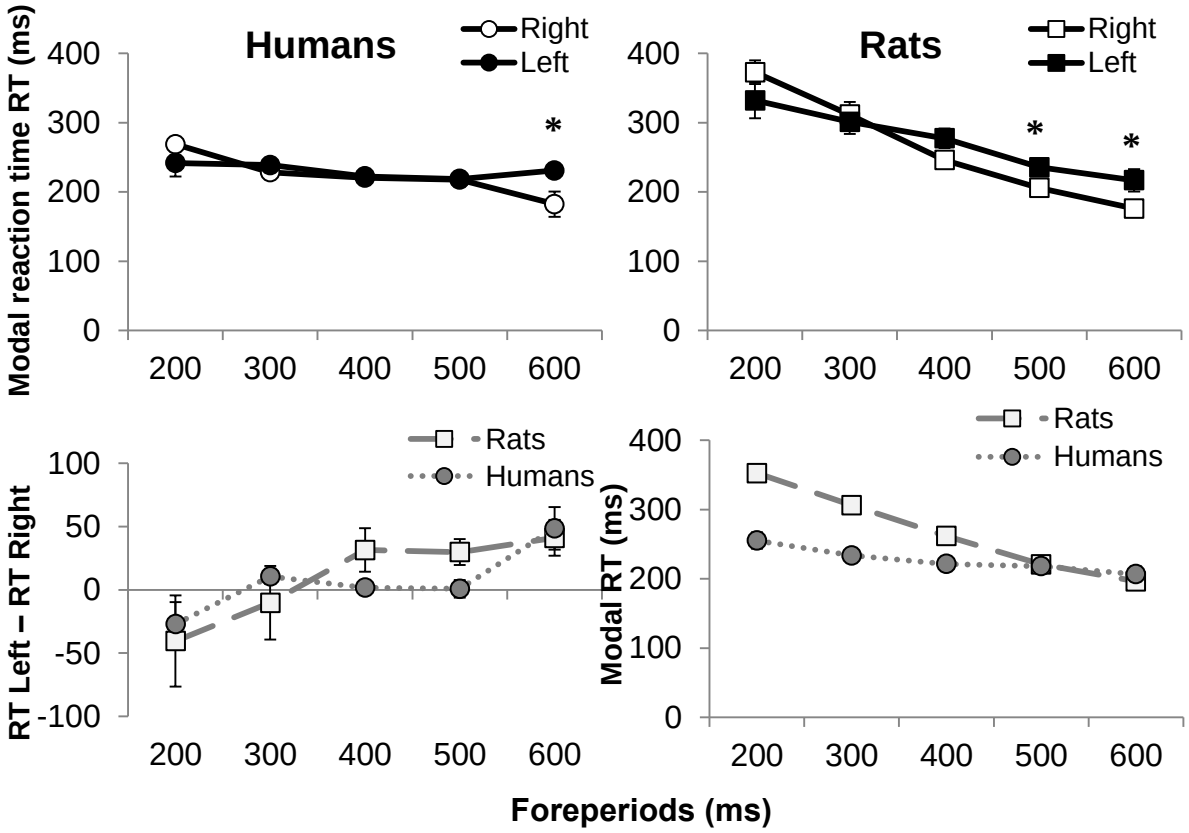

Figure 3.3. *Modal reaction time (±SEM) in milliseconds across foreperiods for rats and humans. The upper row shows the mode RT for right and left sides across foreperiods for humans (left column) and rats (right column). The lower row shows the difference in reaction time to the left side minus the right side (left column) and the average of the reaction time to the left and the right side (right column) across foreperiods for rats and humans. RT was predicted to be slower for right than left targets at shorter foreperiods (unexpected location), while faster for right than left targets at longer foreperiods (expected location). Humans and rats had faster reaction times on the right side at the longest foreperiod (FP 600); RTs were also faster to the right side at FP 500 for rats (***** $p < .05$).*

Movement time (MT). At shorter foreperiods (FP 200 and 300) humans had faster MTs to the right side in comparison to the left side; but at the longest foreperiod (FP 600) they were faster on the left side in comparison to the right side (Side x Foreperiod interaction: $F(4, 136) = 6.70$, $p < .001$, $\eta_p^2 = .17$). Overall, rats had faster MTs to the right side (main effect of Side: $F(1, 23) = 6.41$, $p = .02$, $\eta_p^2 = .22$), and except for the shortest foreperiod (FP 200), as foreperiod

increased, movement times were slower (main effect of Foreperiod: $F(4, 92) = 29.18$, $p < .001$, $\eta_p^2 = .56$).

Incorrect responses and late errors. Figure 3.4 shows the percentage of incorrect responses (upper row) and the percentage of late errors (lower row) for humans (left column) and rats (right column). Incorrect and late errors for humans were not affected by increasing the length of the foreperiod. In addition, there were no differences in incorrect or late errors between the left and the right side across the different foreperiods for humans. For rats, the percentage of incorrect responses was not affected by side; but incorrect responses increased as a function of lengthening foreperiod (main effect of Foreperiod: $F(4, 92) = 4.82$, $p < .001$, $\eta_p^2 = .17$). Sidak's-corrected pairwise comparisons indicated that there were fewer incorrect responses in the shortest foreperiod (FP 200) than in the longest foreperiod (FP 600; $p = .05$). The percentage of late errors was affected by side and foreperiod for rats (Side x Foreperiod interaction: $F(4, 82) = 3.63$, $p = .01$, $\eta_p^2 = .14$). Sidak's-corrected pairwise comparisons indicated that at the shortest foreperiod (FP 200) there were fewer late errors to the right side than to the left side ($p = .01$; see lower row-left column from Figure 3.4).

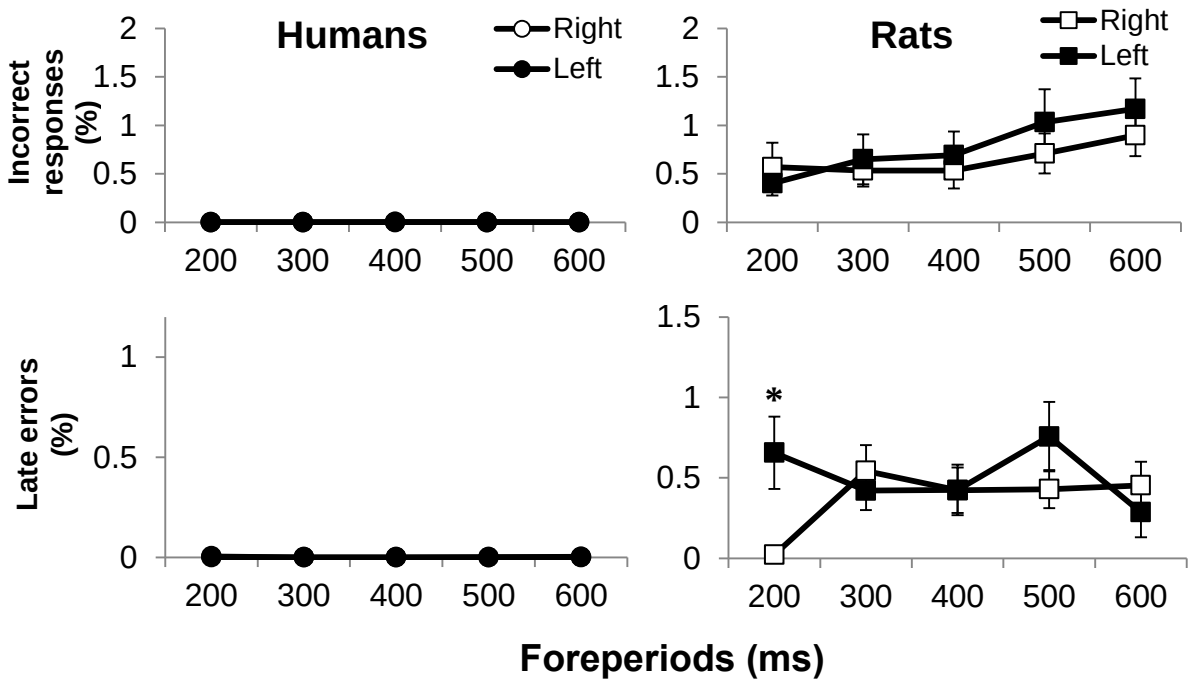

Figure 3.4. *Percentage of incorrect and late errors (±SEM), upper and lower rows respectively, for right and left sides across foreperiods for humans (left column) and rats (right column). Rats had less late errors to the right side, in comparison to the left side at the FP 200 (***** $p < .05$).*

Anticipatory errors. Figure 3.5 shows that anticipatory errors increased as a function of lengthening foreperiod. For humans (main effect of Foreperiod: $F(4, 136) = 76.01$, $p < .001$, $\eta_p^2 = .70$), Sidak's-corrected pairwise comparisons indicated that the foreperiod (FP) 200 had fewer anticipatory errors than FP 400, 500 and 600; FP 300 had fewer anticipatory errors than FP 400, 500 and 600; FP 400 had fewer anticipatory errors than FP 500 and 600, and FP 500 had fewer anticipatory errors than FP 600 (see Table 3.2). For rats (main effect of Foreperiod: $F(4, 92) = 10.74$, $p < .001$, $\eta_p^2 = .32$), Sidak's-corrected pairwise comparisons indicated that FP 200 had fewer anticipatory errors than FP 400, 500; and FP 600 had more anticipatory errors than all the other foreperiods (see Table 3.3).

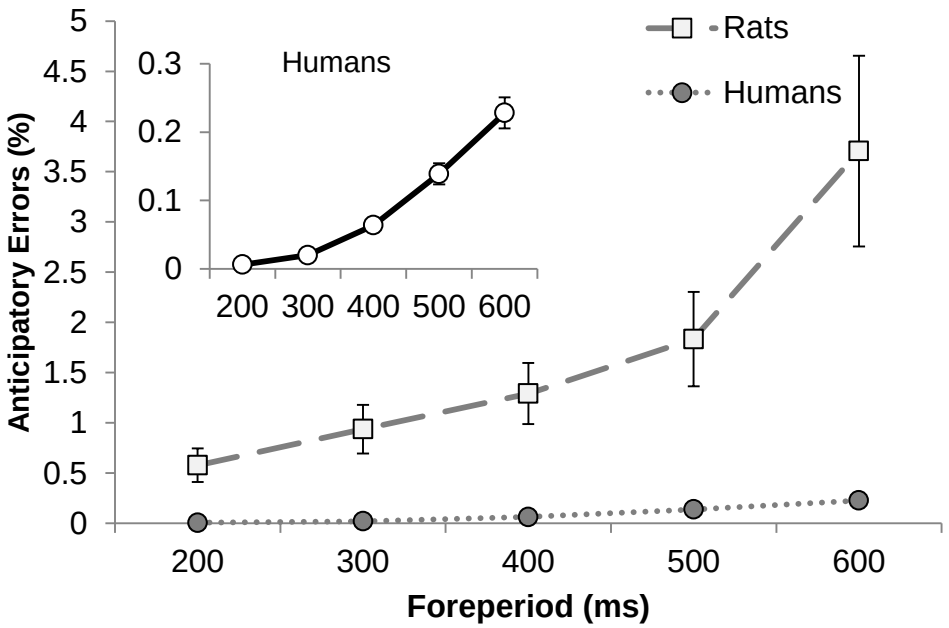

Figure 3.5. *Percentage of anticipatory errors (±SEM) across foreperiods for humans and rats. Insert shows an expanded y-axis scale for the human data. Anticipatory responses increased as foreperiods increased.*

Table 3.2.
Foreperiod Sidak's-corrected pairwise comparisons for anticipatory errors for humans.

Foreperiod	**FP 200**	**FP 300**	**FP 400**	**FP 500**	**FP 600**
	M =.01	*M* =.02	*M* =.06	*M* =.14	*M* =.23
FP 200		.080	**.000***	**.000***	**.000***
FP 300			**.000***	**.000***	**.000***
FP 400				**.000***	**.000***
FP 500					**.000***
FP 600					

Note. ***** The mean difference is significant at the .05 level.

Table 3.3.
Foreperiod Sidak's-corrected pairwise comparisons for anticipatory errors for rats.

Foreperiod	FP 200	FP 300	FP 400	FP 500	FP 600
	M =.58	*M* =.94	*M* =1.29	*M* =1.89	*M* =3.71
FP 200		.55	**.01***	**.03***	**.02***
FP 300			.62	.20	**.03***
FP 400				.57	**.04***
FP 500					**.02***
FP 600					

Note. ***The mean difference is significant at the .05 level

3.4 Discussion

The purpose of this study was to develop an implicit memory task that could be performed by rats and humans. Having a task in which rats and humans show similar response patterns will allow the investigation of animal models of neurodegenerative diseases, such as HD, in which implicit memory is impaired. The performance of rats and humans was compared on a modified reaction time task where the location of the target (spatial probability) changed as a function of the length of the foreperiod (temporal probability). Hence, at shorter foreperiods targets on the left side were more probable than targets on the right side, but as the foreperiods increased the targets were more likely to appear on the right side than on the left side.

One of the main measurements of the task was reaction time, if implicit learning occurred, it was expected that during the task, the reaction time of subjects would gradually become faster on the left side at shorter foreperiods, and have faster reaction times on the right

side at longer foreperiods (i.e., the side were the target was more likely to appear). First, both, rats and humans showed faster reaction times at longer foreperiods, suggesting that both species were sensitive to the conditional temporal probability. That is, if a target stimulus did not appear at the first foreperiod, the probability of the target appearing on the next foreperiod increased, this continued for the following foreperiod, until at the last foreperiod, when, if the target had not, yet appeared, the probability of the stimulus appearing was 100%. Thus, as the probability of the stimulus to appear increased with time, reaction times got faster. These result are consistent with previous studies that observed the same effect in simple reaction time tasks in humans (Näätänen, 1970) and in rats (Brown and Robbins, 1991; Carli et al., 1989), and may reflect processes of response preparation or motor readiness (i.e., the acceleration of reaction time of responses as the delay elapses). Second, reaction times were faster on the right side at the longest foreperiod (the side where the stimuli were more likely to appear), suggesting that both rats and humans were able to learn that at longer foreperiods, the stimuli were more likely to appear on the right side.

Although, humans had fewer anticipatory responses in comparison to rats, the pattern of anticipatory responses of both groups was similar. Both, rats and humans had more anticipatory responses as the foreperiods increased, which suggested that both species were sensitive to the conditional temporal probability. But in this case, motor readiness resulted in premature responses rather than an improved performance. That is, as the probability of the stimulus to appear increased with time, the tendency to respond before the stimulus (anticipatory responses) also increased.

Even though rats and humans showed a similar pattern in reaction time and anticipatory responses in the Spatiotemporal Target Probability Signal Reaction Time Task, there were

differences in their performances during the task. For example, humans did not make incorrect responses or late errors. Nevertheless, incorrect responses for rats increased as foreperiod increased, which suggested that rats were sensitive to the conditional temporal probability, and motor readiness resulted in premature responses rather than an improved performance. In addition, there were no differences between the left and the right side on the number of incorrect responses. It would have been expected that there would be more incorrect responses to the side where the stimuli were more likely to appear (i.e., on the left at short foreperiods and on the right at long foreperiods); however, neither rats nor humans showed this pattern, which suggested that even though both species prepared their responses to respond to the stimuli as the foreperiod increased, responses depended on the location of the light. This was also corroborated by the late responses, as it would have been expected that the less probable location would have had more late responses (as it would take longer to move to the less expected location). However, rats showed a reduced number of late errors to the right side at the shortest foreperiod, which suggested that, even though their reaction times were slower for the right side at the shortest foreperiod, they were able to respond within 2 seconds on the illuminated hole, regardless of whether they were expecting the stimuli to appear on that side.

In summary, the present results showed that rats and humans can learn the Spatiotemporal Target Probability Signal Reaction Time Task efficiently. Although the performance of humans and rats was different in the percentage of incorrect responses and on late errors (i.e., humans did not make incorrect responses or late errors), both groups showed a similar pattern in reaction times. Both rats and humans had faster reaction times to the right side at longer foreperiods (i.e., the side where the stimulus was more likely to appear), which indicates that with practice both species were able to learn that the probability of the stimulus appearing on the left side was more

likely at shorter foreperiods, and at longer foreperiods, the probability of the stimulus appearing on the right side was more likely. Thus, the Spatiotemporal Target Probability Signal Reaction Time Task provides an effective basis for evaluating implicit learning in rats and humans. In addition, the task could facilitate the assessment of short acting treatments in pharmacological studies and the effects of lesions in rodents, these were investigated in Chapters 4 and 5 respectively.

Chapter 4

Selective Effects of D_1 or D_2 Dopamine Receptor Antagonism in the Spatiotemporal Target Probability Signal Reaction Time Task in Rats

Previous studies have reported a loss of striatal D_1 and D_2 dopamine receptors in HD. The purpose of the experiment presented in this chapter was to investigate the effects of D_1 dopamine receptor antagonist SCH-23390 or D_2 dopamine receptor antagonist raclopride on the execution of a reaction time task that evaluated implicit memory by manipulating the probabilities of the location of the stimuli as a function of the length of the foreperiod.

Is implicit memory (the computation of probabilities to predict the location of a stimulus) affected by selective blockade of dopaminergic transmission at the D_1 or D_2 receptors?

Is the performance on the Spatiotemporal Target Probability Signal Reaction Time Task affected differently by D_1 dopamine receptor antagonist SCH-23390 or D_2 dopamine receptor antagonist raclopride?

4.1 Introduction

The previous chapter presented the Spatiotemporal Target Probability Signal Reaction Time Task, a new reaction time task that requires the computation of probabilities to predict the location of the stimuli. At short foreperiods, stimuli were more likely to appear on the left side, than on the right side; however, as the foreperiods length increased, the probability of the stimuli appearing on the left side decreased and the stimuli became more likely to appear on the right side. Rats and humans showed faster reaction times at longer foreperiods on the right side (i.e., the side where the stimuli were more likely to appear), which suggests that both species were able to learn that the probability of the location of the stimuli changed as function of the length of the foreperiod.

This chapter investigated whether and how a selective blockade of dopamine transmission by means of systemically administered receptor antagonists would affect the performance of the Spatiotemporal Target Probability Signal Reaction Time Task. Previous studies have shown that reaction times are sensitive to the blockade of dopamine receptors, and it has been suggested that D_1 and D_2 receptors have different effects in reaction time tasks (Amalric et al., 1993; Baunez et al., 1995; Courtière et al., 2003; Domenger and Schwarting, 2006; Mayfield et al., 1993). For example, while it has been reported that D_2 antagonism increases reaction times (Amalric et al., 1993; Baunez et al., 1995; Courtière et al., 2003), the effects of D_1 antagonism are not clear: some experiments have reported no effect on reaction time after blocking the D1 receptor (Amalric et al., 1993), while others have reported slower reaction times (Courtière et al., 2003) or even decreased response latencies (Mayfield et al., 1993). Therefore, the functional differences between D_1 and D_2 receptors in reaction time tasks are not clear and should be investigated further. In particular and for the aim of this thesis, it has been suggested that the differential loss

and dysfunction of the neurons that express these two dopamine receptors may contribute to the different symptoms of HD (see sections 1.4.1.1 and 1.8.1.1 from the General Introduction). For example, HD patients and preclinical carriers of HD show cognitive deficits induced by reductions in D_1 and D_2 receptor density in the striatum (Backman et al., 1997; Berent et al., 1988; Bohnen et al., 2000; Brandt et al., 1990; Ginovart et al., 1997; Lawrence et al., 1998b). Based on these studies, it was hypothesised that selective blockade of dopaminergic transmission at the D_1 or the D_2 receptor would affect the performance on a reaction time task, which require the computation of probabilities to predict the location of the stimuli. Future research in HD could benefit by incorporating a cognitive task that detects differences between the loss of striatal D_1 and D_2 receptors to evaluate the effects of new treatments. Thus, this experiment investigated if the performance on the Spatiotemporal Target Probability Signal Reaction Time Task was differently affected by dopamine D_1 receptor antagonist SCH-23390 or D_2 receptor antagonist raclopride.

4.2 Method

4.2.1 Animals

The subjects were 8 experimentally-naïve male hooded Lister rats (Charles River, UK Ltd) with a mean *ad libitum* weight of 303 g (range = 289 - 315 g) at the beginning of the experiment. The housing and husbandry conditions were described in the General Methods in Chapter 2.

4.2.2 Apparatus

The apparatus were described in the Methods section of Chapter 3.

4.2.3 Procedure

Rats were tested in the Spatiotemporal Target Probability Signal Reaction Time Task. Training and testing procedures were described in the Methods section of Chapter 3 within the "Behavioural protocol for rats" subsection. Briefly, rats had to respond to lateralised visual stimuli in order to receive food. Rats were required to sustain a nose poke in the central hole until a stimulus on the left or the right side hole appeared after one of the 5 different foreperiods. Foreperiods could have durations of 200, 300, 400, 500 or 600 ms and were followed by a light stimulus of 100 ms on either left or right side. At the short foreperiods, the stimuli were more likely on the left side, but as foreperiods increased, presentation of the stimuli became more likely on the right side (see figure 4.1).

After 116 sessions in the Spatiotemporal Target Probability Signal Reaction Time Task, rats were able to complete 120 correct trials within 45 min.

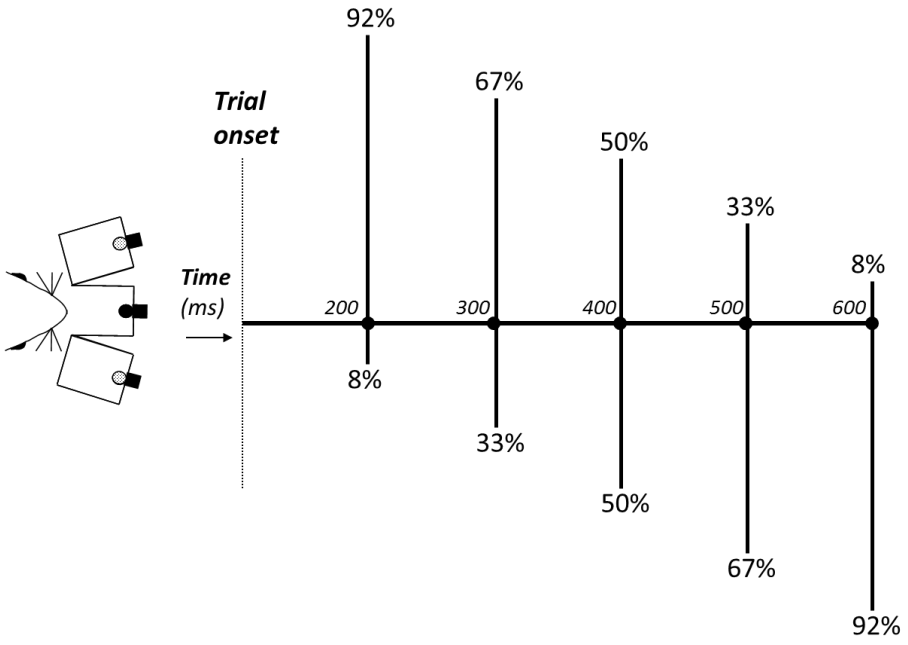

Figure 4.1. *The probability of the stimuli on the left was more likely on short foreperiods; but on longer foreperiods, the probability of the stimuli on the right was more likely. (Figure based on O'Neill, 2005)*

Drug administration. Rats were pseudo-randomly divided into two groups (each group contained four rats). On separate sessions, the first group received one of three doses (0.005, 0.01 and 0.02 mg/kg) of D_1 antagonist receptor SCH-23390 (Sigma Aldrich, UK) or vehicle (0.9% saline) via subcutaneous (SC) injections. The second group received one of three doses (0.05, 0.1 and 0.2 mg/kg) of D_2 antagonist raclopride (Sigma Aldrich, UK) or its vehicle (0.9% saline) via SC injections. Each injection day was followed by one testing day in which no drug was administered. Both drugs were freshly prepared on the day of injection (dissolved in 0.9% saline vehicle and protected from light). The different doses were administered in a semi-randomised order 30 min prior to testing in the Spatiotemporal Target Probability Signal Reaction Time Task (each drug session assessed the three different doses and vehicle). Once all the subjects received the different doses (three drug doses and vehicle) of the same drug, they

were tested for one day with no injection. Finally, the groups were tested in the alternative drug condition (i.e., the first group that received SCH-23390 was tested with the different doses of raclopride and vice versa) using the same protocol. The DA antagonist compounds (i.e., SCH-23390 and raclopride), doses, and method of administration used in this experiment were selected based on Amalric et al. (1993), which have also been previously tested in our laboratory (O'Neill, 2005).

4.2.4 Data Analysis

Data analyses were the same as described in Chapter 3. All analyses were conducted using IBM SPSS (v 21, SPSS Inc., Chicago, IL). The criterion for significance (alpha level) was $p < .05$ in all cases. The data for each drug were analysed separately with a three factor, 4 x 2 x 5 repeated measures ANOVA with Dose, Side and Foreperiod as the within-subject factors. Sidak's-corrected pairwise comparisons for significant effects or interactions were performed. When data violated the assumption of sphericity, Huynh-Feldt corrections were reported.

4.3 Results

Given that the foreperiod and target side were determined on a trial-by-trial basis according to *a priori* probabilities, there was not always precisely the same number of trials in every session. Therefore, animals with incomplete data sets were discarded from the experiment. The final numbers (n) in each group were: SHC-23390 ($n = 7$), raclopride ($n = 7$).

4.3.1 Raclopride

Reaction time (RT). Figure 4.2 shows the effect of raclopride on the modal reaction times across foreperiods. Raclopride slowed reaction times (main effect of Dose: $F(3, 18) = 10.61$, $p < .001$, $\eta_p^2 = .64$. Sidak's-corrected pairwise comparisons indicated that the three doses

of raclopride were different from the vehicle (vehicle vs 0.05 mg/kg, p = .01; vehicle vs 0.1 mg/kg, p = .03 and vehicle vs 0.2 mg/kg p = .003). As foreperiod increased, reaction times were faster (main effect of Foreperiod: $F(4, 24) = 36.74$, $p < .001$, $\eta_p^2 = .86$. Sidak's-corrected pairwise comparisons indicated that the foreperiod (FP) 400 had faster RT than FP 200 and 300; FP 500 had faster RT than FP 200 and 300; FP 600 had faster RT than FP 200, 300 and 400; see Table 4.1). There was a pattern of faster reaction times to the left side at shorter foreperiods and faster reaction times to the right at longer foreperiods (Side x Foreperiod interaction: $F(4, 24)$ 6.02, p = .002, η_p^2 = .50). This is observed on the left column of Figure 4.2, where negative values represent faster reaction times on the left side and positive values represent faster reaction times on the right side. Sidak's-corrected pairwise comparisons indicated that at the shortest foreperiod (FP 200) reaction times were faster on the left side (p = .02), the more probable target location.

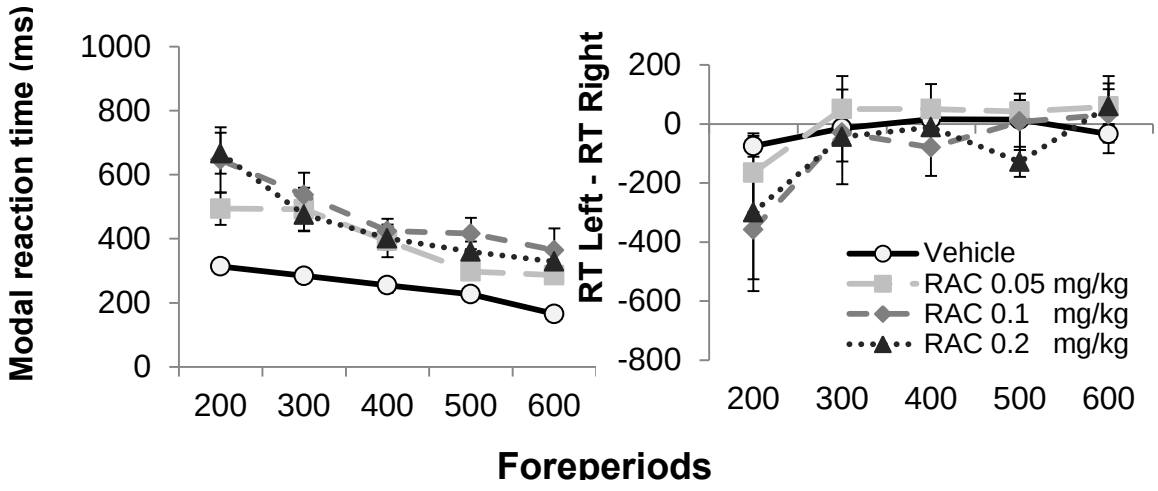

Figure 4.2. *The left column shows the effects of raclopride on modal reaction time (± SEM); in comparison to vehicle, all doses of raclopride increased RT. The right column depicts the same information by side subtraction, which reflects the cost/benefit of conditional spatiotemporal probability. At shorter foreperiods the subtracted values were negative, as RT was slower on the right compared to the left side.*

Table 4.1.
Foreperiod Sidak's-corrected pairwise comparisons for reaction time.

Foreperiod	**FP 200**	**FP 300**	**FP 400**	**FP 500**	**FP 600**
	M =530	*M* =447	*M* =369	*M* =325	*M* =286
FP 200		.482	**.019***	**.003***	**.001***
FP 300			**.017***	**.009***	**.000***
FP 400				.436	**.006***
FP 500					.172
FP 600					

Note. *****The mean difference is significant at the .05 level.

Movement time (MT). Movement time was not affected by raclopride.

Incorrect responses and late errors. The percentage of incorrect responses and late errors were not affected by side, foreperiod or raclopride.

Anticipatory errors. Figure 4.3 shows the effect of raclopride on the percentage of anticipatory errors across foreperiods. Anticipatory errors increased as foreperiods increased (main effect of Foreperiod: $F(4, 24) = 5.50$, $p = .003$, $\eta_p^2 = .48$). Raclopride decreased the percentage of anticipatory errors at the longest foreperiods, but at the shortest foreperiods, there were no differences in anticipatory errors between the different doses of raclopride (Dose x Foreperiod interaction: $F(12, 72)$ 2.50, $p = .01$, $\eta_p^2 = .29$. Sidak's-corrected pairwise

comparisons indicated that at the longest foreperiod (FP 600) anticipatory errors decreased with dose 0.1 mg/kg in comparison to vehicle).

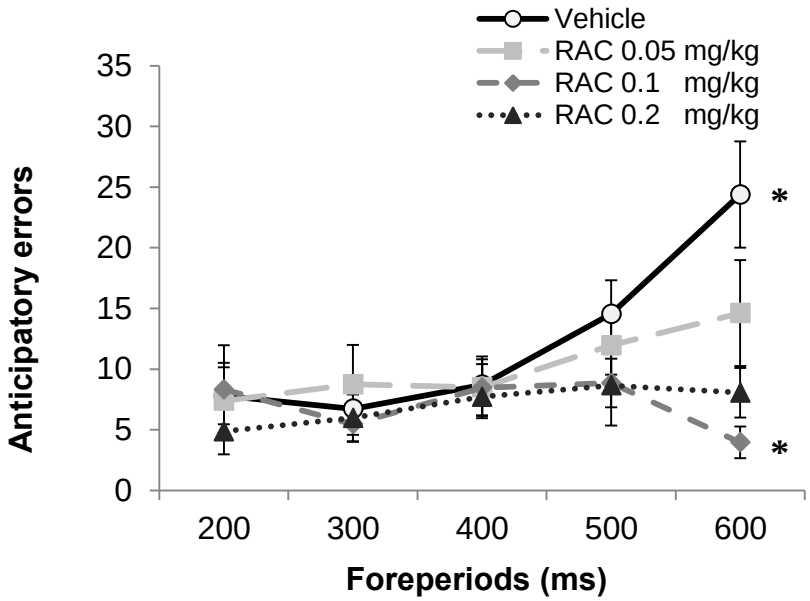

Figure 4.3. *Effect of raclopride on the percentage of anticipatory errors (±SEM) across foreperiods. At the longest foreperiod (FP 600) anticipatory errors decreased with dose 0.1 mg/kg (***** $p < .05$).*

4.3.2 SCH-23390

Reaction time (RT). Figure 4.4 shows the effect of SCH-23390 on the modal reaction times across foreperiods. Reaction times were slower as a function of increasing the doses of SCH-23390 (main effect of Dose: $F(3, 18) = 7.74$, $p = .002$, $\eta_p^2 = .56$. Sidak's-corrected pairwise comparisons indicated that, in comparison to vehicle, 0.01 mg/kg of SCH-23390 slowed RT, $p = .02$). Overall, as foreperiod increase, reaction time were faster (main effect of Foreperiod: $F(4, 24) = 6.90$, $p = .001$, $\eta_p^2 = .54$. Sidak's-corrected pairwise comparisons indicated that FP 600 had faster reaction times than FP 200, 300 and 400; $p = .05$, $p = .05$ and $p = .03$ respectively).

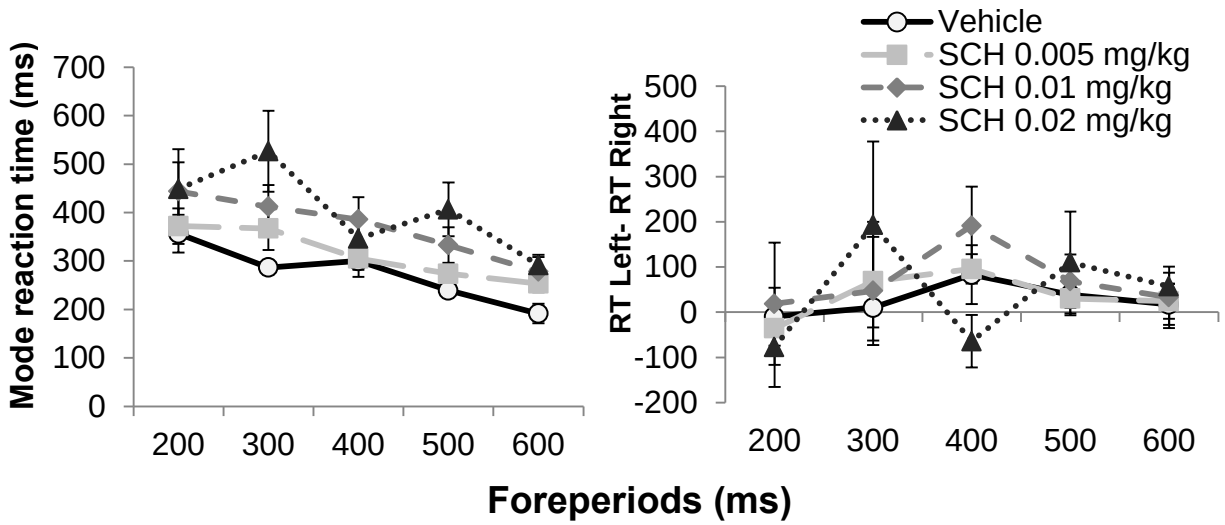

Figure 4.4. *The left column shows the effects of SCH-23390 on modal reaction time (± SEM); in comparison to vehicle, dose 0.01 mg/kg increased reaction times. The right column depicts the same information by side subtraction, which reflects the cost/benefit of conditional spatiotemporal probability.*

Movement time (MT). Figure 4.5 shows the effect of SCH-23390 on movement time (latency from the withdrawal of the nose from the central hole, to the nose poke in either side hole) across foreperiods. MTs were slower as the doses of SCH-23390 increased (main effect of Dose: $F(3, 18) = 6.08$, $p = .01$, $\eta_p^2 = .50$; Sidak's-corrected pairwise comparisons indicated that, in comparison to vehicle, 0.005 and 0.01 mg/kg of SCH-23390 slowed MT; $p = .03$ and $p = .04$ respectively). With vehicle, movement time was faster at shorter foreperiods to the left side (more probable target location) compared to the right side, and at longer foreperiods, MT was faster to the right side (more probable target location) compared to the left side. However, with SCH-23390 at shorter foreperiods, MT was faster to the right side (less probable target location) compared to the left side, and at longer foreperiods, MT was faster to the left side (less probable target location) compared to the right side (Dose x Side x Foreperiod interaction: $F(12, 72)$ 1.91, $p = .05$, $\eta_p^2 = .24$. Sidak's-corrected pairwise comparisons showed that at FP 300, MT was faster

to the left side with vehicle (p = .03; more probable location), but with 0.01 mg/kg of SCH-23390, MT was faster to the right side (p = .04; less probable location) and at FP 200 with 0.02 mg/kg of SCH-23390, MT was faster to the right side (p = .01; less probable location).

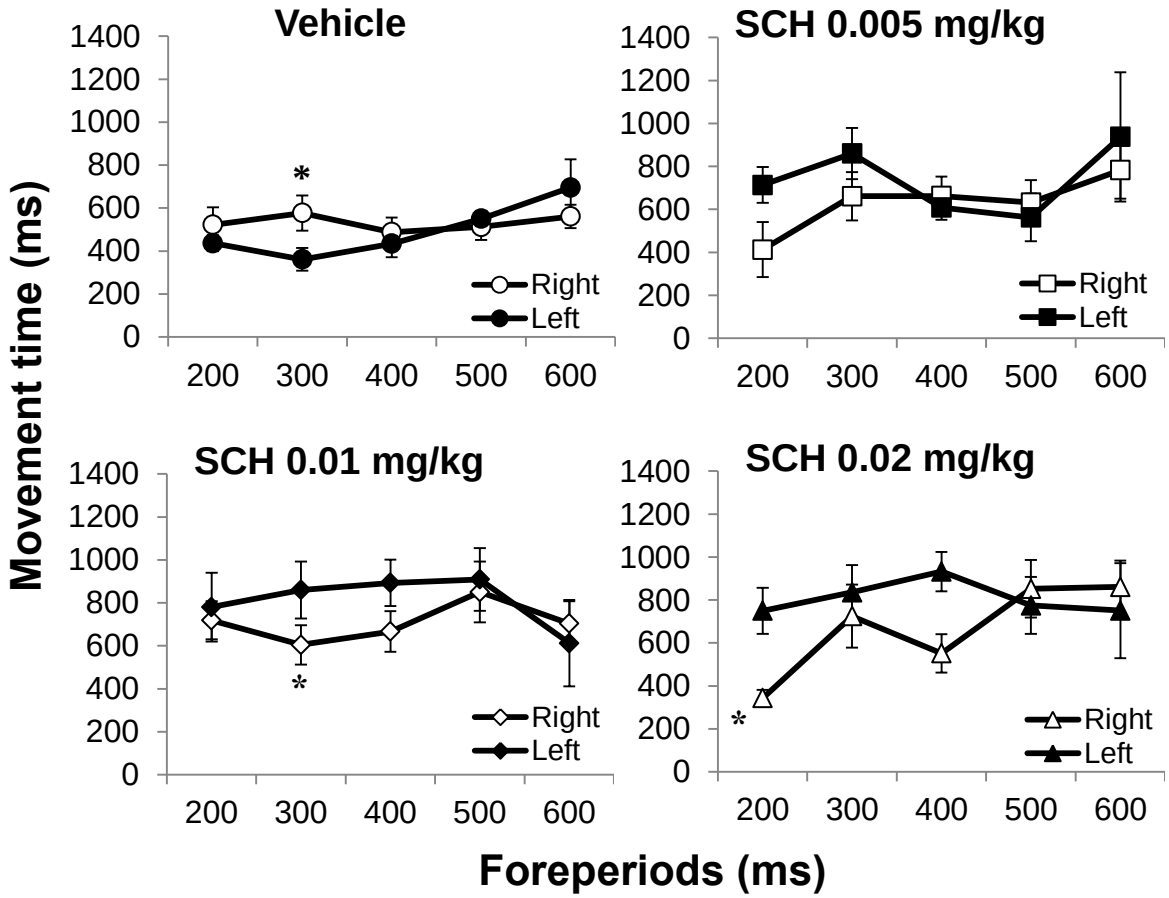

Figure 4.5. *Effects of SCH-23390 on movement time (±SEM) across foreperiods. The upper row-left column shows movement time with vehicle, while the movement time with the different doses of SCH-23390 (0.005, 0.01 and 0.02 mg/kg) are shown in the upper row-right column, lower row-left column and lower row-right column respectively. At FP 300, MT was faster to the left side with vehicle (more probable location), but with 0.01 mg/kg of SCH-23390, MT was faster on the right side (less probable location) and at FP 200 with 0.02 mg/kg of SCH-23390, MT was faster on the right side (* $p < .05$).*

Incorrect responses. Figure 4.6 shows the effect of SCH-23390 on the percentage of incorrect responses across foreperiods. The upper row-left column shows the percentage of incorrect responses with vehicle, while the different doses of SCH-23390 (0.005, 0.01 and 0.02

mg/kg) are shown in the upper row-right column, lower row-left column and lower row-right column respectively. With vehicle, at shorter foreperiods, when the stimulus was more likely to appear on the left, there were more incorrect responses on the right side (less probable target location) compared to the left side, and at longer foreperiods, when the right side stimulus became more likely to appear, there were more incorrect responses on the left side. However, 0.005 mg/kg of SCH-23390 reduced the percentage of incorrect responses to the right side at the shortest foreperiod (FP 200; p = .03), while 0.02 mg/kg of SCH-23390 reduced incorrect responses on the right side at FP 300 (p = .05; Dose x Side x Foreperiod interaction: F(12, 72) 2.07, p = .03, η_p^2 = .26).

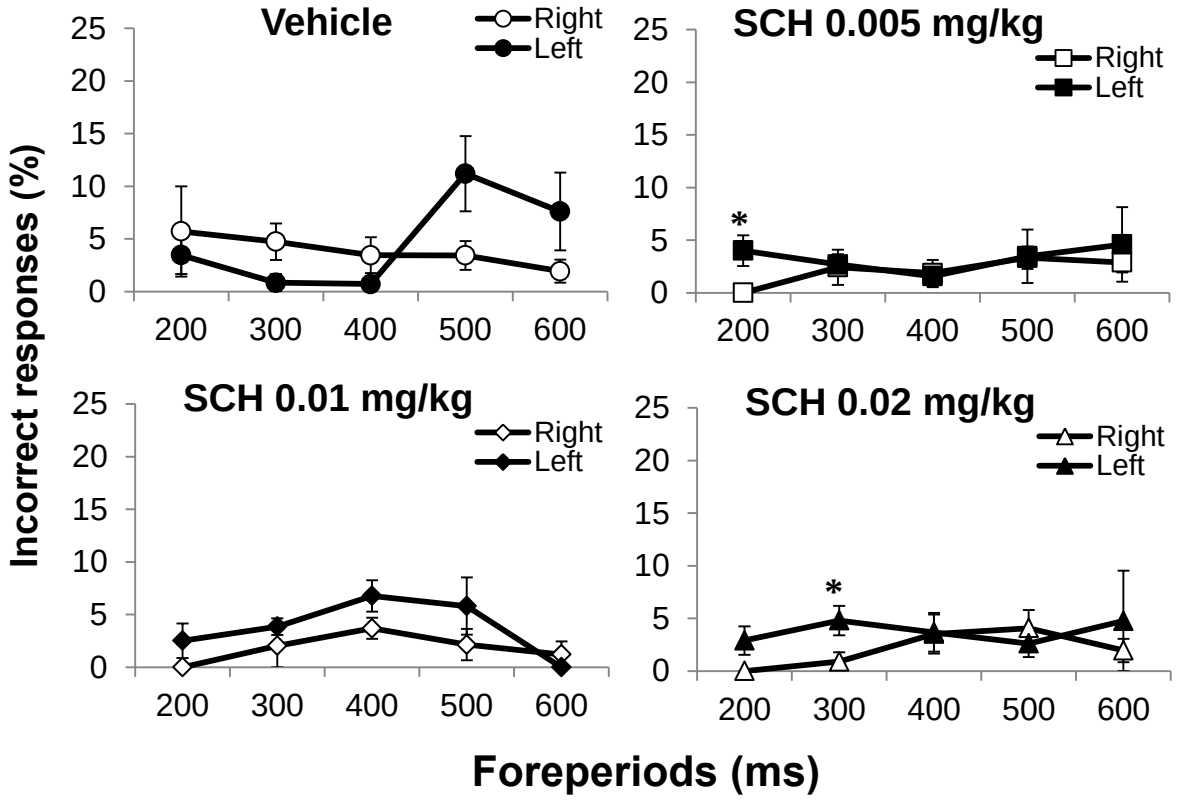

Figure 4.6. *Effects of SCH-23390 on the percentage of incorrect responses (±SEM) across foreperiods. The upper row-left column shows the percentage of incorrect responses with vehicle, while the percentage of incorrect responses with the different doses of SCH-22390 (0.005, 0.01 and 0.02 mg/kg) are shown in the upper row-right column, lower row-left column and lower row-right column respectively. Incorrect responses on the right side decreased at the shortest foreperiod (FP 200) with 0.005 mg/kg of SCH-23390 and at FP 300 with 0.02 mg/kg of SCH-23390 (* $p < .05$).*

Late errors. Figure 4.7 shows the effect of SCH-23390 on the percentage of late errors across foreperiods. SCH-23390 increased the percentage of late errors (main effect of Dose: $F(3, 18) = 6.79$, $p = .003$, $\eta_p^2 = .53$. Sidak's-corrected pairwise comparisons indicated that, in comparison to the vehicle, doses 0.01 and 0.02 mg/kg of SCH-23390 increased the percentage of late errors; $p = .05$ and $p = .01$ respectively). Overall, at shorter foreperiods there were fewer omissions of responses to the right side (less probable location) compared to the left side, and at longer foreperiods, there were fewer omissions to the left side (less probable location) compared

to the right side (Side x Foreperiod interaction: $F(4, 24)$ 4.45, p = .01, η_p^2 = .43. Sidak's-corrected pairwise comparisons indicated that at the shortest foreperiod (FP 200) there were fewer late errors to the right side in comparison to the left side, p = .03).

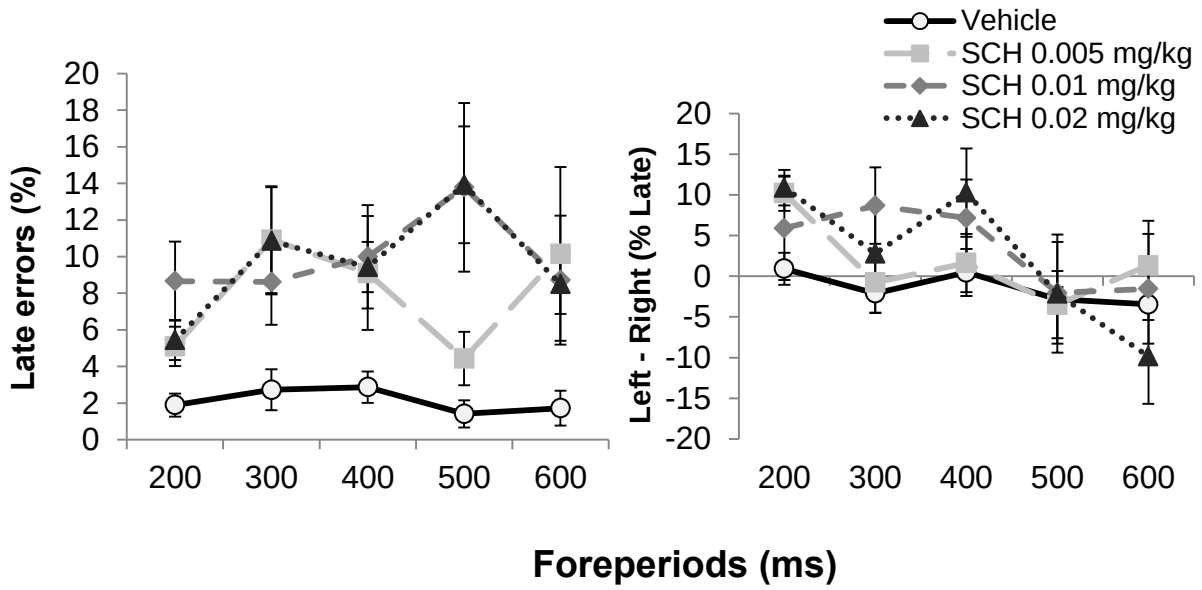

Figure 4.7. *Effect of SCH-23390 on the percentage of late errors (±SEM) across foreperiods. The left column shows the effects of SCH-23390 the percentage of late errors (± SEM); in comparison to vehicle, doses 0.01 and 0.02 mg/kg of SCH-23390 increased the percentage of late errors. The right column depicts the same information by side subtraction. At shorter foreperiods the subtracted values were positive, as there were more late errors to the right side compared to the left side, and at longer foreperiods the values were negative as there were more late errors to the left side compared to the right side.*

Anticipatory errors. Figure 4.8 shows the effect of SCH-23390 on the percentage of anticipatory errors across foreperiods. Anticipatory errors increased as foreperiods increased (main effect of Foreperiod: $F(4, 24)$ = 4.19, p = .01, η_p^2 = .41). SCH-23390 increased the percentage of anticipatory errors at shorter foreperiods, but at the longest foreperiod reduced them (Dose x Foreperiod interaction: $F(12, 72)$ 2.07, p = .03, η_p^2 = .26. Sidak's-corrected pairwise comparisons indicated that, in comparison to vehicle, all doses of SCH-23390 increased

anticipatory errors at foreperiod 400. Vehicle vs 0.005 mg/kg, p = .05; Vehicle vs 0.01 mg/kg, p = .01; Vehicle vs 0.02 mg/kg, p = .02).

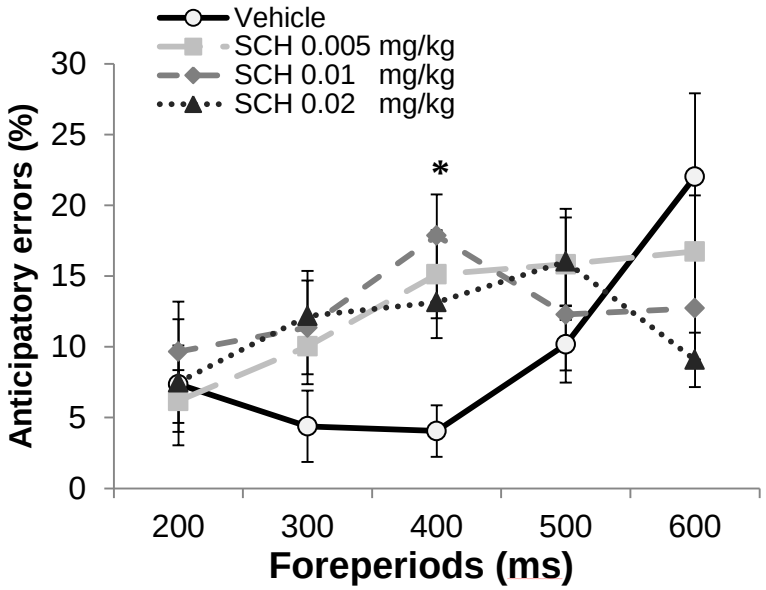

Figure 4.8. *Effect of SCH-23390 on the percentage of anticipatory errors (±SEM) across foreperiods. In comparison to vehicle, all doses of SCH-23390 increased anticipatory errors at foreperiod 400 (* $p < .05$).*

4.4 Discussion

The aim of the present experiment discussed in this chapter was to test the effects of the D_1 dopamine receptor antagonist SCH-23390 (in doses of 0.005, 0.01 and 0.02 mg/kg) or the D_2 dopamine receptor antagonist raclopride (in doses 0.05, 0.1 and 0.2 mg/kg) on the execution of the Spatiotemporal Target Probability Signal Reaction Time Task, a new reaction time task that requires the computation of probabilities to predict the location of the stimuli. At short foreperiods, stimuli were more likely to appear on the left side, than on the right side; however,

as the foreperiods length increased, the probability of the stimuli appearing on the left side decreased and the stimuli became more likely to appear on the right side.

As previously observed, *reaction times* were faster as a function of lengthening foreperiod. D_1 antagonist SHC-23390 or D_2 antagonist raclopride did not affect this pattern. However, reaction times were faster on the left side at the shortest foreperiod (i.e., the side were the target was more likely to appear) only after administration of raclopride. In addition, while all doses of raclopride (0.05, 0.1 and 0.2 mg/kg) slowed reaction times, only one of the doses of SCH-23390 (0.01 mg/kg) slowed reaction times in comparison to vehicle. The highest dose of SCH-23390 (0.02 mg/kg) may appear to slow reaction times in comparison to vehicle; however, with the large variability in the responses, the effect at this dose was not significant. These results may be explained as a result of the sedative effects (Christensen et al., 1984; Gessa et al., 1985; Hoffman and Beninger, 1985) as well as catalepsy (Christensen et al., 1984; Morelli and Di Chiara, 1985) that have been reported with high doses of SCH-23390.

Movement times, as well as incorrect and late errors, were differently affected by blocking D_1 and D_2 receptors. While raclopride had no effect on movement time, incorrect responses or late errors, these were affected by SCH-23390. *Movement time* (latency from the withdrawal of the nose from the central hole, to the nose poke in either side hole) was faster to the left side at FP 300 (the more probable side location) with vehicle, but with 0.01 mg/kg of SCH-23390, MT was faster to the right side at FP 300 (the less probable location), and at FP 200 with 0.02 mg/kg of SCH-23390, MT was faster to the right side (less probable location). This finding suggests that SCH-23390 reduced the response preparation bias to the more probable side at shorter foreperiods.

With vehicle, there were more *incorrect responses* to the right side at shorter foreperiods (the less probable target location), and at longer foreperiods, when the right side stimulus became more likely to appear, there were more incorrect responses to the left side. However, 0.005 mg/kg of SCH-23390 reduced the percentage of incorrect responses to the right side at the shortest foreperiod (FP 200), while 0.02 mg/kg of SCH-23390 reduced incorrect responses to the right side at FP 300. This suggests that SCH-23390 reduce incorrect responses to the least probable target location. SCH-23390 (0.01 and 0.02 mg/kg) increased *late errors*, which suggests that blocking D_1 receptors impaired the rat's ability to initiate responding. Therefore, the time to respond to any side hole took more than 2 seconds after successfully removing the nose from the central hole when the target stimuli were onset.

D_1 and D_2 antagonists had different effects on *anticipatory errors*; all doses of SCH-23390 significantly increased the anticipatory responses at FP 400. However, raclopride (0.1 mg/kg) decreased the number of anticipatory responses at the longest foreperiod (FP 600) in comparison to vehicle. Showing that with raclopride rats were more likely to respond until the presentation of the stimuli, yet they were able to use the information of the length of the foreperiod to respond.

Slowed reaction times as a result of antagonising D_2 dopamine receptors have been previously reported using the D_2 antagonist raclopride (Amalric et al., 1993; Baunez et al., 1995; Domenger and Schwarting, 2006; O'Neill, 2005), the D_2 antagonist eticlopride (Courtière et al., 2003), and high doses (0.1 mg/kg) of the D_2 antagonist haloperidol (Mayfield et al., 1993). However, it has been reported that low doses of the D_2 antagonist spiperone (0.001 mg/kg) and haloperidol (0.01 mg/kg) decrease response latencies (Mayfield et al., 1993). Likewise, there are contradictory results with regard to reaction times after blocking D_1 receptors. For example,

slowed reaction times have also been reported with the D_1 antagonist SKF83566 (Domenger and Schwarting, 2006). The D_1 antagonist SCH-23390 has been reported to also slow latencies (Mayfield et al., 1993) or slow reaction times at short foreperiods (Courtière et al., 2003), although no effect on reaction times has also been reported (Amalric et al., 1993). The results presented in this chapter showed that only all the doses of raclopride slowed the reaction times, which suggests that the D_2 antagonist might be more potent than the D_1 antagonist, a point which is in agreement with previous findings (Amalric et al., 1993; Courtière et al., 2003; Domenger and Schwarting, 2006; Mayfield et al., 1993).

The results regarding accuracy after blocking D_1 or D_2 receptors are also contradictory. While some studies report impairments, others report improvements or no effects (Amalric et al., 1993; Courtière et al., 2003; Domenger and Schwarting, 2006; Mayfield et al., 1993; O'Neill, 2005). Similar to the results of this chapter, Domenger and Schwarting (2006) found that a D_1 antagonist SCH-23390, but not the D_2 antagonist raclopride, increased accuracy in a RT task.

These inconsistencies in reaction times and accuracy can be attributed to the differences in methodology, including test paradigms, types and doses of DA antagonists, and methods of administration (e.g., intraperitoneal, IP, vs. subcutaneous, SC, injections). In the case of D_1, the ranges of doses that have been reported are broad, ranging from 0.005 to 0.15 mg/kg, IP or SC. In the case of D_2 antagonist, they have been reported from 0.01 to 0.20 mg/kg, IP (Amalric et al., 1993; Baunez et al., 1995; Courtière et al., 2003; Domenger and Schwarting, 2006; Mayfield et al., 1993). The DA antagonist compounds (i.e., SCH-23390 and raclopride), doses, and method of administration used in this experiment were selected based on Amalric et al. (1993), which have also been previously tested in our laboratory (O'Neill, 2005).

In summary, D_1 dopamine receptor antagonist SCH-23390 and D_2 dopamine receptor antagonist raclopride affected the performance of the Spatiotemporal Target Probability Signal Reaction Time Task differently. D_1 antagonist SCH-23390 slowed the ability to initiate responding, which slowed reaction times, increased late responses, and slowed movement times but reduced the percentage of incorrect responses to the least probable target location. These result could simply reflect a speed-accuracy trade off, since faster responses also produce relatively more errors, whilst reduced speed (like the one induced by DA antagonists) yield relatively fewer errors. In contrast, D_2 antagonist raclopride only slowed reaction times but did not affect movement time, incorrect responses or late errors, and decreased anticipatory responses. These results suggested that reaction times were slower with both SCH-23390 and raclopride, but only D_1 antagonist SCH-23390 reduced errors to the least probable target location.

Changes in cognitive performance induced by reduction of dopamine binding have also been observed in patients with HD. Brandt et al. (1990) showed that HD patients with reduced D_2 receptor binding in the caudate nucleus presented impairments in tasks that required rapid coordination and set alternation. Reductions in D_1 and D_2 receptor density in the striatum in HD patients have also been correlated with the severity of impairments of executive function, perceptual speed, visuospatial skill, verbal fluency, episodic memory, and reasoning (Backman et al., 1997). These correlations between reduced D_1 and D_2 receptor binding and cognitive deficits have also been reported in preclinical carriers of HD (Lawrence et al., 1998b; Lawrence et al., 1998c). Therefore, having a task that is differentially affected by D_1 or D_2 receptors could evaluate cognitive impairments in patients with HD (and preclinical carriers of the disease) and assess selective treatments.

Altogether, the results presented in this chapter have important implications for the therapeutic use of D_1 and D_2 antagonists in the Spatiotemporal Target Probability Signal Reaction Time Task. First, it suggested that the effects of D_1 and D_2 antagonists could have different effects, and reaction times and accuracy could be dissociated. Second, it provided a new task to potentially evaluate the cognitive changes that have been reported from the reduction in D_1 and D_2 receptors density in the striatum for patients with HD and patients in the preclinical phase. Finally, the task may be used to evaluate therapeutic interventions that selectively block D_1 or D_2 receptors. Therefore, to further investigate the effects of striatal dysfunction in the Spatiotemporal Target Probability Signal Reaction Time Task, the next chapter evaluates an animal model of HD with quinolinic acid lesions of the striatum.

Chapter 5

The Effects of Bilateral Quinolinic Acid Lesions of the Dorsomedial Striatum in the Spatiotemporal Target Probability Signal Reaction Time Task

Deficits in implicit memory in patients with Huntington's disease have been associated with loss of striatal neurons. To examine if the neuronal loss in the dorsomedial striatum (DMS) affects implicit memory (the computation of probabilities to predict the location of a stimulus), rats with bilateral quinolinic acid lesions of the DMS were tested in the Spatiotemporal Target Probability Signal Reaction Time Task. These results enabled us to evaluate the quinolinic acid lesioned rat as a suitable model for cognitive deficits in HD and, in addition, facilitated the identification of task parameters for exploring ameliorative interventions for HD.

Is implicit memory (the computation of probabilities to predict the location of a stimulus) affected by quinolinic acid lesions of the DMS?

5.1 Introduction

The Spatiotemporal Target Probability Signal Reaction Time Task is a new reaction time task that requires the computation of probabilities to predict the location of stimuli. At short foreperiods, stimuli are more likely to appear on the left side, than on the right side. However, as the foreperiod length increases, the probability of the stimuli appearing on the left side decreases and the stimuli becomes more likely to appear on the right. Previous data have shown (Chapter 3) that both rats and humans, had faster reaction times at longer foreperiods on the right side (i.e., the side where the stimuli were more likely to appear), which suggested that both species were able to learn that the probability of the location of the stimuli changed as function of the length of the foreperiod (Chapter 3). In addition, data showed that the task performance could be affected by selective blockade of dopaminergic transmission at the D_1 or D_2 receptors (Chapter 4).

This chapter reports on the testing of an animal model of HD to investigate the effects of QA lesions of the striatum in the Spatiotemporal Target Probability Signal Reaction Time Task. The results presented in Chapter 1, section 1.8.1, showed that patients with HD are impaired in acquiring new motor skills, which suggested that the striatum is necessary for learning implicit tasks. However, the question of whether the striatum, once a task that requires implicit memory has been learned, is necessary for continuing performance in the task, has yet to be addressed. Therefore, the aim of this chapter was to investigate if the performance on the Spatiotemporal Target Probability Signal Reaction Time Task was affected by bilateral QA lesions of the striatum. If the striatum is necessary for continuing performance in the task once it has been learned, it would be predicted that post-surgery the performance on the Spatiotemporal Target Probability Signal Reaction Time Task would be affected.

5.2 Method

5.2.1 Animals

The animals were the 24 male hooded Lister rats (Charles River, UK Ltd), described in Chapter 3.

5.2.2 Apparatus

All phases of the experiment were conducted in a set of four nine-hole operant chambers (Paul Fray Ltd, Cambridge, UK; see the Apparatus section in Chapter 3).

5.2.3 Procedure

Behavioural procedures. Rats were trained and tested in the Spatiotemporal Target Probability Signal Reaction Time Task described in the Methods section of Chapter 3. Briefly, rats had to respond to lateralised visual stimuli to receive food. Rats were required to sustain a nose poke in the central hole until a stimulus on the left or the right side hole appeared after one of five different foreperiods. Foreperiods could have durations of 200, 300, 400, 500 or 600 ms and were followed by a light stimulus of 100 ms on either left or right side. At the short foreperiods, the stimuli were more likely on the left side, but as foreperiods increased, presentation of the stimuli became more likely on the right side (see Figure 5.1). After 140 sessions in the Spatiotemporal Target Probability Signal Reaction Time Task, rats were able to complete 120 correct trials within 45 min.

Surgery. Pre-surgery, rats were pseudo-randomly assigned to the sham-surgery control (n =8) or dorsomedial striatum (DMS) lesion (n =16) group. Data from the two groups were analysed to ensure there were no differences between the groups before the surgery. The surgery protocol was as described in Chapter 2 (General Methods).

Post-test. 12 days after surgery, rats were retested in the Spatiotemporal Target Probability Signal Reaction Time Task.

Histology. Histology was as described in Chapter 2 in the General Methods.

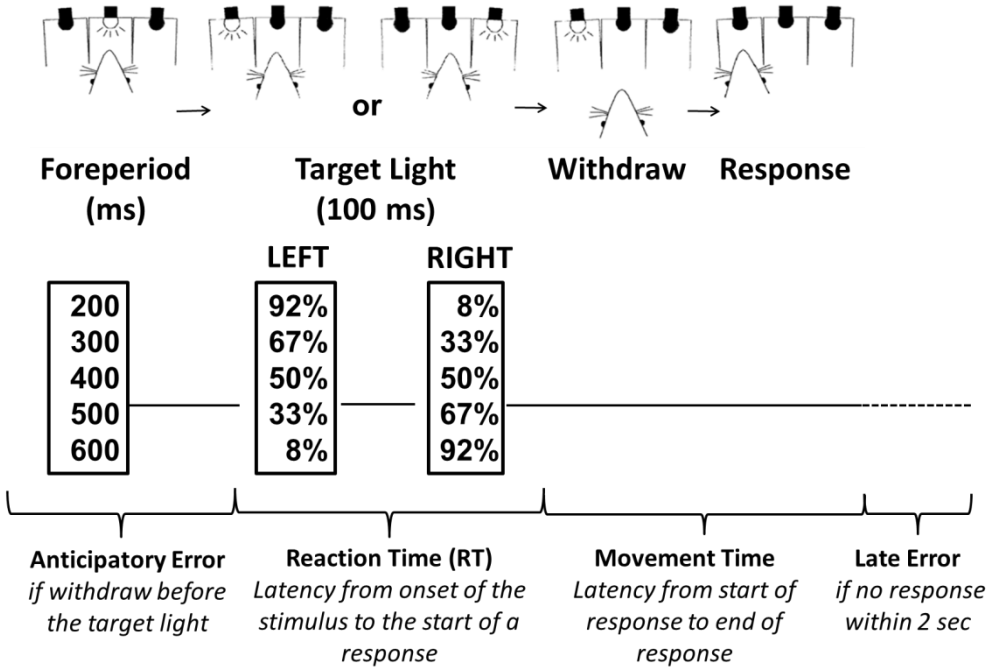

Figure 5.1. *Schematic representation of the Spatiotemporal Target Probability Signal Reaction Time Task. Foreperiods could have durations of 200, 300, 400, 500 or 600 ms and were followed by a light stimulus of 100 ms on either left or right side. At the early foreperiods, the stimuli were more likely on the left side; as time elapsed, presentation of the stimuli became more likely on the right.*

5.2.4 Data analysis

Reaction time, movement time, percentage of incorrect responses, percentage of late errors and percentage of anticipatory errors were calculated as described in Chapter 3.

Statistical analyses. All analyses were conducted in IBM SPSS (v 21, SPSS Inc., Chicago, IL) using the average data collected during the last 13 sessions of the experiment. The criterion for significance was set at $p < .05$ in all cases. A four factor, 2 x 2 x 5 x 2 mixed analysis of variance (ANOVA) with the variables of Surgery (Within-subjects factor with 2

levels: Pre-surgery vs. Post-surgery), Group (Between-groups factor with 2 levels: Lesion vs. Sham control), Foreperiod (Within-subjects factor with 5 levels: 200, 300, 400, 500, 600) and Side (Within-subjects factor with 2 levels: Left vs. Right), was conducted to evaluate if there were significant differences between the two groups on each of the dependent variables. Sidak's-corrected pairwise comparisons for significant effects or interactions were performed. When data violated the assumption of sphericity, Huynh-Feldt corrections were reported.

5.3 Results

5.3.1 Histology

Ten out of 16 DMS lesioned rats were determined to have appropriate bilateral lesions. Figure 5.2 shows photomicrographs of coronal sections from sham-surgery control and DMS lesioned animals. Two out of 8 sham-surgery control lesioned rats showed signs of cell damage, and therefore, were excluded.

The final numbers (*n*) in each group were: DMS lesion ($n = 10$), sham-surgery control ($n = 6$). Figure 5.3 shows a schematic diagram of a series of coronal sections of the rat brain (Paxinos and Watson, 2007), illustrating the extent of bilateral dorsomedial (DMS) striatum lesions.

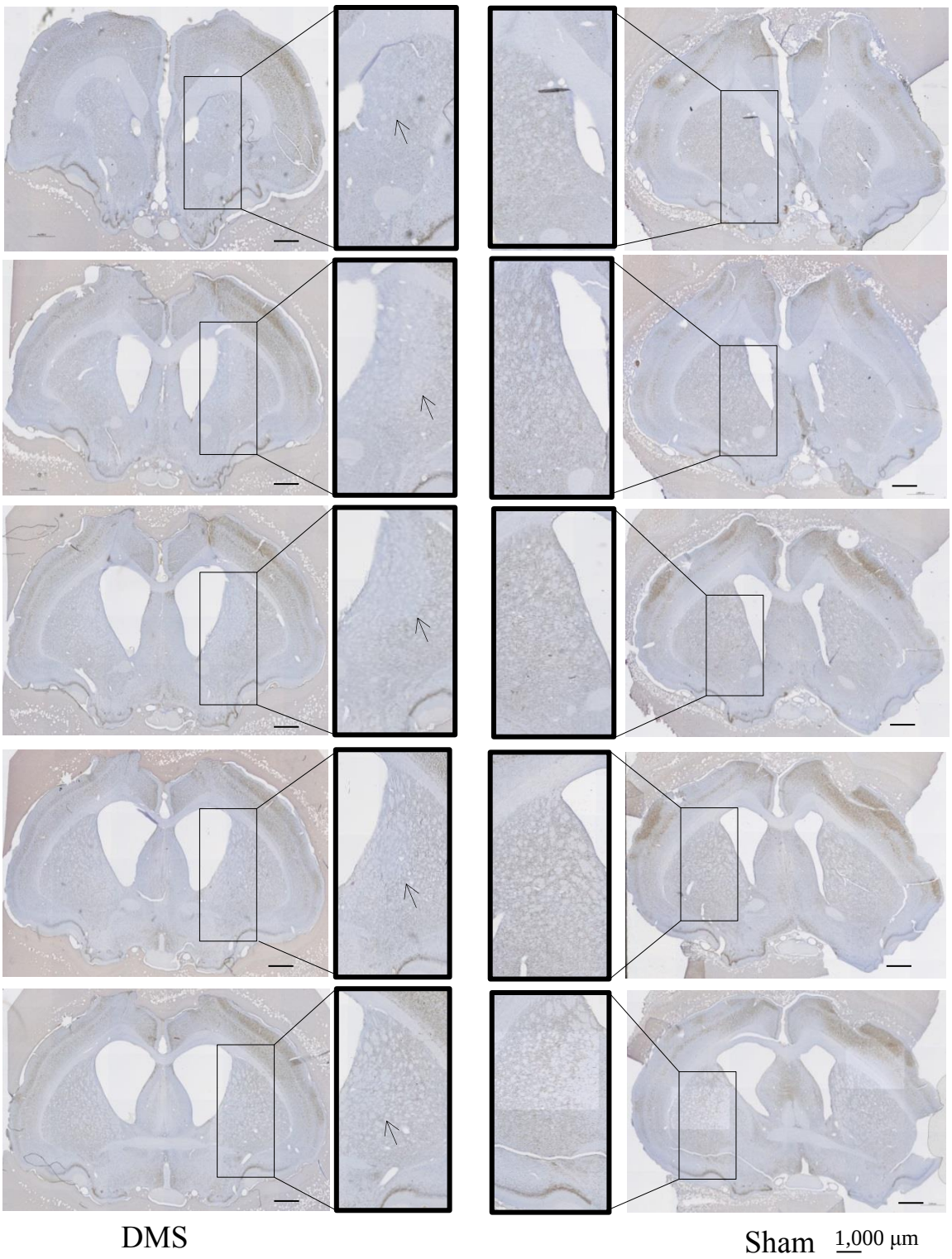

Figure 5.2. *Representative photomicrographs of coronal sections double-stained with NeuN and Cresyl Violet from dorsomedial striatum (DMS) lesion (left), and sham control (right) animals. Sham control rats showed an even distribution of neurons in the striatum whilst DMS lesioned rats showed cell loss. Arrows point to the lesion area. From the top, sections are +1.7, +1.2, +0.7, +0.2, and -0.3 mm from bregma. Scale bar 1,000 µm.*

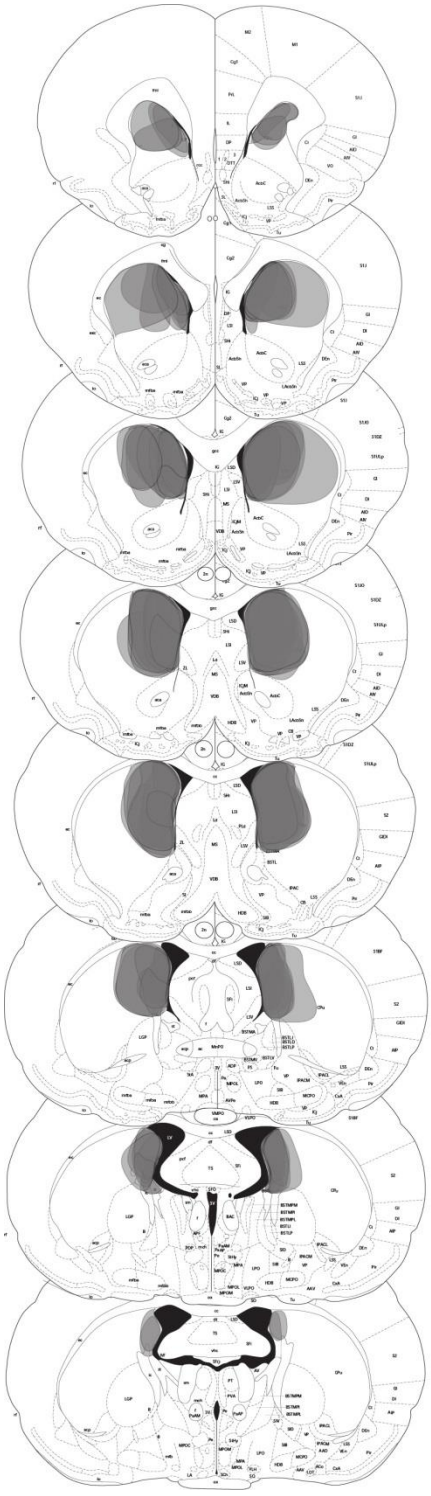

Figure 5.3. *Schematic diagram of a series of coronal sections of the rat brain, illustrating the extent of bilateral dorsomedial (DMS) striatum lesions. Darkness represents coincidence lesions from different animals. From the top, sections are +2.2, +1.7, +1.2, +0.7, +0.2, −0.3, −0.80 and −0.92 mm from bregma (Paxinos and Watson, 2007).*

5.3.2 Behavioural performances

Reaction time (RT). Figure 5.4 shows the modal RTs pre-surgery (upper row) and post-surgery (middle row) for the sham-surgery control group (left column) and for the DMS lesion group (right column). The lower row shows the cost/benefit of varying the spatial probability by subtracting the RT to the right from the RT to the left side. At shorter foreperiods, when the stimulus was more probable on the left, the reaction time was slower on the right (less probable target location) compared to the left side; therefore, the subtracted value was negative. However, at longer foreperiods, when the right side stimulus became more probable, the reaction time was faster to the right compared to the left side; therefore, the subtracted value was positive.

For both groups, reaction times were faster after surgery (main effect of Surgery: $F(1, 14) = 11.34$, $p = .01$, $\eta_p^2 = .45$). Pre-surgery, there was no difference in reaction times between sham-surgery control and lesioned rats; however, post-surgery, reaction times of the control group were faster than those of the lesion group (significant interaction between Surgery x Group: $F(1, 14) = 5.22$, $p = .04$, $\eta_p^2 = .27$).

For the DMS lesioned and the control group, reaction times were faster as foreperiods increased (main effect of Foreperiod: $F(4, 56) = 137.43$, $p < .001$, $\eta_p^2 = .91$. Sidak's-corrected pairwise comparisons revealed that all foreperiods were different from each other). The lower row of Figure 5.4 shows that reaction times were faster to the left side at shorter foreperiods and faster to the right side at longer foreperiods (Side x Foreperiod interaction: $F(4, 56) = 4.32$, $p = .04$, $\eta_p^2 = .24$). Although there was this trend, Sidak's-corrected pairwise comparisons revealed that reaction times to the right side (the more probable target location) were faster than the reaction times to the left side only at the two longest foreperiods (FP 500, $p = .01$; FP 600, $p = .01$). There was a three way interaction between the variables Surgery, Side and Foreperiod ($F(4,$

56) = 3.01, p = .03, η_p^2 = .18). Sidak's-corrected pairwise comparisons revealed that pre-surgery reaction times were faster on the right side for the two longest foreperiods (FP 500, p = .02; FP 600, p = .02) only. Similarly, post-surgery, reaction times were also faster to the right side for the two longest foreperiods (FP 500, p = .01; FP 600, p = .03) only.

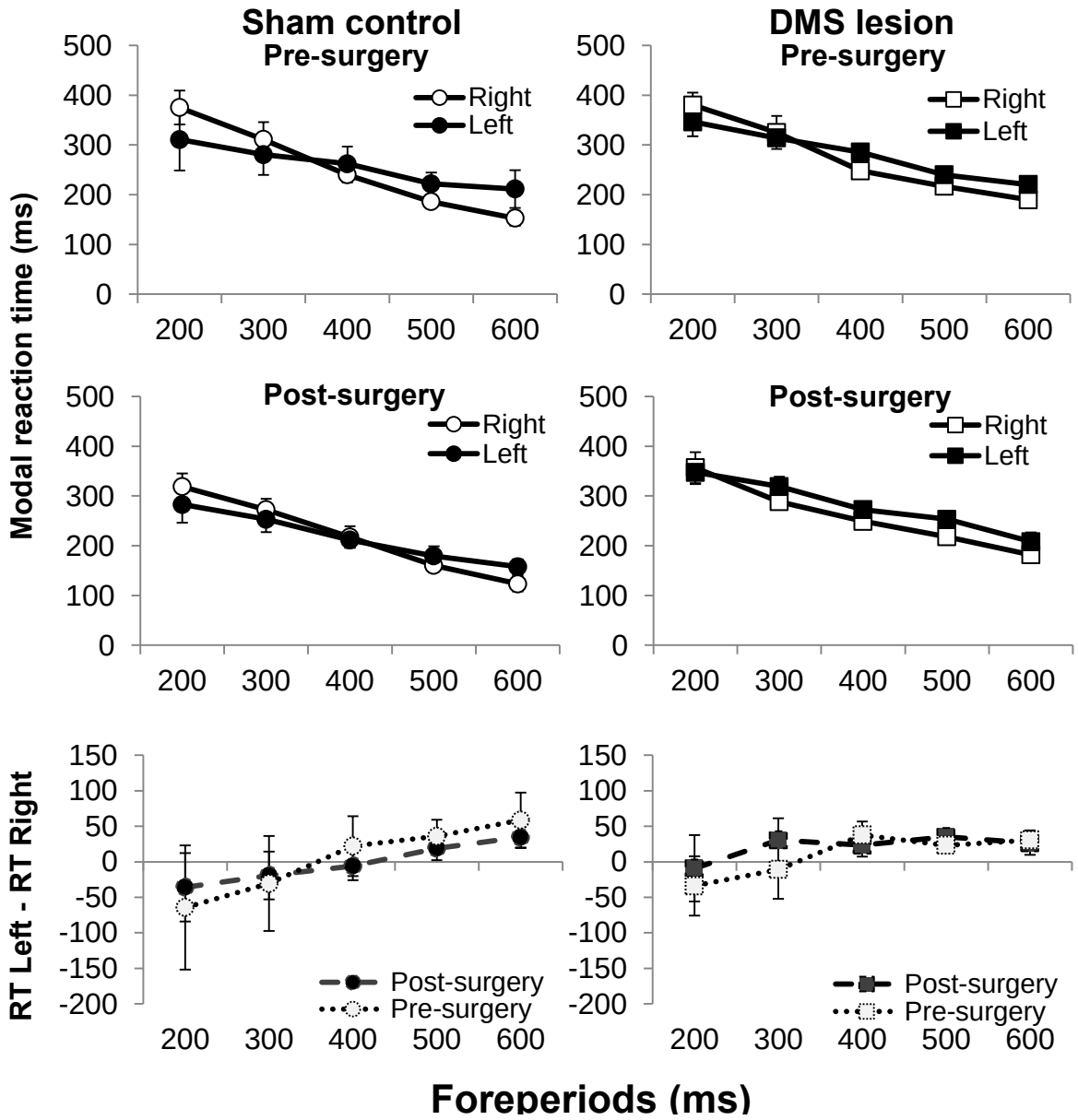

Figure 5.4. *Modal reaction time (± SEM) to left and right targets across foreperiods for sham-surgery control (left column) and DMS lesioned (right column) animals. The upper row shows the mode RT pre-surgery, while the middle row shows RT post-surgery. RT was predicted to be slower for right than left targets at shorter foreperiods (unexpected location), while slower than left targets at longer foreperiods (expected location). The lower row depicts the same information by side subtraction, which reflects the cost/benefit of conditional spatiotemporal probability. At shorter foreperiods the subtracted value is negative, as RT was slower to the right compared to the left side, while at longer foreperiods, the subtracted value is positive, as RT was faster to the right compared to left side.*

Movement time (MT). Figure 5.5 shows the mean movement times (latency of the withdrawal of the nose from the central hole, to the nose poke in either side hole) for the sham-surgery control group (left column) and for the DMS lesion group (right column) pre-surgery (upper row) and post-surgery (lower row). For both groups, movement time was slower after surgery (main effect of Surgery: $F(1, 14) = 7.87$, $p = .01$, $\eta_p^2 = .36$); the longest foreperiods (FP 400, 500 and 600) had the slowest movement times after surgery (Surgery x Foreperiod interaction: $F(4, 56) = 5.22$, $p = .003$, $\eta_p^2 = .27$). Overall, movement times to the right side target were faster than to the left side (main effect of Side: $F(1, 14) = 9.07$, $p = .01$, $\eta_p^2 = .39$) and, as foreperiod increased, movement time was slower (main effect of Foreperiod: $F(4, 56) = 4.32$, $p = .004$, $\eta_p^2 = .24$). There was a four way interaction between the variables Surgery, Side, Foreperiod and Group ($F(4, 56) = 7.42$, $p < .001$, $\eta_p^2 = .35$). Sidak's-corrected pairwise comparisons revealed that for the control group, pre-surgery, movement time was faster on the right at longer foreperiods (FP 500, $p = .02$ and FP 600, $p = .02$; see Figure 5.5 left column- upper row). However, after surgery, movement time was faster on the right side at the shortest foreperiod (FP 200, $p = .02$) and at FP 400 ($p = .02$; see Figure 5.5 left column- lower row). For the lesion group, pre-surgery there were no differences in movement times between the left and right side across the different foreperiods (see Figure 5.5 right column- upper row); however, post-surgery movement time was faster to the right side at the longest foreperiod (FP 600, $p = .04$; see Figure 5.5 right column- lower row).

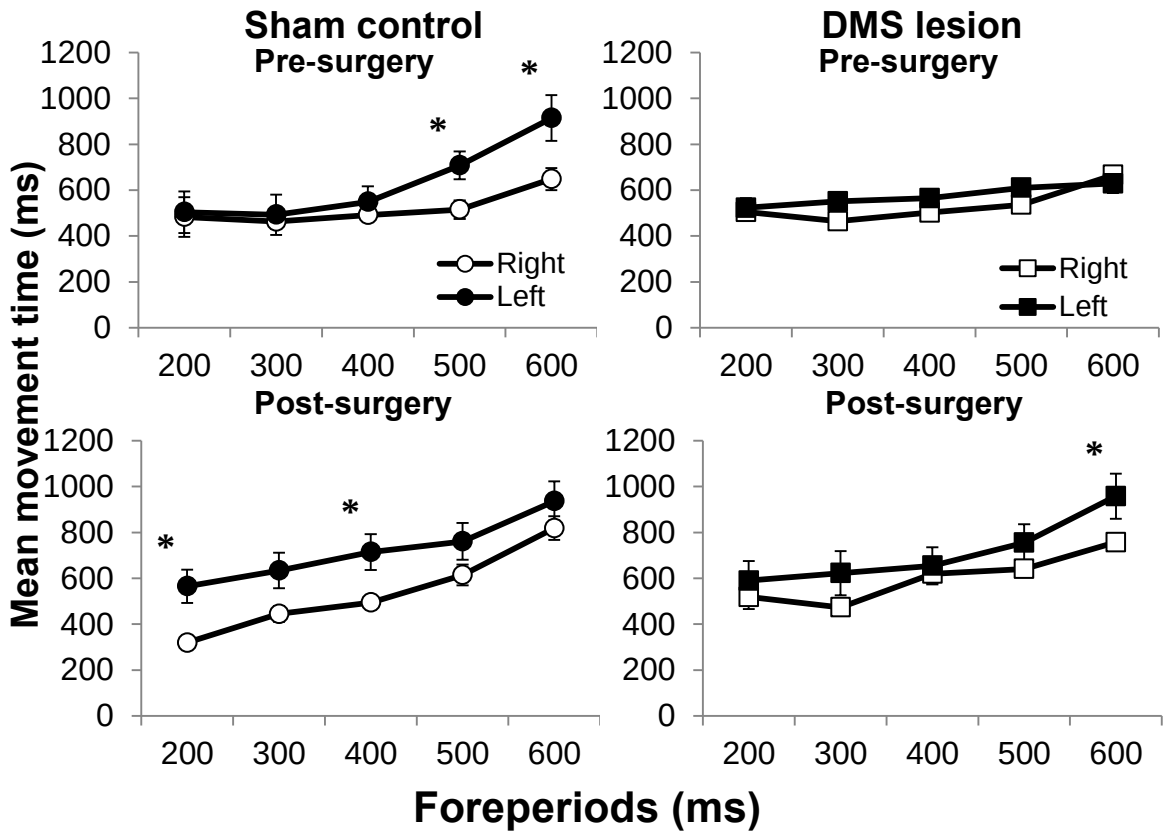

Figure 5.5. *Movement time (± SEM) to left and right targets across foreperiods for sham-surgery control (left column) and DMS lesioned (right column) rats. The upper row shows the mean MT pre-surgery, while the lower row shows MT post-surgery. The left column- upper row shows that the control group, pre-surgery, has faster MT to the right at longer foreperiods (FP 500 and 600). However, after surgery, MT was faster to the right side at the shortest foreperiod (FP 200) and at FP 400 (left column- lower row). The right column- upper row shows that for the lesion group, pre-surgery there were no differences in movement time between the left and right side across the different foreperiods; however, the right column- lower row shows that post-surgery, movement time was faster to the right side at the longest foreperiod (FP 600) for the lesion group (* $p < .05$).*

Incorrect responses. Figure 5.6 shows the percentage of incorrect responses pre-surgery (upper row) and post-surgery (lower row) for the sham-surgery control group (left column) and for the DMS lesion group (right column).

Both groups made more incorrect responses to the left side (main effect of Side: $F(1, 14) = 10.43$, $p = .01$, $\eta_p^2 = .43$) and for both groups, incorrect responses increased as a function of

increasing the length of the foreperiod (main effect of Foreperiod: $F(4, 56) = 17.14$, $p < .001$, $\eta_p^2 = .55$. Sidak's-corrected pairwise comparisons showed that FP 200 had fewer incorrect responses than all the other foreperiods; FP 300 had fewer incorrect responses than FP 500 and 600, and FP 400 had fewer incorrect responses than FP 500; see Table 5.1). At short foreperiods there were no differences in the percentage of incorrect responses between the left and the right side, but at long foreperiods, there were more incorrect responses to the left side (Side x Foreperiod interaction: ($F(4, 56) = 3.80$, $p = .01$, $\eta_p^2 = .21$). Sidak's-corrected pairwise comparisons showed that at the longest foreperiods (FP 500, $p < .001$ and FP 600, $p = .002$) there were more incorrect responses to the left side.

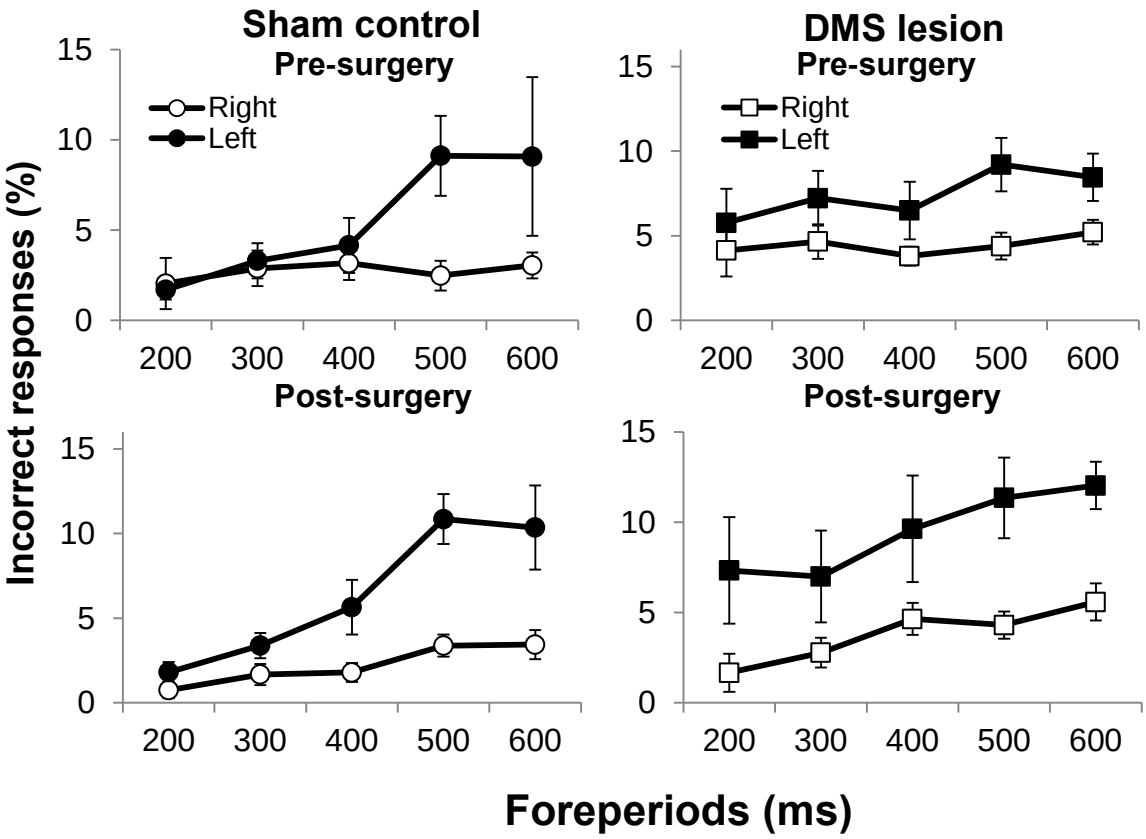

Figure 5.6. *Percentage of incorrect responses (± SEM) to left and right targets across foreperiods for sham-surgery control (left column) and DMS lesioned (right column) rats. The upper row shows the percentage of incorrect responses pre-surgery, while the lower row shows the percentage of incorrect responses post-surgery.*

Table 5.1.
Foreperiod Sidak's-corrected pairwise comparisons for incorrect responses.

Foreperiod	FP 200	FP 300	FP 400	FP 500	FP 600
	M =3.15	*M* =4.11	*M* =4.92	*M* =6.89	*M* =7.13
FP 200		**.039***	**.001***	**.000***	**.003***
FP 300			.113	**.000***	**.037***
FP 400				**.000***	.184
FP 500					1.00
FP 600					

Note. *****The mean difference is significant at the .05 level.

Late errors. Surgery increased the percentage of late errors (main effect of Surgery: $F(1, 14) = 7.54$, $p = .02$, $\eta_p^2 = .35$). Both groups made more late errors to the left side (main effect of Side: $F(1, 14) = 7.56$, $p = .02$, $\eta_p^2 = .35$). For both groups, late errors were affected by increasing the length of the foreperiod (main effect of Foreperiod: $F(4, 56) = 3.68$, $p = .03$, $\eta_p^2 = .21$. Sidak's-corrected pairwise comparisons indicated that FP 200 had fewer late errors than FP 400, $p = .01$ and FP 500, $p = .01$). While at the longest foreperiods there were no differences in the percentage of late errors between the left and the right side, at the shortest foreperiods there were more late errors to the left side (Side x Foreperiod interaction: $F(4, 56) = 3.73$, $p = .04$, $\eta_p^2 = .21$). Sidak's-corrected pairwise comparisons indicated that at FP 200 ($p = .002$), FP 300 ($p = .004$) and FP 400 ($p = .02$) there were more late errors to the left side in comparison to the right side.

Anticipatory errors. Figure 5.7 shows the percentage of anticipatory errors pre-surgery (left column) and post-surgery (right column). Anticipatory errors were not influenced by the

spatial location of the side stimuli because the nose poke was withdrawn from the central hole before the onset of the side target stimuli; therefore, they were analysed without including side.

The percentage of anticipatory errors increased post-surgery (main effect of Surgery: $F(1, 14) = 10.07$, $p = .01$, $\eta_p^2 = .42$). As foreperiod increased, the percentage of anticipatory errors increased (main effect of Foreperiod: $F(4, 56) = 97.24$, $p < .001$, $\eta_p^2 = .87$. Sidak's-corrected pairwise comparisons revealed that all the foreperiods were different from each other). In comparison to the control group, the DMS lesioned rats had higher anticipatory errors at the shortest foreperiods and lower anticipatory errors at the longest foreperiods (Foreperiod x Group interaction: $F(4, 56) = 4.39$, $p = .004$, $\eta_p^2 = .24$). Sidak's-corrected pairwise comparisons showed that the DMS lesioned rats had more anticipatory responses than the control group only at FP 200 ($p = .03$) and FP 300 ($p = .04$). At the shortest foreperiods there were no differences in the percentage of anticipatory responses pre- and post-surgery; however, at the longest foreperiods the percentage of anticipatory responses increased post-surgery (Surgery x Foreperiod interaction: $F(4, 56) = 13.74$, $p < .001$, $\eta_p^2 = .50$). Sidak's-corrected pairwise comparisons showed that anticipatory responses increased post-surgery at FP 500 ($p = .01$) and FP 600 ($p < .001$).

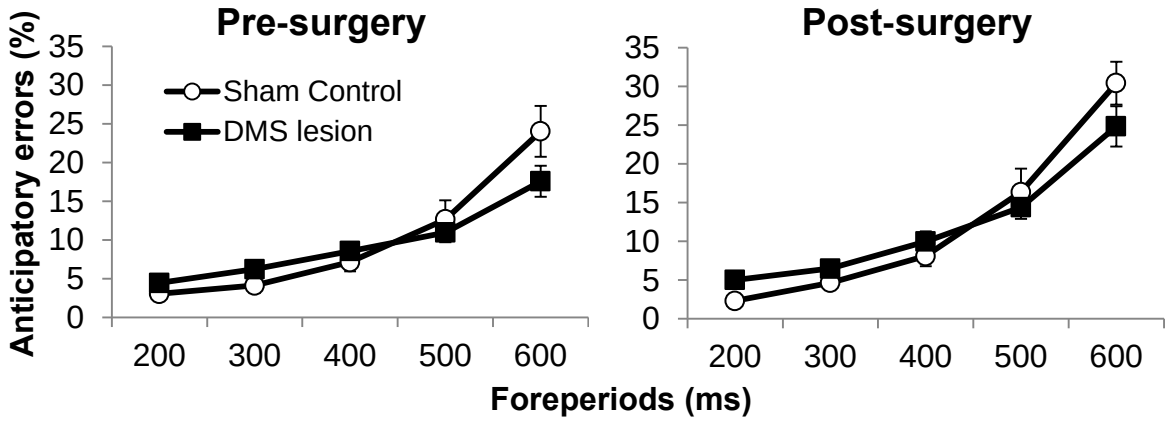

Figure 5.7. *Percentage of anticipatory errors (±SEM) pre-surgery (left column) and post-surgery (right column) at each foreperiod for the DMS lesioned and the control rats.*

5.4 Discussion

The aim of this chapter was to investigate whether the performance on the Spatiotemporal Target Probability Signal Reaction Time Task was affected by bilateral QA lesions of the dorsomedial striatum.

Overall, reaction times were faster as foreperiods increased, which suggested that rats were sensitive to the conditional temporal probability of the occurrence of the stimulus. That is, at the start of the delay, the stimulus could appear at any of the five foreperiods, but as the foreperiods elapsed, the likelihood of the stimulus appearing increased; thus general readiness to respond increased and reaction times were faster. In addition, reaction times were faster to the left side at shorter foreperiods and faster to the right side at longer foreperiods (i.e., the corresponding sides where the target stimulus was more likely to appear depending on the length of the foreperiod), which suggested that the rats were sensitive to the conditional spatiotemporal probability. Reaction times were faster after surgery, as expected, pre-surgery there were no

differences between the lesioned and the sham-control groups. However, post-surgery, reaction times were faster for the sham-control rats, which suggested that lesions of the DMS affected the general readiness to respond. However, the conditional spatiotemporal probability was not affected by the lesions as post-surgery reaction times remained faster to the right side at the longest foreperiods (i.e., the side where the target was more likely to appear).

Movement time (i.e., the time to execute the lateralised response) was affected after surgery for both groups. For the sham-control groups, the MT pre-surgery was faster to the right side at the longest foreperiods; but after surgery the MT to the right side was also faster on the shortest foreperiod. For the lesioned group, pre-surgery, there were no differences in MT between the left and right side across the different foreperiods; however, post-surgery MT was faster to the right side at the longest foreperiod. The faster movement times to the right side, across all foreperiods, could suggest a side bias to prepare movements to the right side regardless of the duration of the foreperiod. The increased percentage of late responses (i.e., inability to respond within 2 seconds) to the left side provides evidence in favour of a possible predisposition to respond to the right side.

As previously observed in Chapters 3 and 4, anticipatory responses increased as foreperiods increased. After surgery, the percentage of anticipatory responses increased at the longest foreperiods, which suggested that surgery enhanced conditional temporal probability. But, in this case, motor readiness resulted in premature responses rather than in an improved performance. That is, as the probability of the stimulus appearing increased with time, the tendency to respond before the stimulus was presented (anticipatory responses) also increased. Lesions of the DMS did not affect accuracy on the task; overall, incorrect responses increased as

a function of foreperiod and there were more incorrect responses on the left side at longest foreperiods.

The results from this experiment, slowed reaction times (i.e., initiation of response) but not movement time (i.e., latency to execute the lateralised response), were in accord with previous studies that investigated the effects of striatal lesions on reaction time performance (Brasted et al., 1998; Brasted et al., 1997; Brown and Robbins, 1989; Hauber and Schmidt, 1994; Mittleman et al., 1988). In addition, these results extend the findings that, even with minor differences in the lesions placements in the DMS, the different volumes of toxin infused, and different lesions modes (bilateral or unilateral), lesions of the DMS impaired rapid initiation of responses triggered by a stimulus.

In summary, the present study showed that rats were able to learn the Spatiotemporal Target Probability Signal Reaction Time Task efficiently, as they had faster reaction times to the left side at shorter foreperiods, and faster reaction times to the right side at longer foreperiods (i.e., the side were the target was more likely to appear). This conditional spatiotemporal probability (probability to respond to the right or the left side as a function to the length of foreperiod) was not affected by lesions of the DMS. Given that, in the present study, the animals were trained on the Spatiotemporal Target Probability Signal Reaction Time Task prior to lesioning the striatum, the results suggested that once a task that requires implicit memory has been learned, the DMS was not involved in sustaining the performance of the task. Further research could investigate if lesions of the DMS disrupt the acquisition of this implicit task; lesioning the animals before the probabilities are introduced on the task, would allow investigating if the striatum is involved in the computation of probabilities to predict the location of a target stimulus that changes as a function of the length of the foreperiod. Furthermore, it

could also be possible that the DMS is not involved in this form of implicit task, but this should be further investigated. Different human studies have suggested that the striatum is not the only neuronal structure that contributes to implicit learning, and various cortical areas (e.g., ventrolateral prefrontal cortex, ventromedial premotor area, medial prefrontal cortex) have been reported to be involved in various stages of implicit learning (Exner et al., 2002; van der Graaf et al., 2006). Consequently, it could be possible that lesions of the prefrontal cortex may impair the computation of probabilities that predict the location of the stimuli in this task. This could also be investigated in the future given that, in addition to the striatum, the cortex of patients with HD also suffers from atrophy. Furthermore the cortical neuronal degeneration leads to excessive thinning of the cerebral mantle of the entire brain (Hedreen et al., 1991). As a new task, further investigation is required to evaluate which neuronal structures are involved in the computation of probabilities to predict the location of the stimuli.

Given that no cognitive impairments were observed in the Spatiotemporal Target Probability Signal Reaction Time Task after lesioning the DMS, the effectiveness of treatments for HD could not be evaluated using this task. Therefore, Chapter 6 investigated another test of striatal function in which potential treatments for HD could be evaluated.

Chapter 6

Goal-Directed and Habitual Responding in Rats with Bilateral Quinolinic Acid Lesions of the Dorsomedial Striatum: Implications for Huntington's disease

The aim of the experiment presented in this chapter was to assess the involvement of the DMS in habitual responding. Using a free scan task, rats had to nose poke a row of five holes to find a target hole selected randomly on each trial. There was no cue that would indicate which hole would produce the reward.

The first part of the experiment used an outcome devaluation procedure to determine if nose poking was affected by the value of the reward or if it was habitual responding. Rats were tested on this procedure pre- and post-surgery.

The second part of the experiment investigated the effects of bilateral QA lesions of the DMS on habitual responding by changing the reward contingencies so that the central hole never produced a reward.

Do bilateral QA lesions of the DMS induce habitual responding?

6.1 Introduction

In an environment which is constantly changing, it is beneficial to have flexible, goal-directed actions; however, monitoring every response reduces the capacity for alternative cognitive processes (Gehring and Knight, 2000). Therefore, habitual, repetitive behaviours are also advantageous, since they reduce attention and decision making resources (Smith and Graybiel, 2013). Habitual, repetitive behaviours can also bring disadvantages, for example when they allow predators to predict behaviour (Desrochers et al., 2010). In this sense, optimal performance of everyday situations requires a balance between goal-directed and habitual behaviours (Balleine et al., 2009; Graybiel, 2008; Yin and Knowlton, 2006).

It has been suggested that goal-directed and habitual behaviours are controlled by distinct learning processes. While goal-directed behaviours involve the formation of action-outcome (A-O) associations to mediate flexible, deliberated actions that are controlled by consequences, habits involve the integration of stimulus-response (S-R) without associating the outcome to those actions in order to mediate inflexible and automatic behaviour (Dickinson, 1985; Dickinson and Balleine, 1994).

To evaluate if a behaviour is goal-directed or habitual, the value of that behaviour's outcome could be reduced (e.g., access to the reward is given before the task). If the performance of the task is unaffected after the value of the outcome is manipulated, then the behaviour is habitual. Conversely, if the performance is affected after the value of the outcome is manipulated (e.g., the rate of responding decreases after the reward is devalued), then the behaviour is goal-directed (Dickinson, 1985; Dickinson and Balleine, 1994).

In addition to having a general role in the initiation and patterning of different behaviours, the striatum has also been associated with habit formation (Packard and Knowlton, 2002; Yin and Knowlton, 2006). In rats, the DMS has been implicated in A-O learning and expression of goal-directed actions (Yin and Knowlton, 2006; Yin et al., 2004; Yin et al., 2005).

Apart from the S-R association that characterises habitual behaviours, Graybiel (1998) proposed that habits are stereotypic, ritualistic behaviours which consist of sequential movements. These ritualistic behaviours are one of the most common obsessions in HD (Novak and Tabrizi, 2010). To study the formation of sequential habits, Desrochers et al. (2010) designed a free-viewing scan task in which monkeys had to scan a grid of dots, one of which was randomly baited. Once the monkey fixated on the target dot, a reward became available. Using this task, Desrochers et al. (2010) found that even though the monkeys were free to move their eyes in any direction to find the target, despite receiving no explicit training to develop a pattern of saccades to scan the grid, monkeys developed a dot-looking visual habit to scan the target grids.

However, even though Desrochers et al. (2010) described the scanning pattern as a habitual behaviour, it remains to be investigated if the behaviour is affected by the outcome. As Dickinson (1985) suggested, if the performance of the task is unaffected after the value of the outcome is manipulated, then the behaviour is considered habitual. This chapter presented a task, adapted from that of Desrochers et al. (2010), which rats nose poked a row of five holes to find a target hole selected randomly on each trial (no cue would indicate which hole would produce the reward) to evaluate habit formation in rats with QA lesions of the DMS.

Changes in goal value were assessed by using a devaluation of the reward, in which rats had free access to the reinforcement in their home cages before testing and the reward was not delivered during the session. It was hypothesised that if habits were formed, the performance on the task would not be affected even though the value of the reward was devalued. In addition, to evaluate if lesions of the DMS would impair goal-directed behaviour (i.e., the association of an action and an expected outcome), in the last phase all of the conditions remained the same, but the central hole never produced a reward. Since there were no cues to indicate the change, the rat had to learn through the task that nose poking into the central hole would never produce a reward. Habit responding would consist of continuing responding to the central hole. Thus, it was hypothesised that if the DMS is required to learn goal-directed behaviours, rats with DMS lesions would show habit responding and would continue responding to the central hole regardless of no further reinforcement for that response. Additional measures such as reaction time, movement time and post-reinforcement pause, were also compared between the groups to evaluate possible movement changes affected by lesions of the DMS. Furthermore, since it has been reported that the most prominent regions where chorea manifests in HD patients are the orofacial regions (Berardelli et al., 1999), the rate of licking the spigot once the liquid reward was available was also compared between the groups.

6.2 Method

6.2.1 Animals

The animals were 16 experimentally-näive male hooded Lister rats (Harlan, UK Ltd) with a mean *ad libitum* weight of 274 g (range = 256 - 321 g) at the beginning of the experiment. The rats were housed in quadruplets in plastic box cages and handled five days a week. After habituation to the conditions of the animal colony, water was restricted to free access of one hour

five days per week. Body weight was monitored on testing days to ensure steady gain. Weights did not fall below 85% of free-feeding weight. Food was available *ad libitum* in the home cage. The experimental testing was carried out five days a week during the light portion of the cycle. Husbandry conditions were described in the General Methods in Chapter 2.

6.2.2 Apparatus

All phases of the experiment were conducted in a set of 4 five-hole nose poke wall operant chambers (Figure 6.1; Med Associates, Vermont, USA). Each chamber (34 x 29 x 25 cm) was enclosed in a ventilated, sound-attenuating cubicle and had a video camera above them (Santec Smart Vision, model VCA 5156; Sanyo Video Vertreib GMbH CO., Ahrensburg, Germany). An extractor fan provided a constant low-level of background noise and a continuous airflow. The floor of the chamber was a stainless steel grid. The rear wall of each chamber was concave and had a horizontal array of five holes. Each hole contained a vertical photocell beam located at the front to detect nose entries. Liquid reinforcer was available through a metal spigot protruding through a hole in the lower centre of the back wall; this contained a lickometer with a photo beam and delivered 0.1 ml of sodium saccharine solution (0.3% w/v) per trial from a computer-controlled speed syringe pump (PHM-100). A light and a tone (EW-233A) indicated the delivery of the reward. The presentation of stimuli and the collection of data were controlled by a computer using the Medstate programming language (Med-PC-IV, MED Associates). The temporal resolution of the instrumental set-up was 2 ms.

Figure 6.1. *Five-hole operant chamber used for training and testing.*

6.2.3 Procedure

Three days prior to experimental testing, the rats were put in a restricted water access; which consisted of the following schedule: an hour of access to sodium saccharine solution (0.3% w/v), followed to an hour of access to plain water.

Training. In the initial two sessions, rats were habituated to the operant boxes during a 30 min session, in which 0.1 ml of sodium saccharine solution was delivered every time the rat licked the spigot. The delivery of the reward (accompanied by a tone and a light) was between 10-60 sec after the onset of licking within a trial, in 10 sec increments.

During this phase and throughout the experiment (unless specified) the rats had restricted access to water between 16:00-17:00 h on Monday to Friday, with free water access from 16:00 h on Friday to Sunday afternoon.

Nose poke training. During the following sessions, the animals were trained to make a nose poke into the illuminated holes to receive the reward. The trial began with all the five holes' lights on. The lights from the holes were lit until the rat made a nose poke in any of the holes; once this occurred, the lights in the five holes were turned off and a light and a tone at the

spigot that delivered reward were turned on. If the rat licked the spigot during the reward signal (light and tone at the spigot), the reward was pumped for 2 sec, delivering 0.1 ml of sodium saccharine solution. Subsequently, the reward signal was turned off and a new trial initiated. If none of the holes were poked after a variable interval of 2, 3 or 4 minutes, the reward signal was turned on. This phase was conducted for a week.

Task training and testing. On each trial of the following sessions, only one of the holes was randomly selected to produce reward. The beginning of the trial was indicated by illuminating the five holes and every second between 1-7 seconds one of the holes was randomly selected to produce a reward after a nose poke. There was no cue that would indicate which hole would produce the reward. Once the rat nose poked in the target hole the reward signal was turned on and the reward was given as described in the previous phase. When the rat collected the reward, the reward signal was turned off and a new trial started. Each session lasted 30 minutes. Figure 6.2 shows a schematic representation of the task.

An incorrect response was registered when the animal licked the spigot before the reward was available or when nose pokes were produced once the reward was available. This phase was conducted for 40 sessions. Once the behaviour was stable, no difference on the trials completed across 5 sessions, rats were tested on the devaluation phase.

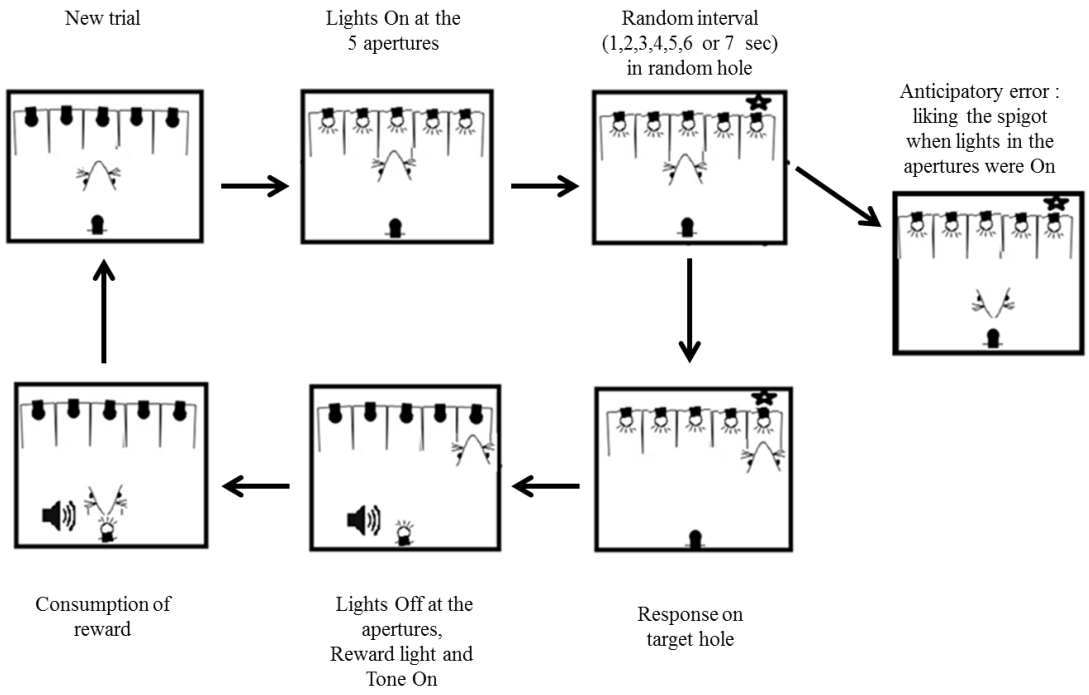

Figure 6.2. *Schematic representation of the task.*

Devaluation phase. An hour before testing, the animals were given 30 min of free access to saccharin solution in their home cages, with the solution removed 30 min before testing. During this phase, testing was conducted in the same way as described before, with the exception that the syringe pumps were blocked so that no reward was delivered during the session. However, all visual and auditory cues remained unchanged. Sessions lasted 30 min and once the rats were placed back in their home cages they were given 30 min of free access of saccharin solution. The volume of saccharin consumed was recorded before and after testing and was calculated from the weights of the bottles. Devaluation sessions were conducted after two regular testing sessions and followed by an additional two regular testing sessions (i.e., Monday and Tuesday were regular sessions, Wednesday was a devaluation session, and Thursday and Friday were regular sessions). This phase lasted 2 weeks.

Surgery. Pre-surgery, rats were pseudo-randomly assigned to the sham-surgery control (n =6) or dorsomedial striatum (DMS) lesion (n =10) group. Data from the two groups were analysed to ensure there were no differences between the groups before the surgery. The surgery protocol was as described in Chapter 2 (General Methods).

Post-surgery testing. After the rats recovered from the surgery (approximately 10 days), they were retested on the task for 17 sessions.

Post-surgery devaluation phase. Rats were retested on 2 sessions of the devaluation phase as previously described for 2 weeks (i.e., a devaluation session was conducted after two regular testing sessions and followed by an additional two regular testing sessions).

Omission of reward on central hole. After the last two regular sessions of the devaluation phase, the omission of the reward on the central hole phase started. In this phase, all of the conditions remained the same as in the testing phase with the exception that the central hole never produced a reward. There were no cues that indicated this change. This phase was conducted for 32 sessions.

Histology. As described in the histology section of the General Methods in Chapter 2.

6.2.4 Data analysis

All analyses were conducted in IBM SPSS (v 21, SPSS Inc., Chicago, IL).

Trials completed. Total number of trials completed by rat per session.

Percentage of pokes in the central hole. This was calculated by multiplying the total number of pokes in the central hole by 100, and dividing it by the total number of pokes in all the 5 holes per session.

Omission of reward on central hole phase. The average of the percentage of pokes in the central hole of the last 2 sessions when the central hole was rewarded (baseline sessions) was

compared to the average of the percentage of pokes in the central hole of the first 2 sessions after the central hole was unrewarded (omission sessions).

Reaction Time. Time to withdraw from reward nose poke hole once reward signal has been presented (ms).

Movement Time. Time between withdrawal of nose poke from the rewarded hole to the first lick at reward spigot (sec).

Post-reinforcement pause. The time between trial onset and the first nose poke (sec).

Lick rate. The rate of licking the spigot once the reward was available.

Statistical analyses. A three factor, 2 x 2 x 2 ANOVA with the variables of Surgery (Within-subjects factor with 2 levels: Pre-surgery vs. Post-surgery), Group (Between-groups factor with 2 levels: Lesion vs. Sham-surgery control), and Session (Within-subjects factor with 2 levels: Baseline vs Devaluation), was conducted to evaluate if there were significant differences between the two groups on each of the dependent measures.

The devaluation values were the average of the two devaluation sessions; the baseline values were the average of the two sessions prior to the devaluation session and the second day following reinforcer devaluation (i.e., the average of the sessions from Monday, Tuesday and Friday from the devaluation phase week) of the two weeks the devaluation phase was conducted. The session following devaluation was not included in the analysis to avoid including skewed data by changes observed during the devaluation session. These values were calculated for the pre-surgery and post-surgery devaluation vs baseline comparisons.

For the omission of reward on central hole phase a two factor, 2 x 2 ANOVA with the variables of Group (Between-groups factor with 2 levels: Lesion vs. Sham-surgery control) and Session (Within-subjects factor with 2 levels: Baseline vs Omission) was conducted. Sidak's-

corrected pairwise comparisons for significant effects or interactions were performed. When data violated the assumption of sphericity, Huynh-Feldt corrections were reported. The criterion for significance (alpha level) was $p < .05$ in all cases.

6.3 Results

6.3.1 Histology

Nine out of 10 DMS lesion rats were determined to have appropriate bilateral lesions. Figure 6.3 shows photomicrographs of coronal sections from sham-surgery control and DMS lesioned animals. Two out of 6 sham-surgery control lesion rats died prematurely.

The final numbers (n) in each group were: DMS lesion ($n = 9$), sham-surgery control ($n = 4$). Figure 6.4 shows a schematic diagram of a series of coronal sections of the rat brain (Paxinos and Watson, 2007), illustrating the extent of bilateral dorsomedial (DMS) striatum lesions.

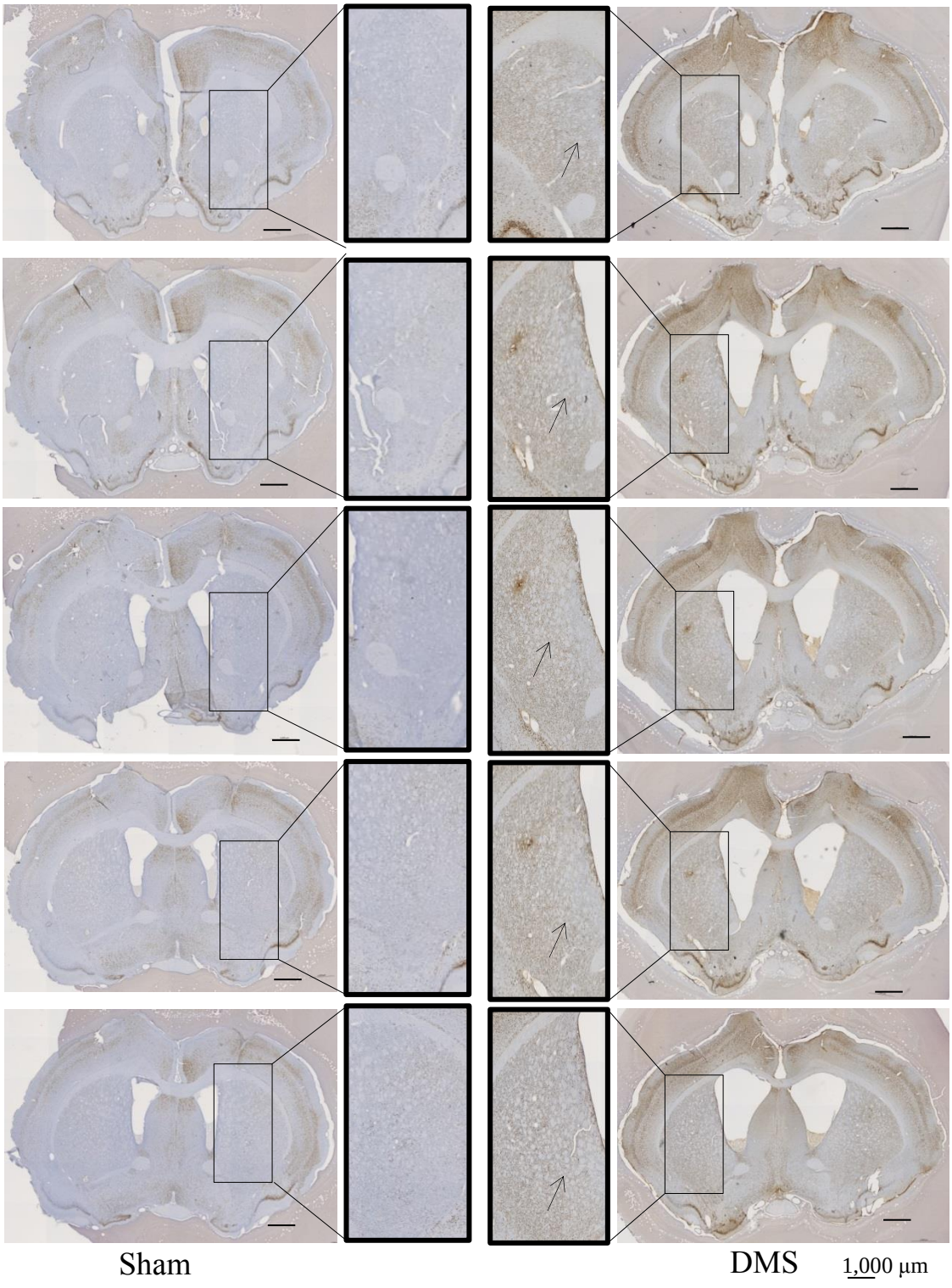

Figure 6.3. *Representative photomicrographs of coronal sections double-stained with NeuN and Cresyl Violet from sham control (left), and dorsomedial striatum (DMS) lesion (right) animals. Sham control rats showed an even distribution of neurons in the striatum whilst DMS lesioned rats showed cell loss. Arrows point to the lesion area. From the top, sections are +1.7, +1.2, +0.7, +0.2, and -0.3 mm from bregma. Scale bar 1,000 µm.*

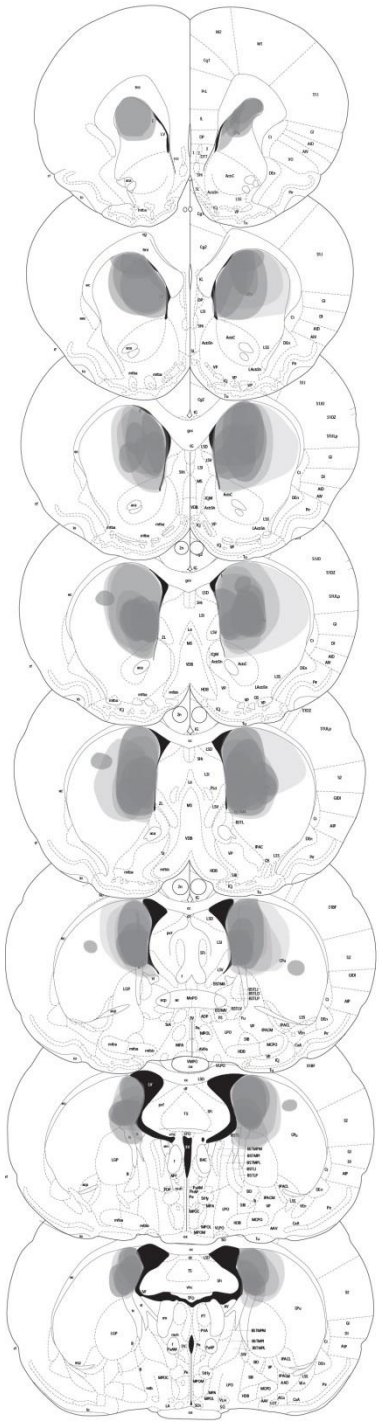

Figure 6.4. *Schematic diagram of a series of coronal sections of the rat brain illustrating the extent of bilateral dorsomedial (DMS) striatum lesions. Darkness represents coincidence lesions from different animals. From the top, sections are +2.2, +1.7, +1.2, +0.7, +0.2, −0.3, −0.80 and −0.92 mm from bregma (Paxinos and Watson, 2007).*

6.3.2 Behavioural performances

Trials completed. Figure 6.5 shows the mean number of trials completed per session for DMS lesion and sham-surgery control rats, pre-surgery and post-surgery. Figure 6.6 shows the mean number of the trials completed on the devaluation and baseline sessions pre-surgery and post-surgery for the two groups.

Both groups completed fewer trials after surgery (main effect of Surgery: $F(1, 11) = 9.12$, $p = .01$, $\eta_p^2 = .46$), and fewer trials were completed on the devaluation sessions in comparison to baseline sessions (main effect of Session: $F(1, 11) = 462.69$, $p < .001$, $\eta_p^2 = .98$). DMS lesions did not affect the number of trials completed on the devaluation or baseline sessions (neither main effect of group nor any interactions were significant).

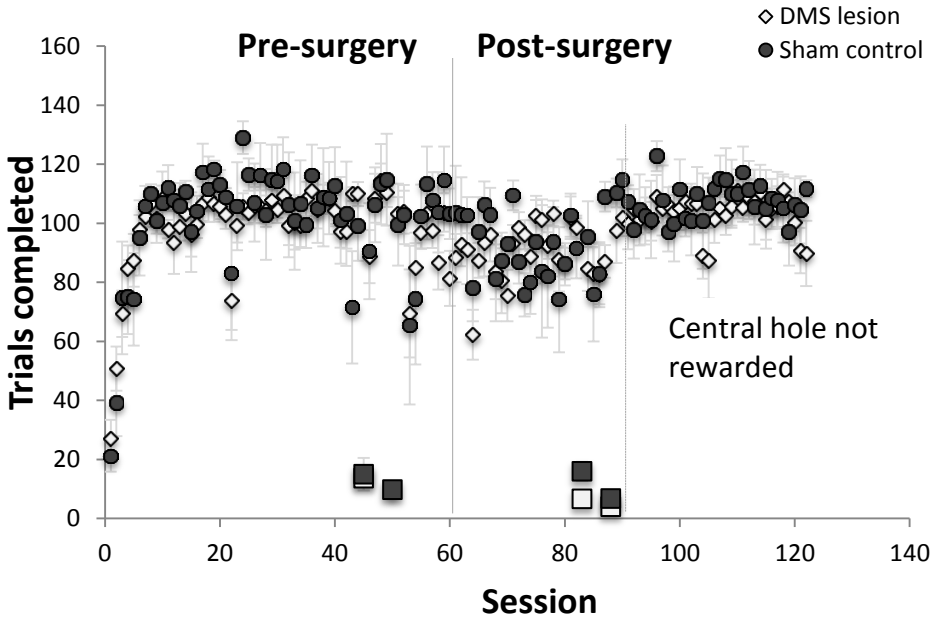

Figure 6.5. *Mean total trials completed (± SEM) for DMS lesion and sham-surgery control rats across all sessions of the experiment, the devaluation sessions are marked with a square (□) symbol.*

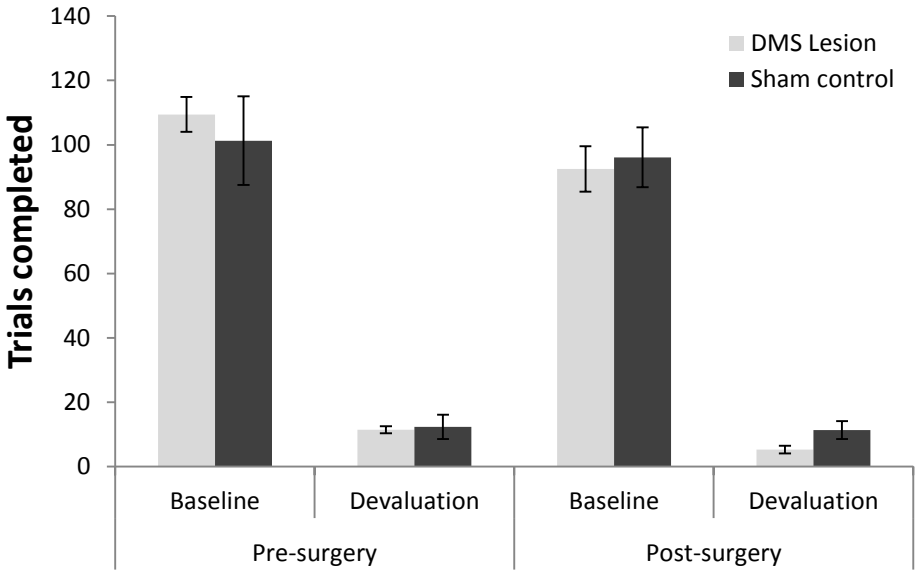

Figure 6.6. *Mean total trials completed (± SEM) for DMS lesion and sham-surgery control rats on devaluation and baseline sessions pre-surgery and post-surgery.*

Percentage of pokes in the central hole. The percentage of pokes in the central hole was not affected by surgery, lesion or by the devaluation sessions (all effects and interactions were not significant). Figure 6.7 shows the percentage of pokes in the central hole for the DMS lesion and sham-surgery control rats across all the sessions of the experiment.

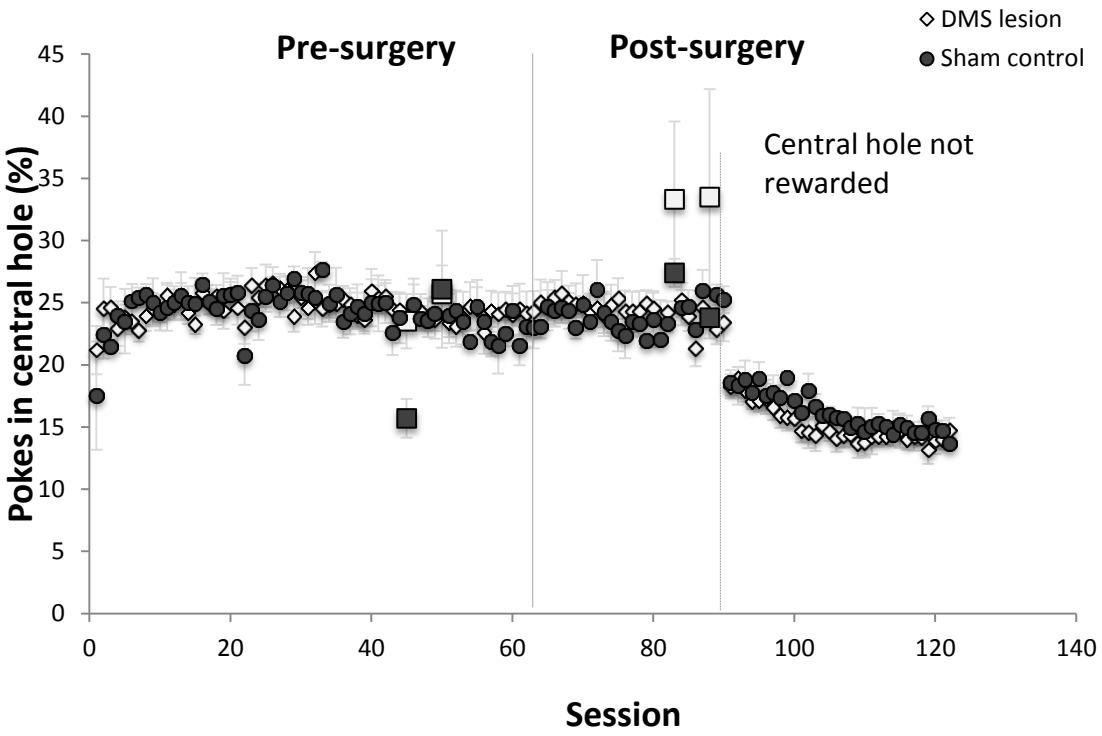

Figure 6.7. *Mean percentage (± SEM) of pokes in the central hole for DMS lesion and sham-surgery control rats across all the sessions of the experiment. The devaluation sessions are marked with a square (□) symbol.*

Omission of reward on central hole phase. Figure 6.8 shows the mean of the percentage of pokes in the central hole for the baseline and omission sessions. Nose pokes in the central hole decreased for both groups when the hole was not rewarded (main effect of Session: $F(1, 11) = 59.26$, $p < .001$, $\eta_p^2 = .84$).

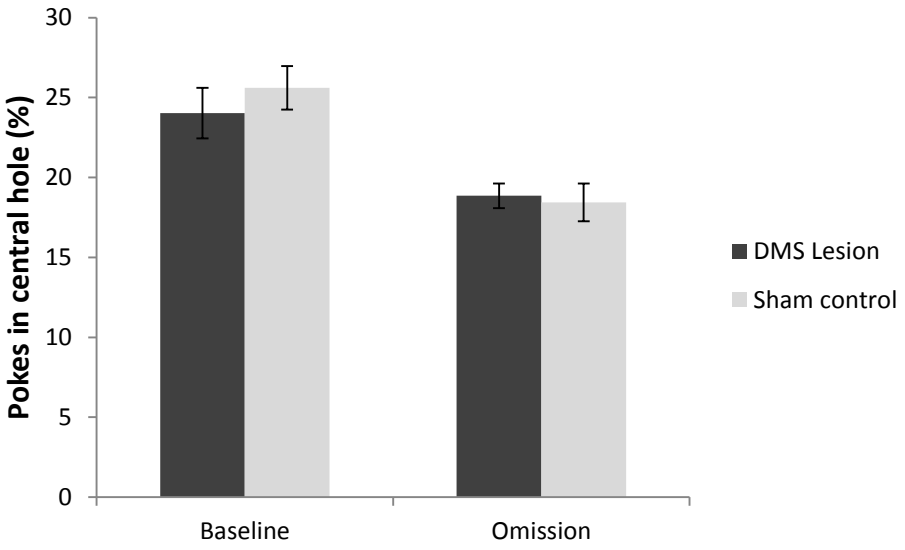

Figure 6.8. *Mean percentage (± SEM) of pokes in the central hole for DMS lesion and sham-surgery control rats on baseline and devaluation sessions.*

Reaction Time (RT). Figure 6.9 shows the mean RTs (i.e., time to withdraw from the reward nose poke hole once the reward signal has been presented) per session for DMS lesion and sham-surgery control rats, pre-surgery and post-surgery. Figure 6.10 shows the mean RTs on the devaluation and baseline sessions pre-surgery and post-surgery for DMS lesion and sham-surgery control rats. RTs were slower post-surgery (main effect of Surgery: $F(1, 11) = 7.53$, $p = .02$, $\eta_p^2 = .41$). Pre-surgery, there was no difference in RTs between sham-surgery control and lesioned rats; however, post-surgery, reaction times of the control group were slower than the lesion group (significant interaction between Surgery x Group: $F(1, 11) = 20.49$, $p = .001$, $\eta_p^2 = .65$). Pre-surgery, there was no difference in RTs between the devaluation and the baseline session; however, post-surgery, reaction times during the devaluation sessions were slower than the baseline sessions (significant interaction between Surgery x Session: $F(1, 11) = 9.25$, $p = .01$, $\eta_p^2 = .46$). There was a three way interaction between the variables Surgery, Session and Group ($F(1, 11) = 21.46$, $p = .001$, $\eta_p^2 = .66$). Sidak's-corrected pairwise comparisons revealed that pre-

surgery the RTs on the devaluation and the baseline sessions were not different between the DMS lesion and the sham-surgery control groups; however, post-surgery, the sham-surgery control group had slower RTs on the devaluation sessions in comparison to the DMS lesion group (p = .01).

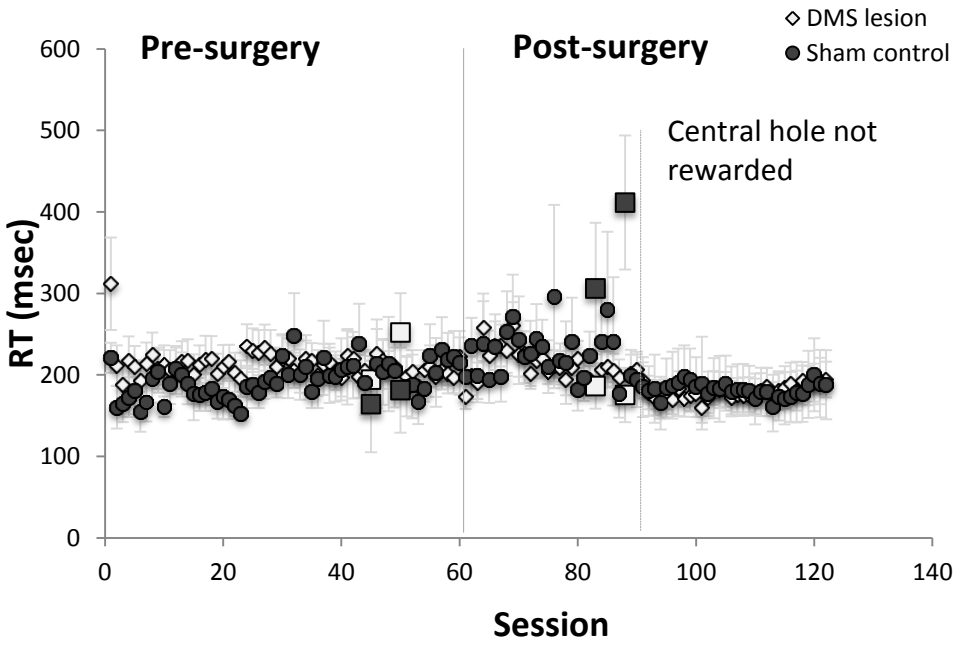

Figure 6.9. *Mean reaction times (± SEM) for DMS lesion and sham-surgery control rats across all the sessions of the experiment. The devaluation sessions are marked with a square (□) symbol.*

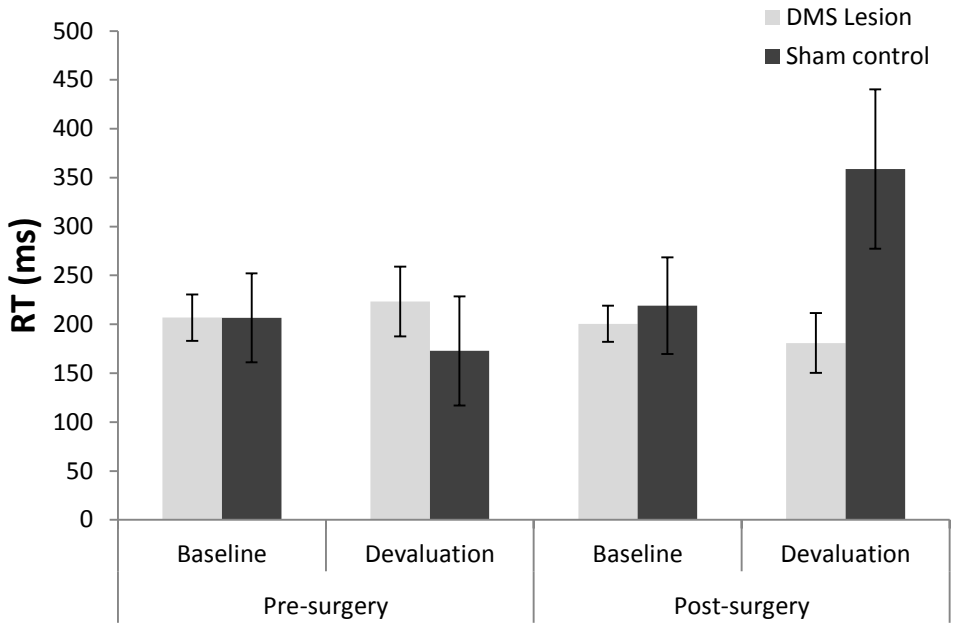

Figure 6.10. *Mean reaction time (± SEM) for DMS lesion and sham-surgery control rats on devaluation and baseline sessions pre-surgery and post-surgery.*

Movement Time (MT). Figure 6.11 shows the mean MTs (i.e., time between withdrawal of nose poke from the rewarded hole to the first lick at reward spigot) per session for DMS lesion and sham-surgery control rats, pre-surgery and post-surgery. MTs were slower on devaluation than baseline sessions (main effect of Session: $F(1, 11) = 9.43$, $p = .01$, $\eta_p^2 = .46$).

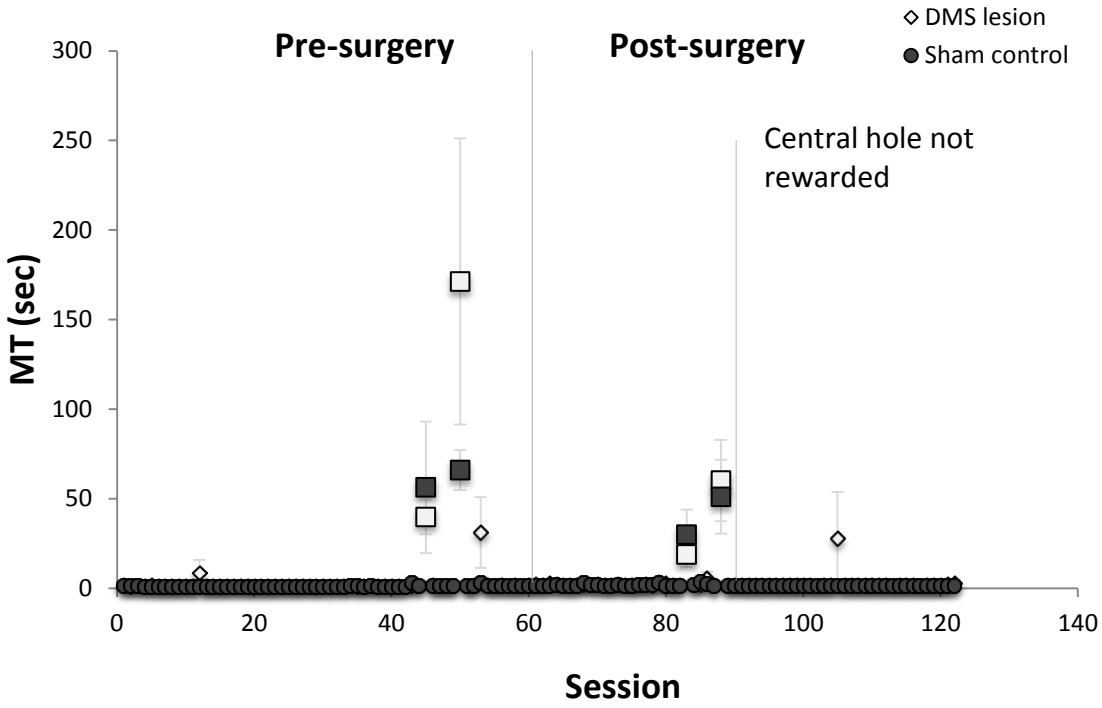

Figure 6.11. *Mean movement times (± SEM) for DMS lesion and sham-surgery control rats across all the sessions of the experiment. The devaluation sessions are marked with a square (□) symbol.*

Post-reinforcement pause (PRP). Figure 6.12 shows the mean PRPs (i.e., time between trial onset and the first nose poke) per session for DMS lesion and sham-surgery control rats, pre-surgery and post-surgery. Figure 6.13 shows the mean PRPs on the devaluation and baseline sessions pre-surgery and post-surgery for DMS lesion and sham-surgery control rats. PRP were longer after surgery (main effect of Surgery: $F(1, 11) = 6.35$, $p = .03$, $\eta_p^2 = .37$). Devaluation sessions had longer PRPs than baseline sessions (main effect of Session: $F(1, 11) = 13.35$, $p = .004$, $\eta_p^2 = .55$). Pre-surgery, baseline sessions had shorter PRPs compared to devaluation sessions; post-surgery, devaluation sessions had longer PRPs than the baseline sessions (significant interaction between Surgery x Session: $F(1, 11) = 5.77$, $p = .04$, $\eta_p^2 = .34$).

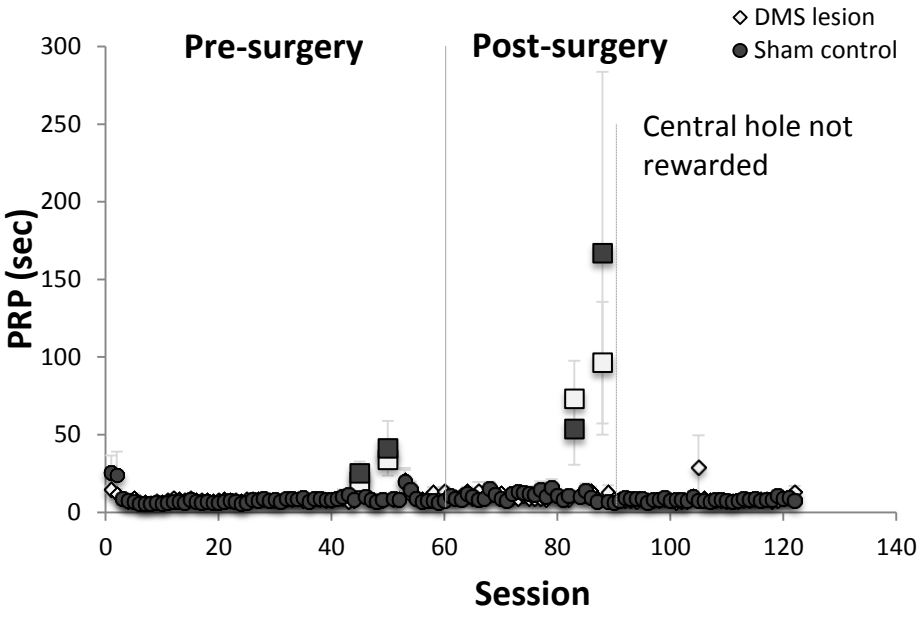

Figure 6.12. *Mean post-reinforcement pauses (± SEM) for DMS lesion and sham-surgery control rats across all the sessions of the experiment. The devaluation sessions are marked with a square (□) symbol.*

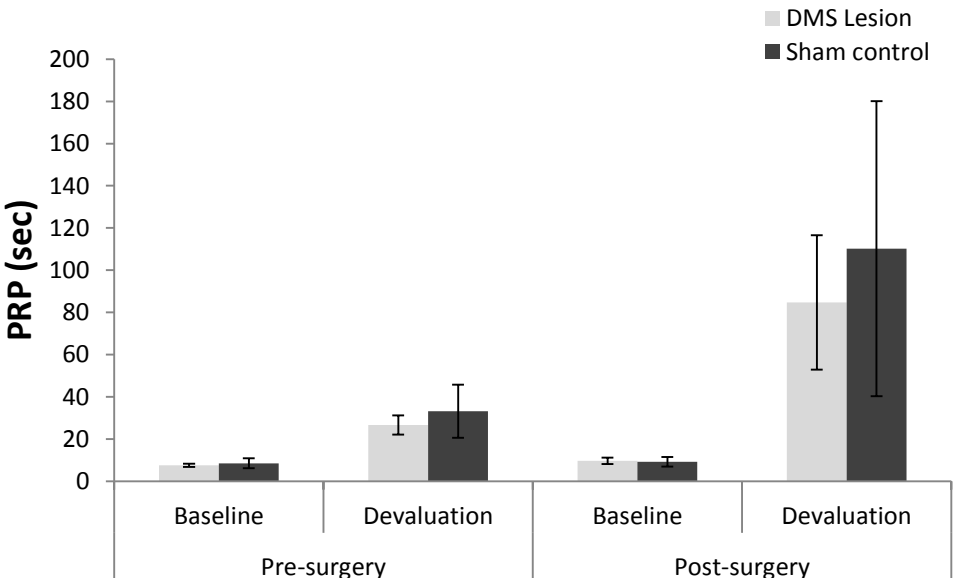

Figure 6.13. *Mean post-reinforcement pauses (± SEM) for DMS lesion and sham-surgery control rats on the devaluation and the baseline sessions pre-surgery and post-surgery.*

Lick rate. Figure 6.14 shows the mean rate of licking the spigot once the reward was available per session for DMS lesion and sham-surgery control rats, pre-surgery and post-surgery. Figure 6.15 shows the mean rate of licking the spigot on devaluation and baseline sessions pre-surgery and post-surgery for DMS lesion and sham-surgery control rats. The rate of licking the spigot was higher during the baseline than in the devaluation sessions (main effect of Session: $F(1, 11) = 393.72$, $p < .001$, $\eta_p^2 = .97$). During baseline sessions, the rate of licking the spigot was higher for the DMS than sham-surgery controls ($p = .05$); however, during the devaluation sessions there was no difference between the groups on the rate of licking the spigot (significant interaction between Session x Group: $F(1, 11) = 6.90$, $p = .02$, $\eta_p^2 = .39$).

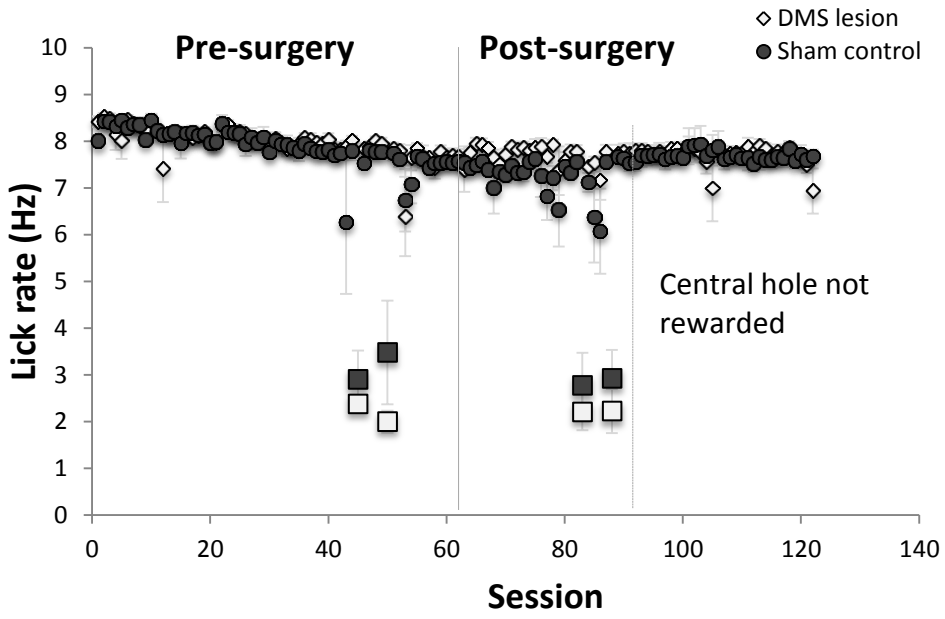

Figure 6.14. *Mean licking rate (± SEM) for DMS lesion and sham-surgery control rats across all the sessions of the experiment. The devaluation sessions are marked with a square (□) symbol.*

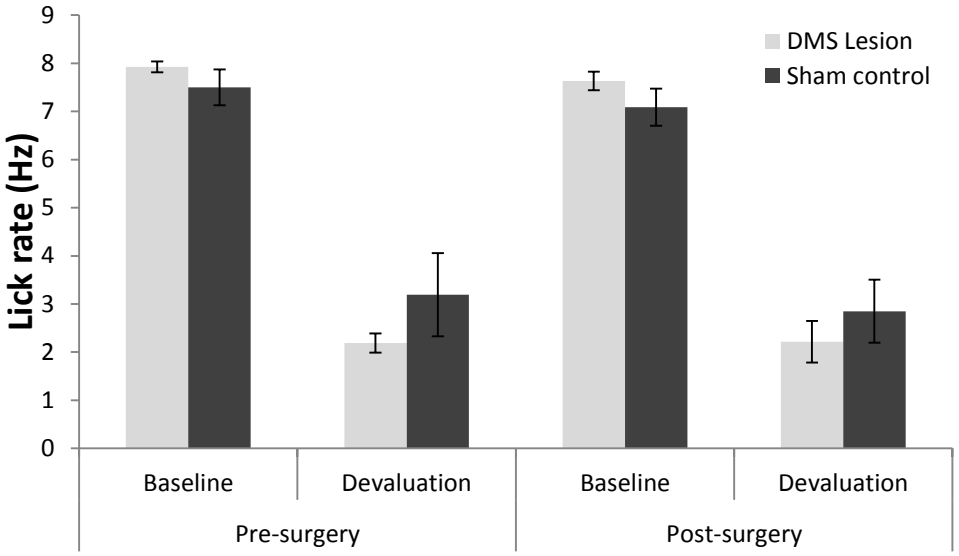

Figure 6.15. *Mean licking rate (± SEM) for DMS lesion and sham-surgery control rats on devaluation and baseline sessions pre-surgery and post-surgery.*

6.4 Discussion

The present experiment adapted the free scan task, proposed by Desrochers et al. (2010), in which rats nose poked a row of five holes to find a target hole selected randomly on each trial. There was no cue that would indicate which hole would produce the reward or when the target would become bated. The aim of this experiment was to investigate the effects of bilateral QA lesions of the DMS on goal-directed behaviour and habitual responding. Changes in goal value were assessed by using a devaluation of the reward, in which rats had free access to the reinforcement in their home cages before testing, and the reward was not delivered during the session. It was hypothesised that if habits were formed, the performance on the task would not be affected even though the value of the reward was devalued. This was not observed, either pre-surgery or post- surgery, both DMS lesioned and sham-surgery control rats completed fewer trials during the devaluation sessions, which suggested nose poking was not habitual responding.

In addition, to evaluate if lesions of the DMS would impair goal-directed behaviour (i.e., the association of an action and an expected outcome), in the last phase all of the conditions remained the same, but the central hole never produced a reward. Since there were no cues to indicate the change, rats had to learn through the task that nose poking into the central hole would never produce a reward. Habitual responding would consist of continuing responding to the central hole. Thus, it was hypothesised that if the DMS was required to learn goal-directed behaviours, rats with DMS lesions would show habitual responding and would continue responding to the central hole regardless of it no longer being reinforced. This was not observed either, as the percentage of responses in the central hole decreased for both the DMS lesioned and sham-surgery control rats, when it did not produce a reward. Given that rats with DMS lesions were able to learn that the central hole no longer produced a reward (i.e., their number of responses in the central hole decreased), it may be suggested that lesions of the DMS do not impair the ability to learn goal-directed behaviours.

Additional measures such as reaction time, movement time and post-reinforcement pause, were compared between the groups to evaluate possible movement changes affected by lesions of the DMS. Furthermore, since it has been reported that the most prominent regions where chorea manifests in HD patients are the orofacial regions (Berardelli et al., 1999), the rate of licking the spigot once the reward was available was also compared between the groups. General motor impairments after DMS lesions should be expected to reduce motor activity. This was not observed in the current experiment. There were no differences between DMS lesioned and sham-surgery control rats in MT, PRP or licking rate; thus DMS lesions did not produce any evidence of general motor impairments. During the devaluation sessions, MTs were slower, PRPs were longer and the licking rate decreased for both groups. However, sham-surgery

control rats had slower RTs on the devaluation sessions post-surgery. Slower RTs (i.e., time to withdraw from reward nose poke hole once reward signal has been presented) during the devaluation sessions could be explained by the fact that no reward was presented, which suggested that sham-surgery control rats learned that the reward signal that would indicate reward would no longer deliver reward during that session.

Previous studies have reported that when the contingency between response and outcome delivery is weakened by using interval schedules of reinforcement, instrumental performance can rapidly become habitual, and also becomes insensitive to outcome devaluation (Dickinson, 1985; Dickinson et al., 1983). This was not observed in the current experiment. Even though uncertainty and unpredictability in the delivery of the reward over a prolonged period of training were used, the results from the current experiment showed that the performance of the task was affected after the value of the outcome was devalued, and when the reward was omitted in one of the holes. These results suggested that nose poking was not habitual and lesions of the DMS did not affect the acquisition of goal-directed behaviours, as it has been previously suggested (Yin et al., 2005).

The results from this experiment are also inconsistent with previous studies that have reported that inhibition of the DMS leads to a loss of behavioural flexibility and outcome sensitivity, and increase habitual responding (Packard, 2009; Packard and McGaugh, 1996; Ragozzino, 2007; Yin et al., 2005). This inconsistency of the results in habitual responding may be due to the differences between the tasks that have been used, and in the operational definition of habit. For example, some studies have used simple operant tasks that required pressing a lever to produce a reward and habits were assessed by comparing the performance once the reward was devalued. Other studies have used maze learning tasks to evaluate place and response

strategies and habits were defined as the inflexibility of always performing the same behaviour of turning to the same side, regardless of where the animal was positioned to start the task (Packard and Knowlton, 2002; Packard and McGaugh, 1996). The new task used in the present experiment, required rats to nose poked a row of five holes to find a target hole selected randomly on each trial. Given that the baited hole changed every trial, it was unpredictable where and when the target would become baited. Therefore, no particular response or sequence of responses would produce a reward, and no fixed S-R habit would be required to solve the task. Thus, it might be suggested that while the DMS was not required for performing the task used in this chapter, the DMS is required in other tasks that involve maze learning or simple operant tasks responses that involve S-R associations. Similarly, studies in patients with neuronal degeneration in the neostriatum, such as HD, have shown impairments in the acquisition of a variety of tasks that require motor skills (e.g., mirror reading, pursuit-rotor tracking, adaptation level during weight judgements), and implicit learning (see section 1.8.1 from the General Introduction). In addition, one of the most common obsessions in HD are ritualistic behaviours (Novak and Tabrizi, 2010). Hence, it was expected that rats with QA lesions of the DMS would present deficits in the current task that could be compared to patients with HD.

In addition, it has been suggested that lesions of the DMS have opposing effects to lesions of dorsolateral striatum (DLS) for balancing habitual and goal-directed behaviours (Smith and Graybiel, 2014). While it has been suggested that the DMS is involved in learning A-O associations, the DLS has been proposed to be involved in S-R learning (Yin and Knowlton, 2006; Yin et al., 2004; Yin et al., 2005). Using the devaluation procedure, Yin et al. (2004) observed that rats with lesions of the DLS reduced their performance after the reward was devalued, while the behaviour of DMS lesioned rats was not different to the sham control rats.

This suggests that the instrumental performance (press a lever) in the DLS lesioned rats was controlled by goal expectancy; therefore, when the value of the reward was decreased, the performance also decreased. But rats with DMS lesions responded habitually, as the performance was not affected when the reward was devalued. Thus, future research should investigate the involvement of the DLS in habitual responding using the task reported in this chapter.

Furthermore, to evaluate if the performance is goal-directed or habitual, in addition of outcome devaluation, the effects of contingency degradation (i.e., rewards are delivered regardless of the behaviour) should be examined in the task used in this chapter to investigate the role of the dorsal striatum in goal-directed and habitual responding.

In summary, this experiment presented a new task to evaluate goal-directed and habitual responding. Both, DMS lesioned and sham-surgery control rats did not show habitual responding in the task, as their performance decreased when the value of the reward decreased. Lesion of the DMS did not change the sensitivity to outcome devaluation, and both sham-surgery control and DMS lesioned rats made fewer responses to the hole that never delivered reward during the last phase of the experiment, which suggested that DMS lesions did not affect the acquisition of a new goal-directed behaviour. From these findings it could be concluded that rats with bilateral quinolinic acid lesions of the dorsomedial striatum do not present deficits as HD patients in goal-directed behaviours, neither showed ritualistic behaviours which are also considered as habits and that are one of most common obsessions in HD (Novak and Tabrizi, 2010).

Given that no cognitive deficits were observed in DMS lesioned rats that could be compared with HD patients; Chapter 7 evaluated if rats with bilateral QA lesions of the DMS

presented similar cognitive deficits in behavioural flexibility as those seen in HD patients in the ID/ED attentional set-shifting task.

Chapter 7

Attentional Set-Shifting in Rats with Bilateral Quinolinic Acid Lesions of the Dorsomedial Striatum

Patients with damage to the striatum arising from Huntington's disease (HD) exhibit deficits in behavioural flexibility in the Intra-Dimensional/Extra-Dimensional (ID/ED) attentional set-shifting task. Patients in early stages of HD and preclinical carriers of the HD mutation showed impairments in ED shift performance; whereas, patients with advanced HD cannot complete the earlier reversal stages and therefore rarely reach the ED stage. To evaluate the QA lesioned rat as a suitable model for cognitive deficits in HD, rats with bilateral QA lesions of the DMS were tested in the attentional set-shifting task.

Do rats with bilateral quinolinic acid lesions of the dorsomedial striatum present similar cognitive deficits in behavioural flexibility as those seen in HD patients in the ID/ED attentional set-shifting task?

7.1 Introduction

One of the cognitive deficits in patients with Huntington's disease is a decrement in behavioural flexibility (i.e., the ability to adjust responses according to the specified context and requirements of varying situations). Attentional set-shifting, which is a process of shifting attention from one perceptual dimension to another, is an aspect of behavioural flexibility that has been assessed in humans using the Wisconsin Card Sorting Test (WCST; Berg, 1948). Deficits on the performance of the WCST have been reported in patients with HD. Josiassen et al. (1983) compared the performance of patients with recently diagnosed HD (diagnosed for one year or less) with moderate HD patients (diagnosed for more than a year to eight years) on the WCST. While both groups were able to infer the first sorting rule without difficulty; when the sorting rule changed, patients with moderate HD showed deficits in behavioural flexibility (i.e., they perseverated in using the past sorting rule). Various imaging studies have suggested that the deficits seen in HD patients in the WCST result from changes in striatal function, rather than in the frontal cortex (Lawrence et al., 1998b, for a review). For example, using high resolution single photon emission computerised tomography (SPECT), Hasselbalch et al. (1992) showed that blood flow is reduced in the caudate nucleus of patients with HD and that there is a positive linear relationship between blood flow in the caudate nucleus and performance of the WCST in patients with Huntington's disease. Deficits in formation, maintenance and shifting of cognitive sets have also been reported in the Intra-Dimensional/Extra-Dimensional (ID/ED) attentional set-shifting task in patients with HD (Lawrence et al., 1998b). Depending on the progression of the disease, patients with Huntington's had shown different impairments in the ID/ED attentional set-shifting task. Patients in the early stage of HD showed impairments in the ED shift (i.e., they had deficits when the task required them to shift responding from stimuli of one perceptual

dimension to stimuli of another dimension) and made more perseverative than non-perseverative errors (i.e., they kept on responding to the previous relevant stimuli dimension, when it was no longer relevant); but were not impaired in set-formation or reversal learning (Lawrence et al., 1996; Lawrence et al., 1999a). However, patients in advanced HD showed reversal impairments (i.e., they continued selecting the previously reinforced stimuli even though it was no longer correct) and fail to complete the task. Patients in advanced stage HD cannot complete the reversal stages and rarely reach the ED stage (Lange et al., 1995). Preclinical carriers of the HD mutation, before the onset of any clinical movement disorder, also showed specific deficits in the ED stage of the ID/ED attentional set-shifting task. Therefore, the ID/ED attentional set-shifting task seems to be a tool that is capable of detecting subtle impairments earlier than many other cognitive tasks and before the development of the movement disorders in HD (Lawrence et al., 1998a).

Given that the ID/ED task has been able to identify cognitive deficits in HD patients even before any motor symptoms (Lawrence et al., 1998a), and since different studies have suggested that the striatum is essential for effective set-shifting (Castañé et al., 2010; Ragozzino et al., 2002b); the aim of this chapter was to evaluate if rats with bilateral quinolinic acid lesion of the dorsomedial striatum, which mimics some aspects of the neural damage in HD, presented similar cognitive deficits in the attentional set-shifting task (Birrell and Brown, 2000) as those seen in patients with HD. Based on the performance on the ED/ID task of HD patients (Josiassen et al., 1983; Lange et al., 1995; Lawrence et al., 1998a; Lawrence et al., 1996; Lawrence et al., 1999b), it was hypothesised that rats with QA lesions of the DMS would show impairments in the ED shift (i.e., they would have deficits when the task requires them to shift responding from stimuli of one perceptual dimension to stimuli of another dimension), would make more perseverative

than non-perseverative errors (i.e., they would kept on responding to the previous relevant stimuli dimension, when it would no longer be relevant), and would have reversal impairments (i.e., they would continue selecting the previously reinforced stimuli even though it would no longer be correct).

7.2 Method

7.2.1 Animals

The animals were the same 24 male hooded Lister rats (Charles River, UK Ltd) used in Chapter 5. Testing was conducted in the light phase of a 12:12 hr. light-dark cycle. Housing and husbandry conditions were described in the General Methods in Chapter 2.

7.2.2 Apparatus

Training and testing sessions were conducted in the attentional set-shifting box, a 69.5 x 40.5 x 18.5 cm adapted plastic housing-cage. The cage was divided into two sections by a Plexiglas panel located at one-third of the length of the cage. The floor of the big section was covered with sawdust and contained a bowl with water in the back corner. The small section was divided with a central divider and each section contained a ceramic bowl with an internal diameter of 7 cm and a depth of 4 cm. Access to the small sections, in which the bowls were placed, was controlled by a removable divider (see Figure 7.1).

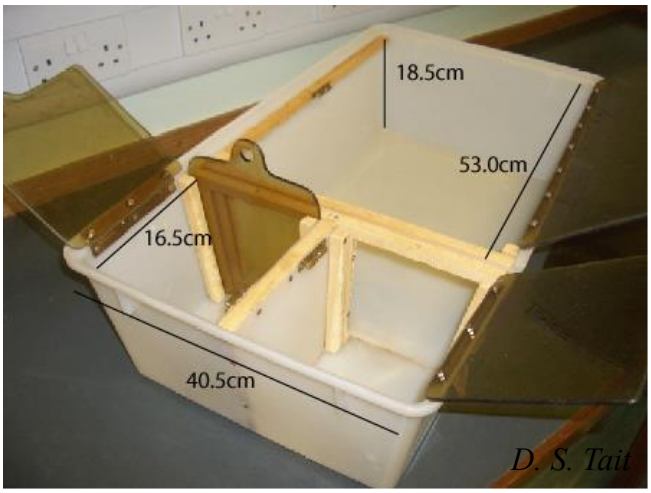

Figure 7.1. *Attentional set-shifting box.*

7.2.3 Procedure

Surgery. The surgery protocol was as described in Chapter 2 (General Methods). Sixteen rats received DMS lesions and eight rats received sham-surgery. After the rats recovered from the surgery (approximately 10 days) testing in the attentional set-shifting task started. In general, one rat was tested per day. The order of testing the rats was counterbalanced.

Habituation. One day before training, rats were given a bowl filled with sawdust and six pieces of food reward (half of a Honey Loop cereal piece; Kellogg Company, UK) in the home cage overnight.

Training. During the following sessions, the animals were trained to dig in bowls filled with sawdust to retrieve the reward. Each trial was initiated by raising the divider that allowed the access to the two bowls. In the initial phase of training both of the bowls were baited. In the first trial the reward was placed on top of the sawdust; once the rats retrieved the reward, the reward was progressively buried further down with each trial. This phase finished when the rats were reliably digging the bowl to retrieve the reward, which typically required six trials in each bowl. In the final stage of the training phase, rats were given two simple discriminations, one

between two odours in sawdust, and one between two digging media with no added odour, with only one of the bowls being baited in each simple discrimination. During the first four trials, the rats were allowed to dig in both of the bowls; this permitted the rats to dig in the baited bowl and learn the cue contingency regardless of whether they first dug in the incorrect bowl. The criterion for a dig used through testing was defined as when the nose or paws of the rat broke the surface of the digging medium. Latencies to respond were recorded and were measured using a stopwatch. Time was recorded from the moment the panel that gave access to the bowls was lifted and stopped when the rat dug in one of the bowls. The trial was scored as *correct* if the rat dug in the baited bowl first; if the rat dug in the incorrect bowl, the trial was recorded as an *error*, but the rat was given the opportunity to dig in the correct bowl to collect the bait. After the fourth trial, once the rat dug in one of the bowls, the access to the other bowl was blocked with a clear plastic panel and a new trial started. If the rat did not dig within 10 minutes, the access to both bowls was closed, the trial was recorded as a *non-di*g and a new trial started. Each stage continued until six consecutive correct responses had been made; the testing phase started the following day.

Testing. In the testing phase of the task, the rats were required to perform a series of seven discriminations in which they had to select a bowl on the basis of a stimulus exemplar in a particular dimension, either odour or digging medium. The exemplars (odour and digging medium pairs) are summarised in Table 7.1.

Simple Discrimination (SD). First rats were presented with a simple discrimination between either two digging media with no added scent, or between two odours mixed in sawdust. This stage provided an early index of general learning ability. For example, in the SD, the only

relevant dimension presented was odour. Therefore, two different odours were presented in sawdust, but the reward was only present in one of the odours.

Compound Discrimination (CD). This stage introduced a second irrelevant dimension to form a compound discrimination stage, but the contingencies of the discrimination remained the same as the SD. This stage showed if the rat was distracted by the irrelevant dimension, and if it remembered the contingencies of the SD. In the example used, digging medium was introduced as the irrelevant dimension, but the rewarded odour used in the SD still identified the bowl that was baited.

Compound Discrimination Reversal (REV1). In the first reversal stage, the discrimination contingency of the CD is switched such that the previously incorrect stimulus becomes correct and the correct stimulus becomes the incorrect. This stage provided a measure of inhibition of responding to the previously rewarded stimuli. In the example, the first reversal required the rats to respond to the bowl with the previously incorrect odour.

Intradimensional (ID) acquisition. In this stage two novel odours and two novel digging media were introduced, but the relevancy of the two dimensions remained the same as in previous stages (e.g., the rat was presented with two different odours and two different digging media but given that, in the example used, odour was the relevant dimension, one of the novel odours identified the baited bowl). This stage measured the abilities to learn a new compound discrimination that was consistent with the attentional set from the previous stages.

Intradimensional Reversal (REV2). The second reversal required the rats to respond to the previously incorrect stimulus. In the example used, rats had to dig in the bowl with the previously incorrect odour. Improvements in reversal learning can be detected in this stage.

Extradimensional (ED) shift. This stage presented new compound stimuli, but the relevancy of the two dimensions was switched. Therefore, if the rats had been required to respond to the odour stimuli in the SD, CD and ID stages, they would now be required to shift their attention, as one of the digging medium stimuli would signal reward in the ED. This stage measured the abilities to learn a compound discrimination that was inconsistent with the attentional set from the previous stages.

Extradimensional Reversal (REV3). The third and final reversal required the rats to respond to the previously incorrect stimulus. Thus, the bowl with the incorrect medium became the bowl with the bait and vice versa.

Examples of the discriminations required to be made are found in Table 7.2. At each stage, the criterion for moving to the next stage required rats to make six consecutive correct responses. Rats were counterbalanced so that the initial SD would be either odour or digging medium.

Table 7.1.

Exemplars used (presented in pairs within each dimension).

	Digging media		Odours	
Training Pairs	M9-Polystyrene pieces	M10-Shredded paper	O9-Mint	O10-Oregano
Pairing 1	M1-Coarse tea	M2-Fine tea	O1-Cinnamon	O2-Ginger
Pairing 2	M3-Sand	M4-Grit	O3-Sage	O4-Paprika
Pairing 3	M5-Coarse shavings	M6-Fine shavings	O5-Turmeric	O6-Cloves

Note. Table adapted from Birrell and Brown (2000).

Table 7.2.
Example of the testing stages and possible stimulus combinations in the attentional set-shifting task.

Discriminations	Dimensions		Exemplar combinations	
	Relevant	Irrelevant	**Rewarded**	Unrewarded
Simple (SD)	**Medium**	Odour	**M1**	M2
Compound (CD)	**Medium**	Odour	**M1**/O1	M2/O1
			M1/O2	M2/O2
Reversal 1 (REV1)	**Medium**	Odour	**M2**/O1	M1/O1
			M2/O2	M1/O2
Intradimensional (ID) shift	**Medium**	Odour	**M3**/O3	M4/O3
			M3/O4	M4/O4
Reversal 2 (REV2)	**Medium**	Odour	**M4**/O3	M3/O3
			M4/O4	M3/O4
Extradimensional (ED) shift	**Odour**	Medium	**O5**/M5	O6/M5
			O5/M6	O6/M6
Reversal 3 (REV3)	**Odour**	Medium	**O6**/M5	O5/M5
			O6/M6	O5/M6

Note. Rats were counterbalanced so that half received digging medium as the initial relevant dimension, whereas the other half received the odour. Stimulus pair order and correct/incorrect stimuli within a pair were also counterbalanced.

Repeat testing. All rats were tested in the Attentional-Set-Shifting task twice. However, the first test was conducted by an undergraduate experimenter and the data were not included in this thesis. The stages were always in the same order while the stimuli were counterbalanced within rats and between tests. The second test started seven weeks after surgery.

Histology. As described in the histology section of the General Methods in Chapter 2.

7.2.4 Data analysis

All analyses were conducted in IBM SPSS (v 21, SPSS Inc., Chicago, IL), using the General Linear Model routine.

Total trials to criterion. Number of trials, in each stage, until six consecutive correct responses were made (p = .0156 considering the six trials and assuming the rat would choose randomly between the two bowls).

Mean shift-costs. Number of trials to criterion in the ED stage minus the number of trials in the ID; this provides an index of the behavioural cost of shifting set that is independent of general learning ability.

In addition to trials to criterion, total errors, number of non-digs and latency to dig were recorded in all stages. Trials to criterion and errors often reveal the same pattern of results; however, trials to criterion have been suggested to be a more powerful measure (Tait and Brown, 2007).

Statistical analyses. Trials to criterion were analysed using a two factor, 2 x 7 mixed repeated measures ANOVA with the variables of Group (Between-groups factor with 2 levels: DMS Lesion vs. Sham-surgery control), and Stage (Within-subjects factor with 7 levels: SD, CD, REV1, ID, REV2, ED, REV3).

Mean shift-cost were analysed using a univariate ANOVA with Group as a between-group factor.

Sidak's-corrected pairwise comparisons for significant effects or interactions were performed. When data violated the assumption of sphericity, Huynh-Feldt corrections were reported, but the uncorrected degrees of freedom were reported. The criterion for significance (alpha level) was $p < .05$ in all cases.

Box plot generation. To effectively display the individual differences in shift-cost, as well as providing central tendency and distributional information, box plots were generated. The central line in each box plot represents the median. The lowest edge of the box is the lower quartile, and the top edge of the box shows the value of the upper quartile. The whiskers on the box show the range between which the lowest 25% and the top 25% of scores fall (Field, 2013). The mean is overlaid on the box plot.

7.3 Results

7.3.1 Histology

Histology was described in Chapter 5. The final numbers in each group were 10 DMS lesion rats and 6 sham-surgery control rats.

7.3.2 Behavioural performances

Total trials to criterion. Figure 7.2 shows the mean number of trials to reach criterion for the DMS lesion and sham-surgery control group in the second test. There was no effect of the lesion on performance of the attentional set-shifting task (main effect of Group: $F(1, 14) = 1.52$, $p = .24$, $\eta_p^2 = .10$). Although all the rats learned to discriminate at the different stages, showing a difference in the number of total trials to criterion in the different stages (main effect of Stage: $F(6, 84) = 6.24$, $p < .001$, $\eta_p^2 = .31$); the pattern was the same for both groups (Stage x Group: $F(6, 84) = 1.80$, $p = .11$, $\eta_p^2 = .11$).

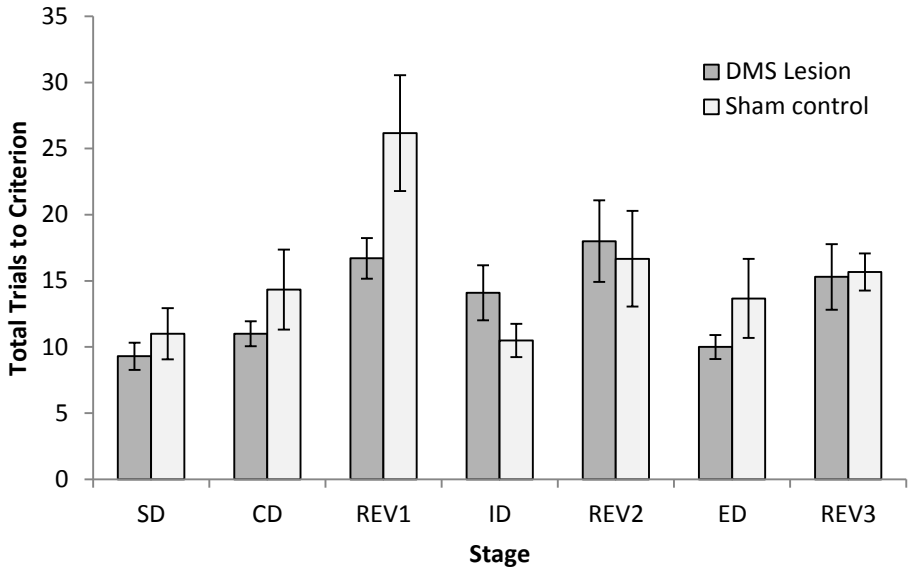

Figure 7.2. *Mean total trials to criterion (± SEM) for DMS lesion and sham-surgery control rats on the standard 7-stage attentional set-shifting task.*

Reversals. Sidak's-corrected pairwise comparisons indicated that DMS lesioned rats required fewer trials to complete the first reversal in comparison to sham-surgery controls (p = .03). The second and third reversal stages in the attentional set-shifting task were not affected by lesions of the DMS. Figure 7.3 shows the trials to criterion on the three reversal stages for DMS lesion and sham-surgery control rats.

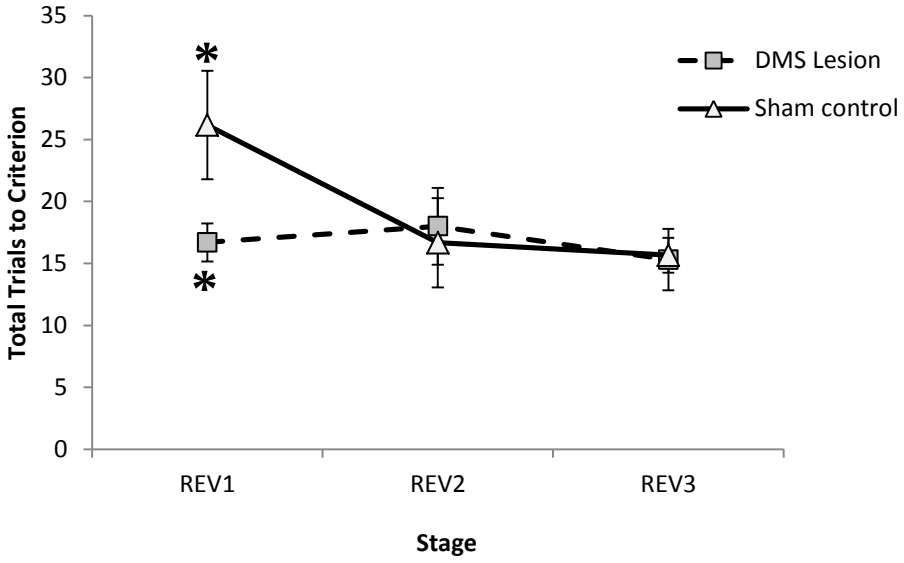

Figure 7.3. *Mean of the Total Trials to Criterion (± SEM) on the three reversal stages (REV) for DMS lesion and sham-surgery control rats on the attentional set-shifting task. DMS lesioned rats required significantly fewer trials than sham-surgery controls to learn the first reversal (* $p < .05$).*

Shift-cost. Figure 7.4 displays the box plot for each group, with the mean overlaid on the box plot. Although there was no difference between the groups in shift-cost ($F(1,14) = 2.89$, $p = .11$, $\eta_p^2 = .17$), sham-surgery control rats showed positive shift-cost on the task (i.e., the number of trials required to learn the ED stage was higher than in the ID stage), which suggested that an attentional set was formed and then needed to be shifted. While DMS lesioned rats showed a negative cost of shifting. It also appears form the box plots that there were considerable individual differences within both groups of rats.

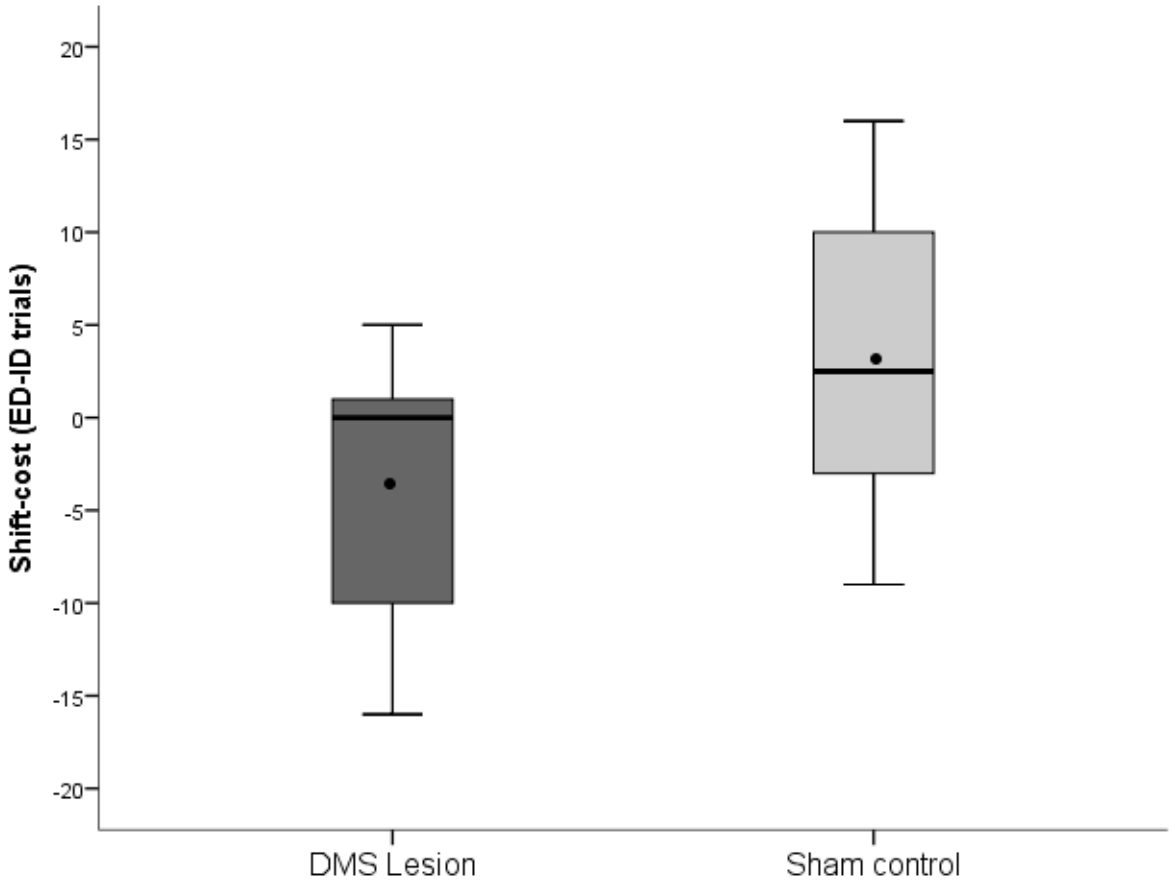

Figure 7.4. *Box plot showing the shift-cost, ED trials minus ID trials, for DMS lesioned and sham-surgery control rats. The central line in each box plot represents the median. The lowest edge of the box is the lower quartile, and the top edge of the box shows the value of the upper quartile. The whiskers on the box show the range between which the lowest 25% and the top 25% of scores fall. The mean is overlaid on the box plot (●).*

7.4 Discussion

The aim of this experiment was to evaluate if rats with bilateral quinolinic acid lesions of the dorsomedial striatum, which mimic some aspects of the neural damage in Huntington's disease, presented similar cognitive deficits in the attentional set-shifting task as those seen in HD patients.

Preclinical carriers of the HD mutation, before the onset of any clinical movement disorder, (Lawrence et al., 1998a) and early stage HD patients showed impairments in the extradimensional shift stage (Lawrence et al., 1996; Lawrence et al., 1999b). Their set-shifting impairments resulted from perseverative responding (i.e., inability to stop responding) to the stimulus dimension that was relevant in the stages before the ED (Lawrence et al., 1999b). Nevertheless, as the disease progresses, advanced stage HD patients showed impairments in the reversal stages (Lange et al., 1995).

In an extradimensional shift, attention to compound stimuli is transferred from one perceptual dimension to another (e.g., from odour to digging media, in the typical rodent version of the task or from line to shape, in the typical human version). Therefore, if the striatum is involved in different forms of flexible behaviour (Castañé et al., 2010; Ragozzino, 2007; Ragozzino et al., 2002b), it would have been expected that the rats' performance in the attentional set-shifting task following lesions of the dorsomedial striatum would have also been impaired. In particular, like patients with HD, it was expected that rats with DMS lesions: 1) would show impaired ED shift performance by continuing to respond to the stimulus dimension that was relevant in the stages before the ED, but that is no longer appropriate in the ED. Therefore, would need more trials than the controls to reach the criterion of six consecutive correct responses in the ED stage; and 2) in the reversal stages, would persist in responding to the previously rewarded stimulus when it is no longer appropriate. That is, in the reversal stage, when the discrimination contingency was switched such that the previously incorrect stimulus became correct and the correct stimulus became the incorrect, the DMS lesioned rats would require more trials to reach criterion than the control rats.

Performance on the ED stage showed that DMS lesioned rats did not require more trials than control rats to complete the stage. Although the difference between the groups was not significant, there was a trend for DMS lesioned rats to require fewer trials to complete the ED stage and more trials to complete the ID in comparison to the control rats; consequently showing diminished or absent shift-costs, which may have arisen because they did not form an attentional set. Individual differences within both group of rats showed that most of the DMS lesioned rats (six out of ten rats) exhibited a negative shift-cost while most of the sham-surgery control rats (four out of six rats) displayed a positive shift-cost. The lack of significant difference between the groups, even though there was a pattern, might result from the reduced number of rats used in this experiment after histological analysis. These results are contrary to what is observed in HD patients, who require more trials than controls to complete the ED stage.

Performance of both groups in the reversal stages showed that the number of trials to reach criterion increased when the stimulus-reward contingencies were reversed (i.e., the previously incorrect stimulus became correct and the correct stimulus became the incorrect). However, DMS lesioned rats did not require more trials to reach criterion in the reversal stages in comparison to the control rats. On the contrary, DMS lesioned rats, required fewer trials to complete the first reversal. Therefore, the results obtained in the present experiment showed that DMS lesioned rats do not present reversal impairments, as they did not perseverate in responding to the previous rewarded stimulus when it was no longer appropriate, at least when assessing reversal impairments under the current conditions. These results are inconsistent with the results seen in patients with advanced HD.

These results are consistent with the findings from Brooks et al. (2006) who reported no difference between the HD homozygous *Hdh*$^{(CAG)150}$ knock-in mouse line and the Wild type mice

in the number of trials to criterion in the reversal stage in the attentional set-shifting task. Likewise, Lindgren et al. (2013) reported no difference between rats with DMS lesions and the controls in the number of total trials to criterion in the reversal stages.

However, the results are inconsistent with reports of reversal learning deficits with DMS inactivation or lesions on other types of reversal learning tests, such as spatial, egocentric response, visual cues, and instrumental spatial discrimination (e.g., Castañé et al., 2010; Ragozzino, 2007; Ragozzino et al., 2002b). Differences between the previous studies and the current study could account for the incongruent results. One difference was that some studies investigated the effect of acute disruption of DMS function using pharmacological agents (Ragozzino et al., 2002a; Ragozzino et al., 2002b), which may be functionally different from excitotoxic lesions. Another difference was that in the previous studies other types of reversal learning tests were used (e.g., mazes and operant boxes). The difference in the way stimuli are sampled and how much can be learned in each trial could account for the incongruent results. In the set-shifting task, the bowls are separated; therefore, the stimuli from the rewarded bowl and the unrewarded bowl are not present at the same time and therefore cannot be compared simultaneously. Thus, learning will be based on the bowl sampled, and unless the rat samples both bowls, the characteristics of the unsampled bowl will remain unknown.

However, in an instrumental two lever spatial discrimination task, like the one used by Castañé et al. (2010), both the rewarded and the non-rewarded levers are present at the same time and could be seen, compared, and processed simultaneously. In each reversal session the previously correct lever became incorrect and the previously incorrect lever became correct. Therefore, after the first session of switching the rewarded lever, if pressing one of the levers was not rewarded, it could be inferred that pressing the other lever would be rewarded. One of the

limitations of using this kind of task for evaluating behavioural flexibility is that once the behaviour of alternating presses on the levers is learned, flexible behaviour may no longer be required. Similarly, on the reversal learning tasks that required rats to make an egocentric response (e.g., always make a turn to the right to receive a reinforcer) or to learn to respond to a visual cue discrimination (e.g., enter the visual cued arm; Ragozzino, 2007; Ragozzino et al., 2002a; Ragozzino et al., 2002b) once the behaviour of turning to the other side or entering to the uncued arm to get a reinforcer is learned, flexible behaviour may not be required. These studies require rats to remember a particular motor response, and stimuli are learned as a whole (i.e., either reinforced or unreinforced). However, given that the baited bowl in the attentional set-shifting task does not depend on location or a particular response, but on odour or tactile properties of the stimuli, learning requires attention to the components of the stimuli present in each bowl. Which may involve learning the relationship between different stimuli components (Ragozzino, 2007). Therefore, the difference of these tasks on the nature of sampling of the stimuli and the availability of the reward between the previous studies and the current study could account for the incongruent results. The findings described above suggest that lesions or inactivation of the dorsomedial striatum impairs reversal learning in tests that require spatial, visual and egocentric response; but reversal learning in the attentional set-shifting task is not affected by quinolinic acid lesions of the DMS, which is inconsistent with the results from advanced stage HD patients.

By the end of this experiment (January 2013), a review of the literature did not reveal any published reports of reversal impairments in DMS lesion rats using the attentional set-shifting task. Therefore, the results presented new findings evaluating reversal learning, set-formation, and set-shifting in an animal model of HD in the attentional set-shifting task. However, after the

completion of the current experiment Lindgren et al. (2013) published a paper that tested DMS lesion rats in the attentional set-shifting task. Nevertheless, the lack of set-formation in the control rats is an important issue that should be considered when interpreting the results from Lindgren et al. (2013). The ID and ED stages of the task are the critical stages for assessing attentional set-shifting (Birrell and Brown, 2000). Cognitively normal rats form attentional sets and require more trials to complete the ED in comparison to the ID stage (i.e., show a positive shift-cost) because the previously relevant stimulus is now irrelevant and a stimulus in a different dimension must be attended to. A positive shift-cost indicates that during the ED stage the animal was attending to the dimension that was relevant during the previous stages, but that is now irrelevant in the ED stage, which suggests that an attentional set was formed and then required to be shifted from. Therefore, an essential component of the task is the presence of a positive shift-cost in the control animals, which was not present on Lindgren et al. (2013) report, but that was observed in the present experiment. However, using a four consecutive ID stage (4ID) set-shifting task, Lindgren et al. (2013) showed that their control rats formed sets, but the DMS lesioned rats did not. Briefly, the 4ID set-shifting task encourages the formation of an attentional set by removing the reversal stages and including four consecutive ID stages before the ED shift stage. Performance improvements over the course of the four ID stages can provide a direct measure of set-formation. In addition, like in the standard 7-stage attentional set-shifting task, it measures the ID/ED difference which investigates set-shifting. Therefore, the standard 7-stage task does not allow drawing conclusions about set-formation in the absence of a positive shift-cost (because it is the relationship between the ID and the ED stages that provides evidence of a cost in shifting and therefore allows to assume set-formation in the presence of a positive shift-cost); but, the relationship between the IDs in the 4ID task, plus the ID/ED difference, can

provide information about set-formation. Thus, an abolished shift-cost from the DMS lesioned rats seen in the standard 7-stage task from this chapter and in the 4ID task from Lindgren et al. (2013), suggest that lesion of the DMS impair set-formation.

Overall, the results from this experiment are inconsistent with the behaviour found in patients with Huntington's disease. Preclinical carriers of the HD mutation (Lawrence et al., 1998a) and patients in an early stage of HD showed impairments in ED shift performance (Lawrence et al., 1996; Lawrence et al., 1999b). Their set-shifting impairments resulted from perseverative responding (i.e., inability to stop responding) to the stimulus dimension that was relevant in the stages before the ED. Results from the current experiment showed that DMS lesioned rats did not require more trials than controls to complete the ED stage. In addition, these results are inconsistent with the results from advanced stage HD patients who showed impairments in the reversals stages (Lange et al., 1995). DMS lesioned rats did not perseverate, more than the control group, in a previously rewarded stimulus when it was no longer appropriate. Given that impairments in set-shifting and reversal learning have been observed in HD patients (Josiassen et al., 1983; Lange et al., 1995; Lawrence et al., 1998a; Lawrence et al., 1996; Lawrence et al., 1999b), DMS lesioned rats should be examined more closely with other procedures to evaluate if quinolinic acid lesions of the striatum is a potential animal model for studying cognitive and behavioural impairments of Huntington's disease. In addition, given that different striatal sub-regions may be involved in different forms of flexible behaviour (Castañé et al., 2010), other dorsal striatum regions should also be examined to determine if they are involved in the attentional set-shifting task.

Therefore, the next chapter used a modified version of the attentional set-shifting task to test the hypothesis that the trend towards a diminish shift-cost observed in the DMS lesioned rats

represented a failure to form attentional set. Furthermore, the next experiment evaluated if selective lesions of dorsomedial and dorsolateral striatum have differential effects on reversal learning and in set-formation in the ID/ED attentional set-shifting task in rats.

In summary, this experiment showed that DMS lesioned rats do not have reversal impairments, but present a non-significant trend towards a diminished shift-cost, which is contrary as to what is observed in HD patients. From these findings, it could be concluded that rats with bilateral quinolinic acid lesions of the dorsomedial striatum do not present the same deficits as HD patients in the ID/ED attentional set-shifting task.

Chapter 8

The Effects of Lesions of the Dorsal Striatum in Reversal Learning, Attentional Set-Formation and Set-Shifting

Three experiments examined the effects of QA lesions of the dorsolateral (DLS) or the dorsomedial (DMS) striatum in reversal learning, attentional set-formation and set-shifting. In Experiment 1, rats were tested in the standard 7-stage attentional set-shifting task. Experiment 2 explored the relationship between reversal learning, attentional set-formation and set-shifting by using two modified versions of the attentional set-shifting task (that differed in the placement of the reversal stages). Experiment 3 introduced a probe stage (which substituted the stimuli of the irrelevant dimension from the preceding stage with novel stimuli) to help identify the aspects of the stimuli to which the rats were attending.

Do bilateral quinolinic acid lesions of the dorsal striatum impair reversal learning, attentional set-formation and set-shifting?

8.1 Introduction

Roberts et al. (1988) showed that distinguishing between stimuli varying along the same dimension (ID stage) as in previous stages requires fewer trials than distinguishing between stimuli varying along a different dimension (ED shift stage). Therefore, in the standard 7-stage attentional set-shifting task from Birrell and Brown (2000), cognitively normal rats require more trials to complete the extradimensional shift stage in comparison to the intradimensional stage (i.e., show a positive shift-cost) because the previously relevant stimulus is now irrelevant and a stimulus in a different dimension must be attended to. A positive shift-cost indicates that during the ED shift stage the animal was attending to the dimension that was relevant during the previous stages, but that is now irrelevant in the ED, which suggests that an attentional set was formed and then shifted from. The experiment from the previous chapter showed that rats with lesions of the dorsomedial striatum had a trend towards a diminished shift-cost (i.e., they did not require more trials to complete the ED shift stage in comparison to the ID stage); raising the possibility that lesions of the DMS impair the formation of attentional set. However, given the structure of the standard 7-stage attentional set-shifting task, it is not possible to make conclusions about set-formation impairments; it is only possible to assume that diminished shift-costs suggest weak or absent set-formation, thus do not require shifting (i.e., change in attentional set). This is because the task does not allow a direct measurement of ***attentional set-formation***, it only assumes that set has been formed by detecting ***set-shifting***. The term ***set*** has been defined as the property of the stimulus that is relevant in a given trial (Rushworth et al., 2005). When ***attentional set*** has been formed, there is a predisposition to attend to the properties of multidimensional stimuli that have predicted reward in the past (Esber and Haselgrove, 2011; Sutherland and Mackintosh, 1971). Therefore, new discriminations that require the same

relevant dimension as the previous stage (e.g., from odour to odour) should be solved faster (i.e., should require fewer trials and less time) than those that require a shift of attentional set (e.g., from odour to digging medium). In ***attentional set-shifting*** the perceptual features contained in a set that predicted reward are no longer relevant to the discrimination. Therefore, the set has to be shifted to the new features that predict reward. As mentioned in the previous chapter, shift-cost is the index of attenuated learning that occurs when the relevancy of the aspects of multidimensional stimuli that used to predict reward change, thus requiring a shift in attention. Therefore, set-shifting can be an index of behavioural flexibility, while set-formation can be an index of behavioural stability.

To test if attentional set-formation is impaired in rats with lesions of the DMS this chapter presents a series of behavioural tasks designed to further elucidate set-shifting performance, with the possibility of drawing conclusions about set-formation. In addition, given that it has been suggested that different striatal sub-regions may be implicated in different forms of flexible behaviour (Castañé et al., 2010), to assess the specificity of the role of the DMS in set-shifting performance, a group with lesions of the dorsolateral striatum (DLS) was added.

The aim of this chapter was to evaluate the contributions of the DMS and the DLS in reversal learning, set-formation and set-shifting in rats.

The first experiment aimed to validate the previous behavioural effects seen in rats with DMS lesion in the standard 7-stage attentional set-shifting task and to evaluate the behavioural effects of lesions in DLS in this task.

The second experiment used a modified version of the attentional set-shifting task to explore the relationship between reversal learning, attentional set-formation and set-shifting in rats with lesions of the dorsal striatum.

The third experiment introduced a probe stage to help identify the aspects of the stimuli the animals were attending to in the intradimensional stages.

Experiment 1. Standard 7-stage attentional set-shifting task

An advantage of the attentional set-shifting task is that it can be used on repeated basis with reproducible results and little to no effect on trials to criterion performance. Therefore, it can be used to evaluate the behavioural effects of a lesion within a group over a series of tests (Tait et al., 2014; Tait et al., 2009; Wallace et al., 2014). This experiment evaluated the behavioural effects of lesions of the dorsomedial striatum or the dorsolateral striatum in the attentional set-shifting task.

8.2 Method

8.2.1 Animals

The animals were 36 experimentally-naïve male hooded Lister rats (Charles River, UK Ltd) with a mean *ad libitum* weight of 307 g (range = 288 - 330 g) at the beginning of the experiment. Testing was conducted in the light phase of a 12:12 hr light-dark cycle. Housing and husbandry conditions were described in the General Methods in Chapter 2.

8.2.2 Apparatus

Training and testing sessions were conducted in the attentional set-shifting task apparatus described in Chapter 7.

8.2.3 Procedure

Pre-surgery testing. All rats were habituated, trained and tested in the standard 7-stage attentional set-shifting task described in Chapter 7. Briefly, habituation consisted of giving to the rats a bowl filled with sawdust and six pieces of food as a reward (half of a Honey Loop cereal piece; Kellogg Company, UK) in the home cage, overnight, one day before training. The animals were then trained to dig in bowls filled with sawdust to retrieve the reward and were then given two simple discriminations stages: one between two odours in sawdust and one between two digging media with no added odour; with only one of the bowls being baited in each simple discrimination (SD). The following day they were tested in the standard 7-stage attentional set-shifting task.

Standard 7-stage attentional set-shifting task. The first stage was an SD between either two odours in sawdust or two digging media with no added odour. The second stage was a compound discrimination (CD), where an irrelevant stimulus dimension is added, but the contingencies of the discrimination remained the same as in the SD. The third stage was the compound discrimination reversal (REV1), where the discrimination contingency of the CD was switched such that the previously incorrect stimulus became correct and vice versa. The fourth stage was an intradimensional (ID) acquisition stage where novel compound stimuli were introduced, but the relevant dimension was the same as in the previous stages. The fifth stage was the intradimensional reversal (REV2), where the incorrect stimulus of the ID became incorrect and vice versa. The sixth stage was the extradimensional (ED) shift, where new compound stimuli were presented and the relevancy of the two dimensions was switched. Therefore, the previously irrelevant dimension predicted reward and vice versa. Finally, the seventh stage was the extradimensional reversal (REV3) where the previously incorrect stimulus

of the ED became incorrect and vice versa (Table 8.1 shows the different stages of this task). Normally, two rats were tested per day.

Surgery. Once all rats were tested in the attentional set-shifting task once, they were pseudo-randomly assigned to the sham-surgery control (n =12), dorsomedial striatum (DMS) lesion (n =12) or dorsolateral striatum (DLS) lesion (n =12) groups. The surgery protocol was described in Chapter 2 (General Methods). Data from the three groups were analysed to ensure there were no differences between the groups before the surgery. Trials to criterion were analysed using a two factor, 3 x 7 ANOVA with the variables of Group (Between-groups factor with 3 levels: DMS Lesion vs DLS Lesion vs sham-surgery control), and Stage (Within-subjects factor with 7 levels: SD, CD, REV1, ID, REV2, ED, REV3). There were no differences between the groups in the performance of the attentional set-shifting task pre-surgery (main effect of Group: $F(2, 33) = 2.55$, $p = .09$, $\eta_p^2 = .13$).

Post-surgery testing: standard 7-stage attentional set-shifting task. After the rats recovered from the surgery (approximately 10 days) they were retested in the standard 7-stage attentional set-shifting task. The stages were always in the same order and the same stimulus pairs were used, counterbalancing both within and between tests for direction of shift, stimulus pair order and correct/incorrect stimuli within a pair. Normally, two rats were tested per day. The order of testing the rats was also counterbalanced.

Histology. This was described in the histology section of the General Methods in Chapter 2.

8.2.4 Data analysis

All analyses were conducted in IBM SPSS (v 21, SPSS Inc., Chicago, IL), using the General Linear Model procedure.

Total trials to criterion. Number of trials, in each stage, until six consecutive correct responses were made (p = .0156 considering the six trials and assuming the rat would choose randomly between the two bowls).

Mean shift-costs. Number of trials to criterion in the ED shift stage minus the number of trials in the ID stage.

Statistical analyses. Trials to criterion were analysed using a three factor, 2 x 7 x 3 ANOVA with the variables of Surgery (Within-subjects factor with 2 levels: Pre-surgery vs Post-surgery testing), Stage (Within-subjects factor with 7 levels: SD, CD, REV1, ID, REV2, ED, REV3) and Group (Between-groups factor with 3 levels: DMS lesion, DLS lesion, sham-surgery control).

Mean shift-costs were analysed using a univariate ANOVA with Group as a between-group factor. Sidak's-corrected pairwise comparisons for significant effects or interactions were performed. When data violated the assumption of sphericity, Huynh-Feldt corrections were reported, but the uncorrected degrees of freedom were reported. The criterion for significance (alpha level) was $p < .05$ in all cases.

Box plot generation. To effectively display the individual differences in shift-cost, as well as providing central tendency and distributional information, box plots were generated. The central line in each box plot represents the median. The lowest edge of the box is the lower quartile, and the top edge of the box shows the value of the upper quartile. The whiskers on the box show the range between which the lowest 25% and the top 25% of scores fall (Field, 2013). Outliers were defined as the values which were between one and a half and three box lengths from either end of the box and were represented by triangles (▲). Extreme values were defined

as the scores that were more than three box lengths from either end of the box and were denoted by asterisks (*). The mean is overlaid on the box plot (●).

8.3 Results

8.3.1 Histology

Six out of twelve DMS lesion rats and nine out of eleven DLS lesion rats (one rat did not recover from surgery) were determined to have appropriate bilateral lesions. Figure 8.1 shows a schematic diagram of a series of coronal sections of the rat brain (Paxinos and Watson, 2007), illustrating the extent of bilateral DLS lesions, and DMS lesions. Four out of twelve of the sham-surgery control rats showed signs of cell damage, so they were removed from the analysis. The final numbers (n) in each group were: DLS lesion (n = 9), DMS lesion (n = 6) and sham-surgery control (n = 8). Figure 8.2 shows photomicrographs of coronal sections from sham-surgery control, DMS and DLS lesioned animals.

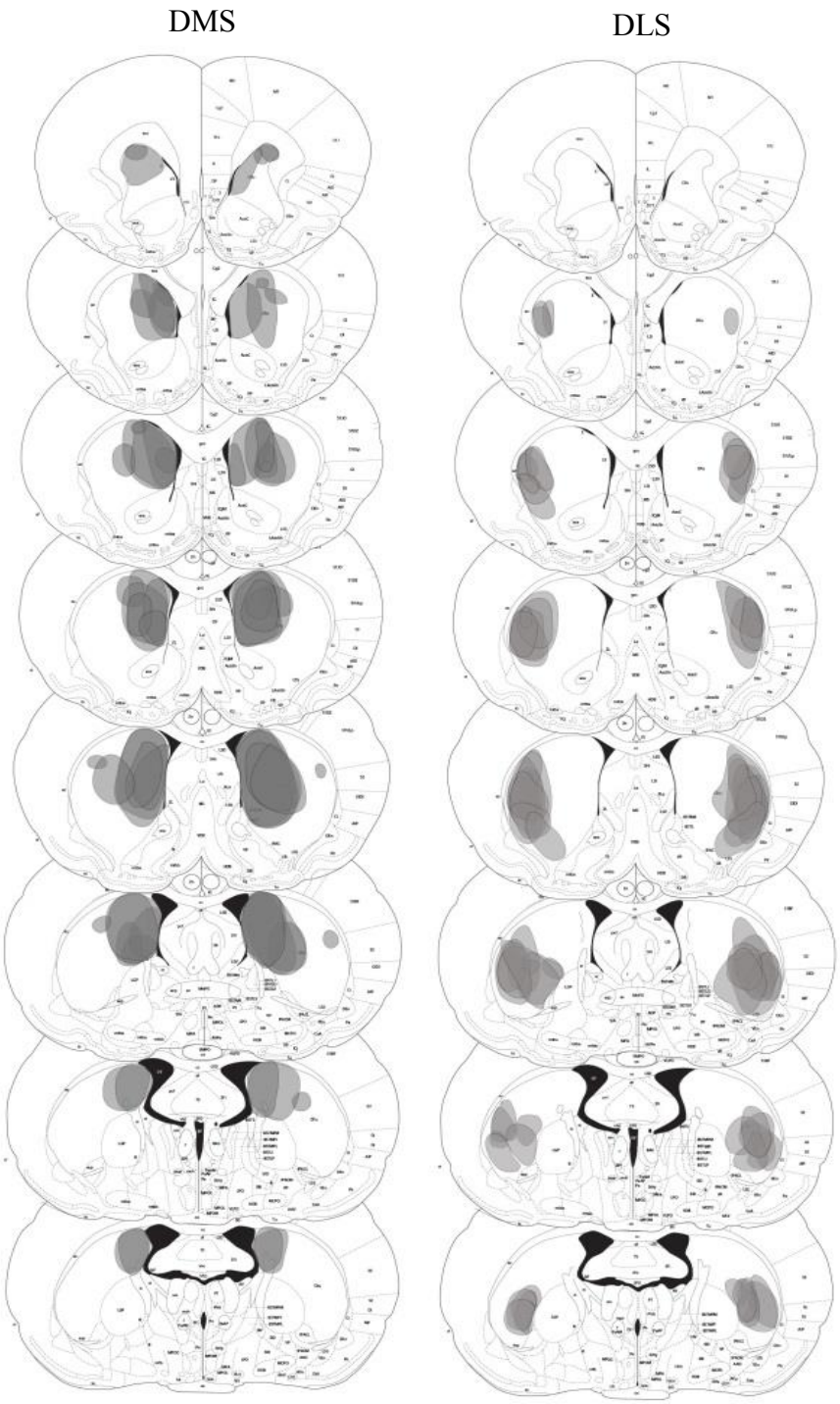

Figure 8.1. *Schematic diagram of a series of coronal sections of the rat brain illustrating the extent of bilateral dorsomedial (DMS) striatum lesions (left) and dorsolateral (DLS) striatum lesions (right). Darkness represents coincidence lesions from different animals. From the top, sections are +2.2, +1.7, +1.2, +0.7, +0.2, −0.3, −0.80, and −0.92 mm from bregma (Paxinos and Watson, 2007).*

Figure 8.2. *Representative photomicrographs of coronal sections double-stained with NeuN and Cresyl Violet from sham control (left), dorsomedial striatum (DMS) lesion (middle), and dorsolateral striatum (DLS) lesion (right) animals. Sham control rats showed an even distribution of neurons in the striatum whilst DMS and DLS lesioned rats showed cell loss. Arrows point to the lesion area. From the top, sections are +1.2, +0.7, +0.2, -0.3, and -0.80 mm from bregma. Scale bar 1,000 µm.*

8.3.2 Behavioural performances

Total trials to criterion. Lesions of the DLS or the DMS affected the performance of the attentional set-shifting task differently (Surgery x Stage x Group interaction: $F(12, 120) = 2.58$, $p < .001$, $\eta_p^2 = .21$). Sidak's-corrected pairwise comparisons indicated that pre-surgery there were no differences between the groups in the performance of the task; however, post-surgery DLS lesioned rats required fewer trials to complete the ED stage in comparison to controls ($p = .04$), and DMS lesioned rats required more trials to complete REV3 in comparison to DLS lesioned rats ($p = .04$). Planned contrasts between the ID and the ED stages showed that control rats required more trials to complete the ED shift stage than the ID stage ($p = .01$). There were no differences between the ID and ED stages for the DMS ($p = .80$) and the DLS ($p = .59$) rats. Figure 8.3 shows the comparison of the mean number of trials to reach criterion for the DLS lesion, DMS lesion and control rats in the standard 7-stage attentional set-shifting task post-surgery.

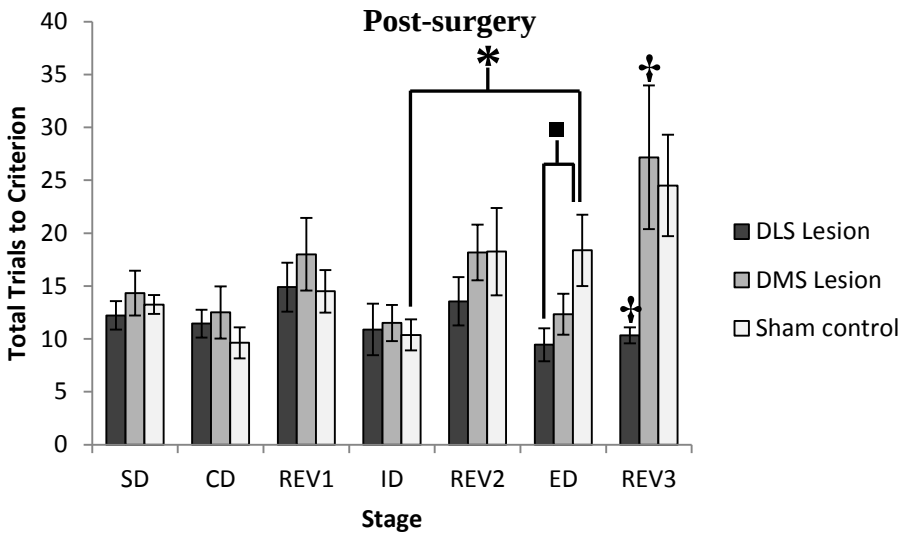

Figure 8.3. *Mean trials to criterion (± SEM) for the three groups post-surgery in the standard 7-stage attentional set-shifting task. Control rats required more trials to complete the ED in comparison to the ID (* $p < .05$), DLS lesioned rats required less trials to complete the ED stage in comparison to the controls (■ $p < .05$) and rats with DMS lesions required more trials to complete REV3 in comparison to DLS lesioned rats († $p < .05$).*

Shift-costs. Figure 8.4 displays the box plot for each group, with the mean overlaid on the box plot. Although there were no differences between the groups in shift-cost ($F(2,20) = 3.13$, $p = .07$, $\eta_p^2 = .24$), the control rats showed positive shift-cost on the task (i.e., the number of trials required to learn the ED stage was higher than in the ID stage), which suggested that an attentional set was formed and then shifted. While DMS and DLS lesioned rats showed a diminished cost of shifting. It also appears from the box plots that there were considerable individual differences within both groups of rats. Moreover, there were two outliers (values that were between one and a half and three box lengths from either end of the box) and an extreme value (value that was more than three box lengths from either end of the box) in the DLS lesioned group, and an outlier in the control group. Removing these rats from the analysis did not change the lack of difference between the groups in shift-cost ($F(2,17) = 3.10$, $p = .07$, $\eta_p^2 = .27$).

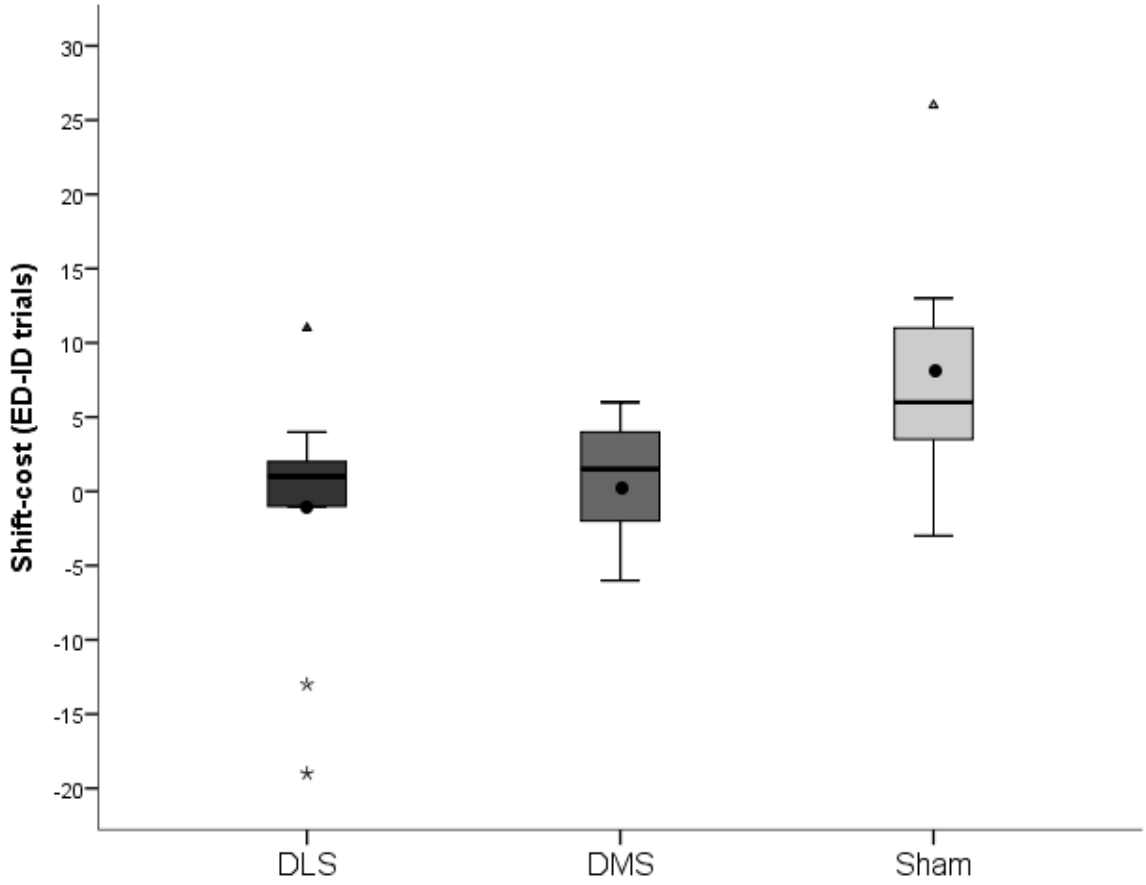

Figure 8.4. *Box plot showing the shift-cost (ED trials minus ID trials) for DLS lesioned, DMS lesioned and control rats. The central line in each box plot represents the median. The lowest edge of the box is the lower quartile, and the top edge of the box shows the value of the upper quartile. The whiskers on the box show the range between which the lowest 25% and the top 25% of scores fall. The values that were between one and a half and three box lengths from either end of the box were considered outliers (▲) and the scores that were more than three box lengths from either end of the box were extreme values (*). The mean is overlaid on the box plot (●).*

8.4 Discussion

The aim of this experiment was to evaluate the behavioural effects of lesions of the dorsal striatum by testing rats with lesions of the DMS and the DLS in the standard 7-stage attentional set-shifting task.

On the reversal stages, there was a difference between DMS and DLS lesions of the performance of the last reversal. DMS lesioned rats required more trials to complete REV3 in comparison to DLS lesioned rats. However, there was no difference on the reversal stages between the dorsal striatal lesioned groups and the control group. Rats with DMS or DLS lesions did not show an increased number of trials to complete the reversal stages in comparison to the control rats. Therefore, the results obtained in the present experiment showed that DMS and DLS lesioned rats do not present reversal impairments, as they did not persevere in responding to the previously rewarded stimulus when it was no longer appropriate. These results are inconsistent with the results seen in patients with advanced HD, but replicate the findings from the previous experiment reported in Chapter 7, which showed that DMS lesioned rats did not show reversal impairments (see the section 7.4 Discussion in Chapter 7). In addition, the results observed in the DLS lesioned group are consistent with the results from Castañé et al. (2010), who found that lesions of the DLS did not impair serial reversal learning of an instrumental spatial discrimination task. These findings suggest reversal learning in an instrumental two lever spatial discrimination task (where the correct response is associated with a particular location in space) or in the attentional set-shifting task (that does not depend on location or a particular response, but requires attention to the components of the stimuli) is not affected by lesions of the DLS.

Regarding set-shifting, a greater number of trials to criterion at the ED shift stage compared to the ID stage (the shift-cost) indicate the flexibility with which an attentional set can be shifted. In this experiment, control rats showed a positive shift-cost (i.e., required more trials to complete the ED in comparison to the ID stage), which suggested that an attentional set was formed and then shifted from. However, the DMS and the DLS lesion groups did not require

more trials to complete the ED stage in comparison to the ID stage and showed no cost of shifting. Therefore, there is no evidence that rats with DMS or DLS lesions either formed attentional set or shifted from one in the standard 7-stage attentional set-shifting task. The diminished or absent shift-cost in rats with DMS lesions from this experiment is consistent with the pattern observed in the experiment from the previous chapter; while the absence of positive shift-cost observed in rats with DLS lesioned rats are the first results to suggest that DLS lesions impair set-formation in the attentional set-shifting task. However, the standard 7-stage attentional set-shifting task does not allow a direct measurement of attentional set-formation; it only assumes that set-formation has been formed by detecting set-shifting. Therefore, it is not possible to conclude that lesions of the dorsal striatum impair attentional set-formation; it is only possible to assume that the lack of positive shift-cost seen in rats with DMS or DLS lesions suggest deficits in attentional set-formation. In order to evaluate if lesions of the DMS or in the DLS impair attentional set-formation, the next experiment used a modified version of the task that, in addition to measuring set-shifting, can provide more information on the formation of attentional set.

Experiment 2. Effects of early reversal vs late reversal stage in set-formation

8.5 Introduction

The results from the previous experiment suggested that lesions of the dorsal striatum impair attentional set-formation. However, the standard 7-stage attentional set-shifting task does not allow a direct measurement of attentional set-formation (it only assumes that set-formation has been formed by detecting set-shifting). To further investigate attentional set-formation in rats, the standard 7-stage attentional set-shifting task was modified to the four intradimensional

(4ID) set-shifting task (Chase et al., 2012). Based on the task used in marmosets (Clarke et al., 2005), the 4ID task for rats removes the reversal stages and consists of four consecutive intradimensional stages before the extradimensional shift stage that can measure the formation of attentional set in rats. Performance over the course of these series of stages has been suggested to provide an index of set-formation (Clarke et al., 2005).

However, patients with HD are impaired in shifting sets and in reversal learning (Josiassen et al., 1983; Lange et al., 1995; Lawrence et al., 1998a; Lawrence et al., 1996; Lawrence et al., 1999b). Therefore, a task that evaluates both cognitive processes would be more appropriate to evaluate if lesions of the dorsal striatum impair the same cognitive processes observed in patients with HD.

An alternative task that measures both set-formation and reversal learning was proposed by Chase (2013). The task is based on the same structure as the 4ID task for rats, which has been shown to be sufficient to elicit set-formation in set-formation-impaired orbital prefrontal cortex lesioned rats (Chase et al., 2012), but includes a reversal stage either before or after the four consecutive ID stages (i.e., before or after an attentional set is likely to have been formed). Therefore, these tasks also allow the investigation of the relationship between reversal learning and attentional set-formation. The rationale of the design of the tasks was that if it is predicted that the main effect of presenting four consecutive ID stages would strengthen attention to the relevant dimension, presenting a reversal stage after attentional set has been formed should require fewer trials than a reversal stage that is presented before the formation of attentional set (i.e., before the first ID stage). This paradoxical effect is known as the overtraining reversal effect, whereby the more an animal is trained to select stimulus X and not Y, the faster it will learn to select stimulus Y and not X on a reversal stage. The additional training strengthens

attention to the relevant cues that predict reward and the tendency to respond to other irrelevant cues weakens. Therefore, during the reversal stage the overtrained animals will learn the reversal faster because they will continue to attend to the relevant cues and learn the new response required relatively quickly. However, animals only trained to a normal criterion cease to pay attention to the relevant cues when they fail to receive reinforcement at the beginning of the reversal stage; therefore, take longer to learn the new response to that stimulus (Sutherland and Mackintosh, 1971).

To test the hypothesis that a reversal stage would be completed in fewer trials after attentional set has been formed, two modified versions of the attentional set-shifting task were used. The difference between the tasks was the placement of the reversal stage (before or after a series of ID stages that promote set-formation). If rats with lesions of the dorsal striatum are unable to form sets after a series of ID stages, the reversal stage for the lesioned rats should require more trials in comparison to the control rats that can form attentional sets.

In addition, the performance across the consecutive ID stages can provide an index of set-formation, which allows the identification of whether the lack of shift-cost seen in rats with dorsal striatum lesions in the standard 7-stage attentional set-shifting task was due to attentional set-formation impairments.

The aim of this experiment was to explore the relationship between reversal learning, attentional set-formation and set-shifting in rats with lesions of the dorsal striatum.

8.6 Method

8.6.1 Procedure

Early vs late reversal stage attentional set-shifting task. Once all rats completed the standard 7-stage attentional set-shifting task, they were pseudo-randomly assigned to the early or late reversal set-shifting task. Both tasks consisted of 11 stages; the difference between the tasks was the placement of the reversal stage. In the *early reversal stage task*, the reversal was the third stage of the task; while for the *late reversal stage task*, the reversal was the seventh stage of the task (Table 8.1 shows the different stages for the two tasks).

Both tasks consisted of an SD, CD, five intradimensional (ID1, ID2, ID3, ID4 and ID5) stages, a reversal stage (located between the CD and ID1 for the early reversal stage task or between the ID4 and ID5 for the late reversal stage task), an ED, ID6 (the relevant dimension from the ED remained relevant) and the original SD. The original SD was presented at the end of the task to control against the possibility that an increase in trials associated with learning the ED were due to issues of fatigue, satiety or memory load, rather than the cost of shifting set. Table 8.2 summarises both of the tasks. The exemplars used in these experiments are summarised in Table 8.3.

Table 8.1.
Stages for each of the modified versions of the attentional set-shifting tasks used in this chapter.

Task	Stages										
7-stage	SD	CD	REV1	ID	REV2	ED	REV3				
Early Reversal	SD	CD	REV	ID1	ID2	ID3	ID4	ID5	ED	ID6	SD
Late Reversal	SD	CD	ID1	ID2	ID3	ID4	REV	ID5	ED	ID6	SD
Probe Task	SD	CD	ID1	ID2	ID3	ID3Probe	ID3	ID4	ED	REV	SD

Table 8.2.
Example of the testing stages and possible stimulus combinations in the early reversal and late reversal stage attentional set-shifting task.

Discriminations	Dimensions		Exemplar combinations	
	Relevant	Irrelevant	**Rewarded**	Unrewarded
Simple Discrimination (SD)	**Medium**	Odour	**M1**	M2
Compound Discrimination (CD)	**Medium**	Odour	**M1**/O1	M2/O1
			M1/O2	M2/O2
Early Reversal (ER task only)	**Medium**	Odour	**M2**/O1	M1/O1
			M2/O2	M1/O2
First Intradimensional (ID1) stage	**Medium**	Odour	**M3**/O3	M4/O3
			M3/O4	M4/O4
Second Intradimensional (ID2) stage	**Medium**	Odour	**M5**/O5	M6/O5
			M5/O6	M6/O6
Third Intradimensional (ID3) stage	**Medium**	Odour	**M7**/O7	M8/O7
			M7/O8	M8/O8
Fourth Intradimensional (ID4) stage	**Medium**	Odour	**M11**/O11	M12/O11
			M11/O12	M12/O12
Late Reversal (LR task only)	**Medium**	Odour	**M12**/O11	M11/O11
			M12/O12	M11/O12
Fifth Intradimensional (ID5) stage	**Medium**	Odour	**M13**/O13	M14/O13
			M13/O14	M14/O14
Extradimensional (ED) shift stage	**Odour**	Medium	**O15**/M15	O16/M15
			O15/M16	O16/M16
Sixth Intradimensional (ID6) stage	**Odour**	Medium	**O17**/M17	O18/M17
			O17/M18	O18/M18
Original Simple Discrimination (SD2)	**Medium**	Odour	**M1**	M2

Note. The two tasks differ in the placement of the reversal stage. In the early reversal stage task, the reversal was the third stage; in the late reversal stage task, the reversal was the seventh stage of the task. Rats were counterbalanced so that half received digging medium as the initial relevant dimension, whereas the other half received odour. Stimulus pair order and correct/incorrect stimuli within a pair were also counterbalanced.

Table 8.3.
Exemplars used (presented in pairs within each dimension).

	Digging media		Odours	
Pairing 1	M1-Coarse tea	M2-Fine tea	O1-Cinnamon	O2-Ginger
Pairing 2	M3-Sand	M4-Grit	O3-Sage	O4-Paprika
Pairing 3	M5-Coarse shavings	M6-Fine shavings	O5-Turmeric	O6-Cloves
Pairing 4	M7-Cotton pads	M8-Cigarette filters	O7-Dill	O8-Coriander
Training Pairs	M9-Polystyrene pieces	M10-Shredded paper	O9-Mint	O10-Oregano
Pairing 5	M11-Course cork	M12-Fine cork	O11-Fenugreek	O12-Tarragon
Pairing 6	M13-Long wire coat	M14-Short wire coat	O13-Cumin	O14-Marjoram
Pairing 7	M15-Beads	M16-Gravel	O15-Thyme	O16-Caraway
Pairing 8	M17-String	M18-Knotted string	O17- Fennel seeds	O18-Chives

8.6.2 Data Analysis

Trials to criterion and mean shift-cost were analysed as described in the previous experiment (section 8.2.4).

Statistical analyses. Total trials to criterion for the early reversal stage task and for the late reversal stage task were analysed separately. Each task was analysed using a two factor, 3 x 11 ANOVA with the variables of Group (Between-groups factor with 3 levels) and Stage (Within-subjects factor with 11 levels). Sidak's-corrected pairwise comparisons for significant effects or interactions were performed. The criterion for significance (alpha level) was $p < .05$ in all cases.

Early reversal stage task. For the early reversal stage task, the following planned contrasts were performed:

ID2 vs ID5: to evaluate set-formation; measured by an improvement (fewer trials to criterion) between the first and the last ID stage. Given that the reversal stage could affect the performance on the stage that preceded it, ID1 was not included in this analysis.

ID5 vs ED: to evaluate set-shifting; measured by requiring more trials to complete the ED shift stage in comparison to the ID5 stage.

CD vs ID1: to evaluate if there was an effect of introducing a reversal stage between these two stages.

SD vs SD2: to eliminate the possibility that an increase in number of trials associated with learning the ED shift stage were due to issues of fatigue, satiety or memory load, rather than the cost of shifting set.

In addition, the difference in the performance on the ID1 stage, the ED shift stage, the reversal stage and shift-cost were compared between the groups.

Late reversal stage task. The following planned contrasts were performed:

ID1 vs ID4: to evaluate set-formation; measured by an improvement (fewer trials to criterion) between the first and the last ID stage.

ID5 vs ED: to evaluate set-shifting; measured by requiring more trials to complete the ED shift stage in comparison to the ID5 stage.

ID4 vs ID5: to evaluate if there was an effect of introducing a reversal stage between these two stages.

SD vs SD2: to eliminate the possibility that an increase in trials associated with learning the ED shift stage were due to issues of fatigue, satiety or memory load, rather than the cost of shifting set.

In addition, the difference in the performance on the ID5 stage, the ED shift stage, the reversal stage and shift-cost were compared between the groups.

Early reversal vs late reversal stage. The number of trials to complete the reversal stage in both tasks was compared to investigate if a reversal stage was acquired in fewer trials after presenting consecutive ID stages.

8.7 Results

8.7.1 Early reversal stage task

After histological analysis the final numbers (*n*) in each group were: DLS lesion ($n = 5$), DMS lesion ($n = 4$) and sham-surgery control ($n = 4$).

Total trials to criterion. Figure 8.5 shows the comparison of the mean number of trials to reach criterion for the DLS lesion, DMS lesion and control group in the early reversal stage attentional set-shifting task. There was no effect of the lesion on performance of the early reversal stage task (main effect of Group: $F(2, 10) = 0.01$, $p = .99$, $\eta_p^2 = .002$). Although the stages were completed differently (main effect of Stage: $F(10, 100) = 3.71$, $p = .005$, $\eta_p^2 = .27$), there were no differences between the performance of DLS lesion, DMS lesion and control rats on the stages of the early reversal stage task (Stage x Group interaction: $F(20, 120) = 0.47$, $p = .97$, $\eta_p^2 = .09$).

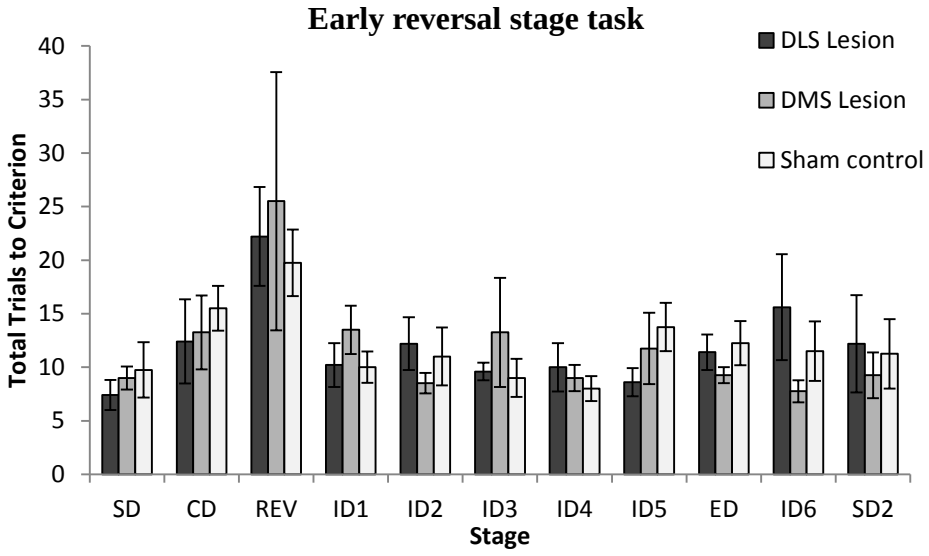

Figure 8.5. *Comparison of the mean number of total trials to reach criterion (±SEM) for the DLS lesion, DMS lesion and sham-surgery control group in the early reversal stage attentional set-shifting task.*

Attentional set-formation. Planned contrast between stages ID2 and ID5, showed that the performance after four consecutive ID stages did not improve (i.e., the number of trials to complete the ID5 stage did not decrease in comparison to the trials to complete ID2) for either of the groups, which suggested that there were no differences between the groups in learning set.

The groups did not show set-shifting as none of the groups required more trials to complete the ED shift stage in comparison to the ID5 stage. The performance on the ED shift stage and the shift-cost ($F(2,10) = 1.80$, $p = .22$, $\eta_p^2 = .26$) was not different between the groups.

There was no difference in the performance of the first and last stage (SD and SD2), which suggested that the performance on the last stages were not due to issues of fatigue, satiety or memory load.

Reversal. Planned contrasts showed that although the number of trials to complete the reversal stage increased in comparison to the previous stage (CD) there were no differences

between the groups in the performance of the reversal stage. In addition, presenting a reversal stage early in the task did not affect the groups differently in the ID stage that followed the reversal (i.e., no difference between the groups on ID1). Finally, the reversal stage did not affect the stage that followed it (there were no differences between the stages presented before and after the reversal stage, CD vs. ID1).

These results suggested there were no difference between the groups in the performance of the early reversal stage task.

8.7.2 Late reversal stage task

After histological analysis the final numbers (*n*) in each group were: DLS lesion (*n* = 4), DMS lesion (*n* = 2) and sham-surgery control (*n* = 4).

Total trials to criterion. Figure 8.6 shows the comparison of the mean number of total trials to reach criterion for the DLS lesion, DMS lesion and control group in the late reversal stage attentional set-shifting task. There was an effect of lesion on performance of the task (main effect of Group: $F(2, 7) = 8.68$, $p = .01$, $\eta_p^2 = .71$). Sidak's-corrected pairwise comparisons indicated that DLS lesion rats required fewer trials to complete the task than control rats ($p = .03$) or DMS lesion rats ($p = .03$). Although the stages were completed differently (main effect of Stage: $F(10, 70) = 2.34$, $p = .02$, $\eta_p^2 = .25$), there were no differences between the performance of DLS lesion, DMS lesion and control rats on the stages of the late reversal stage task (Stage x Group interaction: $F(20, 70) = 1.07$, $p = .40$, $\eta_p^2 = .23$).

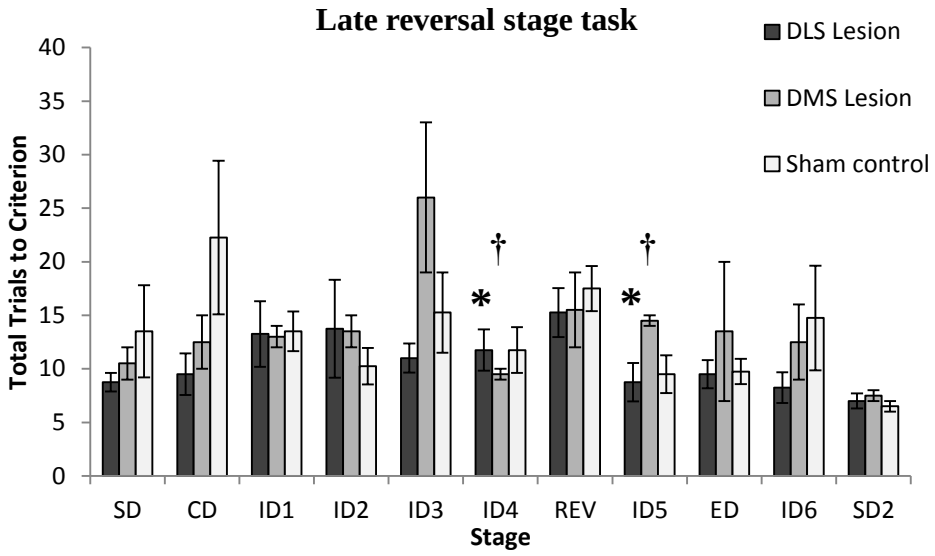

Figure 8.6. *Comparison of the mean number of total trials to reach criterion (± SEM) for the DLS lesion, DMS lesion and sham-surgery control group in the late reversal stage attentional set-shifting task. After the reversal stage DLS lesioned rats (*$p < .05$) required fewer trials to complete ID5 in comparison to ID4 and DMS lesioned rats (†* $p < .05$*) required more trial to complete ID5 in comparison to ID4.*

Attentional set formation. Planned contrast within stages ID1 and ID4, showed that the performance between the first and the last pre-reversal ID stage did not improve (i.e., the number of trials to complete the ID4 stage did not decrease in comparison to the trials to complete ID1) for either of the groups, which suggested that there were no differences between the groups in learning set.

DLS lesioned, DMS lesioned and control rats did not show set-shifting as none of the groups required more trials to complete the ED shift stage in comparison to the ID5 stage. The performance on the ED shift stage and the shift-cost ($F(2,7) = .06$, $p = .95$, $\eta_p^2 = .02$) was not different between the groups.

There was no difference in the performance of the first and last stage (SD and SD2), which suggested that the performance on the last stages were not due to issues of fatigue, satiety or memory load.

Reversal. Planned contrast showed that the number of trials to complete the reversal stage increased in comparison to the previous stage (ID4); however, there were no differences between the groups in the performance of the reversal stage. Furthermore, presenting a reversal stage between ID4 and ID5 decreased the number of trials required to complete ID5 (in comparison to ID4) for the DLS lesioned rats (p = .05), but produced the opposite effect in the DMS lesioned rats (p = .03). However, the performance in the ID5 stage was not significantly different between the groups.

8.7.3 Early reversal vs. late reversal stage

The number of trials to complete the reversal stage in the early reversal task was higher in comparison to the late reversal stage task, which would have suggested that a reversal stage was completed in fewer trials after attentional set has been formed. However, given that none of the groups showed a positive shift-cost, the improvement in the performance of the reversal stage in the late reversal stage task could have been an effect of learning. However, the difference between the tasks to complete the reversal stage was not significant. The lack of significant difference between the tasks, even though there was a pattern, might result from the reduced number of rats used in this experiment after histological analysis. Figure 8.7 shows the mean trials to criterion on the reversal stage for the early and the late reversal stage task for the DLS lesioned, DMS lesioned and control rats.

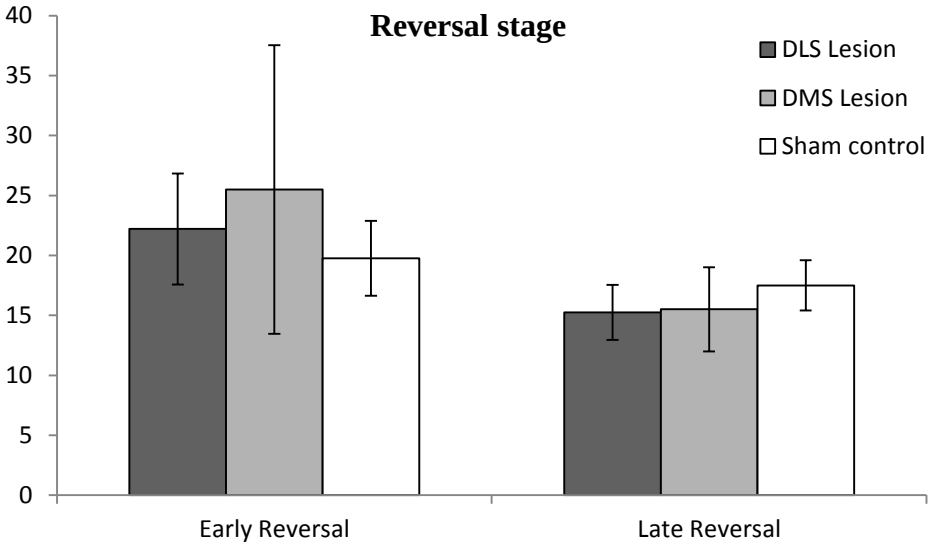

Figure 8.7. *Mean trials to criterion (± SEM)) on the reversal stage for the three groups on the early reversal and the late reversal attentional set-shifting task.*

8.8 Discussion

To explore the relationship between reversal learning, attentional set-formation and set-shifting in rats with lesions of the dorsal striatum, this experiment used two modified versions of the attentional set-shifting task (that differed in the placement of the reversal stage).

In the early reversal attentional set-shifting task, the reversal stage was presented before four consecutive ID stages (i.e., before attentional set formation could be formed); while the late reversal task presented the reversal stage after four consecutive ID stages (i.e., after set-formation could be formed). Based on the overtraining reversal effect (Sutherland and Mackintosh, 1971), presenting additional ID stages before the reversal stage should strengthen attentional focus on the aspects of the stimuli that predict reward; thus, the performance on the reversal stage should be improved after attentional set has been formed (since the animal has learned the relevant cues to which to attend).

The absence of shift-cost observed in rats with DLS or DMS lesions in the standard 7-stage attentional set-shifting task suggested that lesions of the dorsal striatum impaired attentional set-formation. Thus, if the performance on a reversal stage improved after attentional set has been formed and rats with lesion in the dorsal striatum were unable to form sets, the reversal stage for the lesioned rats in the late reversal stage task should require more trials in comparison to the control rats that can form attentional sets.

In the early reversal stage task, although the DLS lesioned and the DMS lesioned group needed more trials, in comparison to the controls, to complete the reversal stage, the difference between the groups was not significant. Therefore, there is no evidence that suggested that lesions of the dorsal striatum impair reversal learning when the reversal stage is presented before 4 consecutive ID stages.

In addition, the reversal stage did not affect the performance of any of the groups on the ID stage that followed the reversal (ID1). These results are consistent with the results from Chase (2013) who showed that when comparing ID1 vs CD (the stages before and after the reversal stage), the performance on the ID1 was not affected by the reversal stage for the sham-surgery control rats.

There was no evidence of attentional set-formation, as the number of trials to complete ID1 and ID5 did not decrease for any of the groups. Although Chase (2013) did not present an analysis of these stages, the graph suggests that there was no difference between ID1 and ID5 for the control group.

Finally, the number of trials to complete the ED shift stage was not higher than the trials to complete the previous ID, which suggested that the groups did not form set, and therefore did

not show a cost of shifting set. These results are inconsistent with the results from Chase (2013) who found that control rats show a shift-cost in this task.

In summary, there were no significant differences between the performance of DLS lesioned, DMS lesioned and control rats on any stage of the early reversal stage attentional set shifting task.

In the late reversal stage task, although the DLS lesioned rats completed the task in fewer trials, there were no significant differences between the performance of DLS lesioned, DMS lesioned and control rats on any specific stages of the task. Although reversing the correct stimulus to the incorrect stimulus and vice versa from the ID4 stage in the reversal stage increased the number of trials for completing the reversal stage, there were no differences in the performance of the reversal stage between the groups. Therefore, there is no evidence that suggested that lesions of the dorsal striatum impair reversal learning after presenting four consecutive ID stages. However, the reversal stage could affect the acquisition of the subsequent stage for the DLS lesioned and the DMS lesioned rats. DLS lesioned rats required fewer trials to complete the ID5 stage in comparison to the ID4 stage (ID prior and post the reversal stage) and DMS lesioned rats required more trials to complete the ID5 stage in comparison to the ID4 stage. Nevertheless, these results cannot be conclusive given the reduced number of rats that were analysed in this experiment after histological analysis. The performance on the ID5 stage showed no statistical difference between the lesioned groups and the control group.

The results from the control group from the current experiment are consistent with the performance of the control rats from the experiment from Chase (2013), where attentional set was not affected after presenting a late reversal stage.

There was no evidence of learning set, as the number of trials to complete the ID1 and the ID4 stages did not decreased for any of the groups and there was no evidence of set-shifting in any of the groups, as the number of trials to complete the ED shift stage was not higher than the trials to complete the previous ID stage. These results are inconsistent with Chase (2013) who found that sham-surgery control rats showed a shift-cost in the late reversal stage task.

To summarise, the results from the late reversal stage task showed there were no differences in the performance of the stages of this task between the DLS lesioned, DMS lesioned and control rats.

Altogether, the results from this experiment showed that there were no differences between the DLS lesioned, DMS lesioned and the control group in set-formation, set-shifting or reversal learning in the early reversal or in the late reversal stage tasks. However, the performance of the control group on the ED shift stage was unexpected as they did not show evidence of an attentional set. Therefore, the lack of difference between the control and dorsal striatum lesion groups cannot be conclusive. In addition, one of the limitations was the number of rats that were eliminated from the analysis since they did not have bilateral lesions, or because they showed signs of cell damage (in the case of the control group). The reduced number of rats that could be analysed compromised the statistical power; therefore, a larger sample size should be evaluated to support the results found in this experiment.

The absence of attentional set-shifting from the control group on the early and late reversal stage tasks is inconsistent with the previous results from Experiment 1 of this chapter; where the same rats were tested in the standard 7-stage attentional set-shifting task and showed a positive shift-cost. The discrepancy in results might be accounted for by the difference in the

number of stages and the characteristics of the stages employed in each task before the ED shift stage. While the standard 7-stage attentional set-shifting task only uses two different pairs of stimuli before the ED shift stage; the early/late reversal stage attentional set-shifting task uses six different stimuli pairs. As every ID stage contains novel stimuli with relevant and irrelevant dimensions, increasing the number of ID stages, each one with novel stimuli, might facilitate the learning of the relevant and irrelevant dimension. Given that the stimuli in the irrelevant dimension are rewarded half of the time (i.e., when they are paired with the correct stimuli), they will likely also gain salience relative to other irrelevant cues (e.g., spatial location). Therefore, when the previously relevant dimension no longer predicts reward on the ED stage, the next most salient cue is the irrelevant dimension (which had previously predicted reward half of the time). Thus the number of trials to complete the ED shift stage may have decreased as the tendency to respond to other irrelevant cues has been weakened. It is possible that the standard 7-stage attentional set-shifting task does not provide enough stages and enough pairs of stimuli to structure the salience of the relevant cues; so the performance on the ED shift stage does not improve even for the control rats. Therefore, the standard 7-stage attentional set-shifting task might be a more sensitive task to detect attentional set-shifting deficits.

Also, the absence of attentional set-shifting from the control group on the early and late reversal stage attentional set-shifting tasks from this experiment are inconsistent with the results on these two tasks reported by Chase (2013). The discrepant results may be explained by the different stimuli that were used in this experiment and the ones that Chase (2013) used. Although some of the stimuli have not being changed, some of the pair stimuli have been substituted for different reasons (e.g., some odours were aversive for the rats and they refused to dig or some of the digging media were too heavy for the rat to dig or some stimuli were too easy

to discriminate) and none of the stimuli used by Chase (2013) or in this experiment have been validated in the standard 7-stage attentional set-shifting task, which is a limitation of these tasks. It is also important to mention that the early and late reversal stage attentional set-shifting tasks have only been used twice. First by Chase (2013) who found set-shifting evidence in sham-surgery control rats (n =8), and the present experiment which did not find attentional set-shifting evidence in sham-surgery control rats (n =8). Given that normal rats, without being exposed to sham-surgery, have not been tested in these two tasks, further research should test unoperated control rats, to validate these two tasks.

The lack of difference between the early and late reversal attentional set-shifting tasks in control rats from this experiment are consistent with the results from Chase (2013) who did not find difference in the performance between these two tasks in control rats. Since the performance of the reversal stage did not improve when rats formed sets (Chase, 2013) or when rats did not form sets (this experiment), there is no evidence to support that there is a relationship between reversal learning and attentional set formation in control rats.

Finally, the reversal stages from both tasks were compared to evaluate if the performance on the reversal stage improved after presenting multiple ID stages. Although there was a trend towards requiring fewer trials to complete the reversal in the late stage reversal task, the difference between the tasks to complete the reversal stage was not significantly different. Thus, presenting multiple consecutive ID stages before the reversal stage did not strengthen attention to the relevant cues that predicted reward and the performance on the reversal stage was not improved. Also, the multiple ID stages did not hinder the learning of the relevant dimension on the ED stage. Both of these results are inconsistent with the predictions of the overtraining

theory, where the more overtraining, the faster the reversal, but the slower a non-reversal shift discrimination is learned (Mackintosh, 1962).

Although these results are inconsistent with the overtraining predictions; these are not the only ones. Since Reid (1953) showed that the more overtraining trials were given to rats on a brightness discrimination task the faster they learned the reversal of the original discrimination, several experiments have tried to study this effect, but the results have been controversial. While some experimental studies have found the same findings from Reid, there are some other studies that have found that overtraining has significantly retarded reversal learning. Nevertheless, the majority of experimental studies have found that overtraining has had no significant effect on reversal learning (Mackintosh, 1969), just like the results found in the present experiment.

A possible explanation for the inconsistency of results from the present experiment and the experiment from Mackintosh (1962) might be the difference in experimental design used for overtraining. Although presenting multiple ID stages could be considered as "overtraining" the relevant dimension, it is important to note that each stage used different stimuli. Therefore, it is not the same design of overtraining used by Mackintosh (1962) where the same stimulus was presented for multiple trials (100% or 200% more trials of the overall mean number of trials to learn the original discrimination). To evaluate if the overtraining reversal effect is observed in the attentional set-shifting task, further research should investigate if presenting the same stimuli for multiple trials on the same stage improves the performance on the reversal stage that follows it.

Overall, the results from this experiment showed that there were no differences between the DLS lesioned, DMS lesioned and the control group in set-formation, set-shifting or reversal

learning in the early reversal attentional set-shifting task or in the late reversal attentional set-shifting task. The absence of set-shifting observed in the DLS lesioned and DMS lesioned rats in the standard 7-stage attentional set-shifting task and the early and late stage attentional set-shifting task could be explained if the rats were performing each stage as novel discriminations. To test this hypothesis, the next experiment used a modified version of the task to help identify the aspects of the stimuli the animals were attending in the intradimensional stages.

Experiment 3. The probe stage attentional set-formation task

8.9 Introduction

The previous experiments showed that in the standard 7-stage attentional set-shifting task, as well as in the early reversal and late reversal stage attentional set-shifting task, when the relevant dimension changed at the ED shift stage, the DLS lesioned and DMS lesioned rats did not require more trials to complete the ED shift stage in comparison to the ID stage; therefore, they did not show a positive shift-cost. Although these results suggested that the dorsal striatum might be involved in attentional set-formation and set-shifting, the specific impairments induced by the dorsal striatum lesions remain unknown. It is possible that the lesioned rats were performing each stage as novel discriminations, which could explain the absence of set-shifting and set-formation. To test this hypothesis, the next experiment, based on the task from Dias et al. (1996b), used a series of intradimensional stages followed by a probe stage to help identify the aspects of the stimuli the animals were attending to in the ID stages. The probe stage consisted of presenting the relevant dimension stimuli from the previous ID, with a new pair of stimuli in the irrelevant dimension (e.g., for medium being the relevant dimension, ID stage: M1,O1;

M1,O2; M2,O1; M2,O2; probe stage: M1,O11; M1,O12; M2,O11; M2,O12), with the reward contingencies of the relevant stimuli remaining the same as the previous ID stage. Therefore, if the rats have learned to distinguish that the compound stimuli are formed by correct and incorrect stimuli (e.g., M1, regardless if it is presented with O1 or O2), then presenting the correct stimuli with new stimuli in the irrelevant dimension (M1,O11 or M1,O12) should not require new learning. Hence, the probe stage should be completed in fewer trials, as the animal should not require additional trials to learn the correct stimulus (i.e., should continue choosing M1 regardless of whether it was presented with O11 or O12). On the contrary, if the rats have completed the stages by learning the stimuli as a compound (e.g., M1,O1 and M1,O2 as rewarded), pairing the correct stimulus with a new exemplar from the irrelevant dimension should require a novel discrimination, and any benefit from learning at the preceding stage should not carry over. Learning the correct stimuli as a compound does not allow a distinction between relevant and irrelevant dimensions. Therefore, a difference between the ID stage and the ED shift stage would not be expected. This might explain the absence of shift-cost that has been observed in rats with DLS lesions and DMS lesion.

To help identify the aspects of the stimuli the animals were attending in the intradimensional stages, a probe stage was incorporated to form a new version of the attentional set-shifting task. If DLS lesioned and DMS lesioned rats perform each stage as an individual stage, without focussing on the relevant stimulus, the probe stage should be solved as a novel ID stage where novel stimuli were presented for the first time. Therefore, the stage will not be completed in fewer trials (as the ‘correct’ stimulus from the previous stage would not be considered such for completing the current stage).

In addition to identifying the aspects of the stimuli attended in the ID stages, this task also evaluated set-formation, set-shifting and reversal learning to help identify possible impairments in the dorsal striatum lesioned animals.

8.10 Method

8.10.1 Procedure

Unlike the standard 7-stage attentional set-shifting task, in which each stage is followed by a reversal stage, the early and late reversal stage attentional set-shifting tasks only have one reversal stage; therefore, some of the exemplars within the stimulus pairs that were used in the preceding early/late reversal stage task were never associated with reward (even partially as irrelevant stimuli). To control against the effects of previous rewarded and unrewarded stimuli associations on the performance of subsequent tasks, the rats were pre-exposed to all the stimuli. Rats were presented twice with each of the digging media (unscented) and with each of the odours (mixed in sawdust) until they retrieved the reward from both of the bowls.

In addition, before testing the rats in the probe stage attentional set-shifting task, all the animals were retested in the standard 7-stage attentional set-shifting task, to ensure there had not been learning effects after the early and late reversal stage attentional set-shifting tasks.

Standard 7-stage attentional set-shifting task. Rats were retested in the standard 7-stage attentional set-shifting task (Table 8.1). The stages were always in the same order and the same stimulus pairs were used as in the first 7-stage attentional set-shifting task, with counterbalancing both within and between tests for direction of shift, stimulus pair order and correct/incorrect stimuli within a pair.

Probe stage attentional set-shifting task. Once all rats completed the standard 7-stage attentional set-shifting task, they were tested in the modified version of the task that included a probe stage (Table 8.1). The task consisted of an SD, CD, three intradimensional (ID1, ID2, ID3) stages, a probe stage of ID3, the original ID3, ID4, an ED, a reversal of the ED (REV) stage and the original SD. Table 8.4 summarises this task. The original SD was presented at the end of the task to control against the possibility that an increase in trials associated with learning the ED shift stage or the reversal stage were due to issues of fatigue, satiety or memory load, rather than the cost of shifting set or reversal learning. The original ID3 was presented after the probe stage, to control for any possible effect of the probe stage on the subsequent ID stage. The exemplars used in these experiments were the same as in the previous experiment, which are summarised in Table 8.3. The stimuli from pair five were always used in the probe stage to substitute the irrelevant dimension.

Table 8.4.
Example of the testing stages and possible stimulus combinations in the probe stage attentional set-shifting task.

Discriminations	Dimensions		Exemplar combinations	
	Relevant	Irrelevant	**Rewarded**	Unrewarded
Simple Discrimination (SD)	**Medium**	Odour	**M1**	M2
Compound Discrimination (CD)	**Medium**	Odour	**M1**/O1	M2/O1
			M1/O2	M2/O2
First Intradimensional (ID1) stage	**Medium**	Odour	**M3**/O3	M4/O3
			M3/O4	M4/O4
Second Intradimensional (ID2) stage	**Medium**	Odour	**M5**/O5	M6/O5
			M5/O6	M6/O6
Third Intradimensional (ID3) stage	**Medium**	Odour	**M7**/O7	M8/O7
			M7/O8	M8/O8
Probe third Intradimensional	**Medium**	Odour	**M7**/O11	M8/O11
(ID3Probe) stage			**M7**/O12	M8/O12
Third Intradimensional (ID3) stage	**Medium**	Odour	**M7**/O7	M8/O7
			M7/O8	M8/O8
Fourth Intradimensional (ID4) stage	**Medium**	Odour	**M13**/O13	M14/O13
			M13/O14	M14/O14
Fifth Intradimensional (ID5) stage	**Medium**	Odour	**M15**/O15	M16/O15
			M15/O16	M16/O16
Extradimensional (ED) shift	**Odour**	Medium	**O17**/M17	O18/M17
			O17/M18	O18/M18
Reversal (REV)	**Odour**	Medium	**O18**/M17	O17/M17
			O18/M18	O17/M18
Original Simple Discrimination (SD2)	**Medium**	Odour	**M1**	M2

Note. The probe tasks consist of replacing the stimuli in the irrelevant dimension with two novel stimuli, whereas the stimuli from the relevant dimension and the reward contingencies remained the same. Rats were counterbalanced so that half received digging medium as the initial relevant dimension, whereas the other half received odour. Stimulus pair order and correct/incorrect stimuli within a pair were also counterbalanced.

8.10.2 Data analysis

Standard 7-stage attentional set-shifting task. Trials to criterion and mean shift-cost were analysed as described in the first experiment from this chapter.

Statistical analyses. Trials to criterion were analysed using a three factor, 2 x 7 x 3 ANOVA with the variables of Test (Within-subjects factor with 2 levels: Post-surgery First Test vs. Post-surgery Repeat Test), Stage (Within-subjects factor with 7 levels: SD, CD, REV1, ID, REV2, ED, REV3) and Group (Between-groups factor with 3 levels: DMS lesion, DLS lesion, sham-surgery control). The rest of the analyses were conducted as described before.

Probe stage attentional set-shifting task. Trials to criterion and mean shift-cost were analysed as described in the first experiment from this chapter.

Statistical analyses. Total trials to criterion for the probe stage task were analysed using a two factor, 3 x 11 ANOVA with the variables of Group (Between-groups factor with 3 levels) and Stage (Within-subjects factor with 11 levels). Sidak's-corrected pairwise comparisons for significant effects or interactions were performed.

Planned contrasts.

ID3 vs ID3Probe: to evaluate if the relevant and irrelevant dimensions were identified; measured by an improvement (fewer trials to criterion) between the ID3 and the ID3Probe stage.

ID1 vs ID4: to evaluate set-formation; measured by an improvement (fewer trials to criterion) between the first and the last ID stage.

ID4 vs ED: to evaluate set-shifting; measured by requiring more trials to complete the ED shift stage in comparison to the ID4 stage.

SD vs SD2: to eliminate the possibility that an increase in trials associated with learning the ED shift stage or the reversal stage were due to issues of fatigue, satiety or memory load, rather than the cost of shifting set or reversal learning.

In addition, the difference in the performance on the ID3Probe stage, the ED shift stage, the reversal stage and shift-cost were compared between the groups.

8.11 Results

8.11.1 Effects of repeated testing in the standard 7-stage attentional set-shifting task

Total trials to criterion. There were no differences between the first and the second test (main effect of Test: $F(1,20) = 3.42$, $p = .08$, $\eta_p^2 = .15$; Test x Group: $F(2, 20) = 2.20$, $p = .14$, $\eta_p^2 = .18$; Test x Stage $F(6, 120) = 2.17$, $p = .06$, $\eta_p^2 = .10$). The effect of lesion on the performance of the task was still present the second time the rats were tested in the standard 7-stage attentional set-shifting task (main effect of Group: $F(2, 20) = 6.75$, $p = .01$, $\eta_p^2 = .40$). The DLS lesioned rats still required fewer trials to complete the task than the controls ($p = .02$) and the DMS lesioned ($p = .02$) rats.

Figure 8.8 shows the mean of the performance of the first and second test on the standard 7-stage attentional set-shifting task post-surgery. Planned contrasts between the ID and the ED stages, of the average of the two tests, showed that control rats required more trials to complete the ED shift stage than the ID stage ($p = .01$). There were no differences between the ID and ED stages for the DMS ($p = .97$) and the DLS ($p = .72$) lesioned rats. DLS lesioned rats required fewer trials to complete the ED stage in comparison to the controls ($p = .02$), and DMS lesioned rats required more trials to complete REV3 in comparison to DLS lesioned rats ($p = .03$).

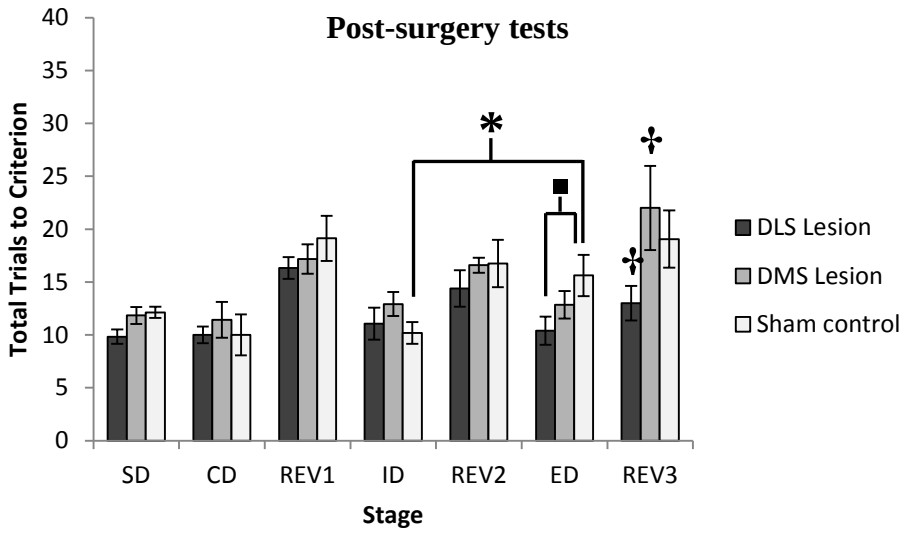

Figure 8.8. *Mean trials to criterion (± SEM) for DLS lesion, DMS lesion and control group in the standard 7-stage attentional set-shifting task (mean of the two tests post-surgery). Sham-surgery control rats required more trials to complete the ED in comparison to the ID (* $p < .05$), DLS lesioned rats required less trials to complete the ED stage in comparison to the controls (■ $p < .05$) and rats with DMS lesions required more trials to complete REV3 in comparison to DLS lesioned rats († $p < .05$).*

8.11.2 Probe stage attentional set-shifting task

Total trials to criterion. Figure 8.9 shows the comparison of the mean number of total trials to reach criterion for the DLS lesion, DMS lesion and control groups on the probe test stage attentional set-shifting task. Lesions of the dorsal striatum did not affect the performance of the task (main effect of Group: $F(2, 20) = 0.40$, $p = .67$, $\eta_p^2 = .04$). Although the stages were completed differently (main effect of Stage: $F(10, 200) = 6.88$, $p < .001$, $\eta_p^2 = .26$), there were no differences between the performance of DLS lesion, DMS lesion and control rats on the stages of the task (Stage x Group interaction: $F(20, 200) = 0.70$, $p = .82$, $\eta_p^2 = .07$).

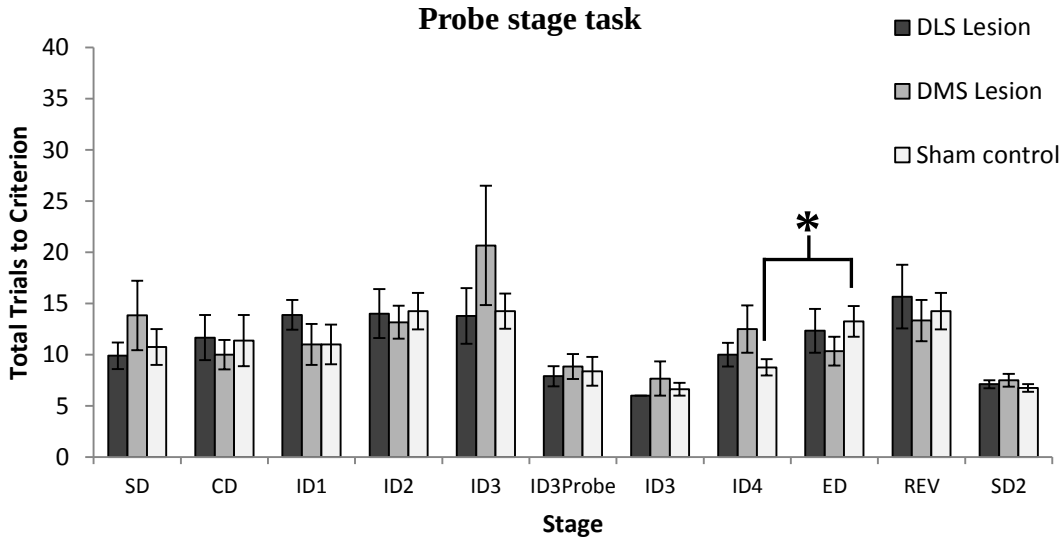

Figure 8.9. *Mean trials to criterion (± SEM) for DLS lesion, DMS lesion and control group in the probe stage attentional set-shifting task. Control rats required more trials to complete the ED shift stage in comparison to the ID4 stage (* $p < .05$).*

Attentional set formation. Although the number of trials between the first and the last ID stages (ID1 vs ID4) decreased for the control rats and the DLS lesioned rats, the difference was not significant for either of the groups; which suggested that there were no differences between the groups in learning set.

Control rats needed more trials to acquire the discrimination requiring an ED shift compared with the immediately preceding discrimination requiring an ID (p = .05), which suggested that they had formed an attentional set and, therefore, required to shift attentional set at the ED shift stage on the test. However, the DLS lesioned and the DMS lesioned rats did not show attentional set-shifting. While the DLS lesioned rats required more trials to complete the ED shift stage in comparison to the ID4 stage, the difference between these stages was not significant. Though, the DMS lesioned rats showed a non-significant trend to complete the ED shift stage in fewer trials in comparison to the ID4 stage (see Figure 8.9). The performance on

the ED shift stage and the shift-cost (main effect of Group: $F(2, 20) = 1.94$, $p = .17$, $\eta_p^2 = .16$) was not different among the groups.

All three groups required fewer trials to complete the last stage in comparison to the first stage (SD vs. SD2), which suggested that the performance on the last stages were not due to issues of fatigue, satiety or memory load.

Probe test. Substituting the irrelevant dimension with novel exemplars had no effect on performance in either control or dorsal striatum lesioned rats. The three groups continued to respond to the previously correct stimulus in the relevant dimension, therefore the ID3Probe stage was completed in fewer trials in comparison to the ID3 stage (see Figure 8.9). Figure 8.10 shows a comparison of the errors made on the previous ID stage and the probe stage. The errors on the probe stage significantly decreased in comparison to the ID3 stage for the three groups, but the difference was only significant for the lesioned groups (DLS: $p = .04$; DMS: $p = .01$).

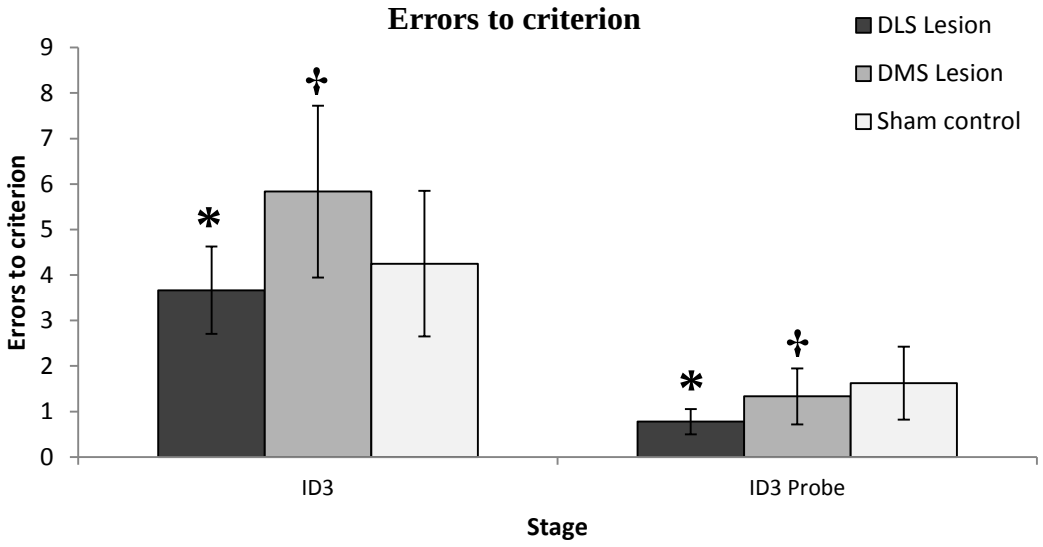

Figure 8.10. *Mean errors to criterion (± SEM) for DLS lesion, DMS lesion and control group on the ID3 and the ID3 Probe stage. The number of errors on the ID3 Probe stage was significantly lower than on the ID3 stage (DLS*****, DMS † $p < .05$).*

Reversal. DLS lesioned, DMS lesioned and control rats did not show perseveration on the reversal stage. Planned contrast showed there were no differences between the groups in the reversal stage.

8.12 Discussion

The aim of Experiment 3 was to introduce a probe stage after a series of ID stages to investigate the aspects of the stimuli that the animals were attending to in the attentional set-shifting task.

Before testing the rats in the modified version of the task, the three groups were retested in the standard 7-stage attentional set-shifting task. In both tasks the pattern of performance did not change. That is, the control group required more trials to complete the ED shift stage in comparison to the ID stage, which suggested that they formed an attentional set to one perceptual

dimension of a compound stimulus and then had to shift from that attentional set to complete the ED shift stage. On the contrary, DLS lesioned and DMS lesioned rats did not require more trials to complete the ED shift stage in comparison to the ID stage and therefore showed a diminished/absent cost of shifting. Therefore, there was no evidence that rats with DMS or DLS lesions formed attentional set in the standard 7-stage attentional set-shifting task. The replication of results confirms that the standard 7-stage attentional set-shifting task can be used on repeated basis with reproducible results and having no learning effects (Tait et al., 2014; Tait et al., 2009; Wallace et al., 2014).

Instead of forming an attentional set to the relevant dimension and learning which of the stimuli within that perceptual dimension was rewarded. It could be possible that the DLS lesioned and the DMS lesioned rats could be solving the discrimination of each stage by combining odour and medium exemplars to form compound discriminations and learn which of the two, out of the four possible compounds, were rewarded (Dias et al., 1996b; Roberts et al., 1988). To test this hypothesis, Experiment 3 introduced a probe stage where the stimuli of the irrelevant dimension were substituted for novel ones.

DLS lesioned and DMS lesioned rats, as well as the control rats, were able to remember the rewarded stimulus, within the relevant dimension, when it was presented with novel stimuli in the irrelevant dimension. This suggests that none of the three groups were solving the stages by learning the two compound discriminations that predicted reward. In addition, the performance of the three groups on the ID stages across the task was very similar. However, the DMS lesioned rats showed a diminished shift-cost, as it was observed on the standard 7-stage attentional set-shifting task, which suggested they were not forming sets.

Shifting an attentional set requires a subject to transfer attention away from the relevant dimension and reengage attention to the previously irrelevant dimension. So it might be possible that the lesioned rats have a limited attentional selectivity and were only able to learn that a dimension is relevant, but might be less capable of learning that a dimension is irrelevant. Therefore, when the irrelevant dimension becomes relevant on the ED shift stage, lesioned animals might only need to learn the new dimension that is relevant, which allowed them to solve the ED shift stage faster. The ability to inhibit a previously learned association in the lesioned animals was observed on the reversal stage, where DLS and DMS were able to learn the new stimulus-reinforcement associations like the control group.

The results from this experiment suggested that the diminished shift-cost observed in rats with DLS or DMS lesions did not result from solving the discrimination of each stage by learning which of the two, out of four, possible compound discriminations (i.e., the combination of odour and medium exemplars) were rewarded.

Overall summary and conclusion

It has been reported that in the attentional set-shifting task, preclinical carriers of HD mutation and HD patients on an early stage show perseveration responding on the ED shift stage (i.e., are unable to stop responding to the stimulus dimension that was relevant in the stages before the ED stage). In addition, advance stage HD patients also show impairments in the reversal stages (Lange et al., 1995; Lawrence et al., 1996; Lawrence et al., 1998b; Lawrence et al., 1999b).

The purpose of this chapter was to test rats with bilateral quinolinic acid lesions of the dorsolateral or dorsomedial striatum in a series of attentional set-shifting tasks to investigate the role of the dorsal striatum in reversal learning, attentional set-formation and set-shifting.

In **Experiment 1**, rats were tested in the standard 7-stage attentional set-shifting task. On the reversal stages, there were no differences between the DLS or the DMS lesioned rats and the control rats, which suggested that lesions of the dorsal striatum do not affect reversal learning as measured by this task. However, as opposed to the control rats, there was no evidence that the DMS and the DLS lesioned groups formed attentional set, as they showed a diminished/absent shift-cost (did not require more trials to complete the ED shift stage in comparison to the ID stage).

Given that the standard 7-stage attentional set-shifting task does not allow a direct measurement of attentional set-formation, it only assumes that set-formation has been formed by detecting set-shifting, **Experiment 2** used two modified versions of the attentional set-shifting task (that differed in the placement of the reversal stages) that, in addition of measuring set-shifting, can provide a better measure of the formation of attentional set. In the early reversal attentional set-shifting task, the reversal stage was presented before four consecutive ID stages (i.e., before attentional set is likely to have been formed); while the late reversal task presented the reversal stage after four consecutive ID stages (i.e., after set-formation is likely to have been formed). The results from the early and late reversal stage attentional set-shifting tasks showed that there were no differences in the performance of DLS lesioned, DMS lesioned and control rats in any stage of these two tasks.

The absence of set-shifting observed in the DLS lesioned and DMS lesioned rats in the standard 7-stage attentional set-shifting task and the early and late stage attentional set-shifting

task could be explained if the rats were performing each stage as novel discriminations. To test this hypothesis, **Experiment 3** used a probe stage to help identify the aspects of the stimuli the animals were attending to in the intradimensional stages. First, the three groups were retested in the standard 7-stage attentional set-shifting task. The results were consistent with the results from the first time the rats were tested post-surgery (i.e., dorsal striatum lesioned rats did not show reversal impairments and showed no difference between the number of trials to complete the ID stage and the ED shift stage). When the rats were tested in the modified version of the attentional set-shifting task that included a probe stage (which substituted the stimuli of the irrelevant dimension from the previous ID with novel stimuli) after three consecutive ID stages, DLS and DMS lesioned rats showed no reversal learning impairments, but they did not appear to have formed attentional set (as there was no difference between the number of trials to complete the last ID stage and the ED shift stage). The performance of the three groups was not affected in the probe stage. When the stimuli of the irrelevant dimension were substituted, all of the rats continued responding to the rewarded stimulus from the relevant dimension of the previous ID stage. Therefore, the three groups did not take as many trials to complete the probe stage as they did on the ID stages, which suggested that the rats were not treating the probe stage as if it was an entirely new discrimination. The results from this experiment suggested that the diminished shift-cost observed in rats with DLS or DMS lesions did not derive from solving the discrimination of each stage by learning the two compound discriminations that were rewarded.

Overall, the results from the three experiments presented in this chapter, and the results from Chapter 7, showed that dorsal striatum lesioned rats were not impaired in the acquisition of discriminations; they were able to inhibit responding to previously rewarded stimuli, showing that they were not impaired in reversal learning. These results are contrary to what is observed in

patients with advanced HD, who show reversal impairments. Given that dorsal striatum lesioned rats did not require more trials to complete the ED shift stage than the ID stage, the ability to set-shift could not be measured, but the results suggested that there was no evidence that they were forming set. These results were consistent across the four different tasks on which the rats were tested.

In summary, the series of experiments presented in this chapter showed that there were no differences between the DLS and DMS lesioned rats in the attentional set-shifting task. Lesions of the dorsal striatum did not impair reversal learning but impaired the formation of attentional set. These results are inconsistent with the results observed in HD patients, who showed reversal impairments and impaired ability to shift attentional set from a previously relevant dimension to previously irrelevant dimension (Lange et al., 1995; Lawrence et al., 1996; Lawrence et al., 1998b; Lawrence et al., 1999b). Therefore, the results from this chapter and the previous chapter, suggested that even though bilateral quinolinic acid lesions of the dorsal striatum in rats mimic some aspects of the neural damage in HD, they do not presented similar cognitive deficits in behavioural flexibility as those seen in HD patients in the ID/ED attentional set-shifting task.

Chapter 9

General Discussion

9.1 Introduction

The purpose of this thesis was to develop methodology by which treatments for the cognitive impairments in HD could be tested. As such, the thesis focused mainly on evaluating rats with quinolinic acid lesions of the striatum, as this manipulation mimics some aspects of the neural damage in Huntington's disease, to try to identify cognitive deficits of HD resulting from cell loss in the striatum. The animal models of HD based on genetic manipulations have, understandably, resulted in a research emphasis on the motor impairments of the models, while – in part because these tend to be mice – the characterisation of the cognitive impairments remains poorly investigated. The reduced number of tasks available for evaluating cognitive impairments in animal models of HD, which are caused by cell loss in the striatum, has slowed the progress for testing treatments for the disease. Currently, the only treatments available for HD ameliorate the motor and psychiatric symptoms, but there are no treatments available for the cognitive decline observed in HD. This suggests there is a necessity to develop and validate new tests of striatal function in which the effectiveness of treatments for HD could be evaluated. Accordingly and based on the cognitive deficits that have been previously reported in patients with HD, this thesis examined the following: implicit memory (Chapters 3-5), habit formation (Chapter 6), reversal learning, attentional set-formation and set-shifting (Chapters 7-8) in rats with QA lesions of the striatum in order, to assess the validity and translatability of these tasks as well as to evaluate the effectiveness of potential treatments for HD. In this concluding chapter, the findings from this thesis will be summarised briefly. Because the results have already been discussed in Chapters 3-8 this chapter considers the implications of these findings in a wider context in order to suggest future avenues of research.

9.2 Summary of results

9.2.1 Role of the dorsomedial striatum in implicit memory

In the first part of this thesis (Chapters 3-5), the role of the striatum in implicit memory was investigated.

The basal ganglia have been reported as a critical brain structure for implicit learning (Paulsen et al., 1995a). In effect, patients with HD, who have neuronal loss of the striatum, exhibit deficits in implicit learning (Knowlton et al., 1996b). Research on implicit learning in animal models of HD has been restricted given that the tasks to evaluate implicit memory have been designed for humans, and thus the behavioural abilities required to perform the tasks cannot easily be tested in animals.

The aim of **Chapter 3** was to develop a task that could be performed by rats and humans as a means of evaluating implicit memory. It has been suggested that the striatum is involved in the calculation of probabilities that predict an outcome (Knowlton et al., 1996b); accordingly, a reaction time task was modified in order to allow the study of implicit memory by incorporating the computation of probabilities to predict the location of the stimuli. In this reaction time task, called the Spatiotemporal Target Probability Signal Reaction Time Task, the location of the target (spatial probability) changed as a function of the length of the foreperiod (temporal probability). At shorter foreperiods, targets on the left side were more probable than targets on the right side, but at longer foreperiods, targets on the right side became more probable than the targets on the left side. If implicit learning occurred, it was expected that during the task participants would gradually have faster reaction times on the left side at shorter foreperiods and have faster reaction times on the right side at longer foreperiods (the more probable location of

the targets), even though they would not have conscious knowledge of the location where the stimuli were more likely to appear. The results from Chapter 3 showed that rats and humans can learn the Spatiotemporal Target Probability Signal Reaction Time Task efficiently. Although the performance of humans and rats differed in the percentage of incorrect responses and late errors (i.e., humans did not make incorrect responses or late errors), both groups showed a similar pattern in reaction times. Both rats and humans had faster reaction times to the right side at longer foreperiods (i.e., the side where the stimulus was more likely to appear), which indicates that with practice both species were able to learn that the probability of the stimulus appearing on the left side was more likely at early foreperiods, and at later foreperiods, the probability of the stimulus appearing on the right side was more likely. Chapter 3 showed that the Spatiotemporal Target Probability Signal Reaction Time Task was an effective task for evaluating implicit learning in rats and humans. Given that the task could facilitate the assessment of short acting treatments in pharmacological studies and the effects of lesions in rodents, these were investigated in Chapters 4 and 5 respectively.

Previous studies have reported a loss of striatal D_1 and D_2 dopamine receptors in HD (Backman et al., 1997; Brandt et al., 1990; Lawrence et al., 1998b; Lawrence et al., 1998c). **Chapter 4** investigated if implicit memory (the computation of probabilities to predict the location of a stimulus) was affected by selective blockade of dopaminergic transmission at the D_1 or D_2 receptors by SCH-23390 and raclopride, respectively. These results suggested that reaction times were slower with both SCH-23390 and raclopride, but only D_1 antagonist SCH-23390 reduced errors to the least probable target location. These results suggested that selective blockade of dopaminergic transmission at the dopamine D_1 and D_2 receptors could have different effects in tasks that require implicit memory, and that reaction times and accuracy could be

dissociated. To further investigate the role of the striatum in implicit memory, **Chapter 5** evaluated rats with QA lesions of the striatum, through the use of the Spatiotemporal Target Probability Signal Reaction Time Task. Implicit memory was not affected by QA lesions of the DMS as reaction times remained faster to the right side at the longest foreperiods (i.e., the side were the target was more likely to appear), which suggests that once a task that requires implicit memory has been learned, the DMS is not involved in sustaining the performance of the task.

Given that no cognitive impairments were induced by lesions of the DMS in the Spatiotemporal Target Probability Signal Reaction Time Task, the effectiveness of treatments for HD could not be evaluated using this task. Hence, Chapter 6 investigated another test of striatal function in which potential treatments for HD could be evaluated.

9.2.2 Role of the dorsomedial striatum in goal-directed and habitual responding

The second part of this thesis (Chapter 6), explored the contribution of the DMS in habit formation.

In addition to having a general role in the initiation and patterning of different behaviours, the striatum has also been associated with habit formation (Packard and Knowlton, 2002; Yin and Knowlton, 2006). Ritualistic behaviours, which are considered as habits (Graybiel, 1998), are one of the most common obsessions in HD (Novak and Tabrizi, 2010). **Chapter 6** implemented a task, adapted from that of Desrochers et al. (2010), as means of evaluating habit formation in rats with QA lesions of the DMS.

Using a free scan task, rats had to nose poke a row of five holes to find a target hole selected randomly on each trial. There was no cue that would indicate which hole would produce the reward or when the target would become bated. Changes in goal value were assessed by

using a devaluation of the reward, in which rats had free access to the reinforcement in their home cages before testing, and the reward was not delivered during the session. It was hypothesised that if habits were formed, the performance on the task would not be affected even though the value of the reward was devalued. This was not observed, neither DMS lesioned nor sham-surgery control rats showed habitual responding in the task, as their performance decreased when the value of the reward decreased.

In order, to evaluate if lesions of the DMS would impair goal-directed behaviour (i.e., the association of an action and an expected outcome), in the last phase of the experiment all of the conditions remained the same, but the central hole never produced a reward. Since there were no cues to indicate the change, rats had to learn through the task that nose poking into the central hole would never produce a reward. Habitual responding would consist of continuing responding to the central hole. Thus, it was hypothesised that if the DMS was required to learn goal-directed behaviours, rats with DMS lesions would show habitual responding and would continue responding to the central hole regardless of it no longer being reinforced. Lesion of the DMS did not change the sensitivity to outcome devaluation, and both sham-surgery control and DMS lesioned rats made fewer responses to the hole that never delivered reward during the last phase of the experiment, which suggested that DMS lesions did not affect the acquisition of a new goal-directed behaviour.

Because no cognitive deficits were observed in DMS lesioned rats that could be compared with HD patients, Chapter 7 evaluated if rats with bilateral QA lesions of the DMS presented similar cognitive deficits in behavioural flexibility as those seen in HD patients in the ID/ED attentional set-shifting task.

9.2.3 Role of the dorsal striatum in attentional set-shifting, set-formation and reversal learning

The third part of this thesis (Chapters 7 and 8) investigated the role of the dorsal striatum in attentional set-shifting.

Patients with damage to the striatum arising from HD exhibit deficits in behavioural flexibility in the ID/ED attentional set-shifting task. Preclinical carriers of the HD mutation (Lawrence et al., 1998a), and early stage HD patients showed impairments in the extradimensional shift stage (Lawrence et al., 1996; Lawrence et al., 1999b). Their set-shifting impairments resulted from perseverative responding (i.e., an inability to stop responding) to the stimulus dimension that was relevant in the stages before the ED (Lawrence et al., 1999b). As the disease progresses, advanced stage HD patients showed impairments in the reversal stages (failing to complete those stages) and therefore rarely reach the ED stage (Lange et al., 1995).

Chapter 7 investigated if rats with bilateral quinolinic acid lesions of the dorsomedial striatum, which mimic some aspects of the neural damage in HD, presented similar cognitive deficits in behavioural flexibility as those seen in HD patients in the ID/ED attentional set-shifting task. DMS lesioned rats and sham-surgery control rats were tested in the ID/ED attentional set-shifting task for rodents, which consists of 7 stages that are analogous to those in the human task. Rats with lesions of the DMS did not show reversal impairments and showed a trend towards a diminished shift-cost suggesting that no sets were formed, which is contrary to what is observed in HD patients. To test if attentional set-formation was impaired in rats with lesions in the DMS, **Chapter 8** presented a series of behavioural tasks designed to further elucidate set-shifting performance, with the possibility of drawing conclusions on the set-formation.

In Chapter 8, three experiments examined the effects of QA lesions of the dorsolateral or the dorsomedial striatum in reversal learning, attentional set-formation and set-shifting. In Experiment 1, rats were tested on the standard 7-stage attentional set-shifting task. DLS and DMS lesioned rats did not have reversal learning impairments, but showed a diminished shift-cost (they did not require more trials to complete the ED shift stage in comparison to the ID stage), which may occur if no set has been formed. Experiment 2 explored the relationship between reversal learning, attentional set-formation, and set-shifting by using two modified versions of the attentional set-shifting task (that differed in the placement of the reversal stages). There were no differences between the DLS lesioned, DMS lesioned and the control groups in the performance of these two tasks. Experiment 3 introduced a probe stage (which substituted the stimuli of the irrelevant dimension from the preceding stage with novel stimuli) to help identify the aspects of the stimuli to which the rats were attending. The three groups continued responding to the rewarded stimulus in the relevant dimension from the previous stage, which suggested that the diminished shift-cost observed in rats with DLS or DMS lesions was not caused by solving the discrimination of each stage by learning the two compound discriminations that were rewarded. Altogether, the four different attentional set-shifting tasks used in Chapter 8 showed that the DLS lesioned and the DMS lesioned rats were not impaired in reversal learning, but they had a diminished shift-cost, which suggests that dorsal striatum lesions disable the formation of attentional sets.

The results from Chapter 7 and 8 suggest that even though bilateral quinolinic acid lesions of the dorsal striatum in rats mimic some aspects of the neural damage in HD, they do not present similar cognitive deficits in behavioural flexibility as those seen in HD patients in the ID/ED attentional set-shifting task.

9.3 Contributions, limitations and future research directions

9.3.1 Spatiotemporal Target Probability Signal Reaction Time Task

This thesis presented a new task that measured implicit memory that could be performed by both humans and rats. Both species were able to learn that the probability of a stimulus appearing on the left side was more likely at early foreperiods; at later foreperiods, however, the probability of the stimulus appearing on the right side was more likely. Consequently, the Spatiotemporal Target Probability Signal Reaction Time Task could be used in future research to evaluate cognitive deficits in implicit learning in patients with HD or in other animal models of HD.

Previous studies have reported that in HD, the cognitive deficits start before the motor symptoms appear, and that subtle cognitive impairments could be among the earliest manifestations of HD (Aylward, 2007; Aylward et al., 2000; Hahn-Barma et al., 1998; Paulsen, 2011; Paulsen et al., 2006; Paulsen et al., 2008; Paulsen et al., 2001b). Deficits in implicit learning and the inability to learn new motor skills or procedures are some of the cognitive changes that have been reported in HD patients (Paulsen et al., 1995a). Future research could evaluate HD patients at different stages of the disease using the Spatiotemporal Target Probability Signal Reaction Time Task. For example, if the task is sensitive in detecting early cognitive deficits in implicit learning in patients at the prodromal stage of HD, the task could be used as a tool for diagnosing and potentially starting treatment that could prevent the decline of cognitive deficits. Conversely, this task could also test patients that have been diagnosed with HD in order to monitor the progression of the cognitive deficits in implicit learning. In addition, the task is not only restricted for evaluating patients with HD, it could also be used to evaluate

implicit learning in diseases or disorders in which implicit learning has been reported to be impaired, such as Parkinson's disease (Heindel et al., 1989) or autism (Kriete and Noelle, 2009).

By showing that rats and humans have a similar pattern of reaction times in the Spatiotemporal Target Probability Signal Reaction Time Task, the task could be used to evaluate implicit learning in different animal models. Although different animal models of HD have been proposed, there are only few tasks available for evaluating cognitive impairments that could be performed by both rats and humans, which have resulted in a slow testing process for treatments for patients with HD. Therefore, the Spatiotemporal Target Probability Signal Reaction Time Task provides a tool for initiating the evaluation of cognitive deficits in HD, which could prove valuable in future investigations of this disease.

Additionally, the results presented in Chapter 4 have important implications for the therapeutic use of D_1 and D_2 antagonists in the Spatiotemporal Target Probability Signal Reaction Time Task. Firstly, because the data presented in this thesis suggest that D_1 and D_2 antagonists could have different effects, and that reaction times and accuracy could be dissociated. Secondly, it provides a new task to potentially evaluate the cognitive changes that have been reported from the reduction in D_1 and D_2 receptor density in the striatum in patients with HD and in preclinical carriers. Finally, the task may be used to evaluate therapeutic interventions that selectively block D_1 or D_2 receptors.

Furthermore, the results from Chapter 5 suggested that once a task which requires implicit memory has been learned, the DMS is not involved in sustaining the performance of the task. However, it remains to be investigated if the DMS is involved in the acquisition of this type of implicit memory task. Lesioning the DMS before the probabilities are introduced in the

task would allow investigation on whether the striatum is involved in the computation of probabilities to predict the location of a target stimulus, which changes as a function of the length of the foreperiod.

Before discussing the implementation of the task, it is necessary to address some methodological points of concern. During the experiments reported in this thesis, for example, the side of stimulus presentation was not counterbalanced. Throughout the experiments, the stimuli at shorter foreperiods, were more likely to appear on the left side, and at longer foreperiods, the stimuli were more likely to appear on the right side. Although there is no evidence to suggest that the results would have been different if the stimuli at shorter foreperiods would have been more likely to appear on the right side and at longer foreperiods on the left side, laterality in rats cannot be ruled out. Therefore, future research should counterbalance the side on which the stimuli are presented at both short and long foreperiods. Another limitation of the task was that the foreperiod and target side were determined on a trial-by-trial basis according to *a priori* probabilities; therefore, there was not always precisely the same number of trials in every session. Methodologically, this concern was not an issue when the data were collected for repeated sessions (like on Chapters 3 and 5) because there were enough trials for all the foreperiods and target sides. However, this was a limitation when the data was collected for a single session, such as in the experiment described in Chapter 4, where data on the effect of each dose were collected only once. Given that there were cases in which a rat did not receive all the targets at all the possible locations/foreperiods, the data could not be included in the analysis, which reduced the power of the experiment. This could have been addressed by having more than one session to test each drug dose. However, the main issue could be addressed by having a

fixed number of stimuli per foreperiod and target side. This would ensure that even within one session all the rats would receive a reasonable number of trials at each foreperiod and side.

Furthermore, future research should investigate other neuronal structures in this form of implicit learning task. Different human studies have suggested that the striatum is not the only neuronal structure that contributes to implicit learning, and various cortical areas (e.g., ventrolateral prefrontal cortex, ventromedial premotor area, medial prefrontal cortex) have been reported to be involved in various stages of implicit learning (Exner et al., 2002; van der Graaf et al., 2006). Consequently, it could be possible that lesions in the prefrontal cortex may impair the computation of probabilities that predict the location of the stimuli in this task. This could also be investigated in the future given that, in addition to the striatum, the cortex of patients with HD also suffers from atrophy. Furthermore, cortical neuronal degeneration leads to excessive thinning of the cerebral mantle of the entire brain (Hedreen et al., 1991). As a new task, further investigation is required to evaluate which neuronal structures are involved in the computation of probabilities to predict the location of the stimuli, which could ultimately become a useful task for testing rats and humans.

9.3.2 Goal-directed and habit formation

In order to continue addressing the issue of the reduced number of tasks available for evaluating cognitive impairment in animal models of HD, Chapter 6 presented a new task to evaluate goal-directed and habitual responding.

The results from Chapter 6 showed that lesions of the DMS did not induce habitual responses (as the performance in the task decreased when the value of the reward decreased), and they did not affect the acquisition of a new goal-directed behaviour (as the responses to the

central hole decreased when it never delivered reward). However, for any given behaviour to be established as goal-directed it should be tested using outcome revaluation (i.e., decrease or increase the value of the outcome) and by evaluating the effects of contingency degradation (i.e., deliver rewards regardless of the behaviour). Behaviours would be considered goal-directed if the performance is affected by these two manipulations (Eilan et al., 1993). Therefore, in addition of outcome devaluation, the effects of contingency degradation should be examined in the task used in Chapter 6 in order to investigate the role of the dorsal striatum in goal-directed and habitual responding.

Furthermore, lesions of the DMS have opposing effects to lesions of the DLS for balancing habitual and goal-directed behaviours (Smith and Graybiel, 2014). While it has been suggested that the DMS is involved in learning A-O associations, the DLS has been proposed to be involved in S-R learning (Yin and Knowlton, 2006; Yin et al., 2004; Yin et al., 2005). Future research could therefore investigate the role of the DLS in goal-directed and habitual responding in this task.

As habitual behaviour has been proposed as a symptom of HD (Novak and Tabrizi, 2010), the task presented in Chapter 6 has the potential to evaluate goal-directed and habitual responding in different animal models of HD.

9.3.3 Attentional Set-shifting task

Patients with HD show impairments in set-shifting and reversal learning in the ID/ED attentional set-shifting task (Josiassen et al., 1983; Lange et al., 1995; Lawrence et al., 1998a; Lawrence et al., 1996; Lawrence et al., 1999b). The series of experiments presented in Chapters 7 and 8 investigated if rats with bilateral QA lesions of the dorsal striatum, not only mimicked

some aspects of the neural damage in HD, but also presented similar cognitive deficits in behavioural flexibility as those seen in HD patients.

In addition to the standard 7-stage attentional set-shifting task for rodents, Chapter 8 presented three modified versions of the task to test different hypothesis about reversal learning, attentional set-formation, and set-shifting. These tasks are not limited to the evaluation of animal models of HD; rather, they provide alternative tools for testing specific hypothesis based on particular behavioural flexibility deficits, which require further investigation.

Although the different modifications of the attentional set-shifting task have several advantages for investigating reversal learning, attentional set-formation, and set-shifting, they also have limitations that should be addressed in future research.

An advantage of the attentional set-shifting task is that it can be used on repeated basis with reproducible results and without having learning effects (i.e., the ED shift-cost and the reversal costs are consistent between tests). However, the effects of counterbalancing on retesting should be considered. Even though the design for repeated testing tries to counterbalance for direction of shift, stimulus pair order and correct/incorrect stimuli within a pair, the design has some limitations. For example, on the tasks that have multiple ID stages with no reversal stages, only limited combinations for the stimulus pairs order can be used, and the pairs are always followed or preceded (most of the times both) by the same pairs even in different tasks. As a result, there will be cases where some cues will never be rewarded which might affect the performance on following tests.

In addition, increasing the number of stages in the task to test different hypotheses reduced the pairs of stimuli that were tested in this laboratory. The limited number of stimulus

pairs available is also a limitation of the task. The tasks used in Experiment 2 and 3 as presented in Chapter 8, have an increased number of stages that require multiple stimuli. Unfortunately, some of the pairs that were used are relatively new to our laboratory and have not been tested enough to validate them. Because the standard 7-stage attentional set-shifting task always uses the same three pairs of stimuli (pair 1-3 from Table 8.3), and it has not been established if the different stimuli (pair 4-8 from Table 8.3), that are only used in tasks that require multiple ID stages, would produce the same results in the standard task. Before using novel stimulus pairs in other tasks, then, further research should be conducted to determine if the stimulus pairs used in the tasks with multiple ID stages are equivalent to the pairs of stimuli used in the standard 7-stage attentional set-shifting task. In particular, it is important to evaluate that there are no pairs of stimuli that are more difficult to discriminate than others. Having pairs that are harder to discriminate in the ED shift would require more trials to learn than the ID stage; but this would lead to erroneous conclusions about performance shifts because the cost was not associated with attentional set-shifting. In the tasks used in Chapter 8, the stimuli in the same dimension presented as pairs differed as little as possible from each other. Furthermore, the digging media were sufficiently dense to mask the scent of the reward, and the reward was buried sufficiently deep. The results from the reversal stages showed that rats required more trials to complete the reversal stage than the preceding stage; therefore, they were not solving the stages by detecting the scent of the reward.

It is also important to mention that the early and late reversal stage attentional set-shifting tasks have only been used twice. First by Chase (2013) who found set-shifting intact (i.e., an ID/ED difference) in sham-surgery control rats. The experiment presented in Chapter 8 did not find an ID/ED difference in in sham-surgery control rats. The difference in results might be

accounted for by the different pairs of stimulus that were used. As previously mentioned, some of the stimulus pair that Chase (2013) used have been replaced because the rats found them aversive or easy to discriminate. However, these new stimulus pairs should first be validated. In addition, cognitively normal rats (without being submitted to surgery) should be tested in these tasks to establish a baseline and to determine if these tasks are valid as a means of measuring reversal learning, attentional set-formation, and set-shifting. This also applies for the probe task attentional set-shifting task used in Experiment 3 from Chapter 8, which is a new task used in our laboratory.

Another fact that should be considered is that in comparison to the automated CANTAB ID/ED task for humans and nonhuman primates, in the rodent attentional set-shifting task, currently a human observer scores and classifies the behaviour of the rat. Therefore, there is possibility for human error, bias or subjectivity. Although the ambiguity of interpretation of behaviour can be reduced by a standard training system within a research group, there will still be differences between different laboratories. Therefore, the observer bias may contribute to the difference in findings and effect sizes. Although an automated version of the task would eliminate these subjective elements, there are elements in the human ID/ED task that cannot be replicated in rodents. For example, the stimuli presented in bowls in the rodent version are presented in separate compartments and the rat/mouse needs to explore each bowl individually. Comparatively, the visual stimuli in the task for humans are presented simultaneously on the touch screen and can be compared at the same time. In addition, while the rat task requires the discrimination of two perceptual dimensions using different sensory modalities (olfactory, somatosensory and possibly visual), the human task only requires one modality (visual) to solve the discriminations. There have been some attempts to automate the rodent task. Brigman et al.

(2005) used a visual discrimination protocol similar to the one used in the primate version of the ID/ED task where compound visual stimuli were presented on a touch screen. However, the mice tested on the task did not require more trials to complete the ED in comparison to the ID stage (i.e., did not show a positive shift-cost), which suggests that visual stimuli in general may not be generalised by mice to form attentional sets. Likewise, the different tasks in rodents that have used visual stimuli have suggested that texture and odour stimuli are discriminated more easily and are learned in fewer trials in comparison to visual stimuli (Brigman et al., 2005; Izquierdo et al., 2006). Although the current version of the attentional set-shifting presents some limitations, it is nevertheless one of the best tasks available to measure behavioural flexibility in rats.

Given that impairments in set-shifting and reversal learning have been observed in HD patients (Josiassen et al., 1983; Lange et al., 1995; Lawrence et al., 1998a; Lawrence et al., 1996; Lawrence et al., 1999b), DMS lesioned rats should be examined more closely with other procedures to evaluate if QA lesions of the striatum are a potential animal model for studying cognitive and behavioural impairments of HD.

9.3.4 Quinolinic acid lesions of the rat striatum as an animal model of Huntington's disease

Although there is no animal model which simulates all the aspects of a disease such as HD, a model needs to present only some aspects of the disease to be an appropriate model (Willner, 1991). The validity of animal models has been evaluated using three main criteria: face, predictive, and construct validity (Willner, 1986). More specifically, an adequate model of HD should resemble the fundamental motor, psychiatric and cognitive symptoms, as well as the pathophysiology, found in HD patients (face validity), conform to a theoretical rationale, such as

the known genetics of HD (construct validity), and allow us to make predictions about HD, based on the performance of the model (predictive validity). From a pharmacological perspective, predictive validity is the ability of an animal model to respond to treatments in the same manner that humans respond to the treatment, and vice versa (Willner, 1984). Given that currently there are no effective treatments available for HD, this criterion cannot be used to validate the existing animal models of HD.

In rats, excitotoxic lesions of the striatum produce anatomical changes that resemble the pathology of HD (face validity; Brasted et al., 1998; Dunnett et al., 2012; Shear et al., 1998). In addition, it has been suggested that striatal lesions produce behavioural changes that, even though might not be identical to all of the symptoms observed in HD, are analogous (Brasted et al., 1998). In this sense, the face validity of the model has focused on similarly impaired behaviours that have been established as behaviours affected in HD. The results from this thesis showed that contrary to HD patients, DMS lesioned rats did not have cognitive deficits in implicit memory (Chapter 5), and they did not show habitual responding (Chapter 6). In addition, DLS lesioned and the DMS lesioned rats did not present similar cognitive deficits in behavioural flexibility as those seen in HD patients in the ID/ED attentional set-shifting task (Chapter 7 and 8). While patients with HD show reversal impairments and impaired ability to shift attentional set from a previously relevant dimension to previously irrelevant dimension (Lange et al., 1995; Lawrence et al., 1996; Lawrence et al., 1998b; Lawrence et al., 1999b), rats with lesions of the dorsal striatum were not impaired in reversal learning but were impaired forming attentional sets. Consequently, the results from this thesis showed that although QA lesions of the dorsal striatum mimic some aspects of the neural damage in HD, they did not result in the same cognitive deficits observed in patients with HD, at least using the tasks presented in this thesis. Therefore,

future research should evaluate rats with quinolinic acid lesions of the dorsal striatum using other procedures to determine if the model presents the same cognitive and behavioural impairments of Huntington's disease.

Another point that should be consider when using QA lesions of the rat striatum as an animal model of HD, is that even though lesions of the striatum are a controlled and rapid method to induce HD neuropathology, in some occasions the number of lesioned subjects is reduced if they do not have appropriate bilateral lesions, which cannot be determined until the behavioural testing is completed and the histology is finalised. This was a limitation of the present thesis as subjects that did not have bilateral lesions were excluded and the number of lesioned animals used in the analyses was reduced. In addition, it was unexpected to observe that some of the sham-surgery control rats showed signs of cell damage; thus, they were also excluded from the analyses. This damage was unusual, and although the surgery protocol for the control rats has been previously used in this laboratory with no cell damage observed, it could have been possible that the sham-surgery control rats presented cell damage because the needles were contaminated with QA. Thus, future research could avoid this problem by using different syringes and needles for the lesion and the control groups and by making sure the equipment is sterilised between surgeries to avoid cross contamination between the groups.

Furthermore, other animal models of HD (see section 1.7 from the General Introduction) could be evaluated using the different tasks presented in this thesis to continue the search of a reliable animal model of HD in which treatments for the disease could be evaluated.

9.4 Conclusion

Despite the numerous animal models of HD available, most research has evaluated the motor impairments of the models, while the characterisation of the cognitive impairments remains poorly investigated. The low number of tasks available for evaluating cognitive impairments in animal models of HD has slowed the progress for testing treatments for the disease. The new tasks presented in this thesis provide several alternatives that should prove valuable in future investigations. First, the Spatiotemporal Target Probability Signal Reaction Time Task could evaluate patients with potential cognitive deficits in implicit learning, and it could also assess cognitive deficits in animal models in order to potentially test different treatments. Second, the new task presented in Chapter 6 has the potential to evaluate goal-directed and habitual responding in different animal models of HD. Third, in addition to the standard 7-stage attentional set-shifting task for rodents, Chapter 8 presented three modified versions of the task to test different hypotheses about reversal learning, attentional set-formation, and set-shifting. The tasks presented in this thesis not only contribute to the research on animal models of HD; they provide alternative tools for testing specific hypothesis based on particular cognitive deficits in implicit memory, habits, and behavioural flexibility in different diseases or disorders that require further investigation.

Finally, the research from this thesis did not find support for validating rats with QA lesions of the dorsal striatum as a model of HD. Contrary to HD patients, DMS lesioned rats did not have cognitive deficits in implicit memory, and they did not show habitual responding. In addition, DLS lesioned and the DMS lesioned rats did not present similar cognitive deficits in behavioural flexibility as those seen in HD patients in the ID/ED attentional set-shifting task. Similarly to patients with HD, the severity of cognitive deficits in the animal models of a disease

is not universal across individuals; rather, it is dependent on the task. Hence, concluding that a specific animal model is inappropriate for studying HD based simply on the results from the tasks presented in this thesis is perhaps incorrect. This animal model should be examined more closely with other procedures to evaluate if rats with quinolinic acid lesions of the dorsal striatum are a potential animal model for studying cognitive and behavioural impairments of Huntington's disease.

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Appendix

University of St Andrews

University Teaching and Research Ethics Committee

5 July 2013

Ethics Reference No: *Please quote this ref on all correspondence*	PS10120
Project Title:	Sensitivity to Changing Probabilities in a Reaction Time Task
Researchers' Names:	Professor Verity Brown, Dr Eric Bowman, Dr Ines Jentzsch, Ana Garcia Aguirre
Supervisor:	Professor Verity Brown

Thank you for submitting your application which was considered by the Psychology & Neuroscience School Ethics Committee on 2nd July 2013. The following documents were reviewed:

1. Ethical Application Form	05/07/2013
2. Advertisement	05/07/2013
3. Participant Information Sheet	05/07/2013
4. Consent Form	05/07/2013
5. Debriefing Form	05/07/2013

The University Teaching and Research Ethics Committee (UTREC) approves this study from an ethical point of view. Please note that where approval is given by a School Ethics Committee that committee is part of UTREC and is delegated to act for UTREC.

Approval is given for three years. Projects which have not commenced within two years of original approval must be re-submitted to your School Ethics Committee.

You must inform your School Ethics Committee when the research has been completed. If you are unable to complete your research within the three year validation period, you will be required to write to your School Ethics Committee and to UTREC (where approval was given by UTREC) to request an extension or you will need to re-apply.

Any serious adverse events or significant changes which occur in connection with this study, and/or which may alter its ethical consideration, must be reported immediately to the School Ethics Committee and an Ethical Amendment Form submitted where appropriate.

Approval is given on the understanding that the 'Guidelines for Ethical Research Practice' (http://www.st-andrews.ac.uk/media/UTRECguidelines%20Feb%2008.pdf) are adhered to.

Yours sincerely

M.P. Latimer

Convenor of the School Ethics Committee

Ccs Prof Verity Brown (Supervisor)
School Ethics Committee