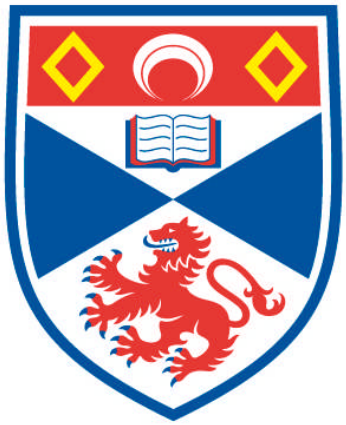
THE CONTRIBUTION OF THE SUBTHALAMIC NUCLEUS TO EXECUTIVE FUNCTIONS IN RAT

Shuang Xia

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



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The Contribution of the Subthalamic Nucleus to Executive Functions in Rat

This thesis is submitted for the degree of

Doctor of Philosophy

by

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at the

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September 2014

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Abstract

Lesions of the subthalamic nucleus (STN) alleviate the cardinal signs of idiopathic as well as MPTP-induced Parkinson's disease in primates. For this reason, the STN is a target for clinical treatment of Parkinson's disease using deep brain stimulation. Despite its small size, the STN plays a vital role in the cortico-basal ganglia-thalamic network. However, the functional features of the STN have yet to be fully uncovered. The research presented in this thesis examines the functions of the STN by measuring behavioural changes resulting from STN lesions in rats performing executive abilities.

In the first experiment, a 'signal change' reaction time task was developed and the performance of humans and rats was compared. The main findings were that although humans and rats used different strategies in the task, the task did challenge the ability to inhibit unwanted responses. In the second and third experiments, the effects of bilateral lesions of the STN on performance of two variants of the 'signal change' task were examined. Rats with the STN lesions were able to inhibit responses when under stimulus control, but were less able to inhibit responses that were not under stimulus control. In the final experiment, the effects of lesions of the STN on inhibitory control in a non-motor, cognitive domain were examined. Rats with STN lesions were not impaired on reversal learning, suggesting intact inhibition of previously rewarded responses. The rats with STN lesions did show impairments in selective attention which resulted in an inability to form an attentional set.

Together, these findings challenge the conventional view that the STN simply plays a global inhibitory role. Rather, the contribution of the STN to inhibitory control is more complex and neither the motor nor the cognitive effects of the lesions are easily explained simply as a failure of inhibition.

General Introduction

Subthalamic nucleus (STN) is a key structure in the Basal Ganglia and is considered as a target for efficient treatment of Parkinson's disease. Functions of STN have been studied in animal models in the last two decades; however, there are still a lot of questions remaining unanswered. In this chapter, previous studies of STN functions in rats will be presented, findings will be summarized and remaining questions will be highlighted.

1.1. Why the subthalamic nucleus?

The basal ganglia have been confirmed to be a critical structure in motor control and functional disruptions within the basal ganglia could induce motor disorders, such as Parkinson's disease and Huntington's disease. With the knowledge of pathways within the basal ganglia, it was noticed that the activity of the STN is abnormal in Parkinson's patients. Since then deep brain stimulation (DBS) in the STN has been involved as a common treatment target for Parkinson's disease, especially for the motor symptoms (Kumar et al., 1998; Fang et al., 2006; Temel et al., 2006). Along with the motor function, the involvement of the STN in limbic and cognitive functions has been proposed and examined in both Parkinson's patients (Boller et al., 2014; Castrioto et al., 2014; Wu et al., 2014) and animal models (Temel et al., 2005). Given the relatively difficult access to patients, observations from patient studies are relatively limited. This makes the preclinical study in animal models an important method for understanding the function of the STN. However, the number of studies of STN function in lab animals, including the rat, is much lower than for studies of other brain areas such as prefrontal cortex. Furthermore, the range of sources of such studies is also very narrow. The STN, as a major input structure to, as well as an important relay within, the basal ganglia, has not been studied enough to fully reveal its role. The present thesis builds on previous work in this and other labs to understand the functional contribution of the STN by studying the effect of STN lesions on complex animal behaviours in different tasks. By exploring the role of the STN, we hope to shed some light on the understanding of the basal ganglia network.

1.2. Background

Although described as a distinct basal ganglia structure in 1960s, the knowledge of the STN has not been updated rapidly. Some aspects, unsurprisingly including the anatomy and connectivity of the STN, have been largely established and accepted for at least a decade (for example, see reviews Hamani et al., 2004; Temel et al., 2005). Therefore, this introduction will focus

more on those aspects of STN function that are less researched and less well established.

The introduction will include three major sections. Firstly, I will give a brief summary of the current understanding of the pathways within the basal ganglia and the important position of the STN. The second part will focus on relevant behaviour tasks, including reaction time tasks and attentional tasks. This provides important knowledge for a better understanding of the behavioural tasks and the animal's performance in the current study. Finally, the main part of the introduction will summarise and critically analyse past studies of STN function in rats. Several significant studies will be described in detail, including critically evaluating the authors' interpretation of the behavioural results. These previous studies provide essential background of the current study and the deficiencies and questions left by the previous studies are the main motivation of the current series of empirical studies.

1.3. Neural circuits and anatomy

The function of the STN is usually discussed in the context of the pathways within the basal ganglia. The classical model of the basal ganglia was developed more than two decades ago (Delong et al., 1984; Albin et al., 1989; DeLong, 1990) and, although still maintains the basis for most of our current understanding of basal ganglia function, is somewhat obsolete. In the past few years, with the development of a range of new techniques, the available data have increased at a stunning speed. Under these circumstances, some aspects of the classical model are challenged and need to be updated and enriched. In this section, I will introduce both well-documented knowledge and the new findings and remaining questions that involve the STN, which include neural circuits and anatomy.

1.3.1. Basal ganglia pathways

When the mode of the basal ganglia was first introduced, it included two pathways – 'direct' pathway and 'indirect' pathway (Albin et al., 1989; Figure

1.1a). The direct pathway originates from the cells of striatum, which express D1 receptors, project inhibition onto cells of SNr and GPi. SNr and GPi are constantly in connection with the thalamus and in an attempt to inhibit the thalamus. So the end-result is lack of inhibition of the thalamus and increasing thalamocortical activity. In the opposite end is the indirect pathway, which also starts from the striatum, but from another type of cells expressing D2 receptors. These cells project inhibitory axons onto the cells of the GPe. GPe constantly inhibits the STN, and STN, in return, always stimulate SNr and GPi. The end-result is an inhibition of the thalamus and, therefore, decreased thalamocortical activity. The traditional view held that the BG function via the balance between the two pathways. A decade ago, a third important pathway – 'hyperdirect' pathway was introduced into the model. STN receives excitatory inputs directly from motor-related cortical area and projects diffusely onto GPi cells (Nambu et al., 2002; Aron and Poldrack, 2006; Jourdan et al., 2010). Therefore, the hyperdirect pathway works as an alternative direct cortico-BG link, which is probably as important as the corticostriatal–GPi pathway, especially in motor control (Nambu, 2004; Leblois et al., 2006; Isoda and Hikosaka, 2008a).

In the past few years, the model has continually been enriched with new anatomy discoveries. The 'direct', 'indirect' and 'hyper-direct' pathways, although remain valid, now only represent a subset of connections between basal ganglia nuclei and between basal ganglia and external structures. The current view holds that those connections are a series of partially segregated parallel projecting re-entrant loops (Redgrave et al., 2011). A recent model of this more complex system is illustrated in Figure 1.1b (Obeso and Lanciego, 2011), from which we could see the STN has become a vital role in the system.

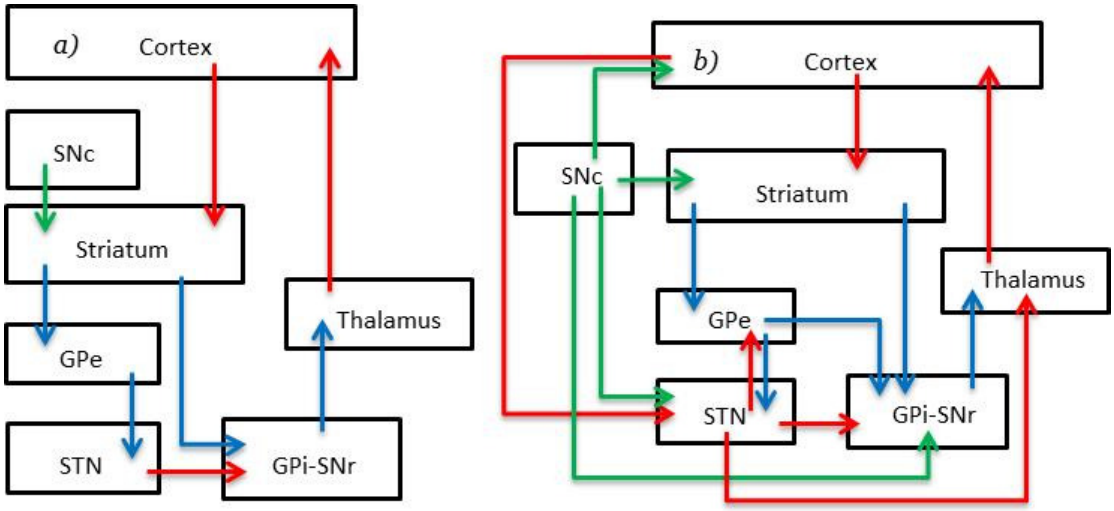

Figure 1.1. a) Albin-Delong classic model of basal ganglia (Albin et al., 1989; DeLong, 1990). b) Recent model of basal gangliawith more complex organizations (simplified from Obeso and Lanciego, 2011).

1.3.2. Functional divisions of the STN

Early researches have shown that subregions of the basal ganglia nuclei (e.g. striatum, thalamus, pallidum) receive signals from functionally segregated regions of the cerebral cortex, which are associated with limbic (emotional), associative (cognitive) and sensormotor functions (McGeorge and Faull, 1989; Nakano, 2000; Nambu et al., 2002). The same segregation model has been applied to primate STN as well (Parent and Hazrati, 1995; Joel and Weiner, 1997), with the dorsolateral third of the STN designated to sensorimotor function, the ventromedial part designated to associative function and the medial tip to limbic function. With advanced techniques, recent study in private has confirmed that the subregions of the STN are topologically divided with overlaps rather than anatomically separated with distinct boundaries (Haynes and Haber, 2013). Limbic cortical areas project to the medial tip of the STN, associative areas project to the medial half and motor areas to the lateral half. Limbic projections terminate primarily rostrally and motor projections more caudally.

In rodent, experimental evidence of functional division of the STN is limited. A common view holds that there are two major subregions in rat STN. The medial part is connected with the associative and limbic functional part of the pallidum, and the lateral part with sensorimotor functional parts of the basal ganglia and related sensorimotor cortical areas (Groenewegen and Berendse, 1990; Heimer et al., 1995). Given that the current study is concentrated on the executive and cognitive functions that involve the STN, all lesions that were done in this thesis were limited to the medial part of the STN. However, given the small size of the STN in rats, we should bear in mind that impairment in motor functions might occur in some rats where the lesions are not well-targeted.

Neurochemistry of the STN

Dopamine

Previous studies have demonstrated the effects of topical and systemic application of dopaminergic agonists on STN activity, and revealed contradictory results. In studies using topical administration of dopaminergic agonists, while some authors addressed that dopaminergic agonists exert an excitatory effect through the activation of D1 receptors (Mintz et al., 1986; Kreiss et al., 1996), others advocated that D1, D2 and non-specific agonists decreased STN activity (Campbell et al., 1985; Hassani and Féger, 1999).

The systemic administration of D1 agonists increase STN activity, but only when D2 receptors are co-activated, whereas D2 agonists do not exert significant effects (Ni et al., 2001). Since most of structures that give rise to STN afferents are also modulated by dopamine, the dopaminergic agonists clearly cause a complex cascade of responses and therefore the exact role played by each structure is uncertain.

Glutamate

Several subtypes of ionotropic and metabotropic glutamate receptors are found in rat STN (Clarke and Bolam, 1998). Current knowledge about subsynapses localization of each receptor holds that AMPA and NMDA

receptors are prominent at postsynaptic locations, while Group II and III mGluRs contribute to the presynaptic regulation of glutamatergic activity (Galvan et al., 2006). While the complexity of glutamate signalling and postsynaptic potentiation is not yet fully revealed, it is clear that a combination of multiple glutamate receptors subtypes mediates a complex signalling pathway in the STN.

GABA

GABA plays a major role in several aspects of the STN physiology, modulating its firing rate, pattern of discharges and bursting activity. GABA receptors in the STN are subdivided into three subtypes: $GABA_A$ and $GABA_C$ are ligand-gated ion channel receptors, and $GABA_B$ is a G protein-coupled receptor. $GABA_A$ and $GABA_B$ are identified in rat STN with different locations and functions. $GABA_A$ receptors have a low affinity for GABA; they desensitize very quickly (Macdonald and Olsen, 1994) and are able to mediate fast phasic inhibition (Mody et al., 1994). $GABA_B$ receptors have much higher affinity for GABA, which makes them capable of detecting extrasynaptic neurotransmitter spill (Jones et al., 1998); they are localized to mediate slow long term inhibition (Mody et al., 1994). Within the STN, GABAergic activity occurs mainly through the activation of postsynaptic $GABA_A$ receptors.

1.4. Experimental animal studies of the subthalamic nucleus

Despite the important role of the STN in Parkinson's disease, published work in lab animals examining the STN is very limited. The majority of publications investigating the effects of lesions on behavioural tasks are authored by one person (C. Baunez). Consequently, it must be conceded that current opinion on the function of STN is based on her reported results and interpretations. However, there are outstanding issues and questions that are yet to be addressed. In this section, previous work on the STN will be summarized and critically analysed. These studies involve STN lesions induced by neurotoxin, alone and in combination with striatal dopamine depletion

(Parkinsonism model), unilateral or bilateral, concentrated on entire STN or partial STN, and also STN dysfunction induced by high-frequency stimulation.

1.4.1. Do STN lesions restore function or merely mask one deficit with another?

Based on the rationale that inactivation of the STN ameliorates the motor symptoms of dopamine depletion in patients with Parkinson's disease, several groups investigated whether this would be true in the rat with striatal dopamine-depletion. Baunez et al., (1995) conducted a simple reaction time task to study the effects of bilateral STN lesions in rats with striatal dopamine depletion. Rats were trained to press the lever to initiate a new trial; after a varied interval a light stimulus was presented and rats were required to release the lever as soon as they detected the light. Reaction time, from the onset of the light stimulus to the release of lever, was recorded. The maximum allowed reaction time was 600ms, after which it would be recorded as an omission. Responses made before the onset of the light stimulus were recorded as premature responses.

Two weeks of preoperative performance were compared with five weeks of postoperative performance, with the mean performance for each week used as a data point. Each week contained five consecutive sessions, with 100 trials in each session per rat. The authors reported decreased correct responses in the rats with STN lesions and dramatically increased premature responses. One thing to notice is that since the lesion resulted in a large increase in premature responding, it necessarily (once a response had been made prematurely, a trial could not then be completed correctly) decreased the number of trials that were completed 'correctly', since the total number of trials in one session was fixed. Therefore, the decreased correct responses did not necessarily reflect worse accuracy (the portion of correct responses out of total completed responses).

The authors also reported shorter reaction times in rats with the STN lesions comparing to the control rats. However, the difference between the two groups seemed to diminish over the five consecutive sessions. An interesting

pattern is noticed from the reported data, although not mentioned by the authors: the lesion rats responded with faster RTs early in the week, and became progressively slower, while the control rats appeared to be slower at the start of the week and became faster (Figure 1.2). This pattern is not unusual (unpublished observations) – at the weekend, food portions can be more generous to reflect the lack of opportunity to 'work' for food and sometimes rats with restricted access gain weight over the weekend. It might be interesting to examine whether this is the case for STN rats, and if not, why not. Nevertheless, regardless of this, the RT changes can be described as, at best, unstable and the report of 'decreased RTs' might be overstated and a more subtle deficit may be masked. To support this view, Baunez et al., (2001), suggested that their previous conclusion that RT was speeded in the lever release study (Baunez et al., 1995) could be due to the limited number of trials completed.

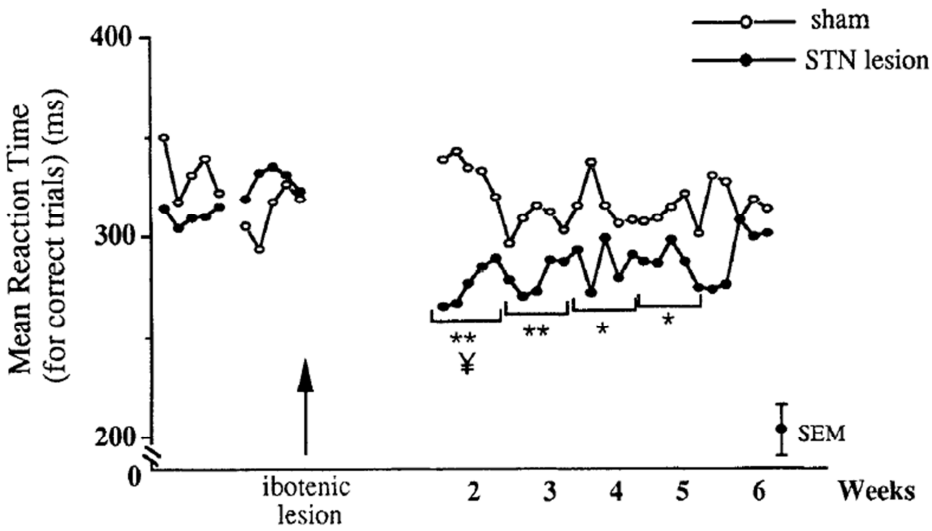

Figure 1.2 shows the effects of STN lesions on reaction times. Mean correct RTs and SEMs are illustrated for the sham (n = 9) and STN lesion (n = 10) group, before and after the surgery. (Baunez et al., 1995)

In rats with striatal dopamine depletion, they reported increased delayed responses and also longer mean RTs, and these were lowered down to baseline after the STN lesions. Based on these results, they claimed that the akinesia

caused by striatal dopamine depletion was masked by the hyperkinesia caused by STN lesions. However, it is not possible to distinguish between this hypothesis (additive impairments of akinesia and hyperkinesia) and the alternative hypothesis that the lesion 'rebalanced' the output pathways, so restoring function to both.

Phillips and Brown (1999) took a different approach and published results that might be contradictory. In their study, rats were required to poke the nose into the central of an array of three holes and hold for varying delays, after which light stimuli were presented on both sides, indicating the target responding location. The rats were either with unilateral striatal dopamine depletion, or a unilateral STN lesion, or both lesions on the same side. Reaction times, percentage of correct, incorrect and premature responses were recorded, and data were collected from 8 sessions (about 800 trials each rat) pre- and post- surgery. As reported by Baunez, Phillips and Brown also reported longer mean RTs in the rats with striatal dopamine depletion and this impairment disappeared after the STN lesions. However, in rats with only unilateral STN lesions, the mean RTs and reaction time distributions were reported unchanged. These results seemed to rule out the possibility that the STN lesion induced a hyperkinetic state, which cancelled the akinesia resulting from the striatal dopamine depletion. This conclusion was also supported by observed response bias in different groups. The rats with dopamine depletion alone made more ipsilateral responses, while the rats with combined lesions made more contralateral responses. The rats with STN lesion alone, however, did not show any response bias.

The coordinates used for the STN lesion were essentially the same as reported in the Baunez study (DV coordinate was slightly different with Phillips used -8.5mm while Baunez used -8.35mm from the skull) and the pictures of brain sections reported in both article showed similar location and size of the STN lesions. Although it remains possible that the difference between the two groups' work is from the different severity of the lesions (bilateral vs unilateral), still there is no evidence showing that the difference between the effect of a unilateral lesion and a bilateral STN lesion would be

quantitative. In fact, the increased percentage of premature responses reported in Phillips and Brown (1999) was as severe as in Baunez *et al.*, 1995. So far, the effect of STN lesions on RTs seemed to be uncertain and might differ between tasks.

Baunez and colleagues also tested the effect of STN lesions on rats with striatal dopamine depletion in the 5-choice serial reaction time task (5-CSRT) (Baunez and Robbins, 1999). 5-CSRT for rats was first developed to measure visuospatial attention (Carli et al., 1983). Rats are required to monitor an array of five apertures and respond to the light stimulus presented in one of the five holes. Accuracy of responding to the correct location within a limited time is used as an index for attention capacity. They found that rats with striatal dopamine loss by itself were not impaired on accuracy or premature responses, but showed longer reaction time, higher percentage of omission and perseverative responses. However, these deficits were not alleviated by the additional STN lesions. Interestingly, the increased premature responses after STN lesions disappeared in rats with combined lesions, which had not been observed in any of the previous tasks (Baunez et al., 1995; Phillips and Brown, 1999).

As mentioned earlier in the introduction, high frequency deep brain stimulation (DBS) in the STN has been widely used to alleviate symptoms in PD patients. This is also observed in animal PD models (Fang et al., 2006, 2010; Rizelio et al., 2010). Studies that report similar symptom-relief from DBS and ablative lesion imply the view that the two manipulations result in similar changes in neuron activities in downstream structures – GABAergic projections to the thalamus is decreased and therefore the thalamus-cortex projection is disinhibited. However, this view is violated when DBS fails to give similar outcomes. For example, the above-mentioned 5-CSRT task was done with STN HFS (high-frequency stimulation) instead of excitotoxic lesions in rats with striatal dopamine depletion (Baunez et al., 2007), but the restored performance on premature responses was not replicated. An earlier exception was reported by (Desbonnet et al., 2004), and the authors suggested that the neuronal cell stimulation might not simply be “inhibitory” and its effects might

be mediated by more complex mechanisms than lesions of the same brain area. A recent research article (Dorval and Grill, 2014) gives their opinion: the authors found that neuronal entropy in both the globus pallidus and substantia nigra pars reticulate (SNpr) was significantly increased and functional information transmission was greatly deduced in the basal ganglia following parkinsonism. Further, DBS in the STN lowers the entropic noise floor and enable more information transmission, therefore, partially restores the functions. Another study demonstrated that STN-HFS alleviated the motor symptoms in rat PD model by inducing increased striatal dopamine release and dopamine contents (He et al., 2014). Both studies above suggested that the STN stimulation does not improve Parkinsonism symptoms via changing the performance of downstream structures, which is different from STN lesions.

1.4.2. Does the STN clear a 'response buffer'?

Baunez and colleagues published a study using STN lesioned rats comparing performance of simple (SRT) and choice (CRT) reaction time task (Baunez et al., 2001). The CRT procedure was similar to the one used in Phillips and Brown (1999) study: rats were required to hold a nosepoke in the central hole for varied delays and then the brightness of the light on both sides changed simultaneously with a tone trigger stimulus presented. Rats then responded to the location indicated by the brightness level. For the SRT procedure, the task was essentially the same, except the light stimulus was given at the beginning of each trial and stayed on throughout the foreperiod, going out 300ms before the occurrence of the tone. In short, the information was given in advance. Pre-operative baseline performance showed that RTs on the 'SRT' procedure were shorter than the 'CRT' procedure, suggesting a benefit of having advanced information. In both the 'SRT' and 'CRT' procedures, rats showed faster RTs on longer delays, indicating a 'motor readiness effect'. Comparing the pre-and post-operative data, they reported slower RTs in all groups, but particularly in the STN lesioned rats – in contrast to their previous work (Baunez et al., 1995). They further reported that with STN lesions, rats no longer showed an RT advantage (shorter RTs) in the 'SRT' procedure, from which they concluded that the STN was important for using

advanced information in response selection. However, there are several outstanding issues that might compromise the conclusions. They used between-subjects design with one group only trained and tested on SRT procedure and the other group only on CRT procedure, which ended up with four groups dissociated by lesion conditions. After surgery, the control groups also showed increased RTs, which suggested an effect simply from the surgery but not the lesion. Moreover, the lesioned CRT group showed longer RTs, which clearly suggested that the increased RTs in the lesioned SRT group was not entirely due to the failure of using advance information. Given these issues, the conclusion that RTs were increased in STN lesion rats, particularly on the CRT procedure, has to be considered tentative.

Another finding they emphasized was the increase in premature responses, combined with a change in premature response bias in rats with STN lesions. They suggested that in a 2-alternative forced choice with a 50% probability of a response to either side, normal rats will alternate on a given trial from the previously rewarded response. This was seen when rats made premature responses and they suggest this is analogous to "inhibition of return" of attention (Posner and Cohen, 1984). For the STN lesioned rats, however, they observed reduced alternations and a tendency to perseverate on the same side as the previously rewarded response. However, in the SRT procedure, both control and STN lesioned rats were more likely to make premature responses to the appropriate (cued) location, albeit that this was slightly decreased in the first 2 post lesion sessions for the STN lesioned rats. This maintained premature response bias towards the cued location clearly suggested the rats with STN lesions could and did still use advanced information to predict potential target. Given these observations, we would expect an interaction between the two premature response biases: towards the cued location and towards the previous rewarded location. Analysis of this interaction would help to confirm if the STN lesioned rats were surely prefer the previous rewarded location; however, this analysis was not reported in the article.

Based on their findings, Baunez et al., (2001) proposed a hypothesis that STN lesions might impair the operation of a 'response buffer', which explained

the function of the STN during a response selection. They suggested a 'buffer-like' mechanism which holds a selected response in readiness until the appropriate time, and which needs to be reset after performance to allow the subsequent response. With STN lesions, the 'buffer' cannot be reset and therefore leads to a conflict between the previous and current responses. This conflict results in longer reaction times and high percentage of premature responses to the previously rewarded location. However, according to the model, conflict should only occur when the subsequent response is different from the previous one, which means the prolonged reaction times and reduced accuracy should only apply to the alternated responses. Although the data was not contradictory, the author did not provide data analysis in terms of this prediction; there is also no further study from the same or other groups based on hypothesis predicted by this model. Therefore the conclusion that STN lesioned rats have deficits in response selection needs more supportive evidence.

1.4.3. Is the STN necessary for 'stopping' or inhibition of responses?

The ability to inhibit unwanted actions is essential for response control. In human studies, it has been identified that cortico-basal ganglia network plays a crucial role in the process and the STN has been established as a key structure (Aron and Poldrack, 2006; Ballanger et al., 2009; Benis et al., 2013).

Eagle et al., (2008) tested rats in a stop-signal reaction time (SSRT) task, in which subjects were required to make a "go" response – a rapid response from left lever to right lever – in most of trials (80%) and a "stop" response – refrain the right lever press when a stop signal (a tone) was presented – in a small portion of trials (20%). Rats were trained and tested on Zero Delay (baseline, no delay between the onset of "go" signal and the "stop" signal) and then tested on 6 different delays (SSD) pre- and post-surgery. SSRT was estimated using the race model, based on the percentage of correct stop and the reaction time distribution for "go" responses. A race model proposes the idea that several processes are in race against each other, whichever reaches the 'finish line' (i.e.,

a process terminates) first, the corresponding response (the process output) will be initiated. Logan and Cowan (1984) first formalized a race model describing the "go" and "stop" processes in the stop signal task. The "go" process triggered by the "go" signal and the "stop" process triggered by the "stop" signal are in competition. If the "stop" process finishes before the "go" process, the response will be successfully stopped; otherwise, the "go" response will be made. The stop-signal reaction time (SSRT) is the time the "stop" process takes to finish, which is unobservable but could be estimated by the race model. Three assumptions are necessary for the SSRT estimation: 1) the "stop" process is independent from the "go" process, 2) the "stop" process is independent from the SSD and 3) the unstopped responses on "stop" trials and normal "go" responses are from the same distribution.

Normal rats could successfully stop 80% trials on Zero Delay and this percentage went lower as the SSD increase. STN lesioned rats made faster "go" responses, which seemed to be consistent with Baunez et al., (1995). Rats with STN lesions made significantly more errors (fail to stop) on Zero Delay. Besides, their absolute percentage of successful stopping was flat across all SSDs, which seemed like their ability to stop was independent of delay. The authors interpreted these as that the STN lesions impacted the rats' stopping attempt (starting of a stopping process). However, the adjusted percentage of successful stops, which attempted to control for changes in baseline performance that were unrelated to SSRT, was not affected by STN lesions. This seems to suggest that once started to stop, the stopping performance for STN lesioned rats falls into the same range as normal rats. This hypothesis should lead to unchanged SSRT in the lesioned rats; however, although reported as unchanged, the estimated SSRTs were not reliable because the speeded "go" responses challenged the assumption of the race model.

Premature responses were not measured in the SSRT task since there was no delay between the left lever press and right lever presentation. However, an extended 'limited hold' (LH, the duration of the right lever presence) was introduced for stop trials. STN lesioned rats made more responses during LH than control rats, which meant they were less able to hold a stop response.

Impulsivity is also an example of impaired inhibitory control. It is a behaviour tendency to act prematurely, with little or no foresight, or consideration of the consequences. Compulsivity is mentioned sometimes, which is very similar to impulsivity, but more emphasizes on persistent, repeated actions. In the 5-CSRT task, premature response made during the inter-trial interval (ITI) is used as the index of impulsive control, while persistent presses on the food magazine and repeated nosepokes into the holes during the time-out are a measurement of compulsivity. Previous studies showed that rats with STN lesions exhibited impaired impulsive and compulsive control (Baunez and Robbins, 1997a, 1999; Chudasama et al., 2003) in the 5-CSRT task. However, this finding is not supported by the data from a delay discounting reward study, in which the rats with STN lesions surprisingly inhibited impulsivity and waited for greater reward (Winstanley et al., 2005). Another study (Uslaner and Robinson, 2006) confirmed this finding and further extended the exploration to dissociate between impulsive actions and impulsive choices. Their results suggested that the STN lesions increased impulsive actions, but decreased impulsive choices (impulsive decision making). Compulsivity was also observed in rats with STN lesions in a peak-interval timing task (Wiener et al., 2008), with a high response rate in the late phase of a trial when responses could not yield any reinforcement.

In summary, these studies demonstrate that with STN lesions, rats' abilities to stop or inhibit unwanted responses are obviously affected. However, so far the observed behaviours are not sufficient enough to reveal which specific deficit is caused by STN lesions.

1.4.4. Is the STN involved in attention?

As has mentioned above, 5-CSRT task for rats was widely used to measure visuospatial attention (Muir et al., 1996). Rats are required to monitor an array of five apertures and respond to the light stimulus presented in one of the five holes. Accuracy of responding to the correct location within a limited time is used as an index for attention capacity. The standard task usually sets the inter-trial interval to 5s, the length of stimulus to 0.5s and the limited period to

5s. Manipulations are usually implemented (e.g. variable short and long ITI, shorter stimulus, shorter LH) to increase the challenge of the task and to explore subject's capacity.

Baunez & Robbins (1997) tested rats with bilateral STN lesions on the 5-CSRT task. The STN lesioned rats were impaired on baseline: they showed lower accuracy, higher percentage of premature responses and omissions, and longer response latencies. Stimulus was prolonged to 4s and ITI was shortened to 2s and rats were tested on these conditions again. Although the long stimulus and short ITI improved rats performance, STN lesioned rats were still impaired compared to control rats. The authors first argued that the STN lesioned rats were impaired because they could not appropriately oriented to the five locations. However, they then ruled out this possibility because 1) even under long stimulus conditions the lesioned rats still never reached the normal level 2) accuracy was not improved with reduced perseverative panel pushing and 3) short ITI did not exacerbate the STN deficits, which would be expected if it was caused by perseverative panel pushing. Therefore, they concluded that the disrupted performance was a result of impaired attention after STN lesions.

As a follow up study, Chudasama et al., (2003) examined the effects of disconnecting the mPFC (medial prefrontal cortex) and the STN in rat, with the prospection that the hyperdirect pathway (cortico-subthalamic projection) is involved in the cognitive function of the STN. The same 5-CSRT task was tested in rats with either disconnected lesions of the mPFC and STN (DISC), unilateral STN lesion, unilateral mPFC lesion, or ipsilateral mPFC and STN lesions (IPL). The DISC group showed very similar impairments as seen in bilateral STN lesioned rats, which were significantly different from the other 3 lesion group and the control group. Both the unilateral lesioned groups showed mild impairments, such as increased premature responses and perseverative responses. Interestingly, they reported no behavior difference in the IPL group, compared to the control group. Based on this finding, they concluded that the previously observed deficits after bilateral STN lesions were due to disrupted cortico-subthalamic projections. Furthermore they proposed that attention and

other executive functions required in the 5-CSRT task rely on the cortico-subthalamic projection in the basal ganglia.

In terms of using visuospatial attention, Phillips and Brown (2000) examined the effect of unilateral STN lesions on a 2-alternative forced choice task with uninformative visual cues. Within a single session, equiprobable cues (dim light) preceded a target (bright light): cues were either both-side (bilateral); on the same side as the subsequent target (valid cue); on the opposite side as the subsequent target (invalid cue) or there was no cue. All rats, even the STN lesioned rats, exhibited faster reaction times after a valid cue than the other three conditions, indicating that attentional orienting towards visual stimuli was intact. The unilaterally lesioned rats made more anticipatory errors and were more likely to direct responses following an anticipatory error towards the contralateral side. However, once the response was under target control (i.e., the response was made after the target was presented), there was no effect of lesion on side of the response, which was consistent with the previous study (Phillips and Brown, 1999). Moreover, there was no significant change in mean reaction time or in the reaction time distributions after the STN lesions in any condition.

In a recent review by Baunez and Lardeux (2011), studies using 5-CSRT in rats were summarized as the only evidence for the role of STN in attention. Of particular notice is that all the studies discussed in this section, including the one from Brown lab, only examined visuoperceptual attention in rats, but not cognitive attention. Since it has been widely believed that the STN is directly connected with frontal cortex, we would expect its role in more complicated, advanced functions, such as cognitive attention and executive functions. However, there is barely any reported study exploring the role of STN on executive functions in rats.

With these studies, there remain several questions about the effects of the STN inactivation in rats: whether it changes rat's performance on reaction times, whether it impairs rat's response selection, and the role of the STN in

executive functions. In this thesis, rats with the STN lesions were tested on several different procedures and answers to these questions were explored.

1.5. Behavioural paradigms in the current studies

Two major behavioural paradigms – reaction time tasks in an operant chamber and the bowl digging task as used to measure attentional set-shifting– are used in this thesis to examine animal's motor and non-motor functions. The understanding of the performance of normal subjects on these tasks and the psychological processes underlying their responses is necessary for further understanding the nature of the behavioural changes after brain damage. In this section, the principles, relevant theories and previous studies of the reaction time tasks and attentional set-shifting task will be introduced.

1.5.1. Reaction time tasks: Sequential Effects

Experimental trials could be considered both as discrete trials and in the context of surrounding trials. Previous studies have demonstrated that the surrounding trials could be manipulated to affect animal's expectation and therefore their performance on discrete trials. In reaction time tasks, the effects from surrounding trials are referred as sequential effects. In this sub-section, several dominant sequential effects are described.

1.5.1.1. Repetition priming effect

The repetition effect is one of the well documented sequential effects. The repetition of stimulus or response, or both, usually affects subject's performance in a beneficial way (e.g. quicker reaction time, higher accuracy). However, the locus of the repetition effect is under debate. Previous studies delineated six possible hypotheses concerning the locus of repetition effect (Pashler and Baylis, 1991; Campbell and Proctor, 1993): a. the perceptual speedup hypothesis predicts that responses to the physically identical stimuli should be speeded up, regardless of whether the stimulus-response mapping remains the same; b. the response execution hypothesis predicts that repetition

effect will occur across trials involving the same motor responses; c. the categorization hypothesis predicts that different instances with the same identity (e.g. A and a) will cause repetition effect, while other elements in the same category (e.g. B) will not; d. the highest link hypothesis predicts that when each category of stimulus is mapped to an unique response, the repetition of the same categorizable mapping is sufficient for the repetition effect; e. the shortcut hypothesis predicts a direct link between the lowest level of stimulus identity and the response associated with it and only the repetition of this link will speed up responses; f. the inclusive links hypothesis suggests that the response is linked with all stimulus features that associated with it and a repetition of any of the links will result in a repetition effect.

Different experiments were conducted to examine these hypotheses (Pashler and Baylis, 1991; Campbell and Proctor, 1993; Hübner and Druey, 2006a). Results show that when the stimuli are uncategorized, only the repetition of both stimulus and response will cause repetition effect. When the responses are category-mapped, repetition of the same category will cause a repetition effect, although smaller than the repetition of the same stimulus; moreover, the more the instances in the same category share in common, the greater is the repetition effect. The repetition effect also occurs even if the two consecutive responses are not physically identical. It is merely necessary that the two responses shared a common feature and this feature repeated. For instance, in two consecutive trials T1 and T2, stimuli S1 and S2 were both mapped to index fingers, or to the most left finger, for left and right hand. Repetition effect was observed in both situations, even though these responses were carried out by different effectors (left vs right hand). This effect is called response category repetition effect, which demonstrates that responses are coded abstractly in terms of response features. In summary, the repetition of a salient feature of stimulus set or response set seems to be necessary for a beneficial repetition effect. This is in line with the inclusive links hypothesis.

Repetition effect is more complicated when there is more than one rule involved. In a dual-task number-categorization experiment (Hübner and Druey, 2008), two tasks, using the same set of numbers as stimuli, were mixed in one

session. In T1 subjects had to judge for numeral parity and in T2 for magnitude. One of the two fingers (index and middle) of their left hand and right hand was used to respond to S1 and S2. For one group of participants the use of spatial response categories (RL-mapping) was induced, and for another group the use of finger-type response categories (IM-mapping) was induced. During testing, S1 and S2 could be the same task or switch between the two tasks. Results showed that response category repetition effect was interacted with task switching: when the same task was repeated, reaction times were shorter on response category repetition than category alternation; when the task was switched, on the contrary, response category repetition leaded to slower reaction times. In a nutshell, response category repetition caused a cost instead of benefit when the task switched. This is reversed repetition effect is highly consistent in task switching experiments, which use the same set of stimuli but more than one task in the same session (Monsell and Rogers, 1995; Ward et al., 2001; Mayr and Kliegl, 2003a).This finding challenges the above mentioned hypothesis of repetition effect and implies a top-down process prior to the stimulus-response pathway.

Another interesting observation is reported by Williams (1966). Participants were asked to make judgement whether the stimulus (red or green light) on the given trial was the same as the previous trial. Results showed that when the relation of two consecutive stimuli was conflict with the relation of two consecutive judgements (responses), participants were much slower. For instance, for a sequence of red-green-green, the stimuli for the last two trials were the 'same' while the judgements were 'different'; the second response would be slower than the second response for sequence green-green-green, where the relation between stimuli and responses was the same. This phenomenon shares some commonality with the task switching effect: although the rule keeps the same in this task, the task representation subjects adopt changes. Along with some other experiments (Kleinsorge, 1999; Notebaert and Soetens, 2003), it was hypothesized that "any change of a task feature that is part of the task representation participants adopt leads to a disruption of repetition-based facilitation".

Besides the different modifications of stimulus-response mapping, simply changing the response-to-stimulus interval (RSI) could change the repetition effect. Previous studies have shown that when the RSI increases, the repetition effect changes from a benefit-only pattern, induced by automatic facilitation (AF), to a cost-benefit pattern, due to strategic expectancy (SE). The strategic expectancy was used called subjective expectancy, which implies that when the stimulus/response is congruent with subject's expectation, the response will be speeded up; vice versa. The boundary value of the RSI between AF and SE varies significantly across different tasks; it could be as short as 100ms or as long as 800ms (Soetens et al., 1985; Cho et al., 2002; Jentzsch and Sommer, 2002).

1.5.1.2. Inhibitory after-effect

As mentioned above, previously executed responses could influence subsequent performance. Interestingly, previous inhibited responses could also influence subsequent performance. For instance, in the SSRT task and other analogous tasks that involve inhibition, inhibition of interfering stimuli results in longer reaction times in the subsequent trial when the inhibited stimulus now becomes the target. This effect is usually referred as inhibitory after-effect. One hypothesis suggests that this is because the residual inhibition of the stimulus needs to be overcome before the currently related response can be initiated.

The inhibitory after-effects have been the focus of intensive research since decades ago, and it has been found that successful inhibition on the previous trial leads to longer reaction times on the next Go trial, especially when the similarity between the previously ignored stimulus and the current target stimulus is high (Verbruggen et al., 2008a). The longer reaction times have also been observed after unsuccessful inhibitions (Rieger and Gauggel, 1999a), but some studies only reported this where the unsuccessfully-ignored stimulus is repeated on the current Go trial (Verbruggen and Logan, 2008a). Some mechanisms have been proposed to explain the inhibitory after-effect, the mainly two of which are between-trials adjustment and repetition priming.

Some other hypotheses resemble the repetition priming, such as theory of automatization and episodic retrieval theory. Unfortunately, until now, no single mechanism has been able to explain all findings. They seem to work well together and rely on the experimental contexts. Moreover, the inhibitory after-effect is not always inhibitory; some studies have observed that inhibition on the previous trial actually facilitates the subsequent inhibition (Bissett and Logan, 2012). However, this facilitatory after-effect has not been well studied.

1.5.2. Reaction time tasks: Race Models

A race model proposes the idea that several processes are in race against each other, whichever reaches the 'finish line' (i.e., a process terminates) first, the corresponding response (the process output) will be initiated. Logan and Cowan (1984) first formalized a race model describing the go and stop processes in the stop signal task. The "go" process triggered by the "go" signal and the "stop" process triggered by the "stop" signal are in competition. If the "stop" process finishes before the "go" process, the response will be successfully stopped; otherwise, the "go" response will be made. The stop-signal reaction time (SSRT) is the time the "stop" process takes to finish, which is unobservable but could be estimated by the race model. Two independence assumptions are necessary for the SSRT estimation: 1) the "stop" process is independent from the "go" process and 2) the "stop" process is independent from the stop-signal delay (SSD, interval between the "go" signal and the "stop" signal). Some simulation studies have shown that in practice the two independence assumptions are not always satisfied, however, estimations based on these assumptions are still quite accurate (Band et al., 2003).

The race model has also been used to describe and predict performance in signal change tasks, in which an extra signal is presented to indicate the change of the target location. In this case, three processes are in competition: Go1 process induced by the original stimulus, Stop process induced by the stop signal and Go2 process induced by the new stimulus. Sometimes only the new stimulus is presented, which requires participants to both stop and change their response. In the latter case, one question arises as whether an

independent Stop process is involved and if yes, whether the Go2 process starts the same time as the Stop process or after the Stop process has finished.

Camalier et al. (2007) addressed this question using an double step saccade task. In their study, subjects were required to make saccade to a target (one of eight possible locations), and on some trials the target changed location before the initial saccade was made and subjects needed to make a saccade only to the new location. Camalier proposed three race models. The GO-GO model assumes a race between GO1 and Go2 processes, while no Stop process is involved (two-goal model). The GO-STOP-GO model assumes that a STOP process terminates GO1 process first, and GO2 process starts when the STOP process has finished (serial three-goal model). The GO-GO+STOP model also assumes that a STOP process, but GO2 process starts the same time with the STOP process (parallel three-goal model). Data of both humans and macaque monkeys were fitted into each of the three models. Results showed that the two three-goal models fit the data equally well, and much better than the two-goal model. Although the experiment could not discriminat between the two STOP models, it still demonstrated that a Stop process is necessary in the response change task.

Considering the important differences between stopping eye movements and hand movements (Logan and Irwin, 2000; Boucher et al., 2007; Emeric et al., 2007a), the conclusions of Camalier et al. (2007) might not generalize to stopping and changing hand movements. Therefore, Verbruggen et al., (2008) conducted a study to see whether a three-goal model also fit into manual movements stopping and changing in the stop-change paradigm. The GO-GO, GO-STOP-GO, and GO-GO+STOP models proposed by Camalier et al. were tested, along with a manipulation of the delay between the stop signal and the change signal, which were considered to be useful for distinguishing between the different three-goal models. Results showed that the three-goal models fitted the data much better than the two-goal model, which once again confirmed the involvement of a Stop process in the stop-change task. However, although with more manipulations, the study still could not distinguish between the subtypes of the three-goal model: the serial model and the parallel

model with a limited capacity shared between Stop and Go2 processes fit the data equally well. Nevertheless, this study at least confirmed that, although with some important differences between eye movement and manual movement stopping, a similar Stop process is shared in both types of performance.

Although race models have been used to describe and predict performance on different tasks, they have a very serious limitation: the race models consider each trial as independent event and do not take effect of trials sequence into consideration. This limitation might cause a huge difference between the predictions from the race model and the actual observations. (Emeric et al., 2007) suggested the orignial race model could be extended to account for sequential effect. A simple way is to adjust the finishing lines of each process between trials. For example, successive Go1 responses could decrease the finishing time of the Go1 process, and this leads to decreased probability of successfully cancelling the movement.

In summary, with the current observations of sequential effects and the understanding of the race models in choice reaction time tasks, and along with the measurements (e.g. reaction times, accuracy) of the tasks, we could better understand the psychological processes underlying the behaviour performance. This will further help us to figure out the deficit caused by a lesion in a particular brain area, which is an efficient way to understand the function of the brain area.

1.5.3. Attentional set-shifting tasks in rats

A major reason for the lack of studies on rat's cognitive function might be the lack of appropriate tasks. The rat version attentional set-shifting task employed in this thesis was developed and first described in Birrell and Brown (2000). While the attentional set-shifting paradigm is designed to test attentional shifting, it is also dependent on other cognitive functions such as discrimination between different stimuli, memory for the stimuli, associative learning and behavioural stability ('rule-maintenance'). In addition, the rat set-

shifting task exploits natural foraging tendencies, making it a species-appropriate task to examine cognitive and executive functions in rat.

"Forming a set" means that subjects develop a predisposition to selectively attend to one dimension of multidimensional stimuli (such as 'odour' or 'medium'), which has been reinforced with reliable feedback (reward or no reward) (Owen et al., 1993). This process involves attention to the relevant dimension while ignoring the irrelevant dimension. The ability to shift the set – set-shifting – is measured by the difference between acquisitions of two discriminations: intra-dimensional discrimination, where the reward associated (relevant) dimension of the compound stimulus is consistent with previous stages, and extra-dimensional acquisition, where the reward associated dimension is not consistent. As in reaction time tasks, surrounding trials in the attentional set-shifting tasks are rather important, since they are the key to decide which dimension/stimulus is consistently relevant.

The ID and ED stages are formally similar: new exemplars from each of the relevant and irrelevant dimensions are presented and the rat has to find the food and learn which exemplar predicts its location. The difference between the ID and ED stages is solely the preceding history – what was the rat attending to when learning previous discriminations? The theory predicts that it will take more trials for subjects to complete the ED stage than the ID stage when the subjects have established an attentional set because they will default to the assumption that the previously relevant dimension remains relevant and they will not initially attend to the previously irrelevant dimension. A set only needs to be shifted when it has first been formed: if there is no attentional set, the ED and the ID stages are essentially the same. In the absence of evidence of set-formation, no conclusions can be drawn about a subject's ability to shift set.

Several reversal stages are included in the standard attentional set-shifting task. On reversal stages, the relevant dimension maintains the same while the reward-related stimulus is now negatively correlated. Reversal learning is an index of behavioural flexibility, which examines the ability of shifting from an old rule to a new rule. Impaired reversal learning might be due to

perseveration on the previously rewarded responses, which possibly suggests a deficit in inhibitory control; it might also be due to 'learned non-reward' (Tait and Brown, 2007), which means the prior exposure to a stimulus that is unrelated with reward hinders subsequent learning of that stimulus. Lesions in brain areas might cause one or the other of the two impairment and both are observed as retarded reversal learning. However, by manipulating the correlated or uncorrelated stimuli on a further step, we are able to find out which impairment makes the most contribution.

The rat version attentional set-shifting has been used to study the roles of brain areas and neurotransmitters in cognitive processes that underneath task performance. Particularly, it has been used to study frontal cortex, which is known to be strongly related with cognitive functions. A ground-breaking finding is the functional double-dissociation between the effect of OFC and mPFC lesions. Rats with mPFC lesions were significantly impaired only on ED stage (Birrell and Brown, 2000), while rats with OFC lesions were only impaired on reversal stages of the attentional set-shifting task (McAlonan and Brown, 2003). Moreover, the rats with OFC lesions did not demonstrate a positive ID-ED shift cost, which implied a suspicious set-formation. A recent study (Chase et al., 2012) pushed the usage of this task further and demonstrated that with multiple ID stages, rats with OFC lesions could eventually form set and subsequently perform bad ED. As previously mentioned in the introduction, the STN receive direct projections from the frontal cortex, but the projection sources are still unclear. After demonstrate the effects of STN lesions on rat's performance on the attentional set-shifting task, we might obtain some clues on the functionally segregated cortico-subthalamic system by comparing the effects of the STN and the PFC lesions.

1.6. Statement of the aims of the thesis

The main motivation of the experiments in this thesis is to specify the nature of the deficits seen in rats with STN lesions on certain behavioural tasks.

In Chapter 3 the newly developed signal-change reaction time task is examined in healthy rats and human participants. This task is designed to test subject's ability of stopping and re-programming a response when the target stimulus changed. Performances are compared between humans and rats, focusing on the sequential effects in particular. Similarities and differences between humans and rats are interpreted. With the help of previous literatures, mainly in human studies, we could obtain a fully comprehension of what are actually measured by the task and how rats are doing the task.

In Chapter 4 the effects of bilateral medial STN lesions in rats are investigated using the same task described in Chapter 3. Chapter 5 is a replication of Chapter 4, but with a modified behavioural task, which included more manipulations that allow us to more clearly distinguish the processes involved in the task. The main experimental questions are 1) whether STN lesioned rats are able to inhibit and reprogram a response before they have started to move and 2) whether STN lesions will affect rats' ability of control a pre-potent response bias (previously rewarded response). Based on the previous studies, our hypothesis is they will make more mistakes when they need to alternate their responses within and between trials. Besides, we expect to see increased anticipatory responses.

In Chapter 6 the same STN lesions are employed, and rats are tested on a serial of attentional set-shifting tasks. According to the previous study in our lab, the STN lesioned rats are expected to show a lack of positive shift cost, which implies the failure of forming an attentional set. A more important aim of the current experiment is to explore the reason that the STN lesioned rats cannot form a set. The results will shed some light on the role of STN in cognition and executive control.

General materials and methods

This section explains the common materials and methods of the following experiments, which include behaviour habituation and training, surgery and histology procedures and apparatus. Materials and methods that are different from the general procedures will be explained in each experimental chapter.

2.1. Animals

All animals used in the following experiments were male Lister-hooded rats from a breeding establishment Charles River (UK). All rats were experimentally naïve before training. Some control rats tested on set-shifting task in Chapter 6 had been tested on the signal change reaction time task in Chapter 4, but there was no evidence showing prior experiment had any impact on following experiments. All work described in this thesis was performed under the authority of Project and Personal License issued by UK Home Office, and in accordance with the requirements of the United Kingdom Animals (Scientific Procedures) Act 1986.

During the whole procedure of the following experiments, rats were socially caged in groups of up to four in home cages measuring approximately 50 x25 x30 cm. Post surgery, rats were single housed in smaller home cages measuring 40 x 19 x 23 cm for one to three days and then put back into groups. In some situations, some rats were kepton single-housed until the end of experiment. The cages contained one clear, plastic tube fixed to the top of the cage, for rats to climb and sit in, and also some chewable materials (e.g. wooden bars, tent-shape cardboard, etc.). The holding rooms were on a 12 h light/dark cycle (lights on at 7am) and training and testing were carried out in the light phase. Both the holding rooms and testing room were maintained at 21 ± 2°C, with a humidity of 55 ± 10%.

Rats were maintained on a restricted diet of 15-20 g standard lab chow, from at least one week before the start of an experiment until the end of that experiment, though they always had free access to water. The purpose of food control was first to prevent rats becoming over weight throughout experiments; and second to increase their motivation for food rewards during testing. Body weight was monitored weekly to guarantee a healthy and steady gain, which should not fall below 85% of free-feeding weight. Start weights and end weights of animals in each experiment were listed in the table below:

2.2. Apparatus

Figure 2.1 shows the internal construction of a 9-hole operant chamber used in Chapter 3, 4 and 5. On the rear wall, 2.5 cm from the grid floor, was a horizontal array of nine poke-holes, of which the three at the centre were used in the present experiments and others were capped. Each response hole had a white LED at the rear to provide light stimulus and a photoelectric cell at the entrance to detect breaks in a vertical infrared beam. Rodent reward pellets (45mg, TestDiet, Richmond, IN, USA) were delivered to a food hopper, which contained a light and was occluded by a vertical hinged panel. The food hopper was allocated on the wall opposite the response holes and was connected to a micro-switch that controlled its openings. Auditory stimuli were displayed by a loudspeaker located on the ceiling of the chamber. House light was on throughout the testing, except for the 1s time-out following an error. The chambers were housed individually within a sound-attenuating box with a fan providing ventilation and background white noise as well.

Chapter	Numbers	Start weights	End weights
Chapter 3 & 4	28	320~360g	460~560g
Chapter 5	22	290~320g	400~560g
Chapter 6	24	275~325g	410~470g

Table 2.1 shows the start and finish weights of all rats used in this thesis

2.3. Training

Behavioural training involved in Chapter 3, 4 and 5 followed the steps described below:

1) Habituation

Rats were habituated to the operant chamber and rewarded for single press on the food hopper. This continued until rats were able to collect 100 reward pellets within a 30 min session, which usually took no more than 2 sessions.

2) Associative conditioning

The central hole was illuminated and the other holes were capped. Rats were required to make a nose-poke into the central hole and stayed in this position for a fixed period of time (foreperiod). Successful maintenance resulted in an auditory signal, a brief (0.1s) tone sound, which indicated a reward.Early withdrawals were recorded as anticipatory errors and resulted in a time-out (house light off for 1s and no reward delivered). The foreperiod was increased gradually over days, according to the individual rat's performance, from 0.1s to 0.5s. Generally rats could reach 0.5s level in two weeks and could complete 100 correct responses within a 30 min session.

Figure 2.1 shows a 9-hole operant box used in the following experiments. The three central holes are in use while the other holes are capped by transparent glass.

3) Simple choice

Three holes, the central hole in the array and adjacent holes to the left and right (hole 4, 5, 6 from left to right), were uncapped. At the end of 0.5s foreperiod, simultaneously with the auditory signal, a light stimulus was presented in either the left or right hole. Rats were required to withdraw the nose from central hole and respond into the target hole, which initially stayed illuminated until a response was made, and reduced to 200ms at the end of training. Rats were trained on this stage until they were reliably completing 120 correct trials within a 30min session.

After rats finished the initial training, specific behavioural tasks were introduced, which are described in each experimental chapter.

2.4. Measurements

Reaction time (RT) and movement time (MT) were recorded during training and testing. Reaction time was the time from the onset of the target stimulus to the withdrawal of the nose from the central hole. Movement time was the time from the withdrawal of the nose to the response made in either side hole. Reaction time and movement time were combined to give response latency. Timing resolution is 10ms.

A trial was designated “correct” if rat withdraw the nose after the onset of the stimulus and made a poke into the correct target hole; “incorrect”, on the other hand, if the response was in the wrong hole. Withdrawal before the light stimulus was recorded as “anticipatory error”. Trials were designated as “omission” if there was no response after 1.5s from nose withdrawal. All kinds of errors resulted in a time-out interval (TOI, house light off for 1s and no food reward).

Generally, only trials which follow a correct trial were taken into analyses. These trials were further categorized into different conditions depending on trial types, position in the trial sequence and trial outcomes. Repeated measures ANOVA were used in most of the conditions. When significant

interactions between the factors were found in the “omnibus” ANOVA tests, simple main-effects analyses were conducted with additional ANOVA tests restricted to the relevant factors and levels.

Data analyses were done in IBM SPSS Statistics 19 and SigmaPlot 12 on PC.

2.5. Surgery

2.5.1. Toxin

Ibotenic acid (IBO), naturally occurring in the mushrooms Amanita, is a powerful neurotoxin that is used to cause excitotoxic lesions of the brain. It is believed to act as an efficient glutamate receptor agonist, which results in prolonged depolarization at pre- and post-synapse sites finally leading to cell death. It has been proved long time ago that IBO induces lesions more selectively and is less toxic to animals (Jarrard, 1989; Schwarcz et al., 1979).

2.5.2. Material

Custom made glass micropipettes, instead of metal needles, were used to administrate toxin into the STN in the present experiments. The reason is that previous STN lesion studies using metal needle consistently report additional damages to surrounding areas (e.g. ZI, EP) and also track damage to ventroposteriomedial thalamus, which impede conclusions about the effects of the STN lesions. In contrast, glass micropipette can restrict lesions to the specific nucleus.

Micropipettes were prepared from borosilicate capillary tubes (1.16-1.19mm outside diameter, 0.49mm bore, 90mm long). The tubes were heated at the centre and pulled apart to produce two pipettes. The ends of the pipettes were then break down to approximately 30μm under microscope guidance. For surgery, the pipettes were marked by 1mm intervals, between which the internal volume equalled to 100nl.

2.5.3. Surgery procedure

Anaesthesia was induced by isoflurane (5% for induction, maintained at 2%-1.5% throughout surgery) and oxygen (1.5 L/min throughout surgery). 5mins prior to surgery, rats were injected with 0.05µL carprofen (s.c.) to reduce pain during recovery and 0.4µL diazepam (i.p.) to prevent post-operative self-harm. Rats were placed in a Kopf stereotaxic frame fixed with non-traumatic ear bars and with the tooth bar set at -3.3mm. A midline incision was made along the scalp, the skin and tissues were retracted, and burr holes were made in the skull at the appropriate stereotaxic coordinates.

Rats in lesion group received injections of 200nl of 0.06 M ibotenic acid in the STN bilaterally, at coordinates AP -3.8 mm; ML ±2.3 mm; DV -7.8 mm (from dura) (Paxinos& Watson, 1986). Rats in control group received bilateral injections of 0.1 M phosphate buffer (PB) in the STN. Solutions were injected manually via a micropipette and the needle was left in situ for a further 3 min. The wound was closed using sterilized metal wound clips afterwards and the rat was placed in a recovery cage with a heating pad underneath.

STN lesions cause impulsive behaviour, such as an intense chewing behaviour. It has been observed previously that rats accidently injured themselves severely during post-operative recovery and had to be sacrificed. Therefore, chew sticks and twines were provided to prevent rats gaining access to their paws and care was taken until this chewing behaviour dissipated, which could take up to 6 hours.

2.6. Histology

On completion of the behavioural task, rats were injected with a lethal dose of Dolethal (0.8 mL) and perfused intracardially with 0.09% phosphate buffered saline (PBS) followed by 4% paraformaldehyde in PBS. The brains were removed and placed in a 20% sucrose solution overnight. The brains were then sliced on a freezing microtome and one 50µm section was taken every 200µm at the level of the STN. The sections were then double-stained for NeuN and with Cresyl violet to map the areas of cell loss.

2.7. Data analyses

Trials were classified into different types based on current and previous trial outcomes (4 levels: correct, incorrect, anticipatory and omit), current and previous trial types (2 levels: NO-CHANGE, CHANGE), and response repetition (2 levels: repetition and alternation).Number of trials, mean reaction times and movement times were measured and calculated for each trial type. Mean reaction times of correct trials and the percentage of correct, incorrect and anticipatory responses were analysed separately by using a repeated measure analyses of variance (ANOVA), with Surgery (pre and post), Current Stimulus Change, Previous Stimulus Change and Response Repetition as within-subjects factors, and Group (control and lesion) as between-subject factor. When significant interactions between the factors were found in the "omnibus" ANOVA tests, simple main-effects analyses were conducted with additional ANOVA tests restricted to the relevant factors and levels.

Data analyses were done in IBM SPSS Statistics 19 and SigmaPlot 12 on PC.

Effect of sequences in a signal change reaction time task: a comparison of the performance of humans and rats

The speed and accuracy of responding in a reaction time task, including the ability to inhibit or reprogram responses, is influenced by recent event history. Most of the literature on 'sequential effects' reports experiments using human participants. Our intention was to develop a task for rats, in which sequential effects could be observed. If rats show similar sequential effects to humans, then it will be possible to learn more about the neural basis of these effects using pharmacological or lesion approaches. The results showed that rats and humans were different on some sequential effects, which indicated some interesting differences in information processing and task solving strategy for the two species.

3.1 Introduction

Motor (and cognitive) inhibition is common in daily life, where current actions frequently become unsuitable as conditions change. Stopping is often the first step of behavioural adjustment, but it is not the only step in most circumstances. Stopping and changing are usually required in synchrony, as the old response is cancelled in order that a new response can be produced. As has been pointed out in the general introduction, the STN is believed to play a crucial role in response initiation and inhibition. The primary goal of the present research was to develop and examine a task which can test such abilities – response inhibition and initiation –in rats.

A two-alternative forced-choice reaction time task (2-AFC RT task) – NO-CHANGE/CHANGE task – was designed to simulate the situation when a signal changes and a response needs to be reprogrammed. This task was carried out in the 9-hole operant chambers, with a tone indicating the end of a wait period (the foreperiod) and lights being used as directional stimuli to indicate the required response. Two trial types were equi-probable: on 50% of the trials, a single light stimulus indicated the required response position, while for the other 50% of trials, the light stimulus appeared first on one side and then changed to the target side.

In the stop signal reaction time task, it has been observed that responses following an inhibition were slower than responses following trials when the response was not inhibited (Upton et al., 2008). This post-inhibition slowing, or inhibitory after-effect, is one of a number of *sequential effects*, which have been widely observed under different conditions in reaction time tasks. Sequential effects can be inhibitory, such as the post-inhibition slowing, and also facilitatory, such as repetition priming. Repetition priming is when elements in a trial are repeated, for example, a stimulus repeats or the same response is required (frequently, both occur together) and the facilitation manifests as faster reaction time and improved accuracy (Bertelson, 1965; Rabbitt, 1968; Hübner and Druey, 2008).

The current NO-CHANGE/CHANGE task was designed to test the ability to inhibit and reprogram responses within a trial, and also to observe effects of inhibition / facilitation across trials. Previous research on sequential effects has mainly been conducted using human participants. Therefore, the second aim of the current study is to compare humans and rats on the same NO-CHANGE/CHANGE task to reveal similarities or differences in behavioural patterns, such as sequential effects and ability to reprogram in these two species. If rats show similar behaviour to humans, then it will be possible to study the neural basis of these effects using pharmacological or lesion approaches.

3.2 Experiment 1

3.2.1 Methodology

3.2.1.1 Apparatus

The same 9-hole operant chamber was used (see General Methods in Chapter 2) in rats and humans. For the rats, the entire chamber was housed within a sound-attenuating box. For humans, the chamber was located on a desk with the food dispenser and the front wall removed and a button replaced the food magazine.

3.2.1.2 Subjects

Twenty-eight male Lister hooded rats (Charles River, UK) were tested. More information of the animals was provided in Chapter 2. Over the six-month study period, rats were trained in daily 30~60mins sessions between 10:00 am and 5:00 pm.

Eleven healthy young adults (4 males and 7 females, 22 – 41 years old) took part in the current experiment, all had normal or corrected to normal vision. Each participant completed 6 blocks of 120 trials, with a rest of a few minutes between blocks.

3.2.1.3 Behavioural task

Habituation and training were as described in Chapter 2. The NO-CHANGE/CHANGE task is illustrated in Figure 3.1. Trials were initiated by a rat nose/human index finger poke into the central of three open holes; after a fixed foreperiod (400ms) a light stimulus appeared in either of the two side holes. The light stayed on the same side for 200ms on 50% of the trials (NO-CHANGE), on the other 50% of the trials the light changed to the opposite side after 100ms (CHANGE). Subjects were required to respond to the final location of the light.

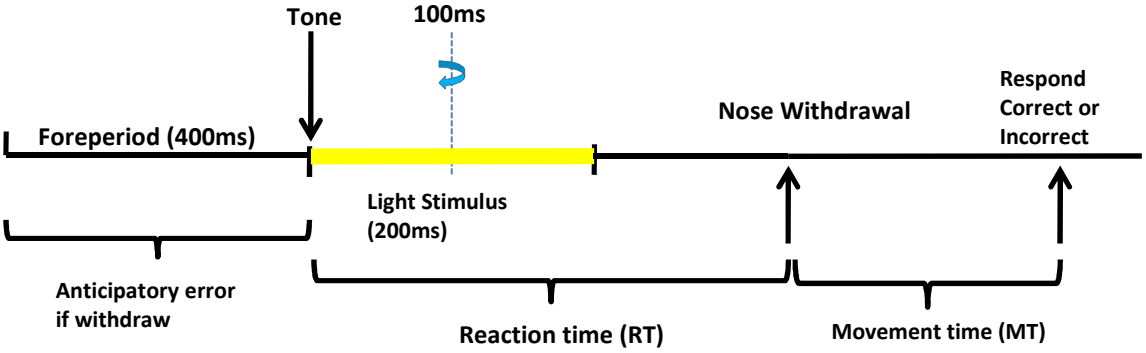

Figure 3.1 illustrates the phases of the signal change reaction time task. After a fixed foreperiod (400ms) a light stimulus appeared in either of the two side holes. The light stayed on the same side for 200ms on 50% of the trials (NO-CHANGE), on the other 50% of the trials the light changed to the opposite side after 100ms (CHANGE).

3.2.1.4 Measurements and Data analyses

Measurements and data analyses were as described in Chapter 2. The ANOVA had three within-subjects factors: previous trial type (*CHANGE* or *NO-CHANGE*); current trial type (*CHANGE* or *NO-CHANGE*); response side (repeated or alternated from previous trial) and one between-subjects factor: Group (human and rat). For analysis of latency (reaction time and movement time), only pairs of correct trials were considered.

3.2.2 Results

3.2.2.1 Inhibition and reprogramming within a trial

The performance of both humans and rats was well above chance, indicating that both were able to inhibit and reprogram responses when the cue changed. Humans made no mistakes on NO-CHANGE trials and very few mistakes on CHANGE trials, therefore accuracy could not be analysed further for humans. Rats made errors on both types of trial, but were significantly less accurate on CHANGE trials (main effect of Trial Type, $F(1,27) = 374.9$, $p < 0.01$, Figure 3.2).

For rats and humans, movement times (MT) were slower on CHANGE trials compared to NO-CHANGE trials (main effect of Trial Type: $F(1,37) = 113.1$, $p < 0.01$, Figure 3.3) although the effect in humans was relatively greater (interaction of Group and Trial Type: $F(1,37) = 19.7$, $p < 0.01$, Figure 3.3). This effect was seen regardless of the Trial Type of the previous trial, although the size of the effect did vary according to the previous Trial Type (details below).

For rats, reaction times (RT) were also slower on CHANGE trials compared to on NO-CHANGE trials; for humans, the effect Trial Type on RT was only statistically significant when preceded by a NO-CHANGE trial (interaction of Trial Type and Group: $F(1,37) = 113.1$, $p < 0.01$, Figure 3.4)

3.2.2.2 Repetition priming effects

For both species, MT was slightly, but significantly, faster when the side of the response repeated compared to when the side of the response alternated (main effect of Response Repetition, $F(1,37) = 7.0$, $p < 0.05$, Figure 3.5; interaction by Group, $F(1,37) = 1.9$, n.s.). The same pattern was seen in the reaction times of rats, but not humans (interaction of Response Repetition * Group, $F(1,37) = 18.0$, $p < 0.001$, Figure 3.5).

As well as being faster, rats were also more accurate when the required response repeated compared to when it alternated (main effect of Response Repetition, $F(1,27) = 45.4$, $p < 0.01$, Figure 3.6).

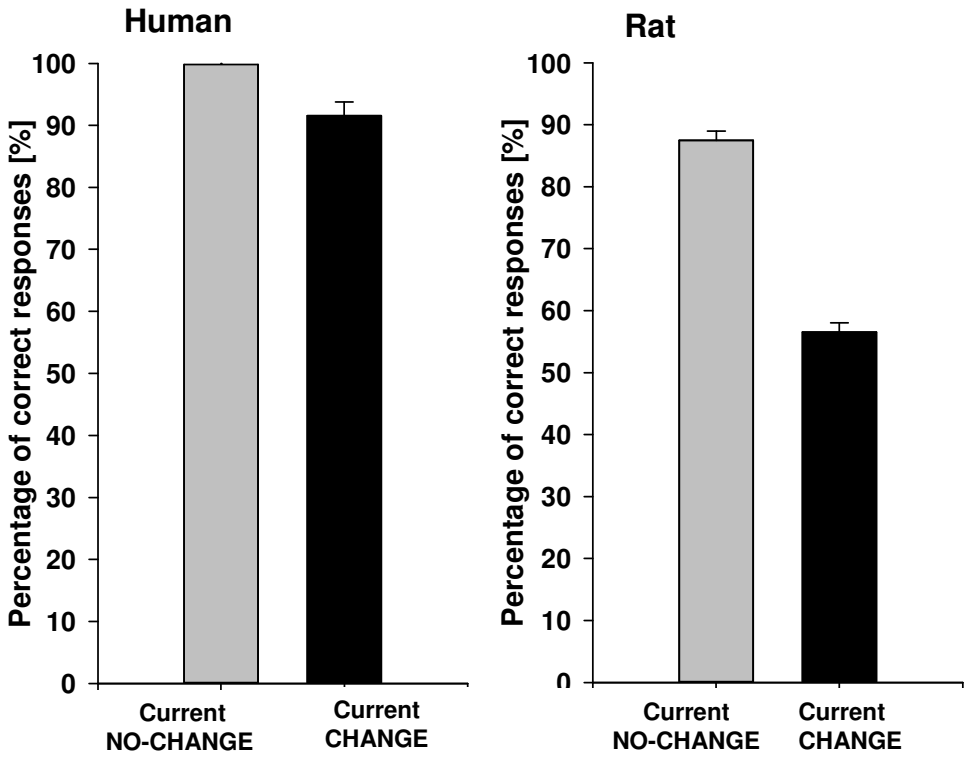

Figure 3.2. Rats made significantly less correct responses on CHANGE trials (Mean = 56.5, SEM = +/- 1.50) than on NO-CHANGE trials (Mean = 87.5, SEM = +/- 1.46). Humans follow the same patern but with higher accuracy on both CHANGE trials (Mean = 91.6, SEM = +/- 2.23) and no errors on NO-CHANGE trials (Mean = 99.9, SEM = +/- 0.04).

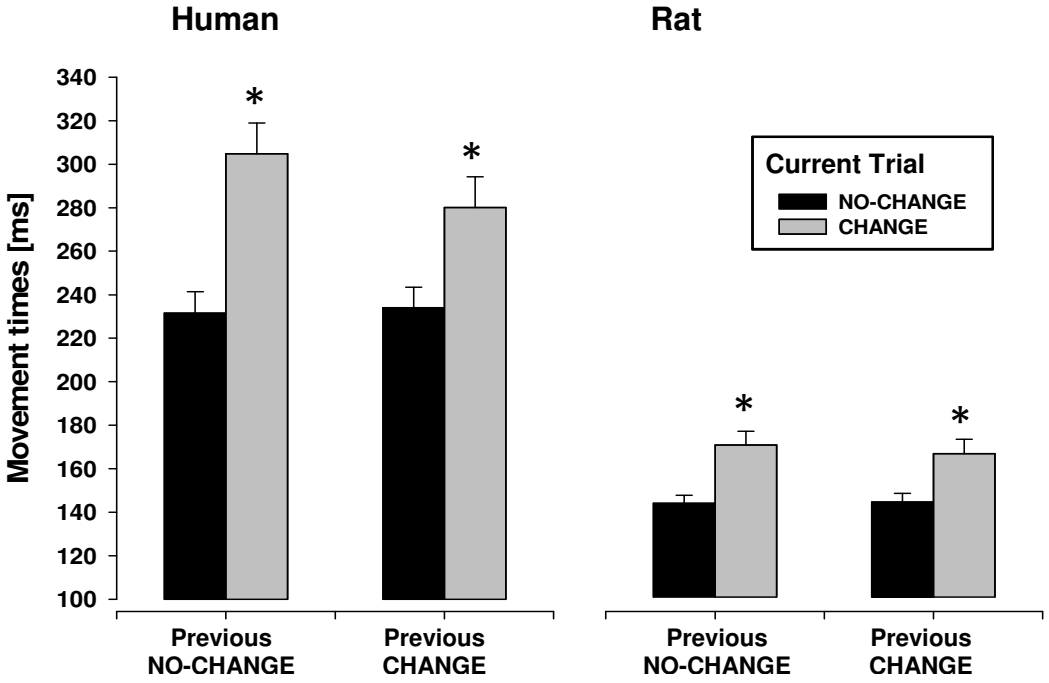

Figure 3.3. Both rats and humans showed faster movement times on NO-CHANGE trails than CHANGE trials. This effect was also modified by the previous trial stimulus change in human, but not in rats. Four bars in the left panel from left to right: Mean +/- SEM 231.5 +/- 9.9, 304.8 +/- 14.2, 234.0 +/- 9.4, and 280.1 +/- 14.1; in the right panel: Mean+/- SEM 143.1 +/- 3.8, 169.8 +/- 6.3, 143.7 +/- 3.9, and 165.9 +/- 6.6.

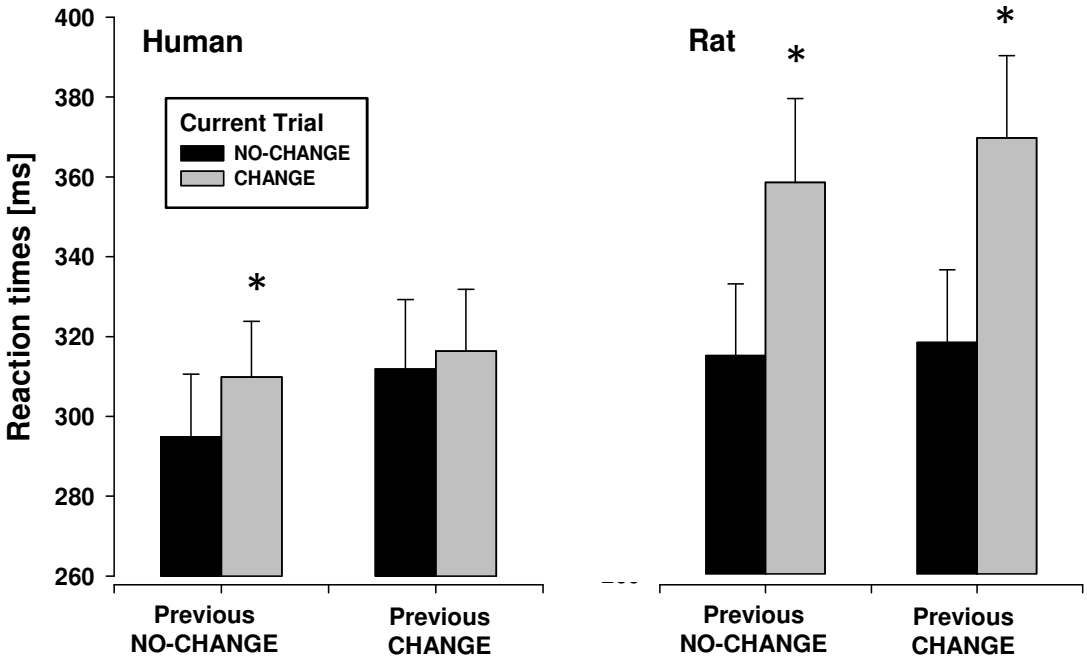

Figure 3.4 Rats showed slower RTs on CHANGE trials compared to NO-CHANGE trials, regardless of the previous trial type. Humans showed an interaction by current and previous trial type: when the previous trial was CHANGE trial, the current CHANGE trial would be facilitated. Four bars in the left panel from left to right: Mean +/- SEM 294.9+/-15.7, 309.9 +/- 14.0, 311.9 +/- 17.4, and 316.4 +/- 15.5; in the right panel: Mean+/- SEM 314.7 +/- 18.0, 358.0 +/- 21.0, 318.0 +/- 18.2, and 369.2 +/- 20.6.

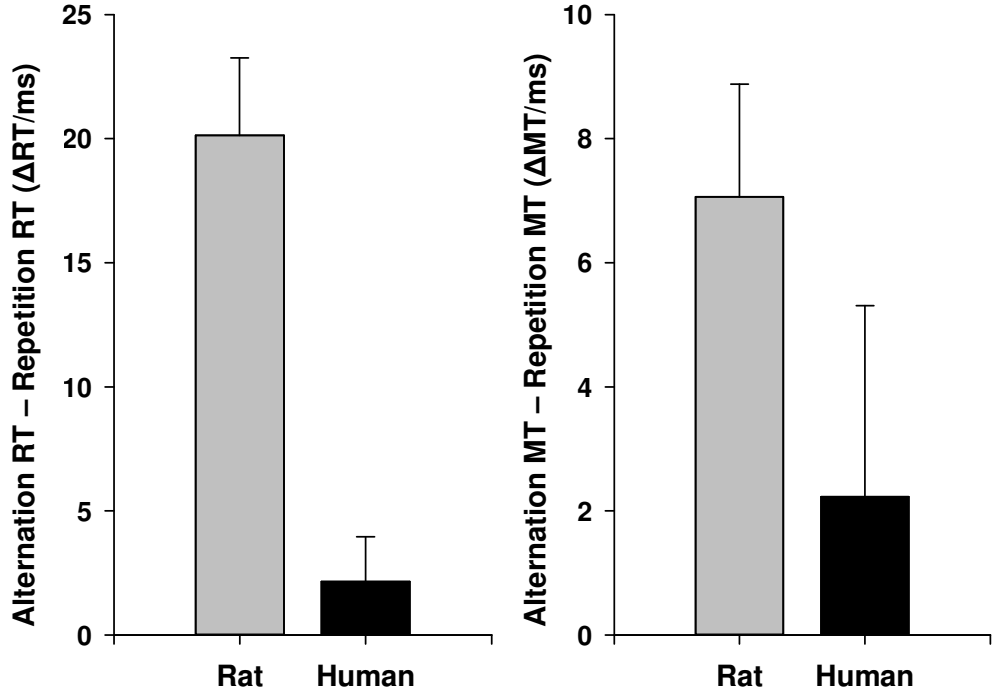

Figure 3.5. Rats showed faster RT and MT on repeated responses than on alternated responses, but human did not show this repetition priming effect. Two bars in the left panel from left to right: Mean +/- SEM 20.1+/-3.1and 2.2 +/- 1.8; in the right panel: Mean+/- SEM 7.1 +/- 1.8 and 2.2 +/- 3.1.

3.2.2.3 Effects of previous inhibition on the subsequent trial

Both humans and rats were slower to initiate a response which had been inhibited on the previous trial, and this was with without respect to the current Trial Type (interaction of Response Repetition * Previous Trial Type on RT: $F(1,37) = 4.4$, $p < 0.05$; interaction of Response Repetition * Previous Trial Type * Group, $F(1,37) = 3.1$, n.s., Figure 3.7). Notably, this effect was only seen for response initiation (RT) and not for response completion (MT).The same effect was also reflected in the response accuracy for rats, with error more likely on trials requiring the execution of a previously inhibited response (interaction of Response Repetition * Previous Trial Type, $F(1,27) = 78.1$, $p < 0.05$, Figure 3.6).

Figures 3.2 and 3.3 show that in humans, but not rats, the effect of Current Trial Type – slower RT and MT on CHANGE compared to NO-CHANGE trials – was diminished when the previous trial was a CHANGE trial. For MT, a previous NO-CHANGE seemed to increase the cost of a Current CHANGE; for RT, a previous CHANGE appeared to decrease the benefit of a current NO-CHANGE (interaction of Current Trial Type * Previous Trial Type * Group for RT: $F(1,37) = 6.3$, $p < 0.05$, Figure 3.4; MT:$F(1,37) = 18.6$, $p < 0.001$, Figure 3.3).

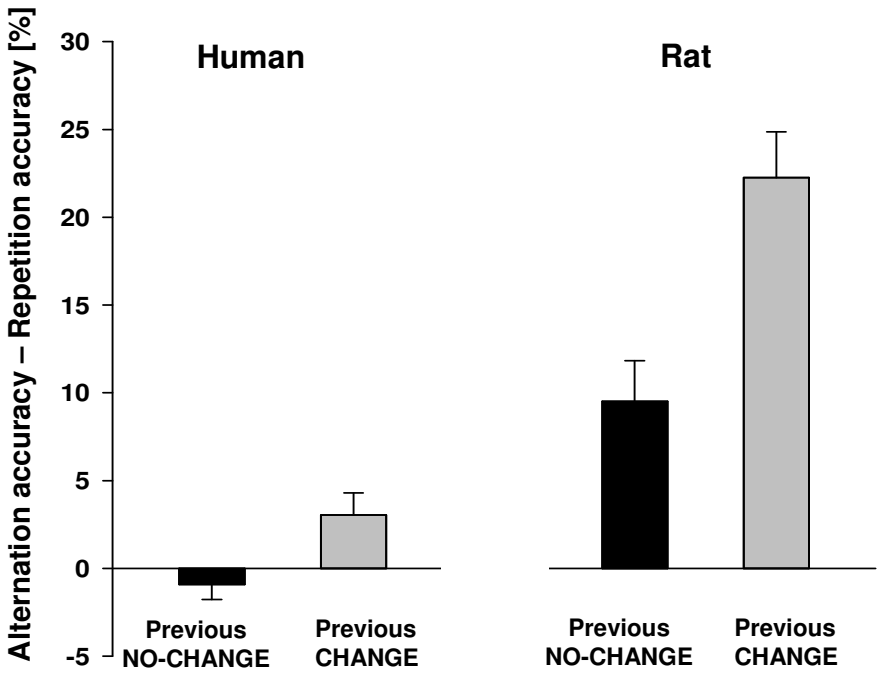

Figure 3.6. Effect of response repetition was significant in rats but not in human participants. However, both humans and rats showed made errors when they were required to respond to the previously inhibited location. Four bars from left to right Mean +/- SEM: -0.9 +/- 0.86, 3.0+/- 1.2, 9.5 +/- 2.3 and 22.3 +/- 2.6.

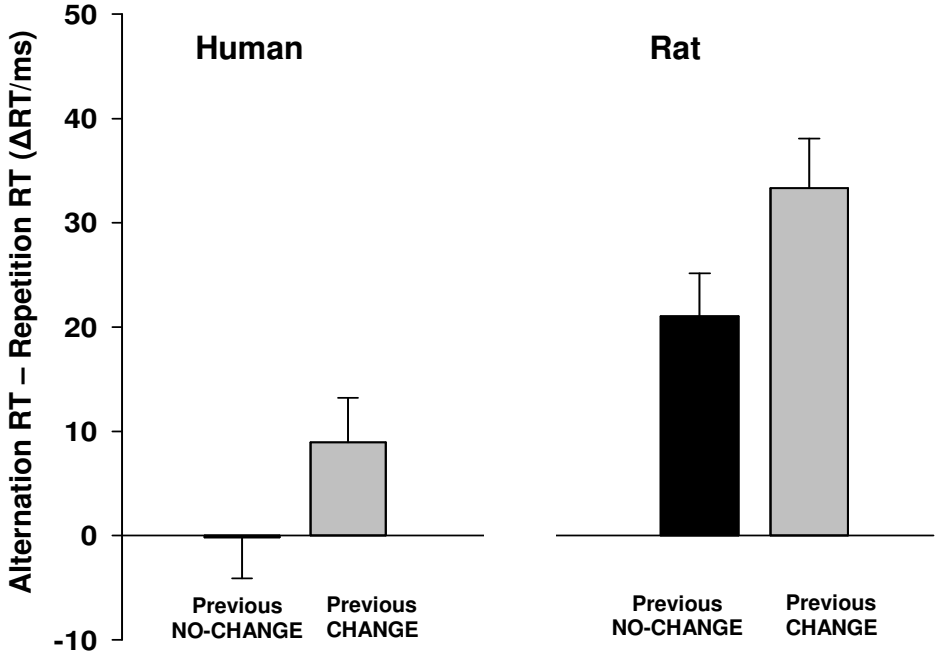

Figure 3.7. Effect of Response Repetition was significant in rats but not in human participants. However, both humans and rats showed slower reaction times when they were required to respond to the previously inhibited location. Four bars from left to right Mean +/- SEM: -0.2 +/- 3.9, 8.9+/- 4.2, 21.0 +/- 4.1 and 33.3 +/- 4.8.

3.2.2.4 Effects of errors on the subsequent trial

As noted above, humans made so few errors, that could not be analysed. However, for rats, there were sufficient instances of correct trials following different types of errors (incorrect, late and anticipatory responses) that it was possible to examine any potential post-error effects. Rats did not show slower RT following any kind of error, as has been reported in RT tasks in human studies. Indeed, rats were faster following an anticipatory error (main effect of Previous Outcome, $F(3,78) = 13.4$, $p < 0.01$; post-hoc Bonferroni test showed post-anticipatory RT were significantly faster than the other 3 conditions, Figure 3.8) .

The repetition priming effect on RT (faster RT when stimulus/response was repeated) disappeared after incorrect responses (interaction of Response Repetition * Previous Error, $F(1,26) = 10.6$, $p<0.01$, Figure 3.10; interaction of Stimulus Repetition * Previous Error, $F(1,26) = 8.7$, $p < 0.01$, Figure 3.9). The repetition priming effect was still seen on MT, which was positive for Stimulus Repetition (main effect of Stimulus Repetition, $F(1,26) = 9.6$, $p < 0.05$; interaction of Stimulus Repetition * Previous Error, $F < 1$; Figure 3.9).

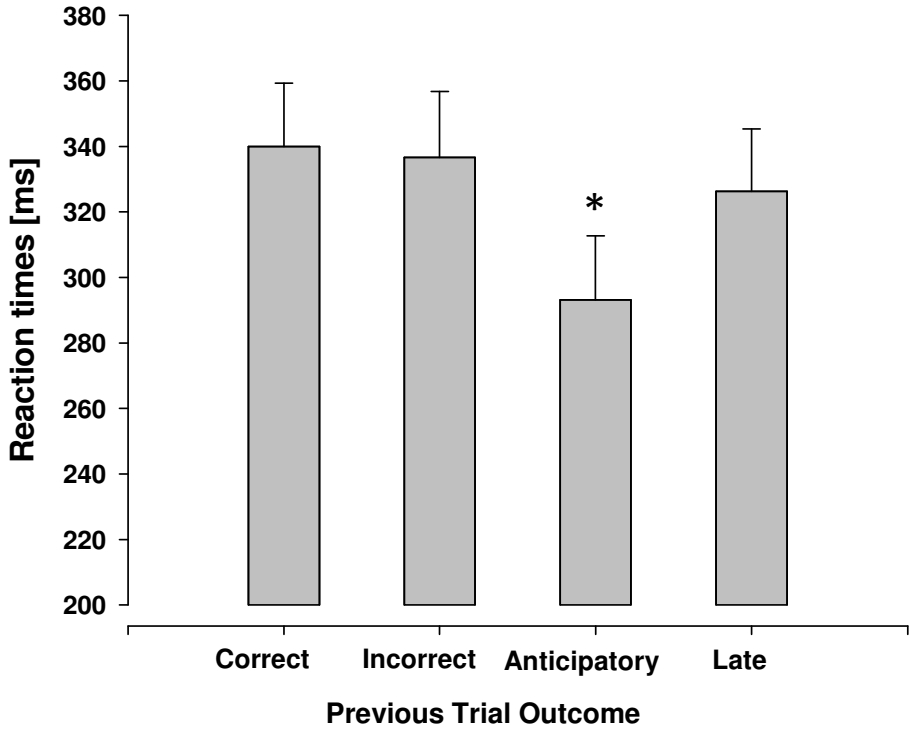

Figure 3.8. RT was significantly faster when rats made anticipatory error on the previous trial. Four bars from left to right: Mean +/- SEM: 340.0 +/- 19.3, 336.6+/- 20.1, 293.2 +/- 19.5 and 326.3 +/- 19.1.

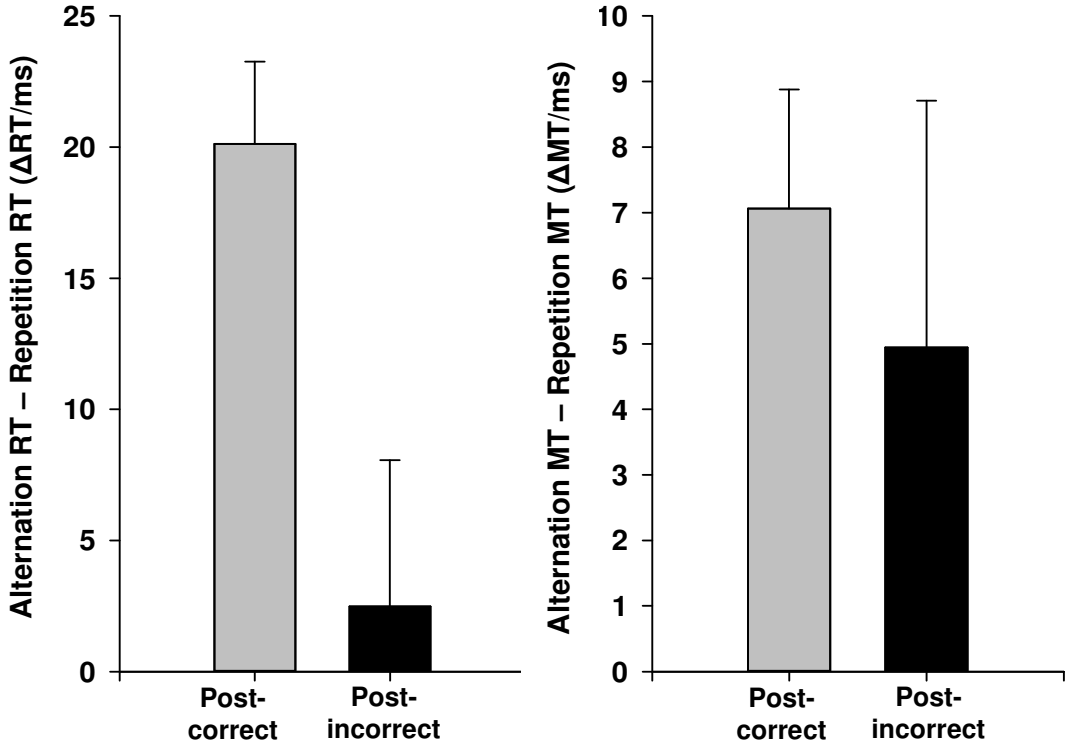

Figure 3.9. When the previous response was incorrect, the current MT, but not RT, still showed a benefit from stimulus repetition. Two bars in the left panel from left to right: Mean +/- SEM 20.1+/-3.1and 2.5 +/- 5.6; in the right panel: Mean+/- SEM 7.1 +/- 1.8 and 4.9 +/- 3.8.

3.2.3 Discussion

In this 2-AFC RT task – NO-CHANGE/CHANGE task, each response was mapped from two types of stimuli: one was a single "NO-CHANGE" stimulus and the other was a dual "CHANGE" stimulus. The effects of current and historical trial events were examined and compared between humans and rats.

Generally, both humans and rats were shown to be able to inhibit and reprogram a response when the cue changes. Rats show a repetition priming effect (faster RT when the stimulus / response repeat on the next trial), as has previously been reported, although not seen here, in humans. For humans, inhibiting any response on trial n-1 slows response initiation on a subsequent NO-CHANGE trial, but speeds RT on a CHANGE trial, perhaps because it facilitates response inhibition. For both humans and rats, inhibiting a particular response on trial n-1 slows initiation of that response on the subsequent trial.

3.2.3.1 Repetition priming effect

For humans in the current study, the repetition priming effect was very small and only seen on MT but not RT, although it has been reported as a robust effect in many human studies (Schvaneveldt and Chase, 1969; Pashler and Baylis, 1991; Campbell and Proctor, 1993; Hübner and Druey, 2006b; Verbruggen et al., 2008a). By comparing our task to other reaction time tasks in human participants, we suggest that the main reason for absence of repetition priming effect is that the current task uses longer inter-trial interval (ITI) (1500ms ~2500ms), or response-stimulus interval (RSI), than the other choice reaction time tasks. Previous studies have confirmed that the repetition effect varies systematically with the ITIs: the repetition effect is usually beneficial when the ITI is relatively short, and the shorter the ITI, the larger the repetition effect; the repetition effect transfers from benefit to cost when the ITI is longer than a particular period (this period varies for different tasks, but usually less than 500ms) (Schvaneveldt and Chase, 1969; Kirby, 1976; Gao et al., 2009). Previous studies also found that the beneficial repetition effect was bigger when there were more alternative responses, and that the effect could be negative when there were only two choices (Hyman, 1953; Shaffer,

1965). The ITI in Pashler & Baylis (1991) was 550ms; in Hübner & Druey (2006) was 50ms~350ms, and the benefits from repetition disappeared at 250ms and even transferred to cost at 350ms. Campbell did find a positive repetition effect at both 100ms and 1000ms, however in this study the S-R mappings were particularly complicated so that a larger repetition effect was predicted and its persistence for 1000ms could therefore have been predicted. In the current task, the ITI is relatively long since the task is self-paced; the S-R mapping is also quite simple, with one stimulus mapped to one response. For these two reasons, finding a small repetition effect in the current task was not unexpected.

Furthermore, in those tasks participants responded only once with each effector for one trial; in our current design, the same effector (index finger) was used to make three actions in each trial: to start the trial, trigger the stimulus and make the response. These two extra actions made by the same effector may have counteracted any potential response repetition effect on the final responding action.

Another probability might be that the mixture of two types of trials (CHANGE and NO-CHANGE) in the same session may have disrupted the cohesion of sequence for each type of trial. This last possibility was tested in a subsequent Experiment 2 (see below).

Rats, unlike humans, showed a robust response repetition effect on both RT and MT and also on accuracy. This may be partially due to the effector the rats use – the whole body. The cost of moving a body is significantly higher than moving a finger, thus it is more likely that rats take advantage from repeating the same response.

Moreover, the rats compared to humans, show a repetition effect at longer ITIs. This indicates that the repetition priming effect in rats has a different mechanism than in humans. For humans, a repetition effect at long ITIs is caused more by subjective expectancy (SE) rather than automatic facilitation (AF) when the ITI is short(Gao et al., 2009); for rats, it seems like they are more controlled by AF even when the ITI is long.

However, the mechanism underlying the repetition priming effect in the current study is still ambiguous. Generally, a repetition effect can be caused by simple stimulus repetition or simple response repetition, and in some situations it can only be caused by stimulus-response repetition. With the analysis of post-error repetition priming effect in rats, we see a positive stimulus repetition priming effect, or we can say a negative response repetition priming effect. However, considering the high possibility that rats make mistakes because they did not see the stimuli clearly, the stimulus repetition/alternation makes less sense for rats. Therefore we suggest that the post-error repetition priming effect is more likely from response repetition than from stimulus repetition, which means rats are slower on completing the response which has been incorrect on the previous trial.

3.2.3.2 Inhibition slows down subsequent initiation of the same response

We found that the inhibition of a response had an inhibitory after-effect on that response but not on the alternative response (interaction of Response Repetition * Previous Trial Type). This was consistent with results from previous studies in the stop-signal paradigm (Rieger & Gauggel, 1999b; Verbruggen, Logan, et al., 2008; Verbruggen & Logan, 2008). We also found that unsuccessful inhibition did not cause any after-effect on the following trial, regardless of whether the stimulus repeated or not. This was different from what has been observed in the stop-signal paradigm, where participants were slower on go responses after a failed stop. The inconsistency is probably induced by the different task designs: the stop-signal reaction time task requires cancellation of all potential responses, therefore, after a failed stop, subjects tend to withhold any response in case of another stop trial; in contrast, the current task requires a quick response on both NO-CHANGE and CHANGE trials, therefore subjects will not slow down for the expectancy of a potential inhibition.

It is noteworthy that this inhibitory after-effect is true in both humans and rats, which suggests that residual inhibition can be carried on from one trial to the subsequent trial in both humans and rats. It also indicates that this after-

effect is controlled by an automatic process that is shared by both humans and rats, rather than a subjective process which is unique for humans.

3.2.3.3 Inhibition reduces the cost of subsequent inhibition

The stop-signal paradigm has shown that in humans, successful stop trials speed up subsequent SSRT (Bissett & Logan, 2012; Morein-Zamir et al., 2007). An evaluated explanation of the post-stop-SSRT-speeding is that the goal priority shifts away from the "go" task towards the "stop" task after a stop trial (Bissett & Logan, 2012). The current study found a similar effect: for humans, previous successful inhibition reduced the effect of Current Trial Type. However, this effect is more complicated than the effect in the stop-signal task and, since a "go" action is always required on the current task, this effect cannot be explained by the goal priority shifting hypothesis.

Figure 3.2 and 3.3 showed that a previous NO-CHANGE seemed to increase the MT of a Current CHANGE, while a previous CHANGE appeared to decrease the RT of a Current NO-CHANGE. They both ended up with a smaller difference between Current NO-CHANGE and CHANGE trials in MT and RT, but caused by different mechanisms. Both situations happen when the previous trial type is different from the current trial type; in other words, the actual current trial type is incongruent with the participant's expectancy. When the previous trial is a NO-CHANGE trial, the participant's expectancy for the subsequent trial will also be a NO-CHANGE trial, therefore they tend to initiate the response as soon as they see the stimulus and this leads to a relatively short RT. If the subsequent trial turns out to be a CHANGE trial, the original response will be cancelled and a new response will be programmed during the MT and this leads to a longer MT. On the other hand, when the previous trial is a CHANGE trial, the participant will more likely expect another CHANGE trial. In this case, they will wait longer to guarantee they see the stimulus clearly (CHANGE or NO-CHANGE) and therefore be slower on initiating a response (longer RT).

This subjective expectancy dependent adjustment is strategic, which is flexible, based on knowledge and can be implemented proactively. Humans are capable of using strategies and explicit up-down controls, while rats more

likely rely on automatic processes, which are less flexible and are driven by the stimulus. The difference explains the fact that rats do not show the same post-CHANGE effect as humans.

3.2.4 Conclusion

The present study confirms that the NO-CHANGE/CHANGE task is feasible for testing the ability of inhibiting and reprogramming a response when the cue changes. It also confirms that humans and rats were influenced by trial sequences differently. For rats, the sequential effects are mainly caused by automatic processes. For humans, the sequential effects are more influenced by subjective expectancy. By understanding the differences, we can avoid over-translation of results between different humans and rats.

The current study leaves a question of whether or not the absence of response repetition effect in humans is caused by the mixture of two types of trials in the same session, and this will be examined in the experiment two by dividing the two types of trials into different sessions.

3.3 Experiment 2

3.3.1 Methodology

Participants were 18 young adults (3 males and 15 females), between the ages of 18 and 30. Apparatus was the same as used in the experiment 1. The whole experiment contained 3 parts: a simple *NO-CHANGE* task, a simple *CHANGE* task and a mixed *NO-CHANGE/CHANGE* task. The order that each participant was tested on the two simple tasks was counterbalanced, and the mixed task was always the last part.

The participants were asked to finish 100 correct trials in the two simple tasks and 400 correct trials in the mixed task, which ended up with about 50 valid trials in each condition in all 3 tasks.

The participants' performance on the mix task in the current experiment was compared to performance in experiment 1, using the same repeated measures ANOVA with the same factors as described above. Repeated measures ANOVA was also used for a comparison between the two simple tasks, with Current Response Side (repeated or alternated from previous trial) and Task (*CHANGE* or *NO-CHANGE*) as within-subject factors.

3.3.2 Results

3.3.2.1 Simple CHANGE or NO-CHANGE tasks

Participants were significantly slower on the *CHANGE* task than on the *NO-CHANGE* task on both reaction times and movement times (main effect of task, $F(1,15) = 104.7$, $p < 0.001$). As expected, no participant made any mistakes in the *NO-CHANGE* task, while the incorrect percentage was around 1% on the *CHANGE* task. However, stimulus/response repetition once again failed to influence participants' performance, on either the simple *NO-CHANGE* or *CHANGE* tasks.

3.3.2.2 Mixed task

The participants in experiment 2 were generally faster on the mixed task than the participants in experiment 1 (main effect of Group, $F(1,25) = 5.1$, $p < 0.05$), which was possibly due to the practice effect from the two simple tasks. It was noteworthy that effects found in experiment 1 were all replicated in experiment 2, only with some differences in the effect sizes. For instance, the participants in both experiments showed that a successful previous inhibition could reduce the difficulty of inhibiting a response on the current trial, but the reduction was so large in experiment 2 that the current *CHANGE* trial could be as fast as a *NO-CHANGE* trial (Group * Previous Trial Type * Current Trial Type interaction, $F(1,25)=14.2$, $p = 0.001$). Response side repetition still did not make any contribution to response latency or accuracy.

By comparing the same conditions between simple tasks and mix task, we found that the participants were faster on simple *NO-CHANGE* task than on *NO-CHANGE* trials of the mixed task, and that they were slower on the simple *CHANGE* task than on *CHANGE* trials of the mixed task. This implied that participants' expectation of the coming trials actually influence their responding times.

3.3.3 Discussion

Experiment 2 replicated the human results in experiment 1 and furthermore confirmed the absence of response repetition effect on the current 2-AFC RT tasks. It helps to eliminate the possibilities that the lack of repetition effect in the NO-CHANGE/CHANGE task is caused by the mixture of two types of trials or by the involvement of response inhibition in the task. Therefore it leaves one possibility as the most probable – that the two extra actions executed by the same effector between the two consecutive responses is the reason that the response repetition effect is diminished in the current task.

Effects of Lesions of the subthalamic nucleus in the rat on performance in the Signal Change Reaction Time Task

Further to the study of normal rats' performance on the 2 alternative forced choice reaction time tasks (2-AFC RT), we introduced the bilateral medial STN lesions into the rats, examining the role of the STN in response inhibition and re-programming, in the dynamic context of alternative stimulus and responses. The results confirmed the previous finding that rats with STN lesions were impaired on preventing anticipatory responses. Results also suggested that STN lesioned rats did not generally perform slower reaction times, as reported in some previous studies. However, pre-surgery difference between the lesion and control groups compromises conclusions about the STN lesions; hence a replication of the present experiment is necessary for any definitive conclusion.

4.1. Introduction

The STN has been implicated in inhibitory control (see Chapter 1). Eagle (Eagle et al., 2008) reported that in the stop-signal reaction time (SSRT) task, rats with STN lesions did not show slower SSRT although they were more likely to fail to stop. They interpreted this as an impairment in 'stopping' that was independent of the SSRT itself. The authors also reported faster 'Go' reaction times, although in another study, reaction times to visual stimuli in a two-choice task were unchanged (Phillips & Brown, 1999).

A consistent finding in rats with STN lesions is a higher percentage of premature responses, which are response made prior to stimulus onset (Baunez & Robbins, 1997; Baunez et al., 2001; Henderson et al., 1999; Phillips & Brown, 2000). This effect implies that the STN is important for withholding a response until an appropriate time point.

As reported in Chapter 3, when dealing with changing information, normal rats are able to inhibit a no-longer-appropriate response and reprogram another response in real time. The experimental question of the current study is that whether STN lesions will stop rats from doing response inhibition and reprogramming efficiently. To explore the answer, we tested rats with bilateral excitoxic STN lesions in the NO-CHANGE/CHANGE task described in Chapter 3.

4.2. Materials and methods

4.2.1. Animals

Eighteen male Lister hooded rats (Charles River, UK) from Chapter 3 were tested in the current study. More details were listed in Chapter 2.

4.2.2. Apparatus

The 9-hole operant chamber (Paul Fray, UK) was used (see the General Methodology in Chapter 2).

4.2.3. Behavioural test

The rats were trained as described in the General Methods section and tested in the NO-CHANGE/CHANGE task as described in Chapter 3.

Pre-surgery baseline data were taken over 2 weeks prior to surgery. The 18 rats were assigned to two groups and latencies and accuracies were analysed using a repeated measure ANOVA with trial conditions as within-subjects factors and Group as between-subjects factor. There was no significant difference between the groups on accuracy or reaction times for any trial types. Thus, performance was stable and surgery was conducted.

4.2.4. Surgery

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

4.2.5. Histology

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

4.2.6. Data analyses

Data analyses were basically the same as described in Chapter 3. The ANOVA had four within-subjects factors: Previous Trial type (*CHANGE* or *NO-CHANGE*); Current Trial Type (*CHANGE* or *NO-CHANGE*); Response Side (repeated or alternated from previous trial); Surgery (pre- and post-surgery); and one between-subjects factor: Group (control vs lesion). Accuracy and reaction time (RT) were used as the measurements of rats' performance. Movement time (MT) was not used in the current analysis because from Chapter 3 we knew that MT generally reflected the pattern of RT, thus gave away no more information than RT, and it was also less reliable than RT. For analyses of RT and accuracy in this chapter, only pairs of correct trials were considered.

4.3. Results

4.3.1. Histology

The lesions were verified by assessing the extent of cell loss in the STN and surrounding areas. Three rats were excluded from the lesion group due to small or unilateral lesions. The remaining six lesioned rats had an average cell loss of 70% ~ 80% of subthalamic neurons, with the lesions focussed on the medial portion of the nucleus, with some intrusion into the surrounding zona incerta and cerebral peduncle. Figure 4.1 illustrates the extent of the smallest and largest lesion and also the typical lesion. Rats that underwent sham-surgery did not have any cell damage in the STN.

4.3.2. Effects of STN lesions

4.3.2.1. Inhibition and reprogramming within a trial

As reported in Chapter 3, rats were able to learn to inhibit and reprogram responses when the cue changed. The accuracy for both NO-CHANGE and CHANGE trials was above chance level, but they made more mistakes on CHANGE trials (main effect of Current Trial Type, $F(1,14) = 172.5$, $p < 0.01$). Rats were also slower on CHANGE trials (main effect of Current Trial Type, $F(1,14) = 63.0$, $P < 0.01$).

STN lesions did not increase incorrect responses on CHANGE trials (interaction of Current Trial Type * Surgery * Group: $F(1,13) = 2.1$, n.s.; Figure 4.2). The lesions also did not change the main effect of Current Trial Type on the RTs (interaction of Current Trial Type * Surgery * Group, $F(1,13) < 1$, n.s; Figure 4.3). There was no evidence of any effect of the STN lesions on rats' ability to inhibit and reprogram responses within a trial when the cue changed.

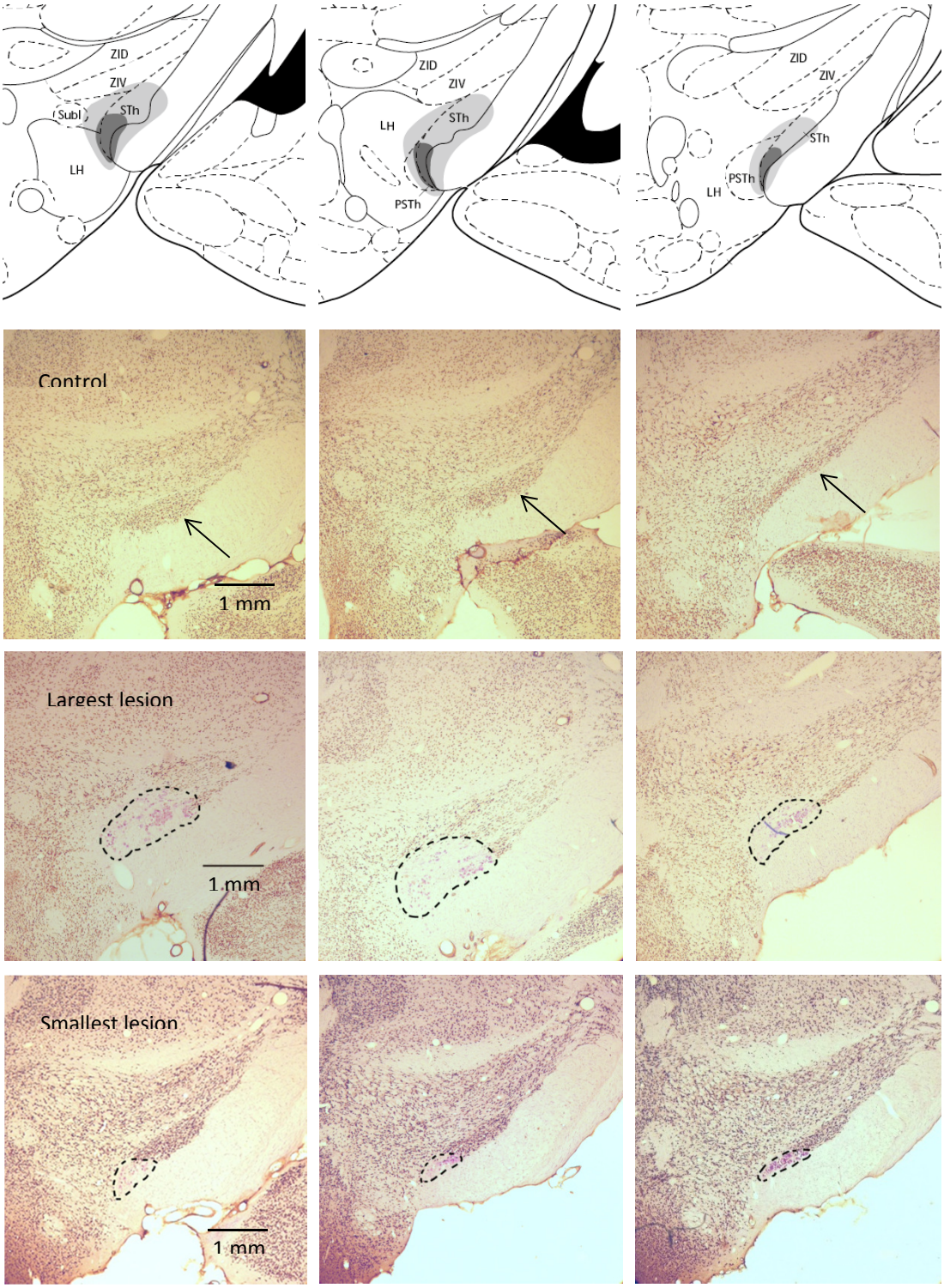

Figure 4.1 shows schematics and examples of photographs of NeuN stained control and STN lesioned rats. Top to bottom: schematics showing the minimum (dark grey) and maximum (light grey) extents of the STN lesions; photograph of a control rat brain; photograph of the largest STN lesions; and photograph of the smallest STN lesions.
STh: subthalamic nucleus; ZID: zona incerta, dorsal part; ZIV: zona incerta, ventral part; LH: lateral hypoth area; SubI: subincertal nucleus.

4.3.2.2. Repetition priming effect

As reported in Chapter 3, as well as being more accurate (main effect of Response Repetition: $F(1,14) = 52.9$, $p < 0.01$, Figure 4.5), rats were also faster when the required response repeated compared to when it alternated from the previous trial (main effect of Response Repetition on RT: $F(1,14) = 24.1$, $p < 0.01$, Figure 4.4).

The effect of alternation of responses on RT was enhanced following lesions of the STN (interaction of Response Repetition * Surgery * Group, $F(1,13)=5.9$, $p<0.05$; Figure 4.4). Further analysis restricted to each group showed that the effect of Response Repetition was changed by Surgery only in the lesioned group and not in the control group (interaction restricted to lesion group: $F(1,5) = 6.9$, $p < 0.05$; control group, $F(1,8) < 1$, n.s.).

Although the effect of surgery was different for the two groups – control performance did not change, but STN lesions enhanced the alternation effect – there was a pre-surgery difference between the groups, such that they did not differ post-surgery. This arose because of the necessity to exclude rats with small or no lesions, which in turn meant that the groups were no longer matched for pre-surgery performance.

The effect of response repetition on accuracy was not significantly changed by the STN lesions (interaction of Response Repetition * Surgery * Group, $F(1,13) =1.7$, n.s.; Figure 4.5), although there was a tendency for accuracy to reflect the RT results. Certainly, as is clear from Figures 4.1-4.4, there was no evidence for a speed-accuracy trade off (i.e., accuracy improving as responses slowed).

4.3.2.3. *Effects of previous inhibition on the subsequent trial*

According to results in Chapter 3, rats should be slower on RTs when the required response was inhibited on the previous trial. However, in the current study, with 15 rats instead of 28 rats, this effect on RT was only approaching significance (interaction of Response Repetition * Previous Trial Type, RT: $F(1,14) = 3.8$, $p = 0.07$). Nevertheless, same as in Chapter 3, this effect was observed again on the accuracy (interaction of Response Repetition * Previous Trial Type, on accuracy: $F(1,14) = 39.7$, $p < 0.05$). The STN lesions did not seem to change this effect, since no interaction by Group or Surgery was statistically significant.

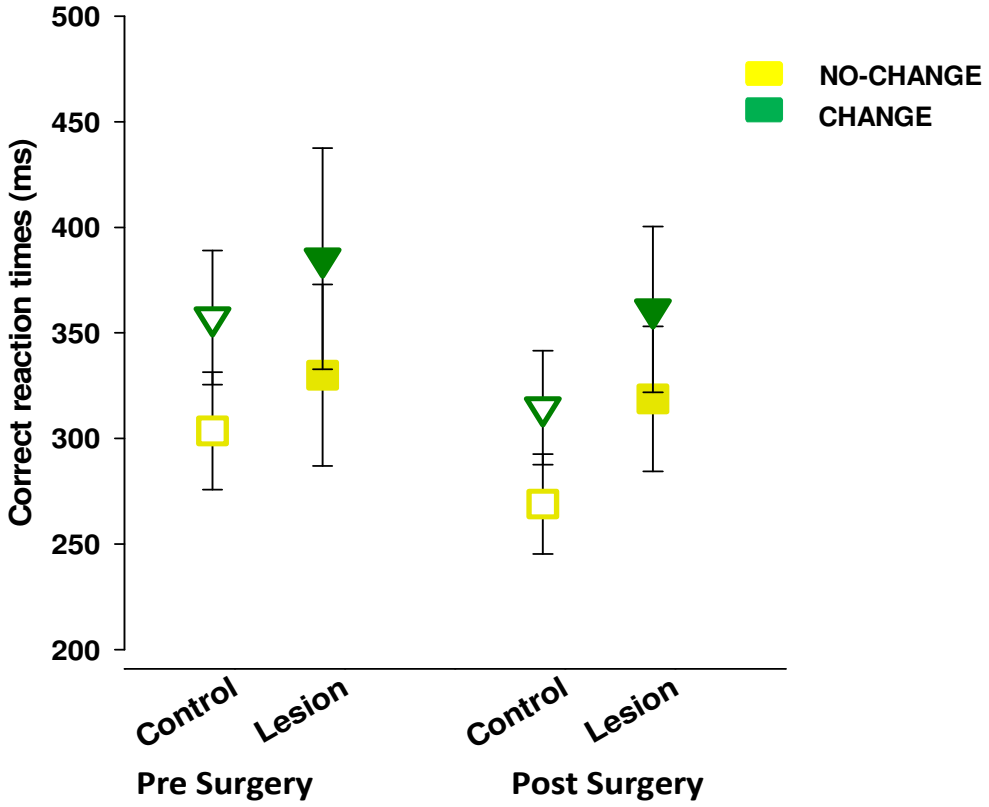

Figure 4.2 shows reaction times of correct responses on CHANGE and NO-CHANGE trials by group and surgery. The STN lesions did not change the main effect of Current Trial Type on RTs, both the control and lesioned rats showed slower RTs on CHANGE trials.

For all rats, even the lesioned rats, the Repetition effect was larger when the previous trial was a CHANGE trial, and even larger when the current trial was also a CHANGE trial (interaction of Response Repetition * Previous Trial Type * Current Trial Type, $F(1,14) = 10.1$, $p < 0.05$).

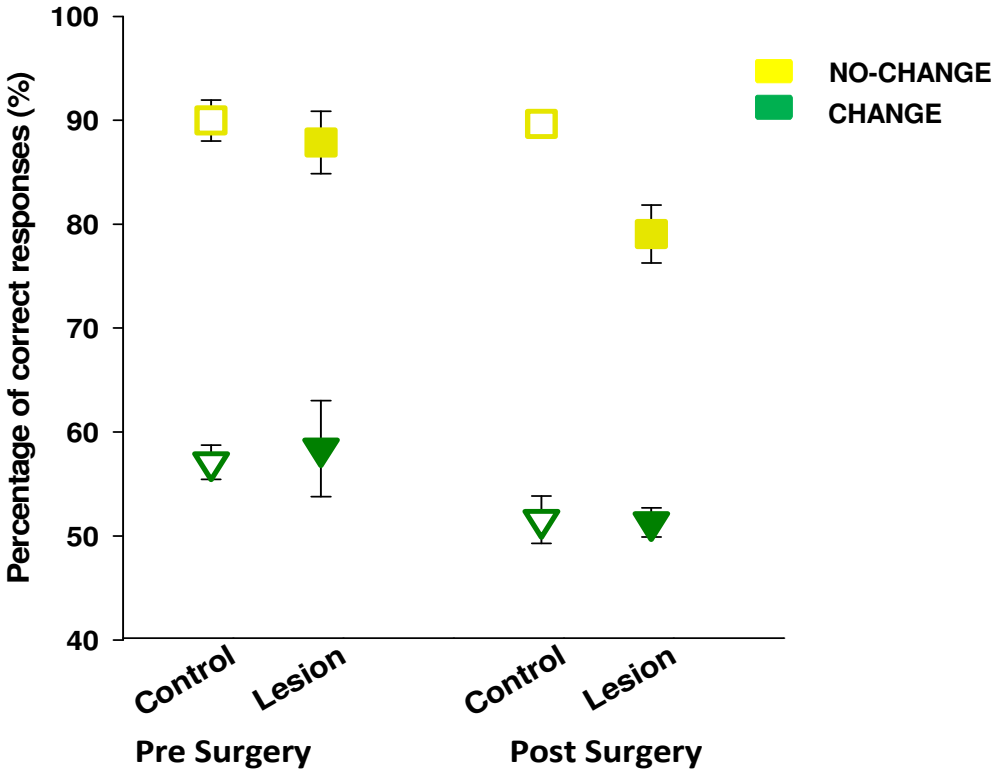

Figure 4.3 shows percentage of correct responses on CHANGE and NO-CHANGE trials by group and surgery. The STN lesions did not change the main effect of Current Trial Type on accuracy, both the control and lesioned rats made more errors on CHANGE trials.

4.3.2.4. Anticipatory probability

As anticipatory responses were, by definition, prior to stimulus onset, they cannot be influenced by the 'current trial type, as this is not known prior to the stimulus onset. Rats were most likely to make anticipatory errors after an anticipatory error and least likely after a correct response (main effect of Previous Outcome, $F(3,42) = 9.9$, $p < 0.01$; Figure 4.6).

The STN lesioned rats generally made more anticipatory errors (interaction of Surgery * Group, $F(1,13) = 5.4$, $p < 0.05$; Figure 4.6).

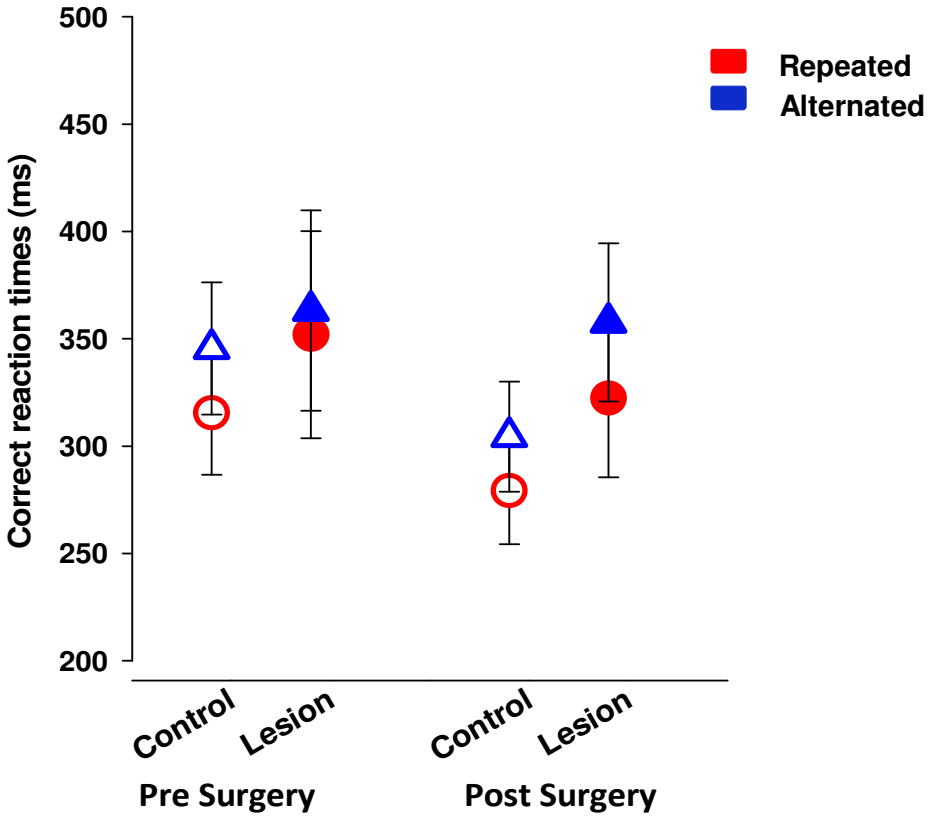

Figure 4.4 shows correct reaction times of repeated and alternated responses by group and surgery. Both the control and lesioned group showed faster RTs on repeated responses. However, the Repetition Priming effect was enlarged in the STN lesioned rats because of even slower RTs on alternated responses. The control rats were equally faster post surgery on both repeated and alternated responses.

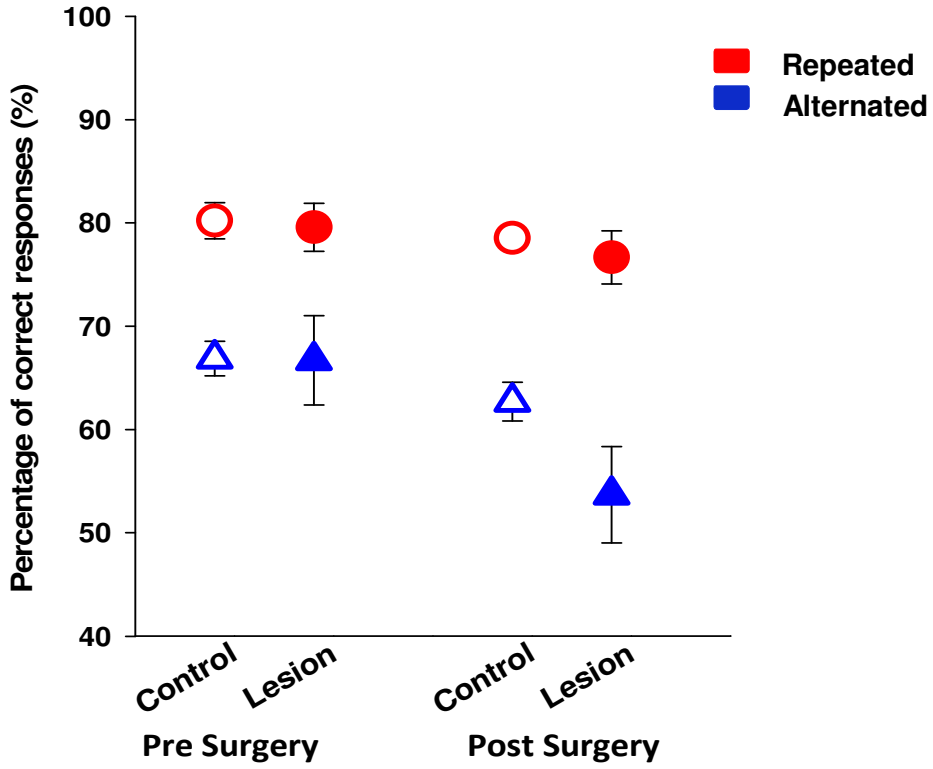

Figure 4.5 shows percentage of correct repeated and alternated responses by group and surgery. Both the control and lesioned group showed higher accuracies on repeated responses. However, the Repetition Priming effect was enlarged in the STN lesioned rats, because of even lower accuracy on alternated responses. The control rats did not change on accuracy post surgery.

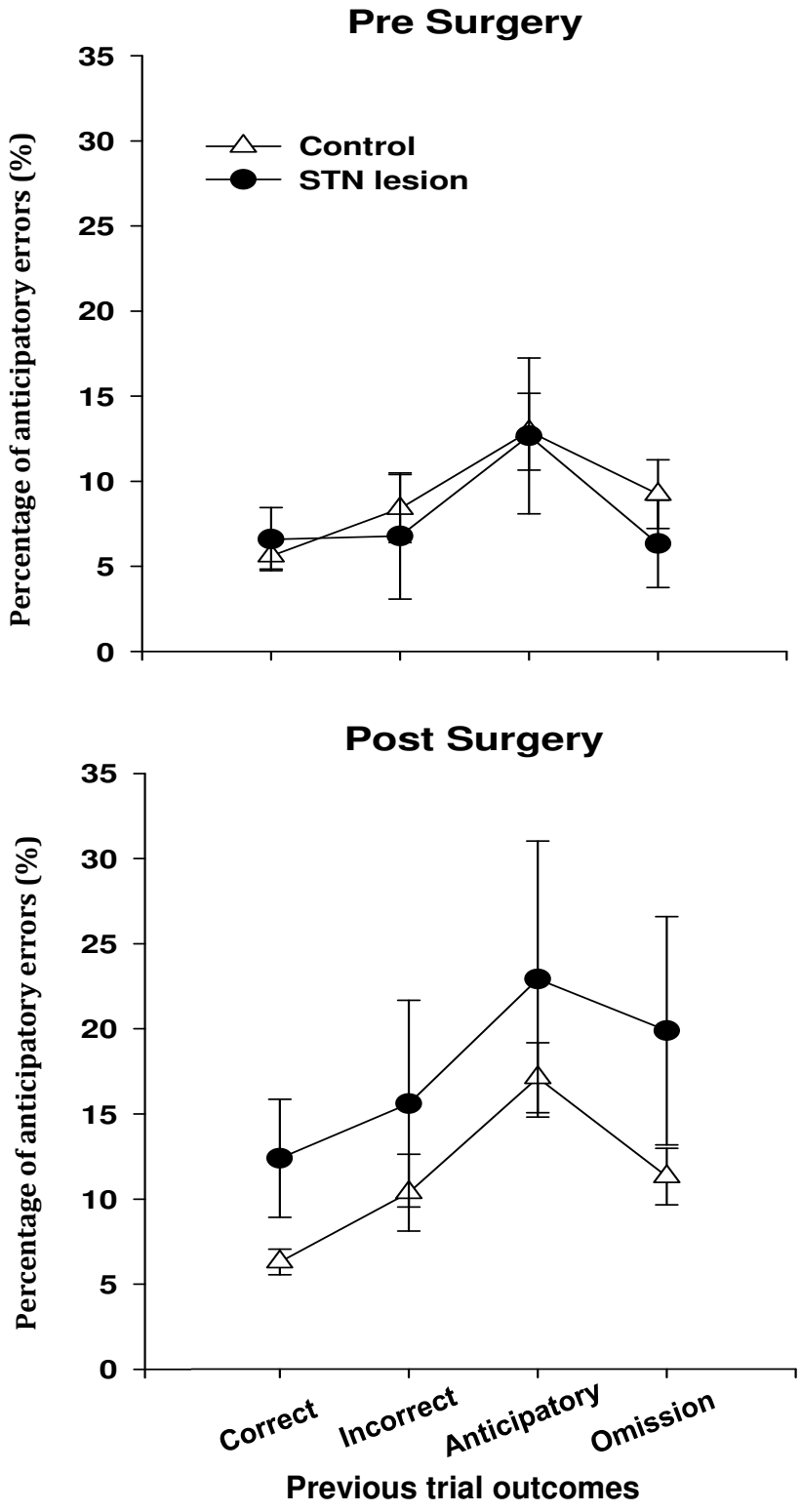

Figure 4.6 shows percentage of anticipatory errors following different types of responses by group and surgery. Rats were most likely to make anticipatory errors after an anticipatory error and least likely after a correct response. The STN lesioned rats generally made more anticipatory errors.

4.4. Discussion

As mentioned in the introduction, previous studies have showed that STN lesioned rats are more likely to make anticipatory errors, have difficulty 'stopping' in the stop-signal reaction time (SSRT) task and 'go' responses are speeded(Eagle et al., 2008b), although reaction times to visual stimuli in a two-choice task are unchanged (Phillips & Brown, 2000). The primary goal of the current study was to investigate whether STN lesioned rats are able to inhibit and reprogram a response if a signal changes before they have started to move.

4.4.1. Baseline performance

In the current study, a two-choice reaction time task was employed in which stimulus changed side on half of trials before any response had been initiated. Pre-surgery data confirmed that a stimulus change disrupted response initiation: this was reflected in slower reaction times and lower accuracy on CHANGE trials. Sequential effects were also found in this task: rats were both slower and less accurate for responses that alternated from the previously rewarded side and moreover, rats were slower and less accurate when they were required to make a response that had been inhibited on the previous trial.

The robust response repetition/alternation effect enabled a useful comparison to be made between the within-trial stimulus change effect and the between trial response priming effect. These effects made the current task a tool to test whether STN lesions would impair the rat's ability to inhibit a potential response and switch to another response within and between trials.

4.4.2. Within-trial response inhibition and re-programing

Given that rats with STN lesions have difficulty in stopping a response in progress in the SSRT task, it was expected that STN lesioned rats might make more mistakes on CHANGE trials comparing to control rats. However, neither

accuracy nor reaction time on CHANGE trials was impaired by the STN lesions. It is likely that task differences explain this discrepancy.

In the SSRT task, a go response (i.e., a rapid movement from the left lever to the right lever) is initiated on every trial and the rats do not need to wait for any signal to initiate a go response. The rat is motivated to 'go' because most go responses are rewarded: a minority of trials require that the rat 'stop', but only after the movement has started. STN lesioned rats complete the 'go' responses more quickly, perhaps because they initiate the movement earlier. It is possible that the STN lesioned rats are less able to stop following a stop signal because they have passed the point of 'no return' sooner than control rats.

By contrast, in the current Stimulus Change task, the rat must wait before making any response, and the required response is unknown until the signal at the end of the foreperiod. This design means that the processing of the signal and response initiation takes place after the onset of the stimulus: this allows more time for inhibition processes to interfere with response initiation process. Moreover, as CHANGE trials comprise 50% of the trials in a session, the cost of an error on these trials is high.

Overall, compared to SSRT task, responses in Stimulus Change task are more under stimulus control, rather than pre-programmed before stimulus onset. This difference is a result of task design and inevitably leads to different results which are not directly comparable, although they are complementary in interesting ways.

4.4.3. Between-trial response bias and alternation

Rats are slower on responses that alternate from the previous rewarded response, compared to responses that repeat. This might because a response becomes 'primed' after it is rewarded and rats are biased towards repeating this response on the next trial.

Although the pre-surgery group difference compromises the interpretation of the effects of the STN lesions, there is nevertheless a trend for rats with STN lesions to be particularly challenged when the response side alternates. This

finding suggests that inhibitory control in rats with STN lesions is compromised for pre-potent response biases which are not under stimulus control.

4.4.4. Effect of STN lesions on anticipatory errors

Consistent with previous studies from our lab and also other labs (Baunez et al., 1995a; Phillips & Brown, 1999; Phillips & Brown, 2000), increased anticipatory errors were observed in rats with STN lesions in the current study. It is noteworthy that the increase was not as great as in the other studies. This is very likely due to the decreased time-uncertainty caused by the fixed foreperiod: the time of stimulus onset is predictable within the trial. In a previous study that used a similar task but with variable foreperiods (unpublished thesis chapter, David Mark Thomson, 2005), rats made significantly more anticipatory errors in baseline performance and the increase in anticipatory errors after the STN lesions was also greater.

All in all, the increased anticipatory errors once again support the hypothesis that when not under stimulus control, STN lesioned rats are impaired on the inhibitory control of pre-potent response bias.

4.4.5. Problematic issues

The lesion group comprised only 6 rats after partial or unilateral lesions were excluded. Furthermore, there was only partial data from 1 of the 6, as the rat developed seizures and was therefore euthanized. This limited sample size compromised the statistical power. For some variables and some conditions, the observed power was lower than the conventional 0.8, thus left a high risk of Type II error, which meant we might miss some positive effects. In addition, the final two groups had pre-surgery differences which resulted in interactions that were difficult to interpret.

4.4.6. Conclusions

In summary, the results showed that the STN lesions did not impact rat's ability to inhibit a response before it has been started; by contrast, the STN lesions did increase the difficulty of alternating responses between two consecutive trials. However, it is a pity that we had a very limited size for the lesion group and also suffered from unmatched pre-surgery performance from the two groups. Therefore, a replication with bigger group sizes is necessary before any conclusion can be drawn about the effects of the STN lesions.

Effects of Lesions of the subthalamic nucleus in the rat on performance in the Signal Change Reaction Time Task (continued)

The current study used a modified signal change reaction time task and further examined the effects of STN lesions on response inhibition and re-programming. The rats with STN lesions made earlier anticipatory responses, though the percentage of anticipatory responses was not significantly higher, relative to the control rats. The lesioned rats were not less able to inhibit an about-to-be-initiated response when the stimulus changed, which suggests that once under stimulus control, STN lesioned rats are as good as controls at response inhibition and initiation. However, the STN lesioned rats were much slower and more inaccurate on alternated responses. This finding suggests that with STN lesions, rats find it more difficult to inhibit the bias towards the previous correct response.

5.1. Introduction

In Chapter 4, it reported that rats with STN lesions are able to inhibit and reprogram a response before the response has started. However, rats with STN lesions seem to be impaired on switching between two alternative responses on consecutive trials. This impairment is particularly great when the required response was inhibited on the previous trial. Unfortunately, the group size was small due to failed lesions and furthermore, after rats were excluded, the pre-surgery performance between the two groups was unmatched. This meant it was difficult to draw firm conclusion about the effects of STN lesions.

Race models (see Chapter 1) have been used to examine and predict human participants' performance in stop, change and dual task paradigms and other reaction time tasks. Results in Chapter 3 indicated that humans and rats perform differently in a signal change paradigm. Therefore, whether the models can account for rats' performance in these tasks is still an open question. In order to check if rats' performance can be fitted into race models, it is necessary to set more than one CHANGE conditions that with different delays. Considering the accuracy on the CHANGE condition was only slightly higher than the chance level, we will add an extra EARLY CHANGE condition relative to the previously used and now referred as LATE CHANGE condition in the current experiment. The EARLY and LATE CHANGE conditions will demonstrate how rats' performance on inhibiting invalid responses is influenced by the delay of information change.

In the current study, effects of bilateral STN lesions were examined again in rats, using an adapted version of the 2-AFC RT task as described in the previous chapters. In the new task, 50% of trials are called NO-CHANGE trials, in which the stimulus will stay on one side for 200ms; 25% of trials are called EARLY-CHANGE trials, in which the stimulus will stay on one side for 50ms and switch to the other side for another 150ms; the rest 25% trials are called LATE-CHANGE trials, in which the stimulus changes at 100ms.

Examining normal rats' performance on this modified task, should reveal how the animal is solving the task and thus provide additional information about the contribution of the STN to 'stopping' and response inhibition.

The primary goal of the current experiment was to test the ability of rats with lesions of the STN to change the response when stimulus changes and the ability to inhibit a pre-potent response bias on a new trial. Another major goal was to explore if the rats' performance on the current change paradigm could be fitted into race models which had been examined in humans.

5.2. Material and methods

5.2.1. Animals

Twenty-two male Lister hooded rats (Charles River, UK) were tested in the current study (see Chapter 2). Over the six-month study period, rats were trained in daily 30~60mins sessions between 10:00 am and 5:00 pm.

5.2.2. Apparatus

The 9-hole operant chamber (Paul Fray, UK) was used (see Chapter 2).

5.2.3. Behavioural test

The rats were trained as described in Chapter 2 and tested on a slightly modified version of the Signal Change Reaction Time Task described in Chapter 3. Instead of two trial types – CHANGE and NO-CHANGE, there were three trial types in the current study – 50% were NO-CHANGE, while the remaining 50% were 'EARLY-CHANGE' (stimulus change side after 50ms; 25%) and 'EARLY-CHANGE' (after 100ms; 25%) (see Figure 5.1).

Data collection and analysis was as described in Chapter 4.

5.2.4. Surgery and histology

As described in Chapter 2.

5.2.5. Data Analyses

Baseline performance was analysed using repeated measures ANOVA with three within-subjects factors: Previous Trial type (NO-CHANGE, EARLY- and LATE-CHANGE); Current Trial Type (NO-CHANGE, EARLY- and LATE-CHANGE); Response Side (repeated or alternated from previous trial). Effects of the STN lesions were analysed with Surgery (pre- and post-surgery) as the additional within-subjects factor and Group (control vs lesion) as the between-subjects factor. Accuracy and reaction time (RT) were used as the measurements of rats' performance and, for RT analysis, only pairs of correct trials were considered.

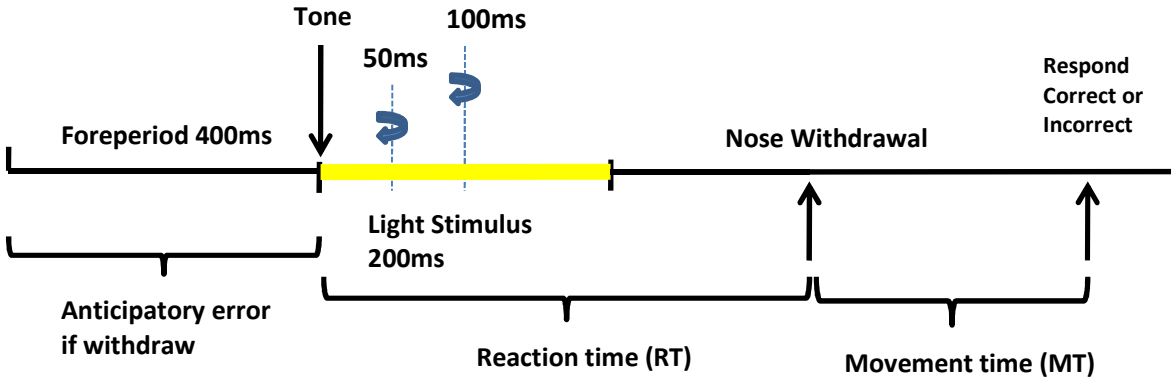

Figure 5.1 illustrates the phases of the signal change reaction time task. After a fixed foreperiod (400ms) a light stimulus appeared in either of the two side holes. The light stayed on the same side for 200ms on 50% of the trials (NO-CHANGE), changed to the opposite side after 50ms on 25% of the trials (EARLY-CHANGE) and changed to the opposite side after 100ms on the rest 25% of the trials (LATE-CHANGE).

5.3. Results

5.3.1. Histology

The lesions were verified by assessing the extent of cell loss in the STN and surrounding areas. Figure 5.2 illustrates the extent of the smallest and largest lesion and also the typical lesion. Four rats were excluded from the lesion group due to insufficient lesion. All other eight rats showed an average cell loss of ~50% of subthalamic neurons, focusing on the medial portions, with no significant damage to relevant surrounding areas, i.e. zonaincerta and cerebral peduncle. Rats with sham-lesion did not show any marked cell atrophy in the respective areas.

5.3.2. Baseline performance

5.3.2.1. Percentage of correct responses

During baseline testing, rats were highly accurate on NO-CHANGE trials (~95%), less accurate on EARLY-CHANGE trials (~86%) and least accurate on LATE-CHANGE trials (~60%) (main effect of the Current Trial Type, $F(2,30) = 296.1$, $p<0.001$, Figure 5.3). They were also more accurate when required to repeat the response from the previous trial, compared to when required the alternative response (main effect of Response Repetition, $F(1,15)= 23.9$, $p < 0.01$, Figure 5.4). This effect was particularly true when the current trial was a CHANGE trial (interaction of Response Repetition * Current Trial Type, $F(2,30) = 10.5$, $p < 0.01$). When the stimulus first indicated a repeated response but then changed side, it was extremely difficult for rats to make the correct response. Replicating the results reported in Chapters 3 and 4, the Repetition effect also interacted with Previous Trial Type (interaction of Response Repetition * Previous Trial Type, $F(2,30) = 6.3$, $p < 0.01$). Initiation of a previously inhibited response was more difficult on the following trial, while the alternate response was initiated more rapidly and more accurately. This

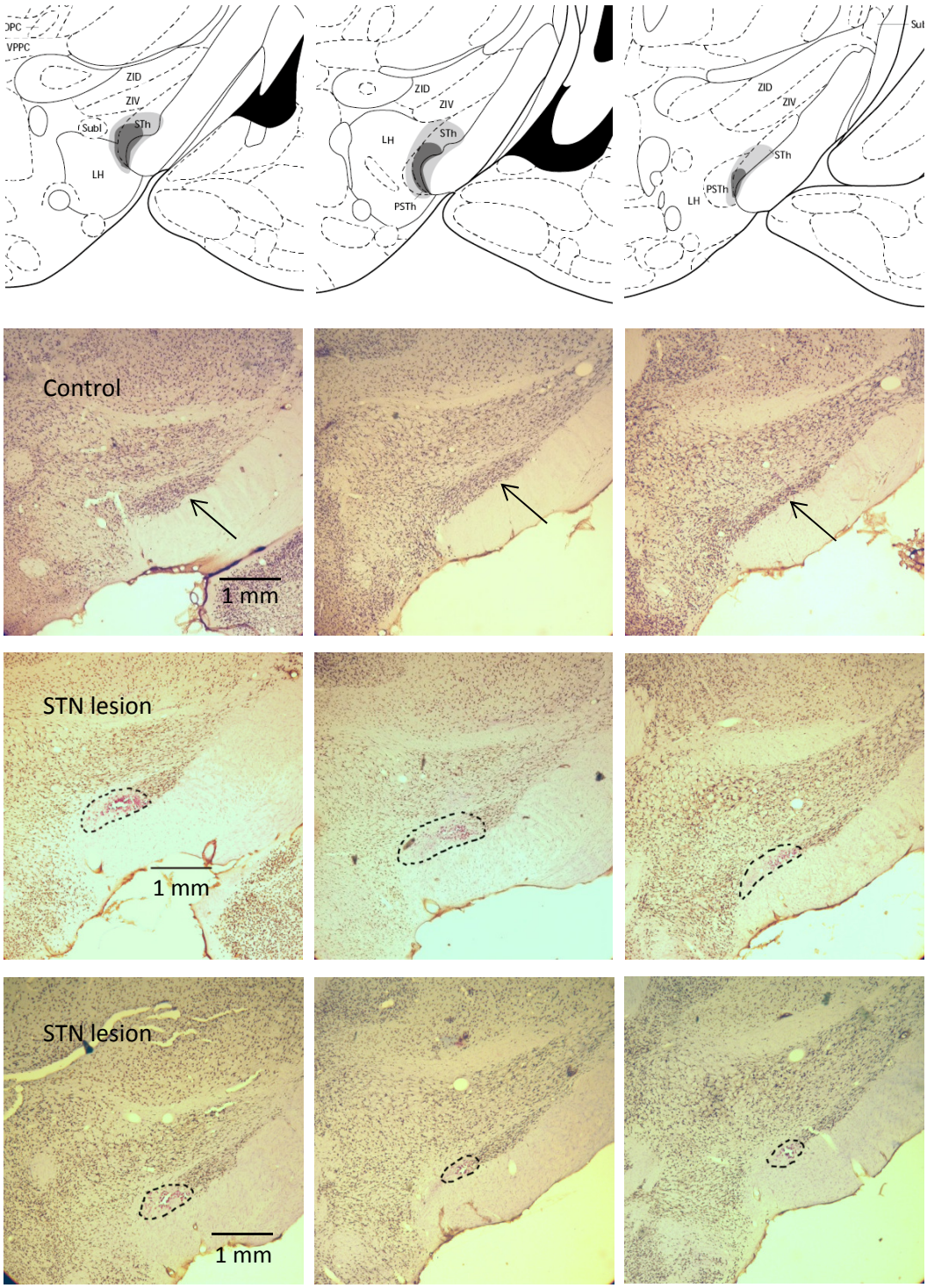

Figure 5.2 shows schematics and examples of photographs of NeuN stained control and STN lesioned rats. Top to bottom: schematics showing the minimum (dark grey) and maximum (light grey) extents of the STN lesions; photograph of a control rat brain; photograph of the largest STN lesions; and photograph of the smallest STN lesions.
STh: subthalamic nucleus; ZID: zona incerta, dorsal part; ZIV: zona incerta, ventral part; LH: lateral hypoth area; SubI: subincertal nucleus.

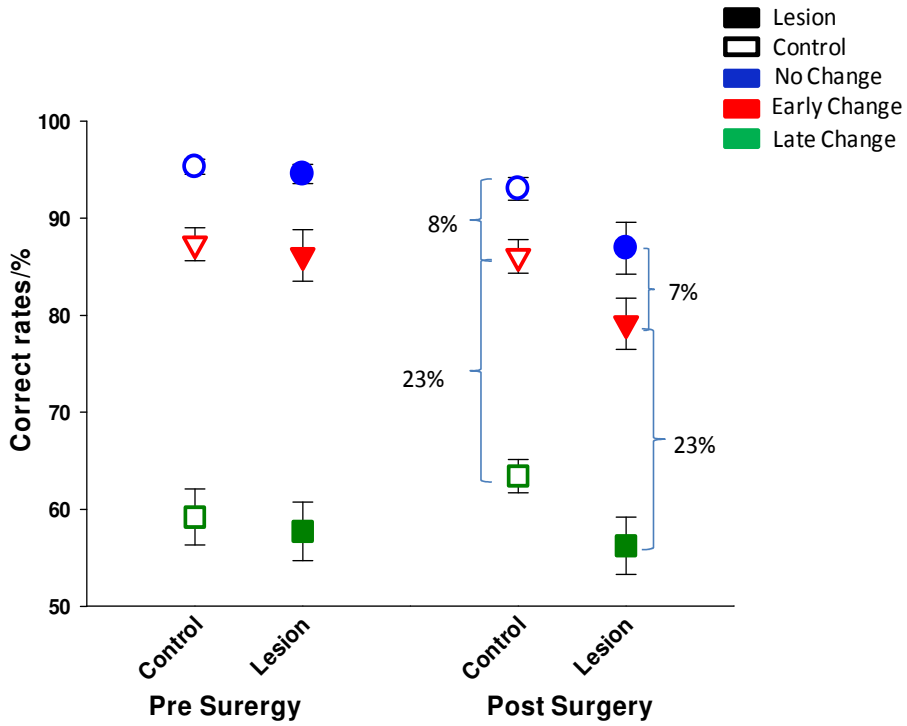

Figure 5.3. shows effects of the Signal Change on Correct Response Rates. The rats were highly accurate in the Non-change trials, a slightly less so in the EARLY-CHANGE trials, while in the EARLY-CHANGE trails were not frequently correct. The cost of the conditions remains the same in correct rates in the lesion group.

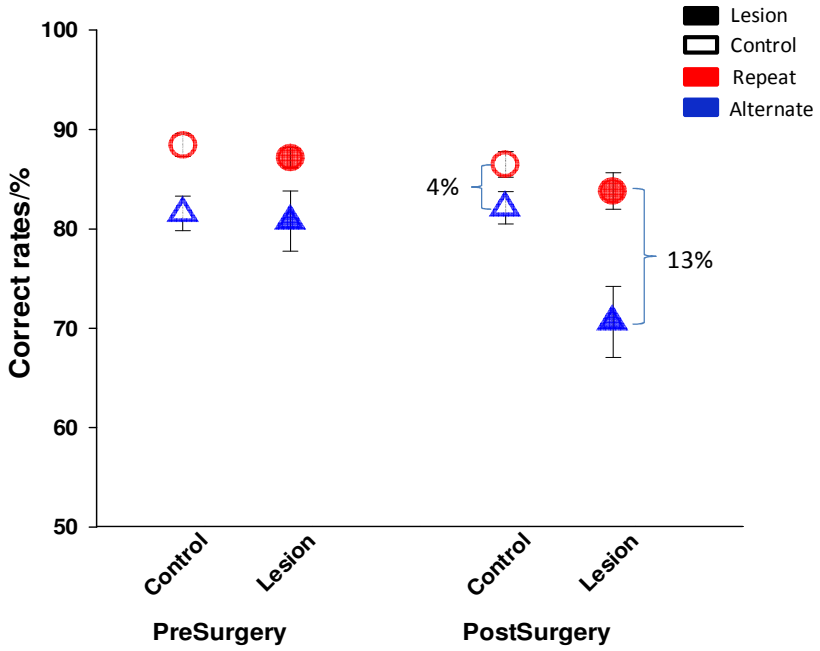

Figure 5.4. shows effects of the Response Repetition on Correct Responses Rates. Rats were more accurate when the response was repeated than alternated, and this effect was enlarged by the STN lesions. To be specific, the STN lesioned rats were much more likely to be wrong on alternations.

two-way interaction was further modified by the Current Trial Type (interaction of Response Repetition * Previous Trial Type * Current Trial Type, $F(4,60) = 3.2$, $p < 0.05$). In other words, the response repetition effect was larger when the previous trial was a CHANGE trial than when the previous trial was a NO-CHANGE trial, and it was even larger when the current trial was also a CHANGE trial. None of these effects interacted with Group, which meant the two groups were fully matched on all aspects before surgery.

5.3.2.2. Reaction times for correct responses

Reaction time for correct responses showed a complementary pattern of accuracy: normal rats performed the fastest mean RT on NO-CHANGE trials, longer for EARLY-CHANGE trials and the longest for LATE-CHANGE trials (main effect of Current Trial Type, $F(2,30) = 29.8$, $p < 0.01$, Figure 5.5). Response Repetition also showed a significant effect, with faster mean RT for repeated responses than for alternated responses (main effect of Response Repetition, $F(1,15) = 13.0$, $p < 0.01$, Figure 5.6). As with Current Trial Type, the Previous Trial Type also had an effect on RT: rats were the slowest following a LATE-CHANGE trial, faster following an EARLY-CHANGE trial and the fastest following a NO-CHANGE trial (main effect of Previous Trial Type, $F(2,30) = 3.5$, $p < 0.05$). In contrast to the effects on accuracy, there was no interaction between these factors on correct RT, although there was a trend for an interaction of Previous Trial Type and Response Repetition ($F(2,30) = 2.7$, $p = 0.08$).

5.3.2.3. Reaction times for incorrect responses

Reaction times for incorrect responses were also influenced by Current Trial Type: they were the fastest on LATE-CHANGE trials and the slowest on NO-CHANGE trials (main effect of Current Trial Type, $F(2,30) = 6.5$, $p < 0.05$). Figure 5.7 shows that reaction times for incorrect LATE-CHANGE trials and correct NO-CHANGE trials had similar distributions, which indicated the main

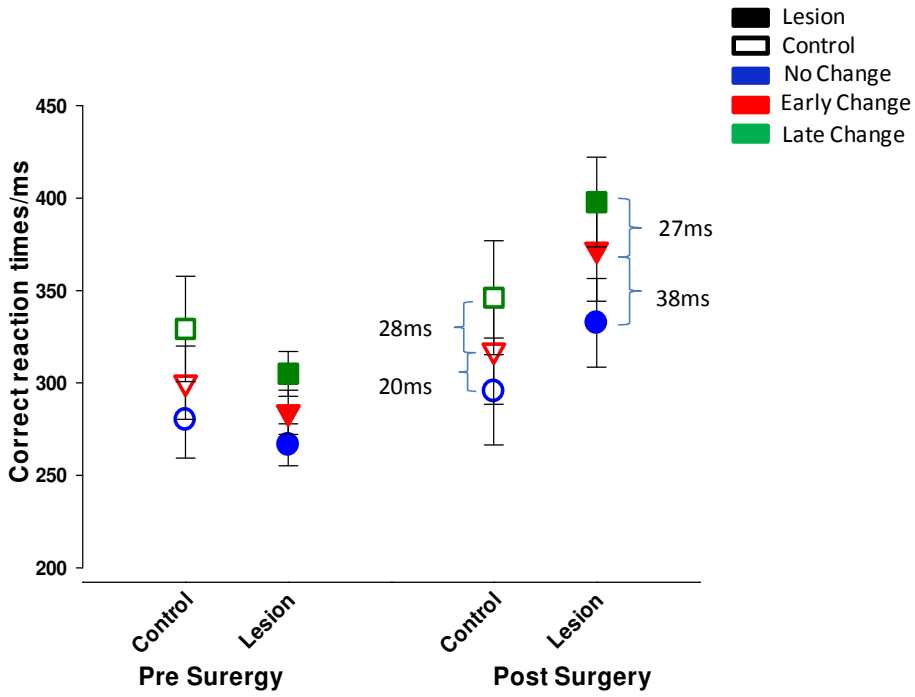

Figure 5.5. shows effects of the Signal Change on Correct Mean Reaction Times. The rats showed a complementary pattern on the reaction times, and still the costs of the conditions remained the same in the lesion group.

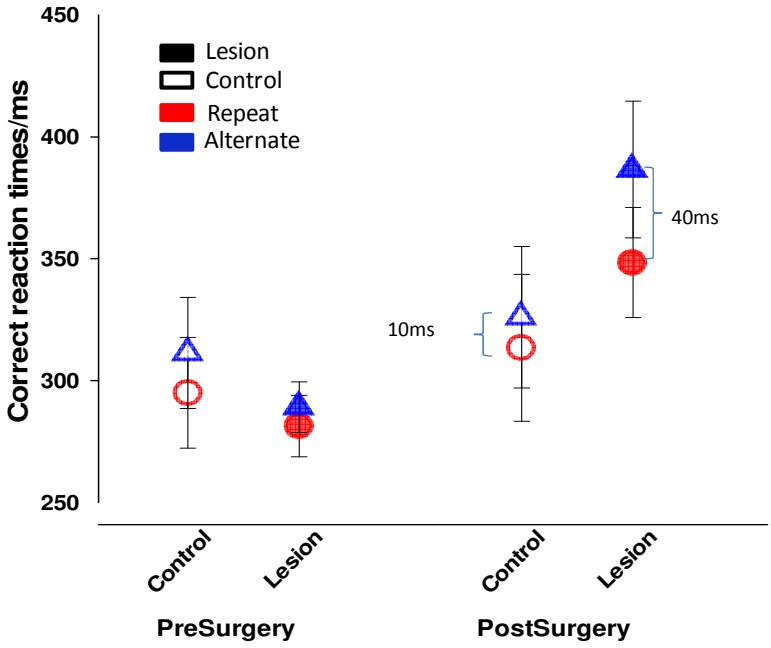

Figure 5.6. shows effects of the Response Repetition on Correct Mean Reaction Times. Rats were faster when the response was repeated than alternated and this effect was enlarged by the STN lesions.

reason for mistakes on LATE-CHANGE trials was that rats considered the trials as NO-CHANGE trials. Modal reaction times for incorrect NO-CHANGE trials were much faster than that for correct NO-CHANGE trials. However, there was a big tail in the distribution, which resulted in slower mean reaction times. The distribution of reaction times for incorrect EARLY-CHANGE trials was similar to the distribution for incorrect NO-CHANGE trials, suggesting a similar behaviour behind these two errors.

5.3.2.4. Anticipatory Errors

Overall average percentage of anticipatory errors was 10.5% for baseline performance. As reported in Chapter 4, the percentage of anticipatory errors was only affected by Previous Trial Outcome (main effect of Previous Trial Outcome, $F(3,42) = 16.7$, $p < 0.01$, Figure 5.7). Rats were most likely to make an anticipatory error when they had made one on the previous trial, and most unlikely to make this error when the previous response was correct.

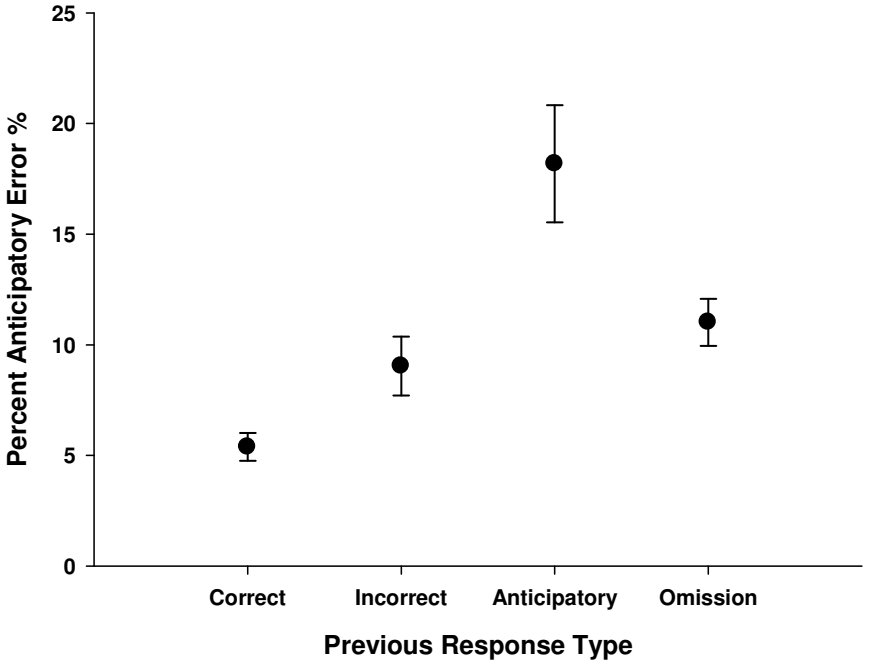

Figure 5.7. shows effect of Previous Trial Type on Anticipatory Error Rates. The rats were most likely to make an anticipatory response when they already made one in the previous trial.

5.3.3. Effect of STN lesions

5.3.3.1. Percentage of correct responses

Overall, rats with STN lesions showed a non-significant decrease in the percentage of correct responses post-surgery (Surgery * Group, $F(1,15) = 4.1$, $p = 0.06$, n.s.). As in Chapter 4, the effect of Response Repetition, but not the effect of Current Trial Type, was affected by the STN lesions (Surgery *Current Trial Type * Group, $F(2,30) < 1$, n.s. Figure 5.3; Surgery *Response Repetition * Group, $F(1,15) = 6.0$, $p < 0.05$, Figure 5.4). Additional analysis restricted to group confirmed that the effect of Response Repetition was enlarged in the STN lesioned group but not changed in the control group (simple main effect analysis of the interaction of Surgery * Response Repetition for the lesion group: corrected-$F(1,7) = 7.4$, $p < 0.05$; for the control group: corrected-$F(1,8) < 1$, n.s.). This was due to a lower accuracy for alternated responses but not any change in accuracy for repeated responses (interaction of Surgery * Group restricted to repeated responses: corrected-$F(1,15) < 1$; to alternated responses: corrected-$F(1,15) = 7.5$, $p < 0.05$). Apart from the effect of Response Repetition, STN lesions did not change other aspects of accuracy.

5.3.3.2. Reaction times for correct responses

As for accuracy, the effect of Response Repetition on RTs was changed by the lesions (interaction of Group * Surgery * Response Repetition, $F(1,15) = 9.0$, $p < 0.05$, Figure 5.6). Additional analysis restricted to groups revealed that the STN lesioned group showed a larger response repetition effect following surgery (interaction of Surgery * Response Repetition restricted to each group, for the lesioned group: corrected-$F(1,7) = 13.5$, $p < 0.05$; for the control group: corrected-$F(1,8) < 1$; n.s.). However, this was not due to a slowing of responses that alternated but neither was it due to faster repeated responses (interaction of Surgery * Group restricted to repeated responses: corrected-$F(1,15) = 2.8$, n.s.; to alternated responses: corrected-$F(1,15) = 3.7$, n.s.). The proportion of longer reaction times seemed to be greater for alternated responses in the STN lesioned rats (Figure 5.9).

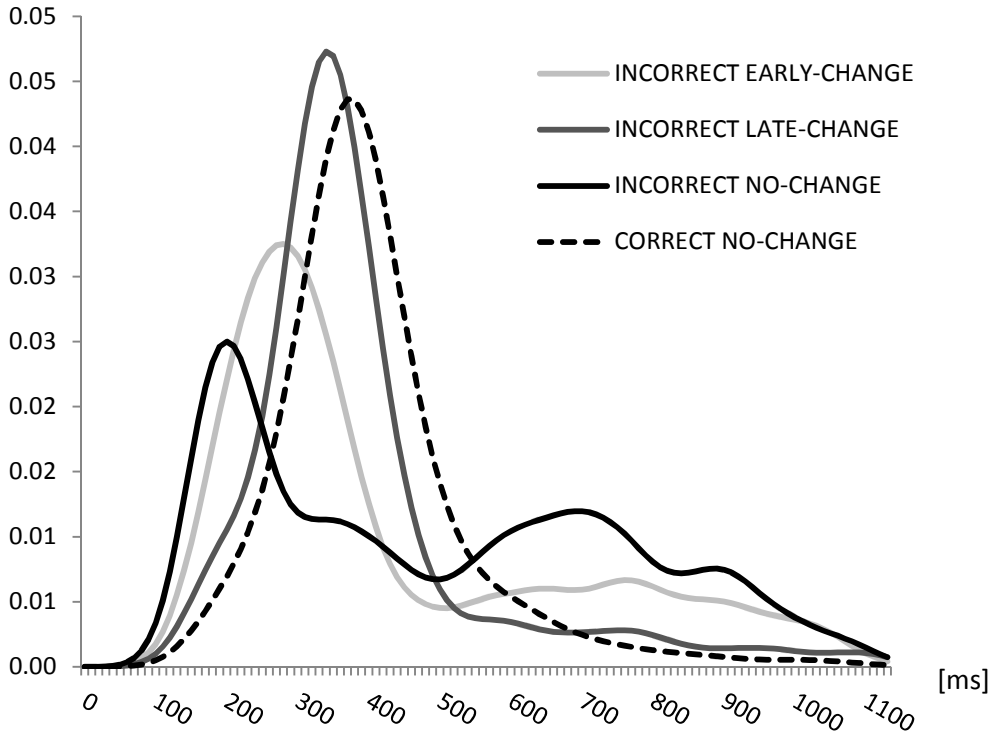

Figure 5.8 shows reaction time distributions for correct NO-CHANGE trials and incorrect NO-CHANGE, EARLY-CHANGE and LATE-CHANGE trials.

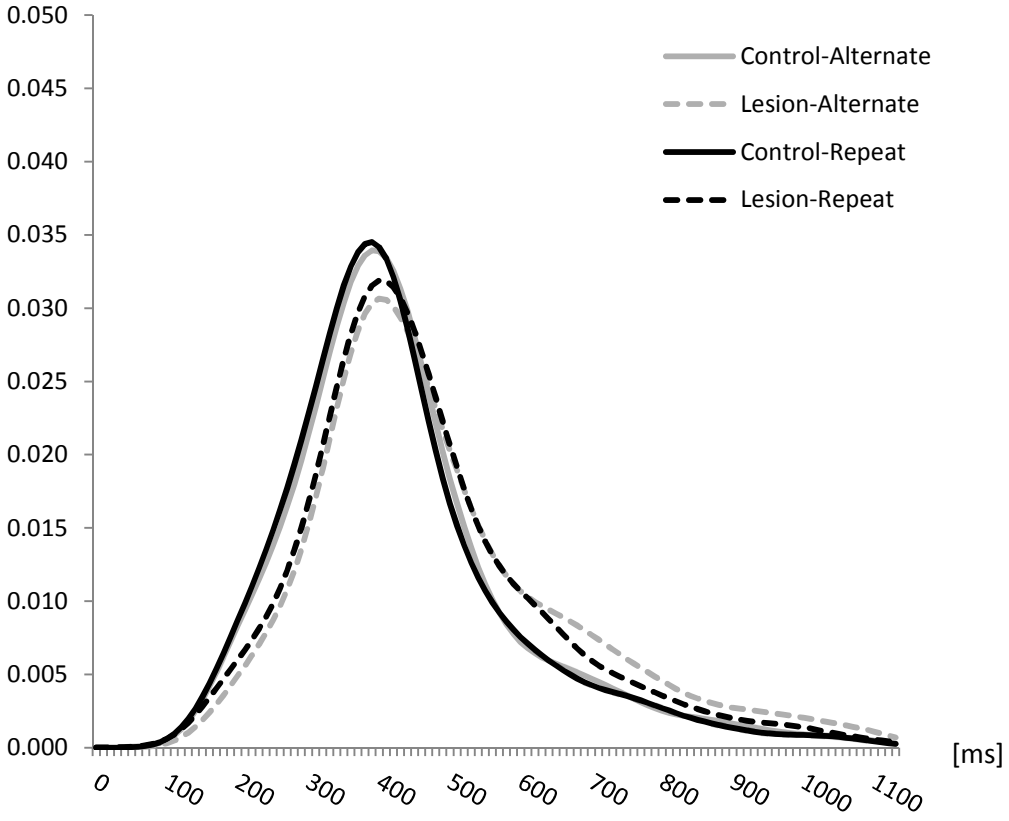

Figure 5.9 shows reaction time distributions for correct repeated and alternated responses for control and STN lesioned rats.

As for accuracy, the STN lesions did not change the effect of the Current Trial Type (interaction of Group * Surgery * Current Trial Type, $F(2,30) = 1.9$, n.s, Figure 5.5), which meant the STN lesioned rats, compared to the control rats, were not slower to inhibit an about-to-be-executed response and switch to another response when the signal changed.

Although normal rats did not show a significant interaction of the Response Repetition and the Previous Trial Type (see Chapter 3 and 4), it was modified by the STN lesions (interaction of Group * Surgery * Response Repetition * Previous Trial Type, $F(2,40) = 3.0$, $p < 0.05$). The STN lesions enlarged the interaction of the Response Repetition and the Previous Trial Type, which meant the STN lesioned rats were particularly slow to initiate a previously inhibited response.

5.3.3.3. Anticipatory Errors

The STN lesion group did not show significantly increased anticipatory errors (interaction of Group * Surgery, $F(1,15) = 2.1$, $p = 0.16$, n.s. Figure 5.10). However, the anticipatory errors seemed to happen in an earlier phase of the foreperiod in the STN lesioned rats than the control rats (interaction of Group *Surgery, $F(1,15) = 6.3$, $p < 0.05$, Figure 5.11).

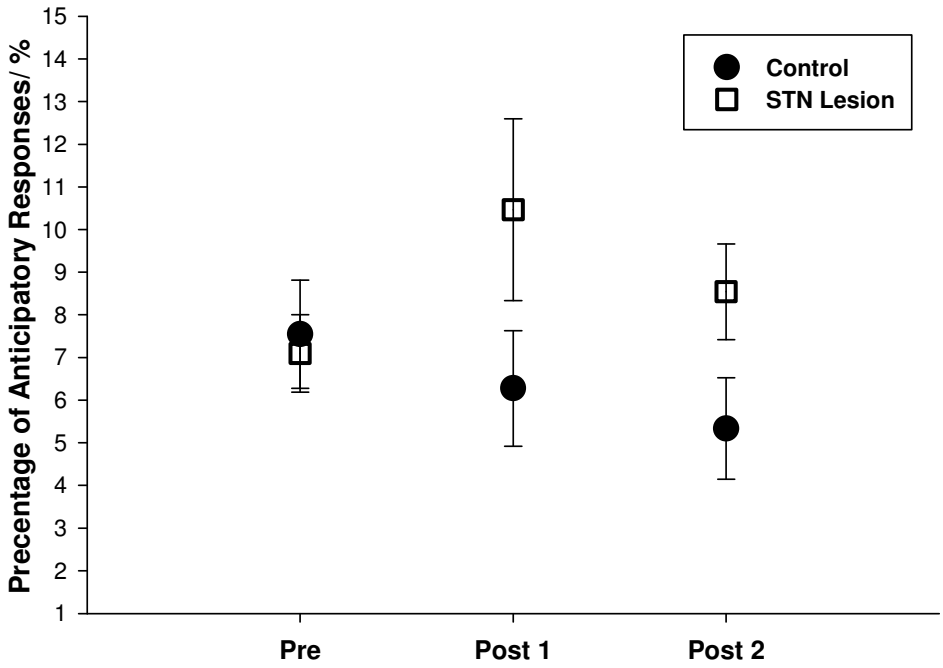

Figure 5.9. shows the percentage of anticipatory responses before surgery and two weeks after surgery. The STN lesioned rats showed a trend of increased anticipatory responses, but the change was not significant.

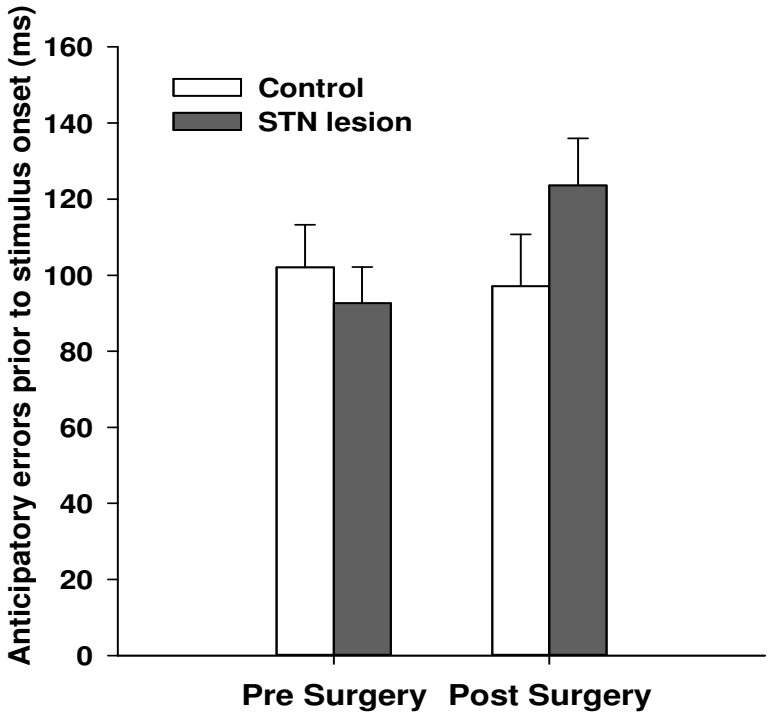

Figure 5.10 shows when the anticipatory responses happened during the foreperiod for control and STN lesioned rats. STN lesioned rats made earlier anticipatory errors than control rats.

5.4. Discussion

5.4.1. Effects of STN lesions

In the present experiment, we largely replicated the results that reported in Chapter 4. STN lesioned rats, like control rats, were slower and less accurate on CHANGE trials (both EARLY and LATE), relative to NO-CHANGE trials. The cost of LATE-CHANGE compared to EARLY-CHANGE was not enlarged by the STN lesions. The STN lesions impacted rat's performance mainly on two aspects: anticipatory responses and between-trials response alternation.

5.4.1.1. Anticipatory responses with the STN lesions

In the present study, Rats with STN lesions did not show a significant increase in anticipatory errors. As previously mentioned, it has been observed that the STN inactivation, from both lesion and DBS, could cause increased anticipatory responses, which reflects a lack of inhibitory control (Baunez et al., 1995; Phillips & Brown, 1999; Phillips & Brown, 2000). There were some exceptions observed which indicate that the increase of anticipatory errors depends on the lesion size, locations and also the task designs (Desbonnet et al., 2004; Wiener et al., 2008).

The lack of increased anticipatory errors possibly because the foreperiod used in the current task was fixed to 400ms, which made the onset of stimulus predictable. Given that an increase of anticipatory errors was found in Chapter 4 with the same foreperiod, it is perhaps more likely dorsolateral STN and surrounding areas lesions account for the deficit. In the current experiment the lesions were relatively small and concentrated on the medial part of the STN.

Although lack of a significantly higher percentage of anticipatory errors, it is noteworthy that the anticipatory errors made by the STN lesioned rats happened earlier in the fixed foreperiod. This is strong evidence that inhibiting premature response was impaired in the STN lesioned rats.

5.4.1.2. Response inhibition and re-programming within and between trials

The current task allowed us to look at the ability of rats with STN lesions to inhibit a response that is under preparation as stimulus change. It also enabled us to look at sequential effects and the influence of previous responses on current response.

It is interesting that we did not find impairment in rats with STN lesions on within trial inhibition. This contradicts the findings from SSRT, which imply that 1) the impairment on SSRT task was due to lateral STN lesions but not medial STN lesions; 2) different inhibition is required for SSRT task and the current 2-choice reaction time task.

Slower and less accurate alternated responses, relative to repeated responses, imply that normal rats have a pre-potent bias towards the previous response. Normal rats are capable of exerting some control over the pre-potent impulses, while rats with STN lesions are impaired on this inhibition. In particular, the repeated responses are not facilitated in rats with STN lesions, while the alternated responses are retarded.

The finding that STN lesions impaired between-trial response alternation but not response reprograming within a trial might imply two different types of inhibition required in these two processes. Within-trial inhibition responds to rapidly changing stimuli and usually happens before the information accumulation reaches any response threshold. Between-trial inhibition, on the other hand, is against a previously executed response and needs to last for a relatively long time (from the end of the previous trial till the response initiation on the next trial).

Our findings – impaired anticipatory error control and ability to alternate responses – suggest that the STN is necessary in inhibiting pre-potent response that is not under stimulus control.

5.4.2. Incorrect responses on the current task

Reaction time for incorrect responses can provide insight into the reasons for those mistakes. Rats make mistakes even on NO-CHANGE trials, although these trials were very simple. A majority of incorrect responses on NO-CHANGE trials have very short reaction times, suggesting these errors to be late anticipatory responses (Figure 5.8). Another considerable amount of incorrect responses have long reaction times, indicating that the rats might have missed the stimulus and made a 'guess' (Figure 5.8). Both late anticipatory responses and guesses are not under stimulus control and cannot be explained in terms of the nature of the task. Of particular notice, these situations apply to both control and STN lesioned rats.

Incorrect responses on EARLY-CHANGE trials might also be the result of two factors. One factor is that they miss the second stimulus and take EARLY-CHANGE trial as NO-CHANGE trial. The other factor is that rats respond so fast that they cannot inhibit the responses, although they realize the stimulus changed. The latter type of mistake is reflected in the long tail on the reaction time distribution: rats had slowed down but still failed to completely stop (Figure 5.8).

Compared to EARLY-CHANGE trials, incorrect responses on LATE-CHANGE trials seemed to have a simpler reason. The reaction time distribution of incorrect LATE-CHANGE trials had a similar shape as correct NO-CHANGE trials (Figure 5.8), implying the main reason for these errors was that rats had started response initiation depending on the first stimulus and were hardly influenced by the second stimulus.

Different shapes of the reaction time distribution for incorrect responses on EARLY- and LATE-CHANGE trials reveal that interference to a response is not always equal during its processing and initiation. There might be a "point of no return" for rat during response initiation, beyond which the decision is irrevocable.

5.4.3. Diffusion model and Race model

According to the diffusion model (an evidence accumulation model for two-choice tasks), starting from baseline level, information will be accumulated towards two response thresholds, whichever is reached first, the corresponding response will be executed (Ratcliff and McKoon, 2008). The amount of information needed to reach the threshold is presented by the distance from the start line to the threshold: when the distance is longer, the time required to execute the response is longer. The reaction time also depends on the speed of information accumulation: the greater the speed is, the shorter the reaction time will be. For a pre-potent response with shorter RT, either the distance to the threshold is shorter, or the information accumulating speed is greater. On the current reaction time task, instead of being reset for each trial, the start line might be left close to the threshold of the executed response. Therefore in the next trial, a repeated response will be faster than the alternated response. For rats with STN lesions, they are even slower and less accurate on alternated responses. One possibility is that the distance between the start line and the threshold for the alternated response is enlarged and the other possibility if that the speed of information accumulation is reduced. For the latter possibility, the reason might be the STN lesioned rats were less sensitive to the stimulus on the alternated location.

The diffusion model has its limitation when applied to the effect of stimulus change for the current task. In a CHANGE trial, the information is first accumulated towards one response. After the stimulus changed, the current information accumulation needs to be stopped and shift to the opposite direction. If the speed of information accumulation and the distance between the baseline and the response thresholds are consistent through a trial, then the reaction times for responses on CHANGE trials should be at least longer than the ones on NO-CHANGE trials by the length of delay between the initial and the second stimulus onset. However, the EARLY and LATE CHANGE trials when measured from the time of the stimulus change, had shorter reaction times than these observed on NO-CHANGE trials. This means the speed of information accumulation needs to be facilitated for the second stimulus or the

baseline needs to be adjusted to be closer for the second response. Neither these changes is assumed in classic diffusion model.

As mentioned in the general introduction, race models are widely used to predict and interpret responses on reaction time tasks. A previous double-step saccade production study (Camalier et al., 2007), where the target visual stimulus changes location before a saccade is made, found the performance of both humans and primates followed several regularities. Firstly, latencies for successfully compensated saccades (correct CHANGE trial), when measured from the time of the step, were shorter than these for no-step trials (correct NO-CHANGE trial). Secondly, the probability of successful compensated saccades decreased with the delay of the step (the time of stimulus change). Thirdly, the non-compensated saccades (incorrect CHANGE trials) had shorter latencies than no-step saccades. They fitted the data into three different race models and demonstrated that an independent STOP process is essential for successfully compensated saccades. Although eye saccades in human and non-human primates are different from body movement in rat on many aspects, these three regularities are also found in our current study.

However, there are also some primary differences between the current study and the saccade study. In the saccade study, the successfully compensated saccades had a common distribution (similar latencies), regardless of various stimulus change delays (Camalier et al., 2007). In contrast, in the current study, when the signal change delay increased by 50ms (EARLY- vs LATE-CHANGE), the reaction time only increased by 20ms ~ 30ms. This suggests that Stop process and/or Go2 process (the process induced by the second signal) takes shorter to finish when Go1 process (the process induced by the original signal) has started relatively earlier. In other words, the longer the subject has prepared for the original response, the faster they complete switch after they detect the changed signal. An important factor is that there are only two alternative responses in the current task, once one of them is suppressed, the other one will be activated. In other words, information processing or evidence accumulation for the two responses is not independent from each other. In contrast, in the saccade study above and also

an hand movement reaction time task (Verbruggen and Logan, 2009), there are more than two potential responses, which means an independent process regarding the new stimulus is required before the response can be initiated.

Moreover, the current results also violate a basic assumption of the race model. The race model assumes that these processes are independent from each other(Logan, Cowan, & Davis, 1984). In this case, disinhibited response (e.g. incorrect response on a CHANGE trial) should be no longer than a correct go response (e.g. correct response on a NO-CHANGE trial), because they are both executed by Go1 process (the Go process induced by the original signal). In the current task, although the reaction times for incorrect LATE-CHANGE trials were not different from the reaction times for correct NO-CHANGE trials, a considerable part of the incorrect responses on Early-CHANG trials were significantly slowed down. This indicated that the Go1 process on an EARLY-CHANGE trial was not independent from, but influenced by the subsequent processes.

In summary, although the diffusion model and the race model can partially explain the rats' performance on the current two-choice reaction time task, neither of them can be fully applied to the current results.

5.5. Conclusion

All rats made some anticipatory errors, but rats with STN lesions made such responses earlier in the fixed foreperiod. Similarly, all rats were slower and less accurate making responses when the response side alternated from the previous trial, but rats with STN lesions had increased difficulty when the side alternated. These findings suggest that inhibitory control in rats with STN lesions is compromised for pre-potent response biases not under stimulus control.

Interestingly, rats with STN lesions were equally able as control intact rats to inhibit an 'about-to-be-initiated response' signalled by a stimulus, and even to reprogram a response when a stimulus changed sides. This finding suggests

that response inhibition and initiation is normal in STN lesioned rats, once they are under stimulus control.

Effect of Bilateral STN Lesions on Attentional Flexibility in Rat

The STN receives input from prefrontal cortex, which has been implicated in shifting of attentional set. It is therefore plausible to suggest that the STN processes this information and makes a contribution to executive functions. The current experiment sought to examine the nature of this contribution. The effects of STN lesions were examined on the standard 7 stage task, and these results informed several follow up studies in which the task was modified to test specific hypotheses about the role of the STN. The results showed that rats with STN lesions do not form an attentional set on the standard 7-stage task, nor when additional ID stages are included. The deficits seem to be alleviated by modafinil, which is a cognitive enhancer.

6.1. Experiment 1

6.1.1. Introduction

To date, most of the published animal studies of the function of the STN have focused on the motor functions, although almost every review talks about the role of STN in cognitive functions (search by *'subthalamic[Title] AND (cognitive OR attention[Title/Abstract]) AND rat'* on PubMed we got 21 results, among which 13 original research articles address cognitive or attentional deficits; search by 'motor' instead, we got 157 original research articles. 6 reviews suggest cognitive or attentional deficits after inactivation of the STN in rats and all of which cite Baunez et al). The paucity of evidence could in part be due to difficulties in measuring cognition in experimental animals, especially rodents, and made particularly more difficult if the animal also has motor impairments.

Previous work in this lab also suggested that there might be STN involvement in executive attention: specifically, Phillips, Blackwell and Brown (unpublished data) found an unexpected impairment on the attentionalset-shifting task in rats with bilateral lesions of the STN. The rats were tested as a control group to verify the hypothesis that inactivation of the STN would eliminate the cognitive deficits induced by striatal dopamine depletion, while having no effect by alone. The data from this experiment is shown in Figure 6.1. As expected, the striatal lesion did result in a deficit on the set-shifting task, with performance of the first reversal significantly impaired relative to unlesioned controls. Also, as predicted, the rats with combined striatal and STN lesions did not differ significantly from controls. However, contrary to expectations, the rats with STN lesions did not perform as controls: rather, they showed increased trials to criterion on the early stages of the test and the expected ED-ID difference was not seen. One possible explanation was that the rats with STN lesions were unable to inhibit a digging response and that this motoric deficit impaired acquisition of the task. Although an explanation in terms of 'dis-inhibition' was consistent with the increase in anticipatory errors seen in the STN lesioned rats on reaction time tasks, there remained doubts.

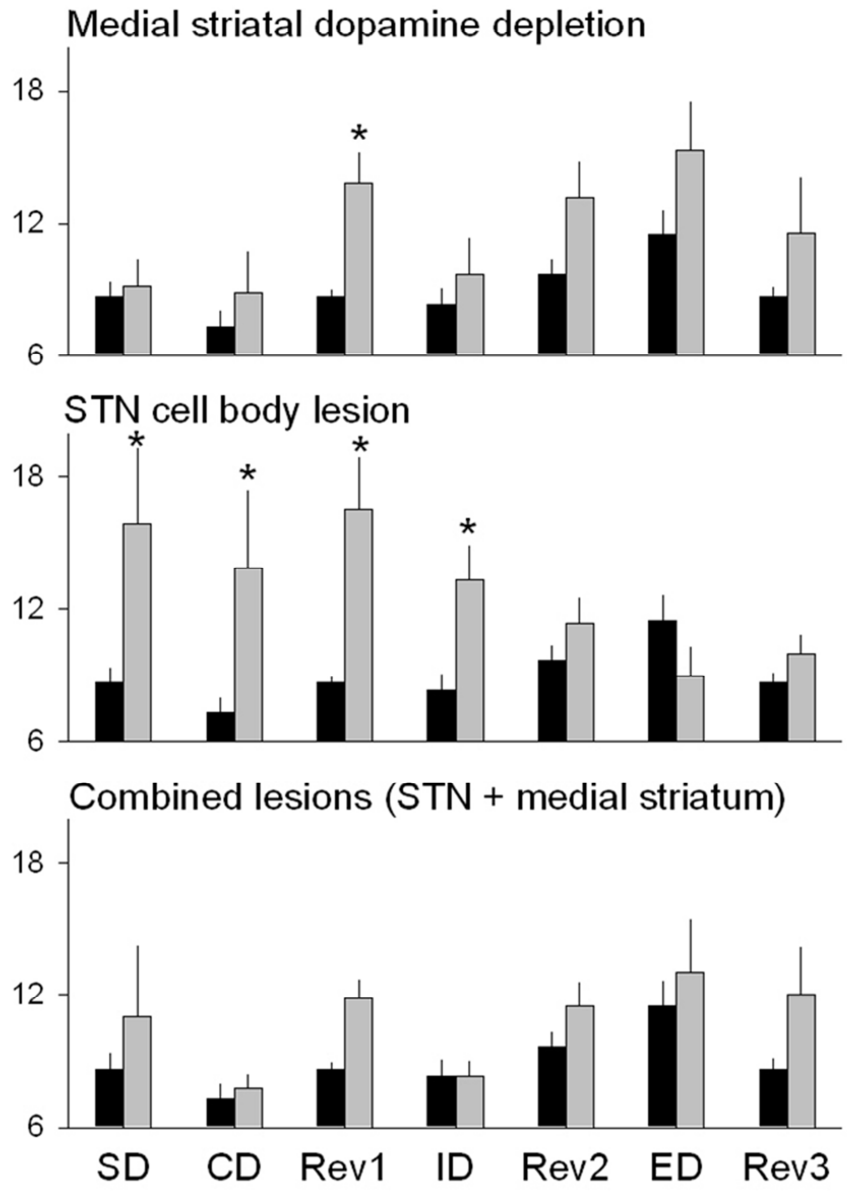

Figure 6.1 shows trials to a criterion (six consecutive correct trials) for each discrimination in the standard 7-stage attentional set-shifting task for the three lesion groups. The data from the combined control group is repeated on all three graphs.

For example, it was not obvious why dis-inhibition of the digging response would spontaneously remit within the few hours of testing on this task, while the effects of an STN lesion in an operant task persist over several weeks. At the time this experiment was performed, it was not known that performance was stable over repeated testing and so the rats were only tested once. Therefore, as the attentional set-shifting task has low motor demands compared to operant tasks, and as there was already a suggestion that performance was not 'normal', there was clearly a reason to explore the pattern of performance of STN lesioned rats in this task.

As has been discussed in the General Introduction, a functional segregation of the STN in rat has been proposed, with the medial portion implicated in cognitive function and the lateral part in motor function (Groenewegen and Berendse, 1990). The lesions in the study described above were relatively large, encompassing the whole of the STN on both sides and also included damage to some surrounding areas. Results from Chapter 5 have suggested that inhibitory deficits are dependent on both the size and the position of the lesions: if the lesion is small and medial, the increase in anticipatory errors is less significant. Therefore, the aim of the present study was to lesion the medial STN to explore the nature of any cognitive impairment, minimizing the possibility of confounding the measures with motor dis-inhibition.

Rats with bilateral medial STN lesions were tested on the standard attentional set-shifting task and also a modified version of task (4ID task, Chase et al., 2012). The primary aim was to examine the role of the STN in attentional flexibility (set-formation and shifting) and further explore the mechanism underlying any potential deficit.

6.1.2. Materials and methods

6.1.2.1. Animals

Thirty-three male, Lister hooded rats were used in the present study, with fifteen rats in the control group and eighteen rats in the STN lesion group. Nine of the control rats were from the control group in Chapter 4: when testing

had been completed in the operant task, they were tested in the set-shifting test. The other six control rats, and all eighteen lesion rats, were experimentally naïve before testing.

6.1.2.2. Surgery and Histology

Surgery and histology was as described in Chapter 2, with one exception. Diazepam was injected immediately prior to the infusion of the neurotoxin, rather than prior to surgery.

6.1.2.3. Apparatus and materials

The attentional set-shifting apparatus was constructed from large homecage, with Plexiglas panels used to divide the cage into two sections at one-third of the length and the smaller section was again divided into two sections with the same size (Figure 6.2). The digging bowls were placed in the two sections. Two removable panels (one large and one small) were used to separate the rat from either or both of the sections. The rat could be given access to the bowls by lifting the panels.

The digging bowls used here were ceramic bowls, with an internal diameter of 7cm and a depth of 4cm. Bowls were filled with a digging medium, which could be scented by an herb or a spice. A list of the digging media and odours are shown in Table 6.1. A food reward – half a Honey Loop (Kellogg Company, UK) – was buried in the centre of the bowl, at least 2cm below the surface of the medium.

Pair	Odour	Medium
1	O1 Cinnamon O2 Ginger	M1 Corse tea M2 Fine tea
2	O3 Sage O4 Paprika	M3 Sand M4 Grit
3	O5 Turmeric O6 Clove	M5 Corse sawdust M6 Fine sawdust
4	O7 Dill O8 Coriander	M7 Cigarette Filter M8 Cotton Pad
5	O11 Fenugreek O12 Tarragon	M11 Coarse Cork M12 Fine Cork
6	O13 Cumin O14 Marjoram	M13 Long Wire M14 Short Wire
7	O15 Thyme O16 Caraway	M15 Ball Bearings M16 Gravel

Table 6.1 *shows the list of all odours and digging medium used in the following experiments.*

Figure 6.2 shows one trial of attentional set-shifting task. Top: the bowls are ready and the rat waits in the holding area. Middle: the large barrier is lifted and the rat investigates the bowls. Bottom: the rat digs in one bowl and the other bowl is blocked.

6.1.2.4. Habituation and training

On the day before training, two bowls were placed in the home cage. Both bowls were filled with normal sawdust with six Honey Loops fully buried at the bottom of each. Rats could usually discover and eat all food within several hours. On the training day, rats were placed in the holding area of the testing box. Two sawdust-filled bowls were placed in the two compartments, with a half Honey-Loop on the surface of the sawdust. The panels were removed to allow rats to investigate the bowls, find and eat the rewards. In the following 4 trials, the rewards were buried in the sawdust gradually deeper until at the bottom of the bowl. If the rats did not uncover the rewards within 1mins, the compartments were closed, the bowls were restored and a new trial started.

For the next stage, the rats were given two simple discriminations (SD): one between two digging medium with no added scent and one between two odours mixed in normal sawdust. For the medium discrimination, one bowl was filled with confetti and the other with polystyrene beads: the food bait was buried in only one of these. For the odour discrimination, sawdust was scented with either mint or oregano, and the rats had to learn which one was baited. Bowls were placed one per compartment, with the side determined randomly for each trial, but there were no more than three consecutive trials with the reward on the same side. The rats had up to 1mins to uncover the reward. If the rat dug in the correct bowl, the latency from the onset of the trial to the digging was recorded and the trial was recorded as correct. The trial ended when the rat got the reward, at which point the barrier was lowered and the bowls were re-baited. If the rat dug in the incorrect bowl, the latency to dig was recorded and the trial was recorded as an incorrect. If this happened within the first four trials at each stage of the testing, the rats were still allowed to "self-correct" and obtain the reward from the correct bowl; after four trials, the incorrect digging ended the trial. Whether the rat dug into the first bowl it approached or whether it investigated both bowls before making the choice was also recorded. The criterion to learn was set as six consecutive correct trials ($p = 0.0156$), which including the first four trials.

In a typical experiment, the first test took place one or two days after training. Subsequent tests did not require re-training.

6.1.2.5. Testing paradigm– standard 7-stage task

The 7-stage task, as first described by Birrell & Brown (2000), comprises seven pairs of discriminations: one simple discrimination (SD) between two odours or two digging medium; one compound discrimination (CD) in which an irrelevant pair of stimuli was added, but with the reward associated exemplars remaining the same as for the preceding SD; one reversal (REV1), in which the stimuli remain the same as in the CD but the correct and incorrect exemplars were reversed; one intra-dimensional discrimination (ID), in which novel stimuli were used but the new correct exemplar was of the same dimension as in the previous CD; the second reversal (REV2), which followed the same rules in the REV1; an extra-dimensional discrimination (ED), in which the second novel stimuli were used and the new correct exemplar was of the other, not the previous relevant, dimension; the third reversal (REV3), where the rules of the REV1 were applied to the ED stimuli. The difference in trials between the ED and ID stage is called the "shift cost", which is an index of set-shifting ability that is independent from general learning speed.

Rats were tested three times on this task. An example of the procedure was shown in Table 6.2. The stages are always in this order while the stimuli are counterbalanced within rats and between tests. Trial outcome, response latency and investigation were recorded for each trial. Non-dig trials were excluded from the final trials to criterion measurement.

Stages	*Discriminanda*	*Mixed with*
Simple Discrimination	M1, not M2	None
Compound Discrimination	M1, not M2	O1 or O2
First Reversal	M2, not M1	O1 or O2
Intra-dimensional Discrimination	M3, not M4	O3 or O4
Second Reversal	M4, not M3	O3 or O4
Extra-dimensional Discrimination	O5, not O6	M5 or M6
Third Reversal	O6, not O5	M5 or M6

Table 6.2 *shows an example of the stages and stimuli of the standard 7-stage task. The stages are always in this order while the stimuli are counterbalanced within rats and between tests.*

6.1.2.6. Testing paradigm – 4ID task

Briefly, the task started with a SD and CD, as in the standard 7-stage task, then there were 4 IDs (ID1, ID2, ID3 and ID4), where different compound stimuli were presented with the relevant dimension remaining consistent. Therefore, on the first six stages rats were required to pay attention to only one dimension of the compound stimuli while ignoring the other. This should facilitate the formation of attentional set and the performance improvements across the four ID stages would provide a direct measurement of set-formation. After the fourth ID, rats were presented an ED stage, where the relevant

dimension switched. Finally, another ID stage (ID5) was presented to control against the possibility that any increase in the trials on the ED stage were not due to fatigue or satiety. An example of this procedure is shown in Table 6.3.

In the 7-stage task, acquisitions are followed by reversals meaning all stimuli are associated with reward at some point in the task. In the 4-ID task, this is not the case: some stimuli are never associated with reward within one testing session. For this reason, rats were planned to be tested only once on the 4-ID task.

Stages	***Discriminanda***	***Mixed with***
Simple Discrimination	M1, not M2	None
Compound Discrimination	M1, not M2	O1 or O2
First Intra-dimensional Discrimination	M3, not M4	O3 or O4
Second Intra-dimensional Discrimination	M5, not M6	O5 or O6
Third Intra-dimensional Discrimination	M7, not M8	O7 or O8
Fourth Intra-dimensional Discrimination	M11, not M12	O11 or O12
Extra-dimensional Discrimination	O13, not O14	M13 or M14
(Fifth) Intra-dimensional Discrimination	O15, not O16	M15 or M16

Table 6.3 *shows an example of the stages and stimuli of the 4 ID task. The stages are always in this order while the stimuli are counterbalanced within rats and between tests.*

6.1.3. Data analyses

Trials to criterion, errors to criterion, latency to dig, and number of non-digs were recorded on all tests. Trials to criterion and errors to criterion usually reveal the same pattern of results, but previous work has suggested that trials to criterion data are more reliable (Tait and Brown, 2007b). Trials to criterion data for the 4ID and standard 7-stage tasks were analyzed separately using repeated measures analysis of variance (ANOVA) tests (SPSS v.19). Stage and, for the 7-stage task, Test were within-subjects factors, and Group (lesion and control) was the between-subjects factor.

When significant interactions between the factors were found in the "omnibus" ANOVA tests, simple main-effects or interactions analyses were conducted with additional ANOVA tests restricted to the relevant factors and levels. The F-values were re-calculated using the appropriate error term and degrees of freedom from the omnibus ANOVA (Winer, 1971). In addition, Hyunh-Feldt corrections were applied when the assumption of sphericity was rejected.

6.1.4. Results

6.1.4.1. Surgery and histology

All 18 rats in the lesion group were observed to be chewing within the 2 hours after surgery, which we have previously found to indicate successfully placed lesions.

The lesions were verified by assessing the extent of cell loss (anti-NeuN staining) in the STN and surrounding areas. Figure 6.3 illustrates the extent of the smallest and largest lesion and also the typical lesion. Possibly because diazepam was given immediately prior to infusion of the toxin, rather than prior to surgery, lesions in the current experiment were much smaller than the ones in Chapter 4 and 5. Eight rats had lesions of the medial STN, and in all cases there was also evidence of damage and calcium deposits in MGP. Four rats showed similar damage in MGP but the STN appeared to be intact. The remaining six rats did not show any visible damage. The eight rats with STN

lesions had an average cell loss of ~30% of subthalamic neurons, and in all case the damage was focussed on the medial portion of the STN, with no significant damage to surrounding areas, i.e. the zonaincerta and cerebral peduncle. Rats with sham-lesions did not show any marked cell atrophy in the respective areas. The tissue was also stained for Tyrosine Hydroxylase (TH), parvalbumin and Glial fibrillary acidic protein (GFAP) in case it was possible to visualize any other damage, however, these stains did not provide additional information over that indicated by NeuN staining.

The limited visible brain damage was surprising, given the chewing behaviour that is generally a reliable sign of a successful lesion and which is not seen in control rats. This chewing behaviour clearly suggests the toxin was infused in the intended location and was exerting an action, although lesions could not be visualized. All the reported analyses in this chapter were first done with the data from all rats included, and then again with only the data from the eight rats with visible STN lesions. Interestingly, the same behavioural effects were seen in the entire group as were seen in the restricted group. This consistency again suggests that the rats without detectable lesions nevertheless may have had compromised STN function.

In the absence of histological confirmation of the lesions, however, the results reported here include only the data from the eight rats with verified lesions.

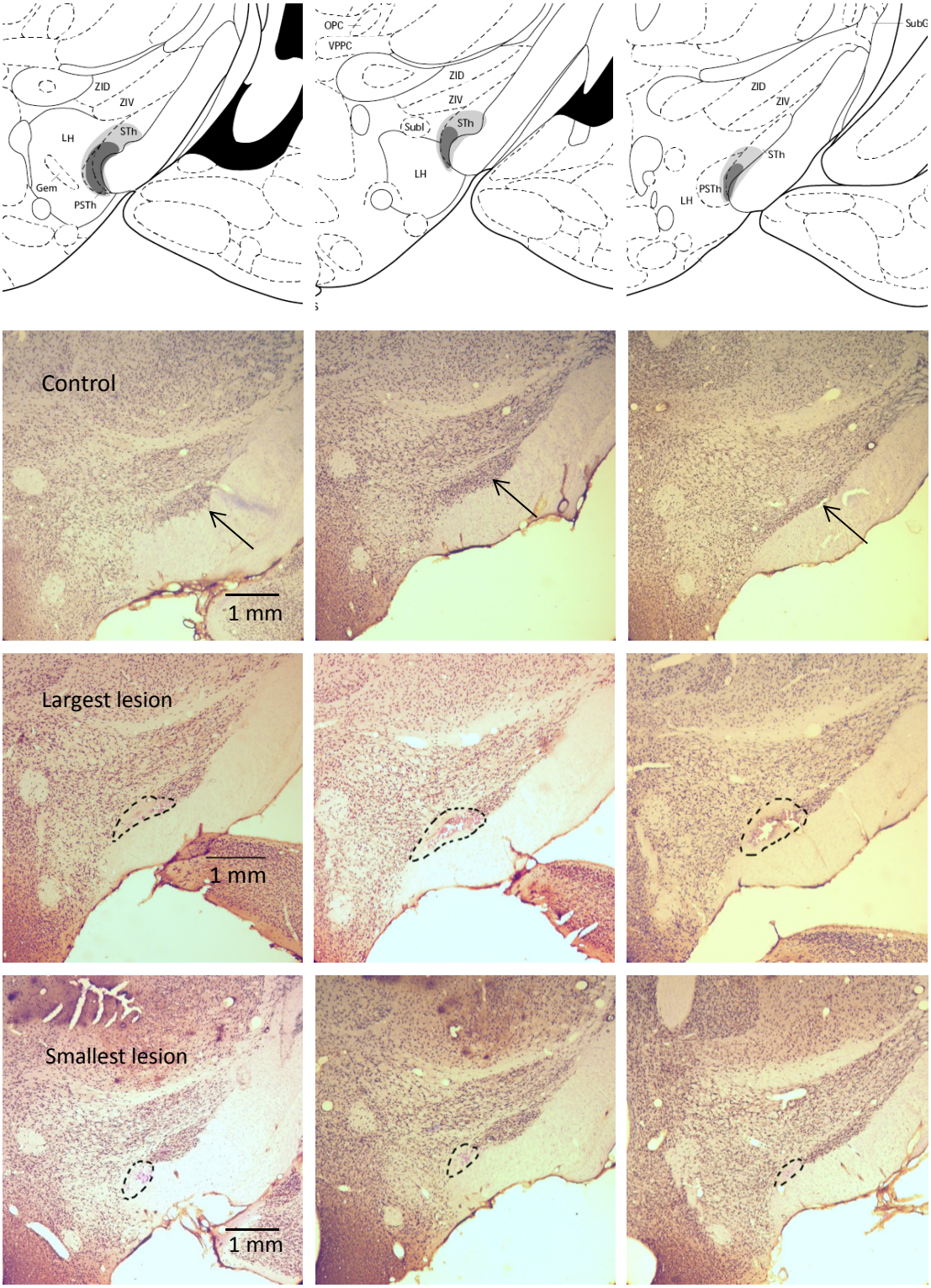

Figure 6.2 shows schematics and examples of photographs of NeuN stained control and STN lesioned rats. Top to bottom: schematics showing the minimum (dark grey) and maximum (light grey) extents of the STN lesions; photograph of a control rat brain; photograph of the largest STN lesions; and photograph of the smallest STN lesions.
STh: subthalamic nucleus; ZID: zona incerta, dorsal part; ZIV: zona incerta, ventral part; LH: lateral hypoth area; SubI: subincertal nucleus.

6.1.4.2. Effects of repeated testing

By the third of the three post-operative tests of the 7-stage task, rats performed with significantly fewer trials to criterion overall (main effect of Test, $F(2,42) = 6.8$, $p<0.05$; post-hoc LSD test: mean number of trials to criterion for Test 1 was 13.7 and for Test 2 was 13.4, compared to 11.4 for Test 3; Figure 6.4). This improvement was seen in both groups, although the control rats performed better on the second and third test while the lesioned rats improved only on the third test (interaction of Test * Group, $F(2,42) = 4.2$, $p < 0.05$). The improvement was irrespective of any particular stage (interaction of Test * Stage * Group, $F(12,252) = 1.3$, n.s.).

6.1.4.3. ID-ED shift costs

Performance of rats with bilateral STN lesions was different to controls on several stages of 7-stage task (interaction of Stage *Group, $F(6,126)= 2.3$, $p < 0.05$, Figure 6.4, 6.5;). Additional analysis showed that, unlike controls, rats with STN lesions failed to show a difference in trials to criterion on the ED compared to the ID stage (ANOVA restricted to ID and ED: interaction of Stage * Group, corrected-$F(1,21) = 1.1$, $p < 0.01$; main effect of Stage restricted to Control: corrected-$F(1,14) = 41.7$, $p < 0.01$; to STN lesion: corrected-$F(1,7) <1$, n.s.). As seen in Figure 6.5, the STN lesioned rats required more trials to reach the criterion at the ID stage and fewer trials at the ED stage relative to control rats, although the difference restricted to each stage was not statistically significant (main effect of Group restricted to ID: corrected-$F(1,21) = 2.8$, n.s.; to ED: corrected-$F(1,21) = 3.9$, $p = 0.09$).

In the 4ID task, the control and lesioned rats also performed differently on several stages (interaction of Stage * Group, $F(7, 147) = 2.6$, $p < 0.05$, Figure 6.6). Control rats gradually performed better over four ID stages, while rats with STN lesions did not show this improvement over multiple ID stages so

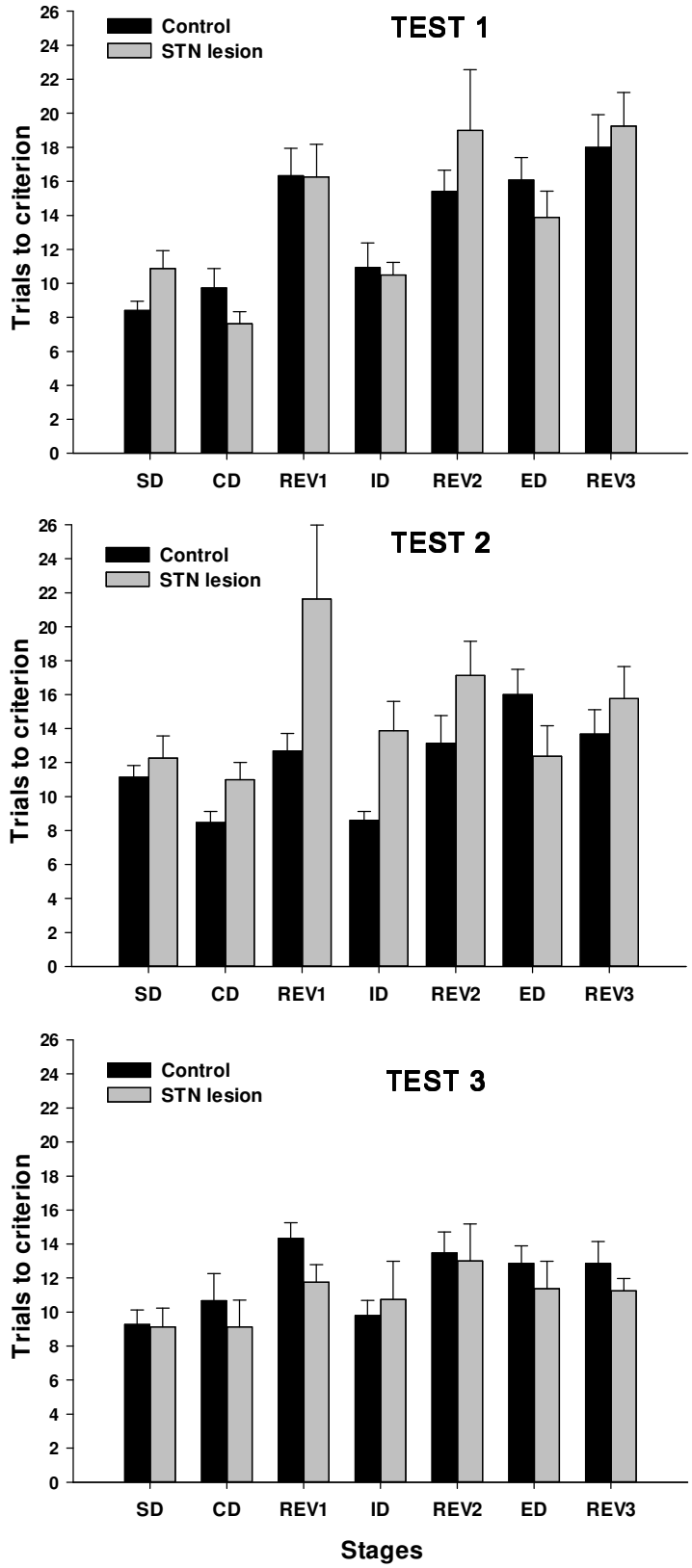

Figure 6.4 shows mean trials to criterion (+SEM) for the STN lesioned and the control rats on three tests of the standard 7-stage task. The control rats showed significant ID-ED difference, while the STN lesioned rats did not.

that ID4 was performed with significantly greater number of trials to criterion (main effect of Group restricted to ID1: $F(1,21) = 1.5$; ID2: $F(1,21) = 2.3$, ID3: $F(1,21) < 1$, all n.s. ID4: $F(1,21) = 13.1$, $p < 0.01$). As for the 7-stage task, STN lesioned rats again failed to demonstrate a positive shift-cost from ID4 to ED (ANOVA restricted to ID4 and ED: interaction of Stage * Group, corrected-$F(1,21) = 6.6$, $p < 0.01$).

Figure 6.7 shows the shift-costs for each group on the two tasks. Clearly, the control rats demonstrated a positive cost of shifting, while the lesion rats were absent of positive shift-costs. Together with the worse performance on ID stages, it implied that the STN lesioned rats had not formed an attentional set during the testing.

6.1.4.4. Pattern of errors on ED

When a rat has formed an attentional set, it would be expected that, when presented with new exemplars, they would first respond to the previously attended dimension, trying both exemplars in turn before considering exemplars in the unattended dimension. This would mean that, having discovered which is the correct bowl from one pair, they would 'follow' the wrong dimension (concluding that it was the irrelevant exemplar that was predicting the location of the food) in the other pair of bowls. Because trial order was fixed, the first two trials were of a single pair of bowls, with the correct bowl first in one location and then in another. On the 3rd trial, the second pair of bowl was presented. We therefore examined the choice on the 3rd trial of the ED stage (see Table 6.4). If a set has been formed, the probability of an error on the 3rd trial of an ED would be significantly higher than 50%. Unfortunately, neither the control group (mean percentage of error = 44%) nor the lesion group (mean percentage of error = 52%) were more likely than chance to make an error on this trial.

Table 6.4 also displays rat's investigation behaviour before a bowl digging. It seems like the control rats prefer to dig into the first bowl they came up to, while the STN lesion rats were more likely to dig after they had investigated both bowls. The percentage of both-bowl-investigation on the 3rd and 4th trial

of ED stage was compared between the two groups. The results showed that this percentage was not different between the two trials, but was higher in the lesion group than the control group (main effect of Group, $F(1,21) = 18.6$, $p < 0.05$;). Theoretically, this difference should also be found on ID stage. However, when comparing the 3rd and 4th trial of ID stages (data not shown), the two groups were not different (main effect of Group, $F(1,21) < 1$).

Group	Rat	Test1	Test2	Test3	4ID
Control	126	X			X
Control	127	X	X		X
Control	128		X	X	X
Control	129	X		X	X
Control	130	X		X	X
Control	131	X	X		
Control	362	X			X
Control	363		X		
Control	364	X			
Control	366	X		X	
Control	369			X	
Control	377				
Control	382				
Control	385		X		
Control	387				X
STN	96			X	
STN	97			X	
STN	98	X	X	X	X
STN	99	X			
STN	107	X	X		X
STN	111		X	X	X
STN	113	X		X	
STN	114	X	X	X	

Table 6. 4 *shows the outcome of the third trial of the ED stage for 3 tests of the 7-stage task and the 4 ID task. Red means error after investigation of both bowls and green means correct after investigation of both bowls.*

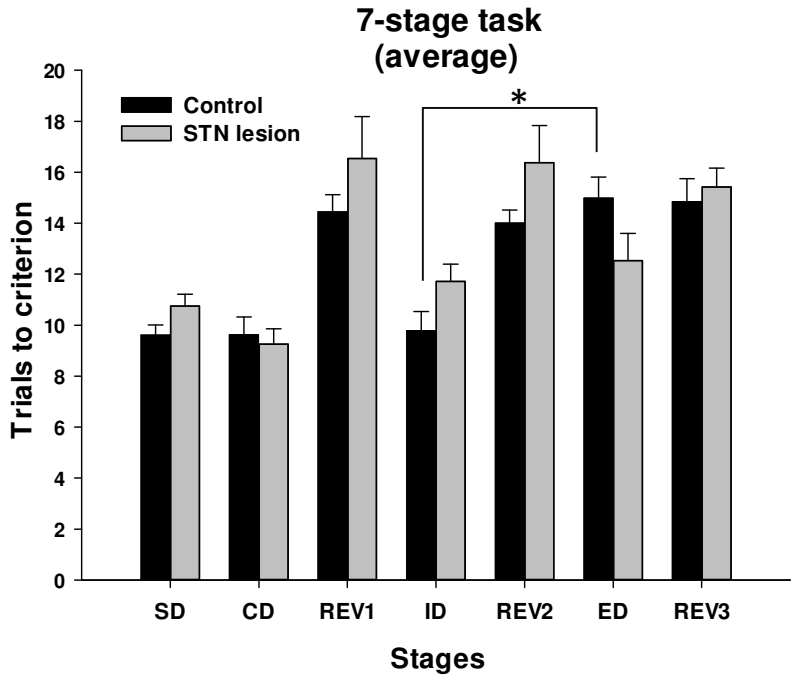

Figure 6.5 shows the average performance of the STN lesioned and control rats on the standard 7-stage task. The control rats showed significant ID-ED difference, while the STN lesioned rats did not.

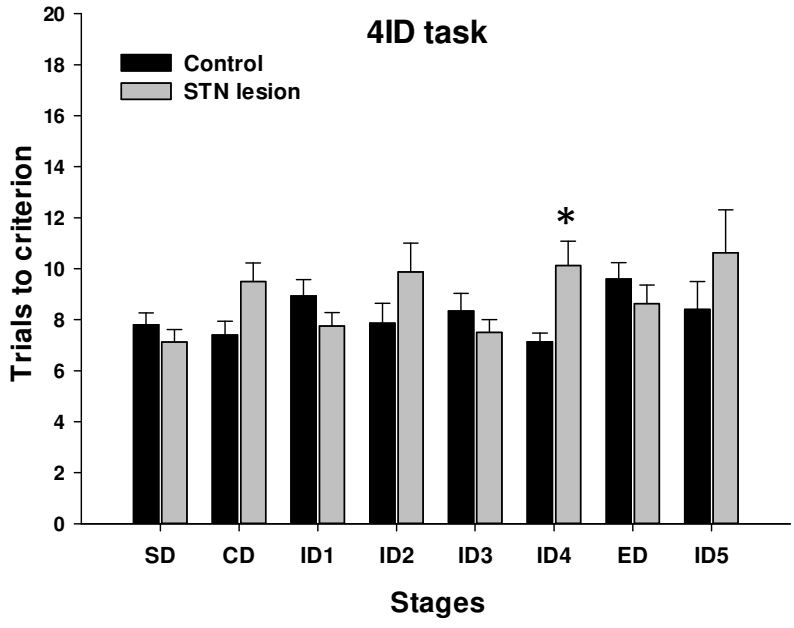

Figure 6.6 shows mean trials to criterion (+SEM) for STN lesioned and control rats on the 4ID task. STN lesioned rats made significantly more mistakes than control rats on ID4.

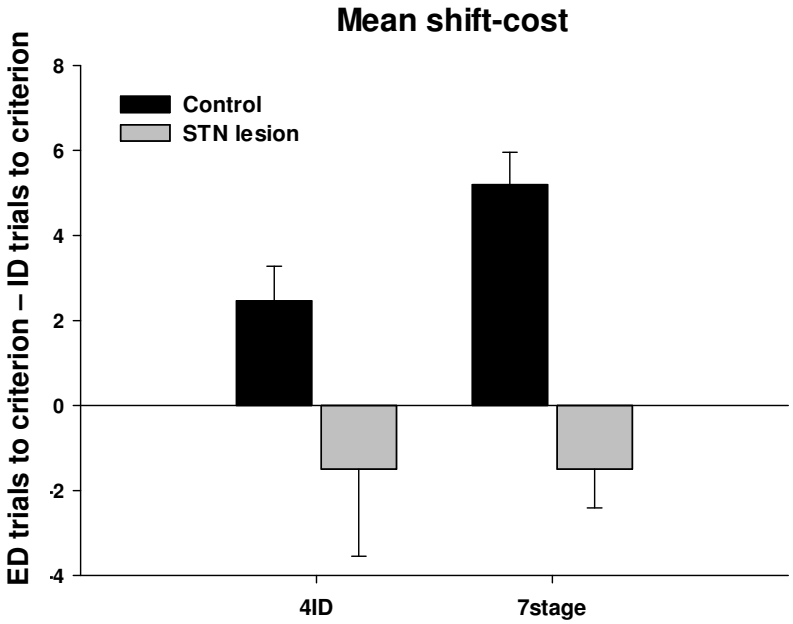

Figure 6.7 shows the shift-costs on the 7-stage and 4ID task for the STN lesioned and control rats. The control rats showed positive ID-ED shift cost on both tasks, while the STN lesioned rats did not on either task.

6.1.4.5. Reversal learning

Rats with bilateral STN lesions performed as well as control rats on all three reversal stages of the 7-stage task (analysis of trials to criterion: main effect of Group restricted to each reversal stage: REV1: corrected-$F(1,21) = 1.4$; REV2: corrected-$F(1,31) = 1.8$; REV3: corrected-$F(1,21) < 1$, all n.s.).

In addition to looking at overall performance, an analysis of type of errors was conducted. Confirming the trials to criterion data, the STN lesioned rats did not make more errors overall than control (main effect of Group, n.s.; interaction of Group * Stage, n.s.). Furthermore, there was no evidence to suggest that the STN lesioned rats made different kinds of errors – for example, they did not make relatively more or fewer perseverative-type errors (continuous digs into the previously baited bowls) or aborted (non-dig) trials prior to a first correct choice.

6.1.5. Discussion

The present study showed that on the 7-stage task, the STN lesioned rats required more trials to learn the ID discrimination and consequently lacked the positive ID-ED shift cost seen in controls. The absence of shift-cost and higher ID stage suggested that the STN lesioned rats were impaired in forming an attentional set. In a previous study, impaired set-formation following lesions of orbital frontal cortex was ameliorated by using multiple ID stages: trials to criterion reduced by a fourth ID and a subsequent ED revealed a positive shift-cost indicative of an attentional set (Chase et al., 2012b). However, in the present study, even four IDs were not sufficient to demonstrate set-formation.

The current experiments did not replicate what Phillips found in the rats whole STN lesions. This difference is possibly due to the size and position of lesions. Although the lesions were very limited in the current experiments, the behaviours were consistent with previous attempts in our lab. This consistence suggests that the medial STN lesions consistently cause impairment in attentional set formation.

The observations from both 7-stages task and 4ID task confirm an important role of the STN underlying one or several cognitive processes involved in attentional set formation. However, the specific deficits induced by the STN lesions that lead to this impairment still remain elusive. For example, even though they failed to form an attentional set after 4 IDs, which was sufficient for rats with lesions of orbital frontal cortex, it is not known whether more ID stages would have been effective. In Experiment 2, we designed a new protocol to simultaneously test several hypotheses to account for specific deficit(s).

6.2. Experiment 2

6.2.1. Introduction

It is possible that there were insufficient stages in either the 7-stage or 4ID task for the rats to form set, but that more stages might be sufficient. Therefore, in this experiment the number of stages was increased again – to 11. In addition, a number of hypotheses about the possible nature of the deficit were tested.

While set-shifting indicates behaviour flexibility, set formation indexes behaviour stability. In terms of set-formation, animals need to learn the valid stimulus-reward relationships, discover and remember the commonalities over trials and stages, and consistently use the learned rule to guide their behaviour in the future. Memory and attention are two keywords in this complex process, either or both of which might be impaired in STN lesioned rats.

Since rats with STN lesions performed simple discriminations as well as control rats, their immediate working memory appears to be intact. However, the deficits could reflect a disruption of short-term memory, such that the rats fail to use information from previous discriminations when solving subsequent discriminations. The positive shift cost seen at the ED stage comes about only because the animal has previous experience of responding to another stimulus feature: if this previous experience is not 'remembered', there will be neither benefit at a subsequent ID stage nor cost at a subsequent ED. The first hypothesis therefore, was that the STN-lesioned animal was solving each stage as if it had no prior information available: as if when the stimuli changed, prior knowledge was reset. To test this idea, we compared the effects of inserting either one or three intervening stages before testing reversal learning. We reasoned that there would be no reversal costs if the prior learning no longer influenced their behaviour.

The ability to attend selectively to distinct elements of complex stimuli is also essential to demonstrate a positive shift cost in the ID-ED task. Animals learn which elements, or dimensions, of a stimulus are relevant and focus

attention on these and away from irrelevant elements. Once animals have learned which dimension/stimulus is relevant (i.e. set has been formed), and they are selectively attending to this, they should not be distracted by any inconsequential changes to the stimuli in the irrelevant dimension. If there is impairment in selective attention, changes to stimuli in the irrelevant dimension would be more salient and potentially distracting. For example, it might be that the rats with STN lesions learn the specific bowls (a combination of all information, e.g. odour, media, depth of bowl, etc.) that are baited, rather than which exemplar within one dimension is reward-related. If this were the case, we reasoned that changing the irrelevant feature of the bowl would change the behaviour of a rat whose attention is not focussed on the relevant exemplar. By contrast, a rat learning a compound discrimination without selective attention to stimulus dimensions would have an advantage if required to learn a bi-conditional discrimination, in which the specific element associated with reward (for example, the odour) depends upon another element (for example, the medium). In this case, we expected that rats with medial STN lesions would show better performance than control rats.

With these manipulations, we tested control and STN lesioned rats on the hypotheses that if STN lesions cause memory or attentional impairments in rats which prevent them from forming attentional set.

6.2.2. Methods

6.2.2.1. Behavioural testing

The new task comprised 11 stages, which include multiple ID discriminations, as well as reversals and an extra-dimensional shift.

The new task starts with a compound discrimination (CD), followed by two intra-dimensional discriminations (ID1 and ID2). Then there is a reversal of ID1 (ID1REV), another two intra-dimensional discriminations (ID3 and ID4), a reversal of ID2 stage (ID2REV), one more intra-dimensional discrimination (ID5) and its distraction probe stage (ID5probe), and then an extra-dimensional discrimination (ED). A SD is put at the end of the task to clarify

that any increase in trial numbers is due to the task design but not the issues of fatigue or satiety. The complete procedure is presented in Table 6.5.

After all rats were tested in the task described above, on a different day, they were tested on a single bi-conditional discrimination, where both the media and the odour were relevant to finding the bait. For instance, a given pair of bowls will contain the same media, but different odours, and the media indicates which odour is baited. For example, if both bowls contain sand, the rat should dig in the one smelling of paprika and not sage, but if both bowls contain grit, then the baited bowl will be sage. Thus the correct odour depends upon the media and so both dimensions are relevant and forming an attentional set to one or other dimension will retard learning.

8 pairs of stimuli were required, so one novel pair was added to the 7 pairs used in the 4ID task. Since in the 4ID task, some stimuli are never associated with reward, prior to testing, the rats were pre-exposed to all stimuli and allowed to dig in them to recover reward. Rats were presented twice with each of the 8 pairs of (unscented) digging media and each of the 8 pairs of odours (mixed in sawdust) and left with the bowls until they had recovered the bait from both of the bowls.

6.2.2.2. Animals

Although all of the lesioned rats were tested, data was only analysed from the eight rats with verified lesions of the STN and six of the control rats. The time interval between the last 4ID testing and the current testing was about three weeks for each rat.

6.2.2.3. Data analyses

As for Experiment 1, trials to criterion were used as measurement of performance. Repeated measures ANOVA were done with a within-subjects factor of Stage (11 levels) and a between-subjects factor of Group. Simple main effects analysis was performed to test the origin or interactions.

Stages	*Discriminanda*	*Mixed with*
Compound Discrimination	M1, not M2	O1 or O2
First Intra-dimensional Discrimination	M3, not M4	O3 or O4
Second Intra-dimensional Discrimination	M5, not M6	O5 or O6
First Reversal	M4, not M3	O3 or O4
Third Intra-dimensional Discrimination	M7, not M8	O7 or O8
Fourth Intra-dimensional Discrimination	M11, not M12	O11 or O12
Second Reversal	M6, not M5	O5 or O6
Fifth Intra-dimensional Discrimination	M13, not M14	O13 or O14
Distractor Probe Stage	M13, not M14	O17 or O18
Extra-dimensional Discrimination	O15, not O16	M15 or M16
Simple Discrimination	M1, not M2	None

Table 6. 5 *shows an example of the stages and stimuli of the 11-stage task. The stages are always in this order while the stimuli are counterbalanced within rats and between tests.*

6.2.3. Results

6.2.3.1. ID-ED shift cost

Rats with STN lesions were different to controls at several stages of the task (interaction of Stage * Group, $F(11,132) = 2.7$, $p < 0.05$, Figure 6.8). Additional analyses showed that the STN lesioned rats required more trials to reach criterion than the control rats on all five ID stages (additional ANOVA restricted to the five ID stages: main effect of Group, corrected-$F(1,12) = 10.7$, $p < 0.05$; interaction of Stage * Group, corrected-$F(4,48) < 1$, n.s.; Figure 6.9).

As in the 7-stage task, rats with STN lesions failed to show a positive shift cost (i.e., ED-ID5); however, the control group also did not show a strong ID5-ED difference (ANOVA restricted to ID5 and ED: interaction of Group * Stage, corrected-$F(1,12) = 3.9$, $p = 0.07$; main effect of Stage, corrected-$F(1,12) < 1$). By looking at individual data, we found that 4 out of 6 control rats showed a positive ID5-ED shift cost, therefore the absence of significant ID5/ED difference was possibly due to small sample size.

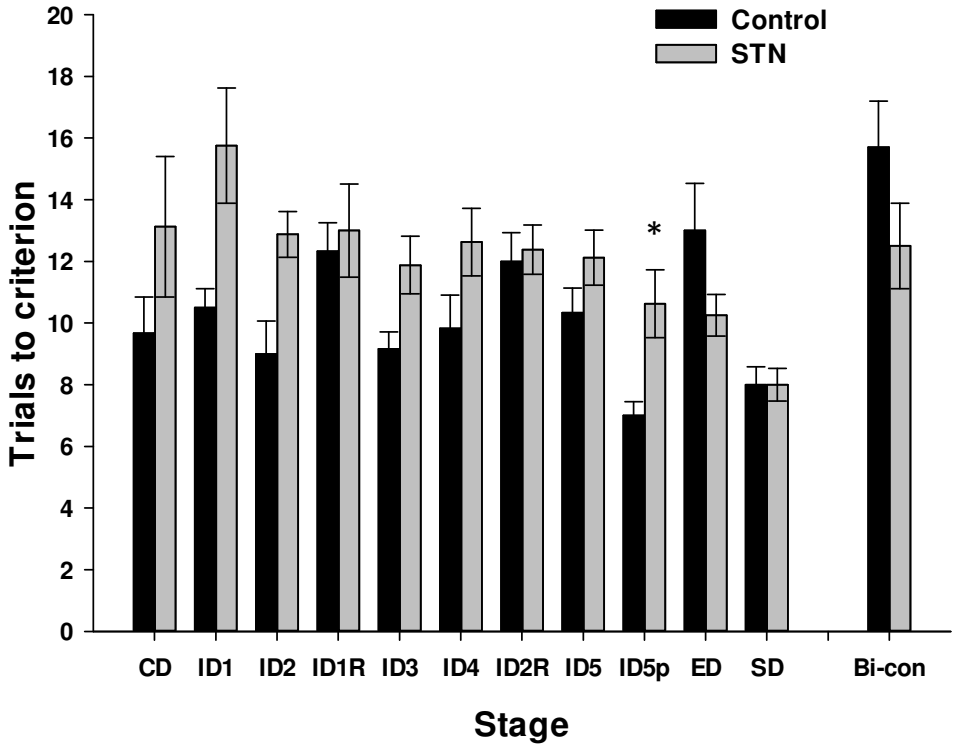

Figure 6.8 shows mean trials to criterion (+SEM) for STN lesioned and control rats on 11-stage task and bi-conditional discrimination. STN lesioned rats did not take more trials on the reversal stages, but took more trials on the probe stage.

6.2.3.2. Reversal cost and pattern of errors

As anticipated, the control group required more trials to reach criterion on the reversal stages comparing to the original discrimination stages. By contrast, when there were either one or three intervening stages, the STN lesioned rats performed the reversals with no additional trials compared to the corresponding novel acquisition stage (ANOVA restricted to two IDs and reversals, interaction of Stage * Group, $F(1,12) = 19.4$, $p < 0.05$, Figure 6.8).

We had predicted that there would be no 'reversal cost' if the rats were treating the reversals as novel discriminations and the pattern of data appeared to support this hypothesis. However, analysis of the pattern of errors on those reversal stages suggested a different conclusion. In the initial trials of a stage with novel stimuli, rats are most likely to dig in the first bowl they encounter because they have no information to suggest that bowl would not be baited. However, in the initial trials of a reversal stage, rats are more likely to dig in the previously rewarded bowl and, if they encounter the previously unrewarded bowl, they will move to and dig in the other bowl. Intriguingly, rats with STN lesions were as likely as control rats to move away and dig in the other bowl if they encountered a previously unrewarded bowl on the first initial trials (see Table 6.6). This is strong evidence that the rats did remember the original discriminations, albeit that they took fewer trials to reverse.

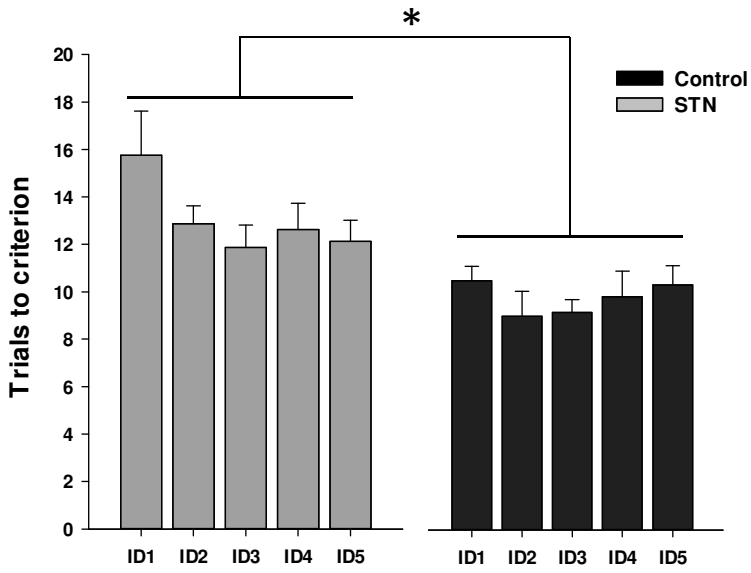

Figure 6.9 shows mean trials to criterion (+SEM) for STN lesioned and control rats on the five ID stages of the 11-stage task. STN lesioned rats required more trials to reach criterion than the control rats on ID stages.

		ID1				ID2				REV1				REV2			
	Group	1st	2nd	3rd	4th	1st	2nd	3rd	4th	1st	2nd	3rd	4th	1st	2nd	3rd	4th
	Control						X	X	X	X	X	X		X	X	X	X
	Control	X				X	X	X		X	X	X	X	X		X	
	Control		X	X		X				X		X		X	X	X	X
	Control		X		X		X			X		X	X	X	X	X	
	Control		X	X			X	X		X	X	X		X	X	X	X
	Control		X	X						X	X	X		X	X	X	X
	STN	X			X	X		X	X		X	X		X	X	X	
	STN	X				X				X			X	X	X	X	X
	STN	X		X				X		X		X	X		X	X	X
	STN	X				X				X	X	X		X	X	X	X
	STN		X	X		X				X	X	X		X	X	X	X
	STN	X	X	X		X		X		X	X	X		X	X	X	X
	STN		X	X			X	X		X		X	X		X	X	X
	STN	X	X	X			X	X		X		X	X		X	X	X

Table 6.6 shows outcomes of the first four trials of the two ID stages and two reversals of the 11-stage task. On ID stages, rats made very few second-investigation errors while on reversals, both control and lesioned rats made this type of errors.

6.2.3.3. Probe distractor and bi-conditional stage

As shown in Figure 6.8, the difference between two groups on the distractor probe stage was significant (restricted analysis on probe stage: main effect of Group, correct-$F(1,12) = 4.9$, $p < 0.05$). On average, the control rats completed the probe stage with no more than two mistakes, while the rats with STN lesions took as many trials to complete the probe stage as they did on the ID stages. This suggests that the rats with STN lesions treated the probe stage as if it were an entirely new discrimination.

The rats with STN lesions were faster on acquisition of bi-conditional discrimination, and this different was marginally significant (restricted analysis on bi-conditional stage: main effect of Group, correct-$F(1,12) = 4.6$, $p = 0.053$). The mean number of trials for the STN lesion group was 12.5, while for the control group was 15.7.

6.2.4. Discussion

The 11-stage task included yet more additional stages before the ED stage, but rats with STN lesions still did not form an attentional set. Unfortunately, although performed better on ID stages than the STN lesion group, the control group also did not demonstrate a strong shift cost.

The current experiment tested two hypotheses about memory and attention in rats with STN lesions. Although rats with STN lesions did not show a positive reversal cost (i.e., they did not require more trials to learn the reversal stage (ID1Rev / ID2Rev) compared to the original discrimination stage (ID1 / ID2), there was clear evidence that they still remembered the original discrimination: they were no less likely than controls to dig first in the previously rewarded bowl. Therefore, we can reject the hypothesis that STN lesions result in impaired short-term memory or that prior experience did not impact on future learning.

Rats with STN lesions performed the distractor probe stage poorly but learned the bi-conditional discrimination stage in fewer trials relative to control rats. This pattern of behaviour supports the hypothesis that the STN

lesioned rats had an impairment of selective attention, such that stimuli were treated holistically and not dimensionally. Rats with OFC lesions are also impaired in forming an attentional set and this has been explained in terms of an impairment in learning 'cue relevancy'(Kim and Ragozzino, 2005; Ghods-Sharifi et al., 2008). However, although OFC lesioned rats failed to form set in standard 7-stage task, they did form set in 4-ID task, and once cue relevancy had apparently been learned, they were impaired at the ED stage, when learning that another cue was now relevant (Chase *et al.*, 2012). The impairment in rats with STN lesions seems different: they seem able to learn that a cue is relevant, but are perhaps less able to learn that a cue is irrelevant. This deficit implies a limited attentional selectivity in rats with medial STN lesions. This is also supported by the better performance of rats with STN lesions on a bi-conditional discrimination compared to control rats. While control rats focus on one dimension and miss the reward-predictive information, rats with STN lesions pay attention to everything and solve the problem faster.

Similar finding have been reported in rats with dorsomedial striatal (DMS) lesions (Lindgren et al., 2013). As rats with STN lesions, rats with bilateral DMS lesions also fail to present positive ID-ED difference on both 7-stage and 4ID task and also do reversals equally well as controls. This deficit in common suggests that the STN lesions and DMS lesions might result in the functional disruption of the same circuit within the Basal Ganglia.

6.3. Experiment 3

6.3.1. Introduction

From the results of Experiment 2, we ruled out the possibility that rats with STN lesions had memory impairment. We also found that they had problems attending to a particular dimension of stimuli, which implied a limited attentional selectivity.

In Experiment 3, we aimed to replicate the set-formation impairment and non-selective attention in rats with STN lesions and extend the research further to see whether a cognitive enhancer would improve performance of rats with STN lesions. The drug we chose was modafinil ("Provigil"), an atypical simulant which was originally used for sleep disorder and has shown benefits for cognitive impairments in a wide-range of disorders. In patients with schizophrenia, modafinil improved spatial planning, attention, working memory and executive functioning (Turner et al., 2004b), but these benefits (specifically to working memory) appeared greater in those with lower baseline cognitive ability (Spence et al., 2005; Hunter et al., 2006). In adults with attention-deficit hyperactivity disorder (ADHD), modafinil improved performance in cognitive domains, including attention, planning and executive control (Turner et al., 2004a). The effects of modafinil in healthy adults so far have been equivocal. Modafinil improved sustained attention, response inhibition and visuospatial planning for the lower IQ group, but not the higher IQ group (Randall et al., 2005). Further support came from Müller et al., (2004) who examined working memory via 'manipulation' and 'maintenance' tasks. Modafinil improved performance in the most difficult manipulation condition for poor manipulators, whereas good manipulators remained constant in their performance. In the maintenance condition, modafinil only improved performance in the long-delay condition for both poor and good manipulators, again indicating modafinil's effect in only the most challenging situation. In addition, modafinil appears to promote rapid switching of attention in conditions that are most demanding (Marchant et al., 2009), whilst has no benefits in other conditions. In animals, modafinil has been seen

to enhance sustained attention (Morgan et al., 2007) and facilitate attentional set-formation in middle-aged rats (Chase *et al.*, 2013, unpublished). Taken together, these findings indicate that modafinil is likely to facilitate pre-existing suboptimal performance in specific cognitive domains and most challenging situations.

Modafinil-induced effects on neurotransmitter systems are related to the activation of receptors and brain pathways that play critical roles in modulating cognitive function. Figure 6.10 shows the target brain areas and neurotransmitter systems of modafinil that are potentially involved in the actions of modafinil as a cognitive enhancer (Mereu et al., 2013). It performs robust effects on catecholamines, serotonin, glutamate, GABA, orexin, and histamine systems. Many of the effects are secondary to catecholamine effects (e.g. NE and DA), with some selectivity for activating cortical over subcortical areas (Minzenberg and Carter, 2008). Overall, these effects in general are beneficial for cognitive processes.

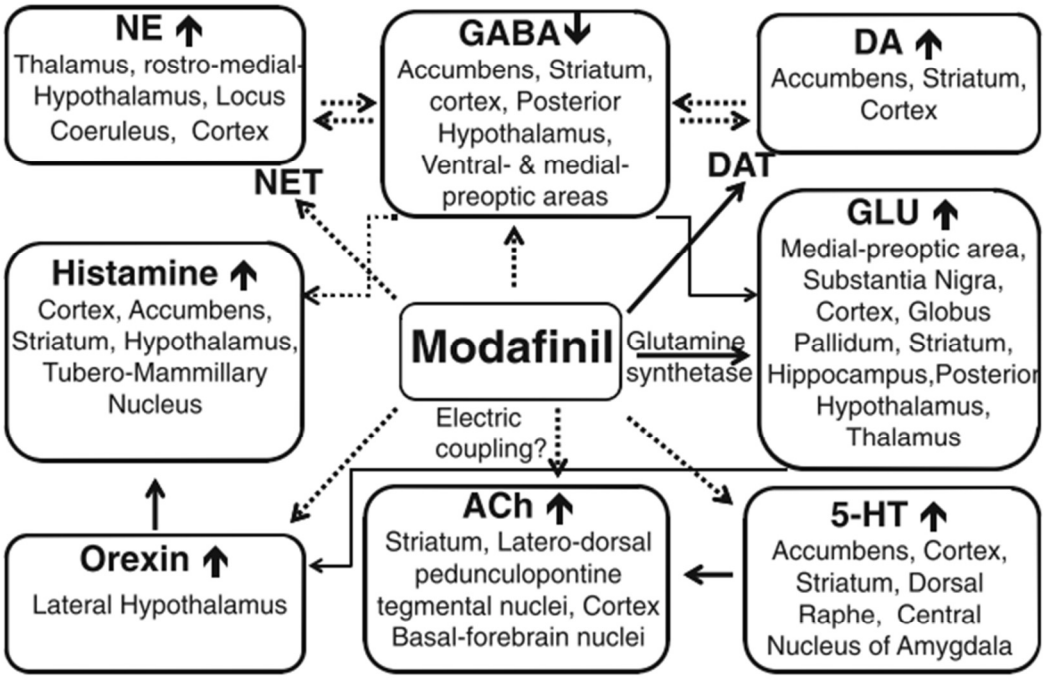

Figure 6.10 Target neurotransmitter systems and brain areas of modafinil's actions as a cognitive enhancer. Solid lines represent direct interactions, while dashed lines indicate indirect interactions or mechanism that has not been fully demonstrated. NE norepinephrine, DA dopamine, GABA gamma-aminobutyric acid, GLU glutamate, NET norepinephrine transporter, DAT dopamine transporter, 5-HT serotonin, Ach Acetylcholine.

The same 18 STN lesioned rats were tested with and without modafinil one a 9-stage task, which contained delayed reversal, extra-dimensional shift, distractor probe stage and bi-conditional discrimination. The hypothesis based on previous studies is that the rats with STN lesions would be facilitated on ID stage and re-build a positive ID-ED shift cost. The impairment on probe-stage would also be attenuated.

6.3.2. Methods

6.3.2.1. Behavioural testing

The new task starts with a compound discrimination (CD), followed by two intra-dimensional discriminations (ID1 and ID2), then two reversal stages (ID1REV and ID2REV), another intra-dimensional discriminations (ID3) and its distraction probe stage (ID3probe), then an extra-dimensional discrimination (ED), and a bi-conditional discrimination (exemplar).

The complete procedure is presented in Table 6.7. The whole task could be completed within 1.5h to 2h. 7 pairs of stimuli are used in the new task, same as in 4ID.

6.3.2.2. Drug

Modafinil was suspended in jellies and given to rats via oral administration (natural eating behaviour). Modafinil jellies were made for each rat, with one jelly contained the amount of modafinil that equalled to 30mg/kg for a particular rat. One jelly was given 30mins before the testing and the second jelly was given 60mins after the first jelly. The interval between two jellies was chosen based on the half-life of modafinil reported by a previous study(Waters et al., 2005a).

All rats were tested once with and once without modafinil, with 9 rats started with modafinil jelly and the other 9 started with pure jelly. Among the 8 rats that have verified STN lesions, 5 were tested on modafinil first and the other 3 were tested on jelly first.

6.3.2.3. Data analyses

Same as in Experiment 1 and 2, trials to criterion were used as measurement of rat performance. Repeated measures ANOVA were done with

Stage and Drug (modafinil or jelly) as within-subjects factors and Order (modafinil first or jelly first) and Group as between-subjects factors. Additional ANOVA tests restricted to certain factor or level were done where needed.

Stages	*Discriminanda*	*Mixed with*
Compound Discrimination	M1, not M2	O1 or O2
First Intra-dimensional Discrimination	M3, not M4	O3 or O4
Second Intra-dimensional Discrimination	M5, not M6	O5 or O6
First Reversal	M4, not M3	O3 or O4
Second Reversal	M6, not M5	O5 or O6
Third Intra-dimensional Discrimination	M7, not M8	O7 or O8
Distractor Probe Stage	M7, not M8	O13 or O14
Extra-dimensional Discrimination	O11, not O12	M11 or M12
Bi-condition Discrimination	O14 in M13, O13 in M14	O14 in M14, O13 in M13

Table 6. 7 *shows an example of the stages and stimuli of task in Experiment 3. The stages are always in this order while the stimuli are counterbalanced within rats and between tests.*

6.3.3. Results

Modafinil influenced rats' performance on several stages (interaction of Stage * Drug * Group, $F(8,80) = 2.1$, $p < 0.05$, Figure 6.11) and the order of drug administration did not change this effect (interaction Stage * Drug * Group * Order, $F(8,80) = 1.6$, n.s.). Further analyses restricted to certain stages were done to reveal specific effects caused by modafinil. According to rats' performance on the previous 11-stage task, if the rats with STN lesioned could benefit from modafinil, then their late ID stage(s) and probe stage should be improved. On the other hand, their ED stage and bi-conditional discrimination should be retarded.

When tested with jelly, the STN lesioned rats were impaired on the ID3 stage, comparing to the control rats (main effect of Group, correct-$F(1,80) = 6.07$, $p < 0.05$); with modafinil, the STN lesioned rats were significantly improved on the ID3 stage (main effect of Group, correct-$F(1,80) < 1$). The rats with STN lesions also seemed to make fewer errors on the probe stage after modafinil, however, this change was not significant. Besides, neither ED stage nor bi-conditional discrimination was significantly affected by modafinil in the rats with STN lesions, although there was both a trend in the predicted direction.

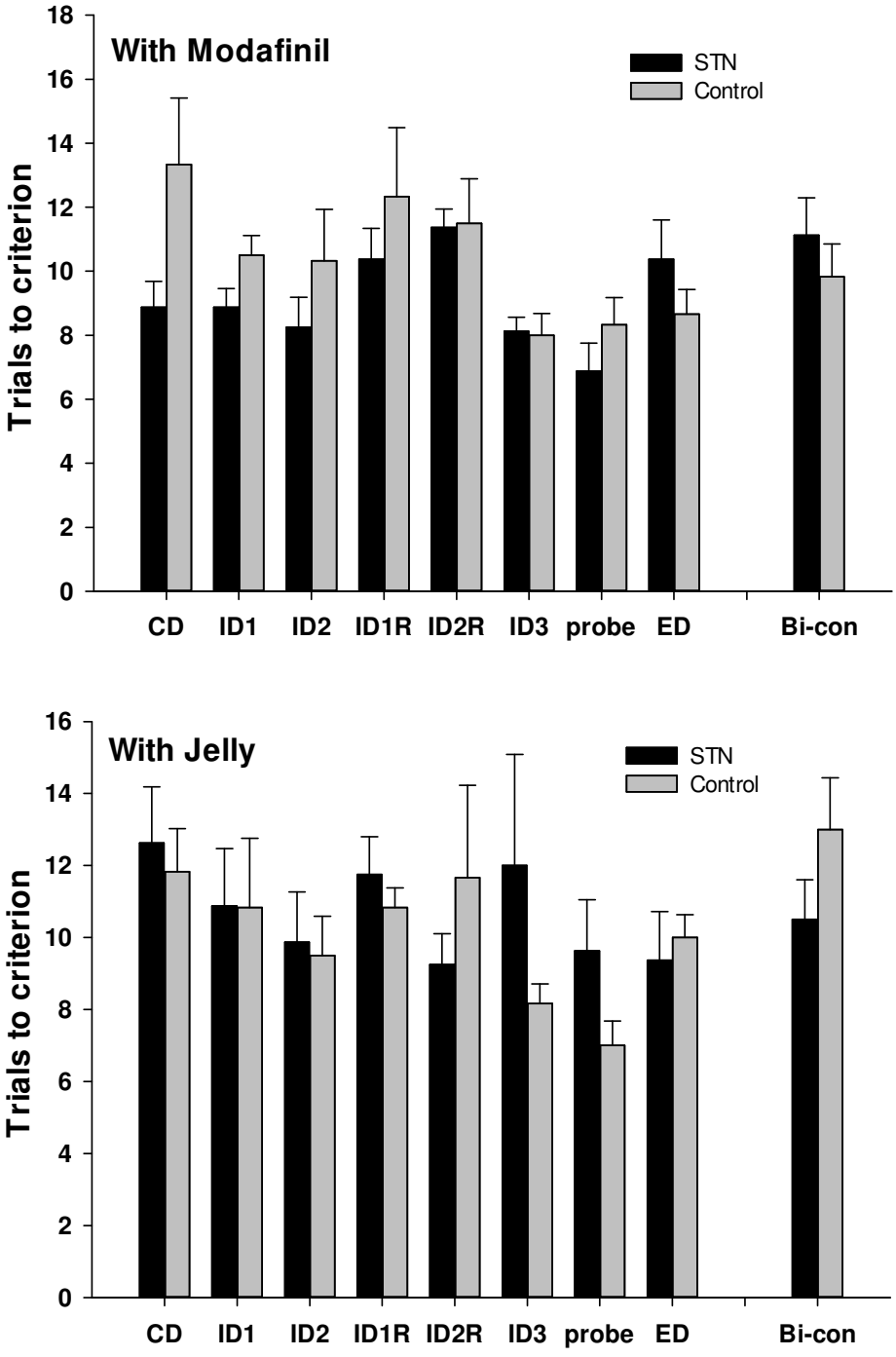

Figure 6.11 shows mean trials to criterion (+SEM) for the control and STN lesioned rats after the consumption of modafinil, comparing with jelly. Modafinil improved performance of the STN lesioned rats on ID3 stage and probe stage.

6.3.4. Discussion

In this experiment we tried a novel way of administering drug into animals. The advantage of hiding drug in jelly is that it guarantees that animals take in the required amount. This is especially necessary for modafinil, since modafinil usually suspends in vehicle solution and cannot be properly administered via injection. Besides, this way causes no harm or pain to animals, comparing to gavage, and unlikely to disturb animal's performance. The dose and interval between drug administration and testing are determined depending on Waters et al., (2005) study, in which modafinil is delivered via gavage. Because of the different methods, the interval between the drug administration and the time drug concentration reaches peak might be slightly different. However, the results showed clearly that modafinil administered via jelly was efficient to affect rats' performance. To figure out the doses and drug-testing intervals that are compatible with traditional methods such as injection and gavage, more studies need to be done in the future.

Previous study in this lab reported that performance of middle-aged rats on attentional set-shifting task was affected by modafinil (Chase, PhD thesis, 2012). Middle-aged rats, which demonstrate reversal impairment, are not impaired at reversals when they first given opportunity to form set. Middle-aged rats can form set, but only with the help of extra ID stages. This suggests that the pre-set reversal deficit might be due to a diminished ability of maintaining attention on the relevant dimension during the reversal. The pre-set reversal impairment is worsened by treatment of modafinil, but meanwhile, the first ID is improved. This double effect suggests that modafinil might make rats more likely attend to both dimensions of the stimuli during the reversal stage. This increases the attention load and lead to worse reversal. However, this extra study about both dimensions help rats to learn the relevant dimension better and in return benefits their first ID stage. The way that modafinil affect rat's performance implied by this previous study is in accordance with its known role as a cognitive enhancer.

The current experiment shows a sign of improved ability of set formation in STN lesioned rats with the treatment of modafinil, which is similar to the

finding in middle-aged rats. However, the expected disrupted reversal is not seen in the current study. Most unfortunately, these changes are not statistically significant in the reported eight rats. One possibility is that the power is not enough, considering eight rats is not a big sample size. As a matter of fact, when including more rats in analysis (the four rats which demonstrated damage in MGP but no visible damage in STN), the effect of the treatment of modafinil becomes statistically significant. As we have mentioned in the Histology part, performance of those four rats on Experiment 1 and 2 did not seem to be different from the eight rats which demonstrated clear STN lesions. In other words, these two groups of rats revealed similar impairments, despite different histology results, and both groups were improved on attentional set formation by the treatment of modafinil.

Frankly speaking, we have not gained enough evidence to conclude whether or not the treatment of modafinil is effective to cure the deficits caused by STN lesions on attentional set-shifting. However, the current results are interesting and inspiring, and this suspending question is worth further study.

6.4. General Discussion

The current study examined the effects of STN lesions on attentional set-shifting task in rat. Experiment 1 confirmed that rats with STN lesions are impaired on attentional set formation. Experiment 2 examined several testable hypotheses about why STN lesioned rats cannot form set: the possibility that STN lesioned rats are memory impaired was excluded. Data from the bi-conditional discrimination suggested that rats with STN lesions seem to attend to both dimensions of the stimuli, while data from distractor probe stage suggested that although rats with STN lesions can learn the correct discriminanda with no problem, they do not get the idea of 'relevant dimension'. This might suggest a perceptual issue in rats with STN lesions – the stimuli are treated as novel when any aspect of them changes. Experiment 3 tested the effect of modafinil, a cognitive enhancer, on the deficits caused by STN lesions on attentional set-shifting task. However, although we see some signs that modafinil improved the performance of STN lesioned rats, no changes were statistically significant. One possibility is that we did not have enough power. Due to the power analysis that had been done before experiment 1, which was based on the size of effect estimated from previous set-shifting tasks studies, 15 rats in each group are considered as enough to get relatively high enough power (0.8). However, due to unverified histology results, only 8 rats were included in the lesion group for the final analyses. This limitation became problematic in Experiment 3, where the numbers of experimental manipulations increased.

Rats with STN lesions were not impaired on reversal learning: STN lesioned rats are able to inhibit previously rewarded responses. The lack of difference between ID and ED replicated in all 3 experiments is indicative of a failure of form an attentional set. The evidence from experiment 3 suggested the impairment is due to an attentional deficit that limited the animals' attentional selectivity. Since there was lack of evidence of set-formation, the ability of set-shifting could not be measured in the current experiments.

General Discussion

The series of experiments described in this thesis examined the effects of lesions of the subthalamic nucleus (STN) on cognitive and motor behaviours in the rat. In the first three experiments, a novel task - the signal change reaction time task – was developed to test specific hypotheses concerning the role of the STN in inhibition of responding. The results confirmed some previously reported deficits, such as increased premature responses, but challenged assumptions about response inhibition.

The STN has also been implicated in cognitive control and, in particular, attention. In the fourth experiment, the self-paced attentional set-shifting task was used to characterize and evaluate cognitive deficits when the motor demands were low. To my knowledge, this experiment was the first attempt to demonstrate a role of the STN in executive functions and to characterize the nature of its contribution.

Together, the empirical work presented in this thesis has generated and tested a number of theoretical questions and hypotheses. The data reported in this thesis, along with interpretations of the data, has increased our knowledge of the functions of the STN and has also raised new questions, as will be explored below.

7.1. Findings

The general protocol used to study the function of a certain brain area includes several steps: select a suitable animal model, choose an effective behaviour task(s), conduct precise lesions, and look for changes in performance relative to controls. Among these steps, the step two is the most flexible and potentially makes the greatest difference between studies. Two key points should be born in mind when choosing or designing an appropriate task. First, it must enable valid interpretation of what the changes in performance actually reveal about the brain functions. Second, the measurements of the task should ideally be comparable between humans and other animals. To fulfil the two requirements, a good understanding of the psychological processes behind the tasks is essential.

The human-rat comparison experiment reported in Chapter 3 was planned and conducted with these goals in mind. The 2-choice reaction time task was designed to measure subjects' ability to inhibit and reprogram responses when the stimulus changes, as inhibitory control has long been regarded as a key function of the STN. The task itself is straightforward and there is a single rule – respond to the final location of the light – so that the experimental participant does not need to be concerned with the particular trial type (i.e., whether the trial is 'CHANGE' or 'NO-CHANGE'). Similar patterns of performance were expected for rats and humans. However, the results did indicate some interesting differences between humans and rats. Human's performance on CHANGE trials benefits if the previous trial was also a CHANGE trial, which suggests that humans consider CHANGE and NO-CHANGE trials as two distinct rules. This is possibly due to the ability to take an overview of the whole experiment, which is either gained from practice or from the instructions given by the experimenter. A similar observation is also made in task-switch paradigms, which usually involve more than one stimulus-response mapping rules (Mayr and Kliegl, 2003; Los and Van der Burg, 2010) . Interestingly, a response repetition effect, which was expected based on previous literature, was not found in the current human participants: they did

not benefit from repeating the previous response. In contrast, rats seemed to be greatly affected by response location switches: when the same response is repeated on the subsequent trial, rats are faster and more accurate compared to when they must make the alternative response. Moreover, although rats, like humans, are slower and less accurate on CHANGE trials, the above-mentioned sequential effect of CHANGE/NO-CHANGE manipulation was not seen in rats. In spite of these important differences, there were many similarities in the behaviour of the humans and rats. Most notably, both rats and humans were slower to execute a previously-inhibited response. This means a within-trial response inhibition leaves a trace in both humans and rats, which carries on till the next trial. These results confirm that the current task fulfils the goal of testing the subject's ability to inhibit and reprogram a response when the stimulus changes. Additionally, it highlights the sequential effects in rats which might be a key point in further studies.

The task developed in Chapter 3 was elaborated in Chapters 4 and 5, to study the effects of STN lesions. Together, the two experiments demonstrated how a bilateral medial STN lesion affected rats' performance on the 2-choice reaction time task. A previous study using a stop-signal reaction time task (Eagle et al., 2008a) in rats suggested that a stopping impairment would be caused by STN lesions. Therefore, the hypothesis was that rats with STN lesions should be impaired on CHANGE trials, as these also seem to require inhibition of an about-to-be-initiated response. Surprisingly, this impairment was not seen in CHANGE trials. This finding suggested two possibilities: first, the inhibitory ability that is required in the current stimulus change task is different from the one in the SSRT task; second, the impairment seen in the previous SSRT study is not due to a general failure of response inhibition. Another surprising finding from the experiments in Chapter 4 and 5 is that rats with STN lesions are impaired when alternating responses on two consecutive trials. This significant finding suggests that STN lesions disrupt a normal within-trial inhibition against a 'win-stay' tendency, which would result in a response bias. It has previously been suggested (Phillips et al) that impairments in inhibition following STN lesions are not seen when the rat is

under stimulus control. The difference between the requirements to inhibit an about-to-be-initiated response within a CHANGE trial and the inhibition of response repetition between trials is that the former inhibition (which is unimpaired by the lesion) is under stimulus control, while the latter (which is impaired) is not under stimulus control.

The studies of reaction time performance suggested that the contribution of the STN to response inhibition is more complex than merely providing a 'stopping' signal. The STN has also been implicated in cognitive control and possibly in attentional control (Baunez et al., 2005). We tested the hypothesis that the STN may be inhibitory in the cognitive domain. Rats were tested in the standard 7-stage attentional set-shifting task. This task includes reversal learning stages, which require inhibiting previously rewarded responses. It also includes an attentional set shift, which requires inhibition of attention to previously relevant stimulus characteristics. Rats with STN lesions were not impaired in the acquisition of discriminations and, interestingly, were also not impaired on reversal learning: STN-lesioned rats are able to inhibit previously rewarded responses. Furthermore, not only were the rats not impaired on acquisition at the ED stage, they were better than controls at this stage, and worse than controls at the ID stage, resulting in no difference between ID and ED performance. As the difference between ID and ED performance is indicative of the presence of an attentional set, this result implied that set had not formed. Subsequent manipulations tested different hypotheses to account for this behavioural pattern and concluded that set-formation following STN-lesions is impaired due to a deficit in attentional selectivity.

7.2. More about the tasks

Although the tasks used in this thesis have provided a lot of information, their usefulness can still be increased by obtaining a better understanding of the psychological processes behind them. However, this will require further modifications to the current tasks.

7.2.1. Signal change reaction time task

The core feature of the signal change reaction time task is that subject needs to change their response rapidly upon the change of the stimulus. To achieve this goal, we assume that subjects inhibit the former response while activating the alternative response. Participants reported the subjective sense of inhibition followed by changing the response; however, there is no direct evidence that this was the case for the rats. Therefore, it remains a possibility that rats perform the task using different strategy (for example, using the simple rule of 'follow the light') which may not require the same inhibitory load as a stop-signal task. If this is the case, although it might appear that the STN lesions do not affect rat's ability to inhibit and change responses, we need to be careful when we extend this conclusion to other tasks where the parameters are different. To make rats' behaviour better resemble humans' behaviour, valuable modifications might include prolonging the length of stimulus; not extinguishing the first stimulus when the second is presented; setting more stimulus change points; and reducing the proportion of CHANGE trials. By doing these rats will be forced to more actively withhold their responses a more well-prepared response, leading to a greater requirement for an efficient inhibition process.

Although the current task is classified as a two-choice task, it is unclear whether, to the rat, it is making a choice between two independent responses or one response albeit with different specifications. In the former case, the inhibition or activation of one response (e.g., 'right') will not affect the other (e.g., 'left') response, while in the latter case the inhibition (or activation) of one response will simultaneously have the opposite effect, and activate (or inhibit) the other response. The former case requires subjects to pay attention to both target locations to gain enough information for an accurate response, while in the latter case information from one target location would be enough. Therefore, these two different ways lead to different distributions of attention and also the total attention load. This is particularly important when fitting the data into race model or other mathematics models, since these models make assumptions about the independence of the processes of the two responses.

7.2.2. Attentional set-shifting task

The attentional set-shifting task is a relatively straightforward task that is now widely used with surprisingly few deviations from the original protocol described by Birrell and Brown (2000). However, this does not mean that there is not scope for refinement. Almost all reports use the criterion for leaning of six consecutive correct trials. This criterion has been theoretically and empirically demonstrated to be a reasonable indicator of learning. However, although it the probability that subjects perform six consecutive trials correctly by chance is 0.015, there is no tolerance for a 'careless mistake' or even an exploratory check and it is possible that subjects have learned the rule even though they make errors.

An alternative would be to use Bayesian probability to test hypotheses about which strategy is being used to guide responses. For instance, subjects might use strategies like win-stay (respond to the previously rewarded location), win-shift (respond to the alternative location after a reward), sticking to left or right side, or respond to the correct/incorrect discriminanda. In this case, the criterion for learning will be when the probability that it is the correct strategy that is driving the behaviour reaches 99% or higher. One advantage of using Bayesian probability is that the estimation of probabilities takes all prior events into consideration and updates after each event. The other advantage is that Bayesian probabilities help to distinguish different hypotheses, which provide us with more information about the nature of subjects' behaviour. To be specific, the probabilities will help us to understand why subjects make errors. Furthermore, Bayesian probability can be used to determine the stimulus configuration of the trial sequence dynamically, selecting the configuration that will most efficiently exclude or confirm a certain hypothesis.

Given these advantages, Bayesian probability might replace the current six-consecutive-trial criterion in future studies. However, this change requires a comparison between the data from tests using the two different criterions.

The most significant contribution of the work described in this thesis is the adaptation of the operant and the bowl-digging paradigms to test specific

hypotheses about inhibitory and attentional control. This approach has contributed not just to improved understanding of the functions of the STN, but has also advanced the psychological understanding of response inhibition, attentional set and the set-shifting task.

7.3. More about different types of inhibition

Most studies of inhibitory control have used stop-signal tasks, which involve stopping of manual or eye movements (Schall and Godlove, 2012). In both human and animal studies, the STN seems to be involved in the performance of stopping (Aron and Poldrack, 2006; Eagle et al., 2008). Therefore, the role of STN has been implicated and emphasized repeatedly in inhibitory control. However, as we mentioned above, the function of STN in inhibition might have been overstated. This is quite possibly due to the fact that there are different types of inhibition and the STN may not be a generic 'inhibitor'.

Inhibition of motor responses can be either general or selective and they can be proactive or reactive. General inhibition is when every potential action from all effectors are inhibited (i.e. left and right hand; hand and eye; etc.), while selective inhibition means inhibiting one thing but not another (i.e. inhibit a left hand, but not a right hand, movement or respond to one stimulus but not another). Reactive inhibition is when subjects are required to inhibit an action on the presentation of a signal, while proactive inhibition is when subjects prepare to stop a forthcoming response tendency. In practice, proactive inhibition allows the inhibitory control to be more selective, when subjects know in advance that they will respond to one stimulus but inhibit a response to another.

7.3.1. Reactive inhibition

The most classic experimental paradigm that used to test reactive stopping is stop signal task, where the stopping response is low probability and unpredictable. Functional and behavioural studies have confirmed that the

STN is involved in performance of the stop-signal reaction time (SSRT) task (see review Aron, 2011). For example, in an fMRI study, high activation of the STN was seen on successful stop trials (Aron and Poldrack, 2006). A later study replicated the results and further found that the STN activation was even greater for stopping errors than for stopping successes (Li et al., 2008). This latter finding suggests that the activation of the STN on stop-signal trials might be more related to the processing of the stop signal than to the execution of the stopping. This interpretation is consistent with the observation that STN lesions in rats did not affect the SSRTs and the stopping impairment that was observed was SSRT-independent (Eagle et al., 2008). Considered along with the present results, the findings suggest that STN does not play a critical role in a stopping process, which determines SSRT, but rather it is involved in other aspects of stopping performance.

7.3.2. Proactive inhibition

While reactive inhibition is triggered by external stimuli, proactive inhibition is invoked prior to the presentation of any stimulus. Using proactive inhibition means subjects are prepared to inhibit forthcoming responses if or when necessary. An example of proactive inhibition is in the Go/No-Go task, when the participant prepares to respond in anticipation of the 'go' signal and so must inhibit any motor preparation that has taken place if the other signal is presented, . The proactive inhibition has been implicated in the "hold your horse" model (Frank et al., 2007), which considers that withholding prepared responses to external stimuli is the default state of sensorimotor reactivity and the brake only releases when an explicit 'go' signal is detected (Ballanger et al., 2009). The proactive inhibition is usually used as a strategy, when the speed is sacrificed for accuracy. In other words, subjects increase successfully stopped responses by slowing down. As an example, when tested on mixed Go/No-Go session and pure Go session, participants perform slower reaction times in the former situation (Ballanger et al., 2009). This was also found in the experiment reported in Chapter 3: human participants were faster on pure NO-CHANGE session than on NO-CHANGE trials in mixed CHANGE/NO-CHANGE session.

An fMRI study has demonstrated that a network for reactive stopping – comprising the rIFC, preSMA and STN – is also activated in a proactive stopping paradigm (Jahfari et al., 2010). This finding suggests that the network for reactive stopping could be activated when a stopping response is anticipated. In other words, proactive inhibition is more accurately characterized as proactive activation of the stopping network. Similar findings of proactive activation of a network for reactive stopping have been reported in several different stopping paradigms, including the Go/No-Go task (Hester et al., 2004; Isoda and Hikosaka, 2008b; Chikazoe et al., 2009). Moreover, the more prepared the subjects are, the greater is the activation of the network, and this leads to slower Go responses when the stop signal does not occur and faster Stop/No-Go responses when the stop signal does occur (Chikazoe et al., 2009; Jahfari et al., 2010).

7.3.3. Global inhibition

It has been observed that reactive inhibition has global effects on the motor system. For instance, the inhibition of a thumb movement causes the suppression of excitability of the muscles of the leg, although the leg movement is not related to the task (Badry et al., 2009). In addition, it has been shown that stopping one effector induces delays in the response executed by another effector (Aron and Verbruggen, 2008). These findings suggest that there must be a global stopping mechanism and, given its massive output to GPi and direct afferent projection from cortical areas, the STN has been implicated in this.

Global inhibition of eye movement might involve different brain areas though. In the anti-saccade task, subjects have to either look toward a visual stimulus (pro-saccade) or away from the stimulus (anti-saccade) depending on a pre-target cue. Pro-saccades are reflexive, automatic eye movements, while anti-saccades involve the suppression of automatic saccade and initiation of an inverted saccade. Therefore, a global fixation or saccade inhibition is required for correct anti-saccades. Previous study demonstrated that in the rostral pole of the monkey superior colliculus (SC), a subset of neurons form part of a fixation system which facilitates active visual fixation and suppresses the

initiation of any unwanted eye movement (Munoz and Wurtz, 1992). These neurons are called 'fixation cells' and they are a 'global inhibitor' of all eye movements, while movement is determined by neurons which are topographically arranged around this area (Everling et al., 1999). Later studies have confirmed that frontal eye field (FEF) also contains distinct populations of fixation and saccade neurons whose discharges are modulated in the anti-saccade task (Everling and Munoz, 2000; Sato and Schall, 2003). Studies also suggested that it is unlikely that FEF and SC alone can account for correct anti-saccades. The caudate nucleus, a major input structure of the basal ganglia was indicated to be involved. Recordings in the caudate nucleus during an anti-saccade task showed that some neurons increased their activities during anti-saccades but not pro-saccades, while some other neurons did the opposite (Ford and Everling, 2009). It further suggested that the discharge pattern of neurons in the caudate might modulate activity in the SC. A microstimulation study concluded that caudate signals were sufficient to suppress saccades and affect saccadic decisions by control ipsilateral and contralateral saccades at the same time (Watanabe and Munoz, 2010).

7.3.4. Selective inhibition

Notwithstanding the evidence for a global stopping mechanism for some circumstances, the brain is clearly capable of selective inhibitory control. However, it is important to distinguish between the observed selective inhibition of a particular behaviour and a brain mechanism for selectivity. Behaviourally selective inhibition is presented as stopping one response while making another response simultaneously or subsequently. This could be achieved by invoking a global stopping mechanism, with the alternative response initiated in a separate process. Therefore the crucial question is whether the brain has a truly selective stopping mechanism?

Aron and Verbruggen (2008) developed a novel stop signal paradigm to dissociate mechanistically global and selective stopping. 60% trials were 'go' trials, in which participants needed to initiate a coupled response with fingers of both hands; in the rest 40% trials a stop signal occurred at some delay after

the onset of go stimulus, and indicated which one of the responses (left or right) they need to stop while continue with the other one. On each trial they presented a cue with the stopping goal ("Maybe Stop Left" or "Maybe Stop Right") or a cue without a specific stopping goal ("Maybe Stop XXX"). The hypothesis was when with foreknowledge (specific cue) participants would use selective inhibition to stop a particular response, if a stop signal occurred, while without foreknowledge (vague cue) participants would use global inhibition to stop all potential movement quickly. Also the stop of one response should have an effect on the alternative (non-stop) response, which they called stopping-interference-effect. They found that with foreknowledge response stopping was slower than without foreknowledge. Moreover, the stopping-interference-effect was smaller for foreknowledge conditions than for no-foreknowledge conditions. These results satisfied the hypothesis and suggested there is a selective stopping mechanism which is dissociated with global stopping mechanism. The author proposed in a later review that the slower selective stopping might relate to use of the indirect pathway which has more synapses than the hyper- direct pathway (Aron, 2011).

7.3.5. Summary

The above-mentioned studies demonstrate different mechanisms for each type of inhibition and the neural basis for them are also different. Although its function is not fully uncovered, the STN seems to be more involved in some types of inhibition than the others. More importantly, its function is diverse in different behavioural paradigms and can be more than inhibitory.

7.4. Conclusions

It is interesting that the current data did not support some of the hypotheses arising from previous studies. Most notably, the intact ability to inhibit an about-to-be-executed response when a stimulus changed was not predicted from previous findings (Eagle et al., 2008). This suggests that the previous view of the role of STN in response inhibitory control might be overstated. The STN lesions also did not change reaction times of the rats,

which had also been predicted by previous research (Baunez et al., 1995, 2001). However, we did find an exacerbated response bias towards the previously rewarded responses, which had been predicted by the *"buffer-like"* mechanism proposed by Baunez et al (2001).

In the cognitive domain, the STN also appears not to be serving an inhibitory function. However, it is clearly involved in attentional selectivity, which impacts the capacity and orientation of attention. Together, these findings indicate that the STN should not simply be referred as a *"global brake"* (Frank, 2006). The role of STN in motor and non-motor executive functions needs to be re-evaluated.

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