ATTENTION & RESPONSE CONTROL:

A NEURAL AND BEHAVIOURAL ENQUIRY

Thesis presented to the Faculty of Science,

University of St Andrews

For the degree of Doctor of Philosophy

by

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DECLARATION

I, Anja Farovik, hereby certify that this thesis, which is approximately 28 000 words in length, has been written by me, that it is the record of work carried out by me and that it has not been submitted in any previous application for a higher degree.

Date 12/01/07 Signature of candidate .

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Cheers, people

ABSTRACT

Goal directed responding is the behavioural expression of the myriad of cognitive processes which are involved in the translation of motivation to a motor program. These processes include learning, memory, expectation, anticipation, working memory and attention. The purpose of this thesis is to explore how and when attention exerts influence over responding and the neural basis of this. The introduction reviews the experimental literature on working memory and attention, noting that most work on attention stands quite separately from studies of working memory, although the contents of working memory may be regarded in some sense as the product of attentional selection. Chapter III describes work developing a new task of working memory. However, rats failed to learn the discrimination and so this line of investigation was not pursued further. In Chapter IV, a different approach was taken. The subthalamic nucleus (STN) is a key structure in the motor output pathways of the basal ganglia. However, a role for the STN in attentional selection, movement and switching has recently been raised. This chapter used a task of divided attention to examine the ability of rats with lesions of the STN to switch attention between modalities. However, an increased attentional load was not associated with an increase in the magnitude of lesion-induced performance deficits, suggesting that this nucleus is more likely involved in motor rather than attentional aspects of behaviour. Previous findings also suggest that an intact STN is important for response preparation in a task used to assess endogenous covert orienting of attention, while the attentional aspect of the task remained intact. Noradrenaline has been implicated in selective attention and in particular to play a role when stimuli are not fully predictable. Chapter V

reports a study to investigate the role of noradrenaline in a test of covert spatial attentional orienting to endogenous cues. Depletions of the ascending noradrenergic system (DNAB) result in impairments supporting a role for this system in selective attention, however, there were no impairments in covert spatial attentional orienting. Previous work has suggested that the basal forebrain cholinergic system is important in covert orienting when the cues are exogenous. Therefore, Chapter VI examined the role of the basal forebrain cholinergic system in the test of covert orienting used in Chapter V, employing endogenous cues. Basal forebrain lesions impaired endogenous covert attentional orienting by abolishing the cost/benefit of the cue on reaction time to the visual target. Systemic injection of nicotine (a cholinergic agonist) did not re-establish directed attention in lesioned animals. This finding is the first to show the neural basis of endogenous covert orienting of attention, and suggest a common neural substrate for both exogenous and endogenous covert attention. Chapter VII considers the work of the thesis as a whole in the context of previous work and suggests directions for further research of this topic.

CONTENTS

CHAPTER I:

GENERAL INTRODUCTION

1.1 In this thesis we will examine the processes by which attention exerts control over responding. Attentional allocation allows for effective execution of motor processes. To date, investigations into the biological basis of attentional processes have shown that distinct anatomical and neurotransmitter systems play a role in attention-guided action. This chapter will give an introduction to the topic investigated herein.

1.2 WORKING MEMORY

From a human cognitive perspective, working memory can be viewed in terms of the model proposed by Baddeley and Hitch (1974). This model is not a unitary system and consists of a central executive with two subsystems termed the visuospatial sketchpad and the phonological loop (Fig. 1.2.). Thus, the proposal by Baddeley and Hitch (1974) stands in contrast to that of Atkinson and Shiffrin (1968) who considered the term as simply short-term storage of information. The model was largely a result of the fact that the proposal by Atkinson and Shiffrin did not appear to be able to account for a range of data, and consequently emphasis shifted towards the functions of this system, rather than the simple storage of information. In the model, the visuospatial sketchpad is used to manipulate visual information, while the phonological loop manipulates information concerned with language. The central executive is not well understood but the idea is that it controls the operation of its subsystems. Due to this unclear nature of the central executive, the study of working memory investigates the components of its subsystems.

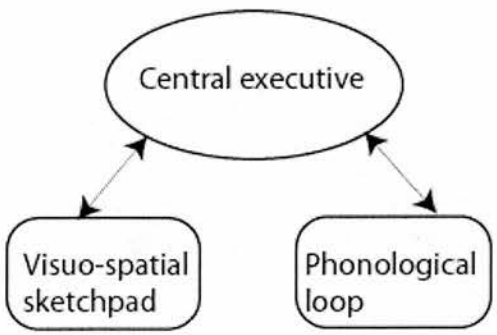

Fig. 1.2. *A schematic illustration of the working memory model by Baddeley and Hitch (1974).*

1.2.1 ASSESSMENT OF WORKING MEMORY CAPACITY IN HUMANS

Working memory tasks in humans commonly assess both maintenance and manipulation of information (Postle *et al.*, 1997; Rowe *et al.*, 2000; Rypma *et al.*,

2002), and are often characterised by the use of verbal working memory (Paulesu *et al.*, 1993; Rypma *et al.*, 2002), although most tasks also tax some aspect of spatial memory, either in the form of remembering spatial locations or internally generated mental rotations of the to-be-remembered information (Lewis *et al.*, 2004). The memory load in working memory paradigms can be easily manipulated. For instance, in the *n*-back task, the subject is presented with a sequence of stimuli (spatial or non-spatial) and has to determine whether a particular stimulus was the same or in the same location as that presented *n*-back in the sequence (Fig. 1.2.1). Memory load can be assessed by increasing the number of items between the matching stimuli, and is therefore a popular task used to measure working memory capacity in both healthy (Cohen *et al.*, 1997; D'Esposito *et al.*, 1998) and clinical samples (Jansma *et al.*, 2004).

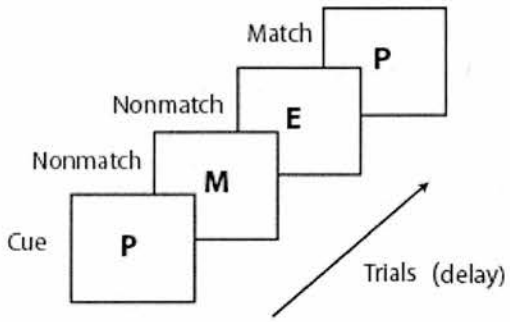

Figure 1.2.1. *An example of a '3-back' continuous n-back task, which includes two letters between the matching stimuli. Subjects are presented with a sequence of letters and have to remember the stimulus (cue) that needs to be matched. Manipulation of working memory load can be achieved by using different levels of the n-back: 1-back, 2-back etc.*

1.2.2 THE NEURAL CORRELATE OF WORKING MEMORY

For many neuroscientists within animal research working memory is often summed up as '...the process of actively maintaining a representation of information for a brief period of time so that it is available for use...' (Ungerleider

et al., 1998 *Proc Natl Acad Sci 95:883-890*). Interpretational difficulties exist, however, in terms of understanding the neurobiological basis of working memory. This difficulty has come about due to a differential emphasis in experimental investigations, particularly between human and animal studies, on the individual features thought to comprise this construct.

Traditionally, the working memory hypothesis has enjoyed widespread popularity in interpretations of the functions of the prefrontal cortex (PFC) (Goldman-Rakic, 1987). This hypothesis maintains that the PFC is organised according to domains of information processing where sensory items required for successful performance on a task are held in "mind" across a short delay (e.g. Goldman-Rakic, 1987) (Fig. 1.2.2).

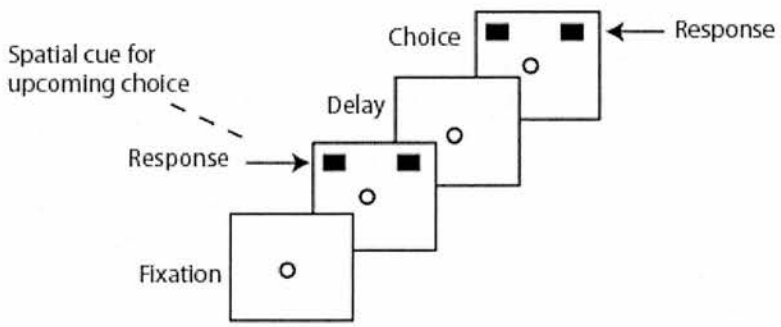

Fig. 1.2.2. *A schematic illustration of an oculomotor delayed-response task used to assess working memory in the monkey. The animal is required to alternate between spatial locations.*

Support for this idea comes from PFC lesion studies in primates showing profound deficits on delayed-response tasks (Funahashi *et al.*, 1993), and neurophysiological findings that show sustained neural activity during delay-dependent tasks (Fuster and Alexander, 1971). With more recent developments in neuroimaging, studies in humans lend support to the idea of an involvement of the PFC in performance reliant on working memory (Jonides *et al.*, 1993; McCarthy *et al.*, 1994). This

working memory hypothesis is further strengthened by interspecies similarities. Experimental findings from lesion studies suggest that the dorsolateral prefrontal cortex (DLPFC) in primates (Passingham, 1985) and the medial frontal cortex in rats (van Haaren *et al.*, 1988; Kesner *et al.*, 1996; Delatour and Gisquet-Verrier, 1999) appear to be crucial for this behaviour. However, there have been diverse and sometimes contrasting conclusions made as to the relative contribution of working memory to PFC function. It appears that the acceptance or rejection of a working memory hypothesis of PFC function largely depends on which characterisation of this construct one adheres to.

Although maintenance of information appears somehow a necessary requirement for certain processes to be carried out in the PFC, this traditional definition of working memory cannot adequately explain the functional significance of the frontal lobes. In light of studies reporting maintenance of information in other cortical areas, such as the inferior temporal cortex (Fuster and Jervey, 1981; Ranganath *et al.*, 2004; Ranganath and D'Esposito, 2005), these results indicate that working memory, according to this definition, is not a hallmark of prefrontal function. Based on neurophysiological findings showing sustained activity in the PFC for individual components of a task, such as cue, delay and upcoming motor response, it has been postulated that the PFC is possibly involved in bridging this gap between cognition and motor acts and that this bridge is made possible via working memory (Fuster, 1990, 1995). This proposal differs to the claim made by others (Passingham and Sakai, 2004), which considered the multifeatural content of sustained activity in neurophysiological and imaging studies as evidence against a working memory hypothesis of PFC function. It was maintained that the PFC is

rather likely to be involved in the *use* of this multifeatural information. As Passingham and Sakai compared experimental evidence according to the view of working memory held by Goldman-Rakic[••] their dismissal of a working memory hypothesis of PFC function appears to be based on only the maintenance component of this construct.

Studies do show differences, however, in the role played by PFC versus other regions in delay-dependent tasks. Neural activity in the PFC, for instance, has been shown to resist interference across a retention interval, while activity in the IT has not (Miller *et al.*, 1996). While delay-dependent activity in other brain regions has been taken as implicating these structures in working memory, others regard the resistance to interference shown specifically by the PFC as a hallmark of this structure (Miller and Cohen, 2001).

1.2.3 SO WHERE ARE WE?

After reviewing the functional role of the PFC in rodents, Kesner (2000) described working memory by way of its relation to rules (see Wise *et al.*, 1996 for a related proposal). This scheme is further supported by electrophysiological findings in the rat, which indicate that neurons in the rat medial PFC are more tuned towards task rules rather than maintaining information across a short delay (Jung *et al.*, 1998). Using this framework, tasks commonly regarded as assessing, for example, spatial working memory, can instead be viewed as rule-governed behaviour of spatial

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[••] It is recognised that there may be some uncertainty as to the exact nature of the working memory model proposed by Goldman-Rakic. Although, delay-dependent activity in the PFC was interpreted as reflecting working memory, it is unclear whether she indeed equates working memory with merely a short-term storage of information across a delay-period.

information. Neurophysiological studies examining prefrontal function in non-human primates suggest an involvement of both (Rainer *et al.*, 1998) and as noted by others (Schoenbaum and Setlow, 2001), it is highly likely that both of these factors are part of the defining characteristics of prefrontal function. Perhaps the cognition assessed in these tasks can be explained without making explicit use of the term working memory, but that does not necessarily imply that this type of memory is not a requirement for this cognition to take place.

1.2.4 INTERSPECIES COMPARISON OF PREFRONTAL CORTEX

It has been argued that the prefrontal region is missing or hardly developed in animals other than the primate, such as the rat (Preuss, 1995). As there are differences between the rat and primate in terms of anatomical structure and connectivity with other brain areas, it is debatable as to whether the rat actually has a similar frontal region and consequently if it possesses the same cognitive complexity commonly associated with this brain area in primates. However, pursuing a rather different approach, Campbell and Hodos (1970) suggested that homology could instead be viewed in terms of functional similarities rather than interspecies comparison of anatomical connectivity and thereby placing emphasis on analogy rather than homology and similar suggestions have been proposed more recently (Brown and Bowman, 2002). Indeed, behavioural studies might help to clarify the discrepancy found with respect to anatomical studies, and in recent years, there have been a growing number of behavioural studies with the aim of functionally delineating the different subregions of the rat frontal cortex. The findings from these studies indicate functional similarities between the rat and primate frontal cortex (see Granon and Poucet, 2000 for a review), and studies with

rats should be capable in providing a useful framework for understanding the neural basis of working memory.

1.2.5 ASSESSMENT OF WORKING MEMORY IN RODENTS

To increase our understanding of the neurobiology of working memory it is of primary importance that tasks used with animals, such as the rat, tax similar features of working memory as tasks used with humans. It is generally accepted that working memory consists not only of keeping information in mind across a short delay, but is also involved in the manipulation of this information. This additional feature immediately creates difficulties for tasks used to assess working memory in rodents, a major source of information concerning the neural basis of cognition and behaviour. The main assumption inferred from performance on various maze tasks, for example, is that the animal does not run down a previously re-visited maze arm because it *remembers* that it has been there previously (see Fig. 1.2.5 for an illustration of common maze tasks used with rats). This explanation, however, carries some limitations to it in light of inter-species comparison of working memory, since it is unclear whether the rat is indeed required to *simultaneously* store and process new incoming information. The rat could perform successfully by using other mediating strategies, such as odour information or various spatial cues. In the T and Y mazes, for example, (Granon *et al.*, 1994; Delatour and Gisquet-Verrier, 1999), animals have a natural tendency to spontaneously alternate and explore the environment, and there are various possibilities as to how they might perform the task. It is likely that rats do use a response strategy in solving this task (i.e. went down left side on previous trial and therefore go right on the subsequent run), but similarly to the radial maze, the use

of odour information as an indicator of which arm was previously visited is possible. Moreover, studies have shown that rats can perform this task with and without cues (Dudchenko, 2001), which strongly indicates that maze tasks can be performed in various ways. This is important because it suggests that interpretation of behavioural performance in rats cannot necessarily be attributed to processes reliant on short-term memory, and even less so, working memory.

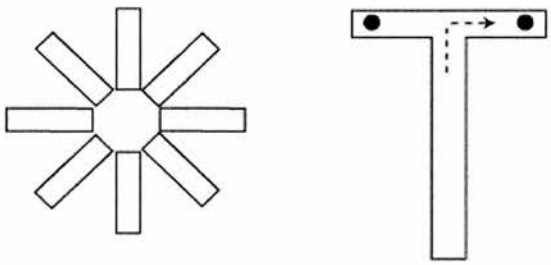

Figure 1.2.5. Left) *Eight-arm radial maze. The rat is placed in the centre and runs down each arm surrounding the centre base. Maze arms can be opened and blocked depending on the objective* ***Right)*** *Delayed alternation on a T-maze. The rat is situated at the bottom end of the T-maze and has to enter one of the arms (left or right). The rat is removed from the maze for a delay period and has to run down the alternate arm on the next trial.*

Automated tasks used in the operant chamber also face difficulties. Let alone the fact that observations have been made that rats can prepare their response before the choice/comparison phase by taking advantage of mediating strategies, such as postural positions (Gutnikov *et al.*, 1994; Chudasama and Muir, 1997), operant tasks only appear to assess the maintenance component of working memory. It is apparent, of course, that rats do show evidence of memory-guided behaviour as reflected in their performance on, for instance, a memory span task (Dudchenko *et al.*, 2000), but it is important to maintain a common conceptual framework of the type of memory under investigation if we are to excel in understanding the neural

basis of working memory. This consensus seems to be particularly needed, as any conclusion reached on the function of the PFC appears to rest on this premise. It would not be worth much to examine the role of working memory in PFC function, if the conclusion reached is based on a different conceptual understanding of the term under investigation rather than the available empirical evidence. It therefore seems necessary to re-examine the current theoretical and experimental framework, where awareness of a common understanding of this construct is considered crucial for appropriate assessment of the neural correlates of working memory.

1.3 ATTENTIONAL PROCESSES

The concept of working memory is closely related to attention. In many respects, the behavioural expression of either memory or attention is a result of the interaction between these two constructs (Fig. 1.3). To be able to attend effectively to environmentally significant events, attended stimuli needs to be stored and processed by working memory, and thus, impairments in attention can result in impairments in learning and memory and vice versa. Considerable effort has been made in trying to elucidate the underlying neuroanatomy and pharmacology of attentional processes. Research has shown impairments associated with attention in normal aging (e.g. McDowd and Craik, 1988) and Alzheimer's disease (Parasuraman and Nestor, 1991; Perry *et al.*, 2000; Rizzo *et al.*, 2000). There are, however, several aspects to attention and the understanding of the neural basis of each of these aspects varies, where some are better understood than others. To understand the biology of these attentional processes it is essential that we are able to study them individually. However, tasks typically assess a combination of these,

and it is a vital aim in current research and task design to delineate these attentional characteristics.

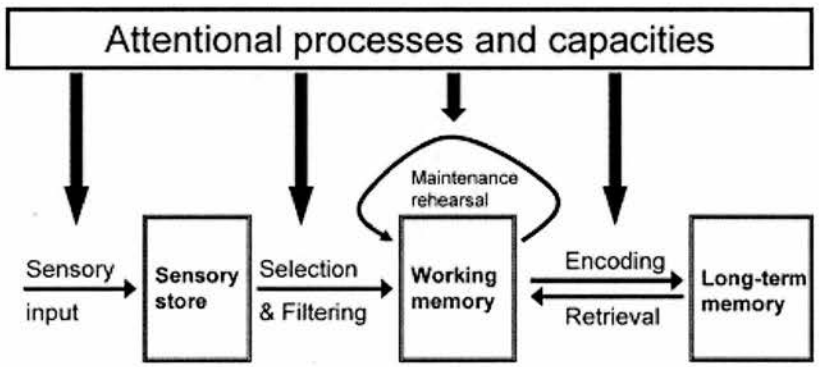

Figure 1.3. *Illustrates the role of attention in learning and memory. At various stages of information processing, from sensory input to output, attentional processes are necessary for the information to be processed effectively. (Figure from Sarter et al., 2003, Neurobiol learn mem 80: 245-56).*

1.3.1 SELECTIVE ATTENTION

Tasks used to assess selective attention typically involve focusing attentional resources on specified stimuli, while at the same time, ignoring other irrelevant and distracting signals. In the animal literature, this is commonly investigated by the infrequent presentation of auditory or visual signals (background noise) that are irrelevant to the performance of the task, and typically reflects a drop in accuracy (e.g. Carli *et al.*, 1983) that is likely due to reflexive orienting of attention towards the distracting signals. Orienting of attention, however, can be either overt or covert. Overt attention is accomplished when we direct our head or eyes to control which stimuli are to be processed, while covert attention is used when we process particular stimuli in the absence of a change in head or eye movements. Covert orienting can be exogenously cued (e.g., a visual event; bottom-up) or endogenously cued (e.g., a 'cognitive' cue indicating the probable target location;

top-down), and can be effectively studied using reaction time tasks, such as the visual orienting paradigm by Posner (1980), and recent research using fMRI techniques indicate that these attentional processes may to some extent be independent (Coull *et al.*, 2000). Covert orienting of attention results in faster reaction times and also fewer errors if attention is directed towards target location by a preceding cue compared to when a cue misdirects attention away from the upcoming target location, presumably by increasing the motor readiness to respond (see Fig. 1.3.1 for an illustration of the Posner task adapted for the rat). This differential effect of the cue on performance is called the 'validity effect' (Posner, 1980) and it reflects the benefit of directed attention and the cost of needing to redirect attention from one location to another.

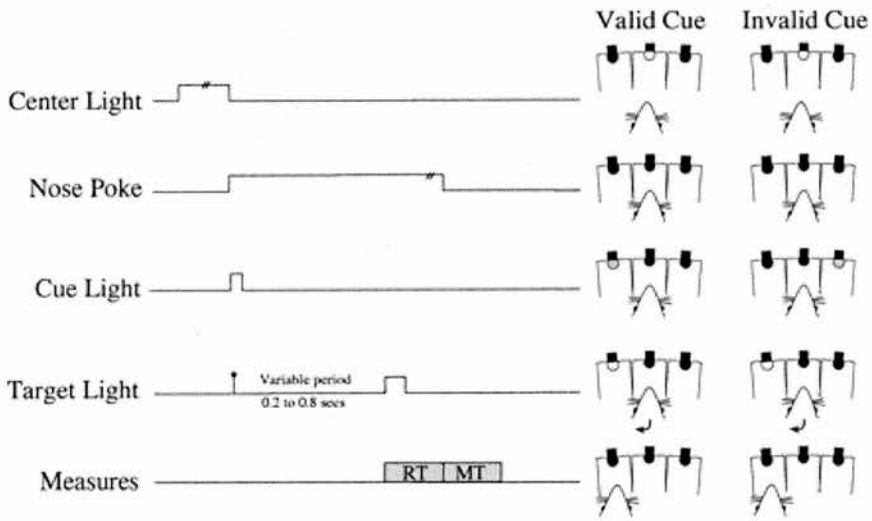

Fig. 1.3.1. *An illustration of the paradigm used to assess exogenous visuospatial covert orienting of attention in the rat (In Ward and Brown, J Neurosci, 1996, pp. 3083).*

An assessment of the ability to redirect attention is also reflected in the attentional set-shifting task, which taxes the ability to shift attention from a previously reinforced stimulus dimension (which no longer is associated with reward) to a

previously irrelevant one (which becomes reinforced). This shift in attention is commonly shown by an increase in the number of trials required to reach criterion, and evidence implicates the prefrontal cortex in this ability to shift attention and adapt to changing contingencies in humans (Owen *et al.*, 1991), monkeys (Dias *et al.*, 1996) and rats (Birrell and Brown, 2000).

1.3.2 SUSTAINED ATTENTION AND VIGILANCE

Sustained attention or vigilance can be viewed as the ability to maintain a state of readiness (attend) to a stimulus in the surrounding environment. In paradigms that assess sustained attention, subjects must maintain focus in order to detect briefly presented targets. The 5-choice serial reaction time task in rodents, which is analogous to the continuous performance task in humans, is typically considered as assessing this attentional aspect. In the task the rat is presented with a brief flash of a visual target and has to nose-poke in the hole that contains the target. Manipulations to the task such as varying the duration of the visual stimulus (Muir *et al.*, 1994) or by introducing distracting bursts of noise (Carli *et al.*, 1983) can be used to assess additional changes in the allocation of attentional resources. The 5-choice RT task, however, does not assess only sustained attention as it requires the animal to monitor several response holes at the same time to detect the spatial location of target, and thus, also requires the animal to divide its attentional resources. In an attempt to overcome such limitations, McGaughy and Sarter (1995), designed a task that required animals to respond to the presentation of visual signals using lever pressing. The rat has to press one lever when presented with a signal and another lever when the signal is absent. In the task, impaired vigilance is evident when event-rate is increased (McGaughy and Sarter, 1995), and

similarly to the 5-choice RT task, the presentation of background noise, by flashing the chamber house light, impairs the ability to discriminate between signal and non-signal events. The development of operant tasks that can be used to assess sustained attention in rats has allowed for exploration into the neural correlate of this attentional aspect, and studies suggest that sustained attentional processing is dependent upon normal cholinergic transmission (Himmelheber *et al.*, 2001; Holley *et al.*, 1995; McGaughy *et al.*, 1996; Nelson *et al.*, 2002). The finding that performance in these tasks is altered after various biological manipulations further supports the validity of the measures used to assess performance in these paradigms.

1.3.3 DIVIDED ATTENTION

Divided attention is considered as the capacity to perform more than one task simultaneously. The idea behind this construct is that our performance is to some extent constrained by limited processing capacity, which is either caused by simultaneous processing of information (parallel processing), or as a result of constant shifts between various activities in the task (serial processing). Evidence from neuroimaging suggests that, to some extent, selective and divided attention activate separate anatomical regions with performance under condition of divided attention primarily activating frontal areas (Corbetta *et al.*, 1991). Knowledge regarding the underlying neural circuit(s) of divided attention, however, is limited because of difficulties in the development of tasks assessing divided attention in experimental animals. The extent to which divided attentional resources is taxed by any given task is also highly dependent on the stimulus characteristics used and levels of processing (automatic vs. controlled).

McGaughy *et al.* (1994) developed a paradigm for use with rodents that is analogous to the cross-modal divided attention task used with humans (Falkenstein *et al.*, 1991; Hohnsbein *et al.*, 1991). The idea is that when the modality of a target stimulus is unpredictable from trial to trial, attention is divided between modalities (e.g. visual and auditory). When the modality is predictable, as when trials only consist of visual or only auditory stimuli, attention can be allocated (selective attention). Reaction times have been reported to be slower in the condition of modality *uncertainty* compared to modality *certainty* with discriminative accuracy remaining unchanged between the selective and divided conditions (McGaughy *et al.*, 1994; Turchi and Sarter, 1997). It is a possibility, however, that the overall increase in reaction times seen in the condition of divided attention relative to selective attention can be attributed to the increase in stimulus repetitions in the modality certainty condition rather than being explained by an attentional account (see Spence and Driver, 1997). Nonetheless, this effect of slower RTs during the condition of modality uncertainty is typically considered to reflect the allocation and division of attentional resources.

1.3.4 BASAL GANGLIA CIRCUITRY

Allocation of attention allows for effective production of motor behaviour. The basal ganglia is a term used to refer to groups of nuclei that play a role in the control and production of movement. The basal ganglia include many anatomical pathways, some more emphasised in the literature than others (Mink, 1996; Wichmann and DeLong, 1996; Levy et al., 1997; Smith et al., 1998) (see figure 1.3.4 for a schematic summary of basal ganglia connectivity). The brain structures that traditionally constitute this system are the striatum (caudate and putamen), the

globus pallidus (GP), the substantia nigra (SN), and the subthalamic nucleus (STN). The pallidum composes the globus pallidus pars externa (GPe) and pars interna (GPi in primates; entopeduncular nucleus (EP) in the rat). Except for the STN, which contains glutamatergic (excitatory) neurons, information through this circuitry is mainly accomplished using GABA (inhibitory). As is true for many brain structures and systems, the exact function(s) of the basal ganglia in behaviour remains a mystery. In an attempt to explain and understand the function of the basal ganglia within a unified framework, several proposals concerning the role of this system have been put forward (Alexander et al., 1986; Mink, 1996; Redgrave et al., 1999). They have in common, however, the fundamental idea that the function of the basal ganglia concerns the control of motor processes.

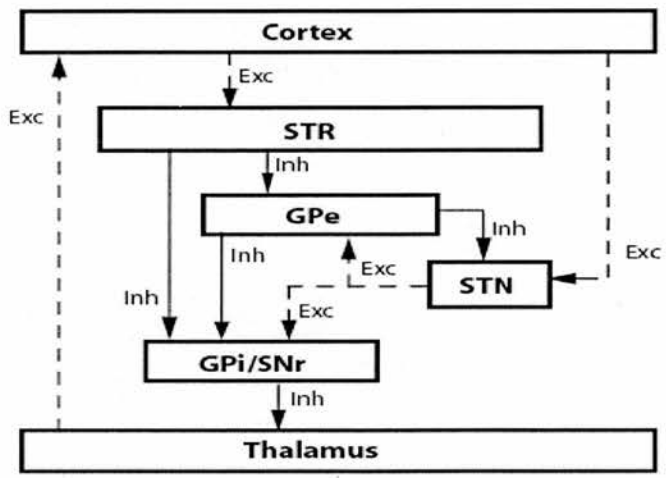

Figure 1.3.4 *A schematic representation of basal ganglia connectivity depicting the major anatomical pathways through this system. Efferent projections enter the system from cortical regions, and information is conveyed to the thalamus via intrinsic connections. The striatum (STR) and subthalamic nucleus (STN) are both considered as input structures of the basal ganglia and send information from cortex to globus pallidus pars externa (GPe) and the output structures globus pallidus pars interna (GPi) and the substantia nigra pars reticulata (SNr). Solid pathways are inhibitory (Inh) and broken ones are excitatory (Exc).*

Traditionally, the striatum was regarded as the major input structure of the basal ganglia (Albin *et al.*, 1989). The striatum receives direct excitatory inputs from the cortex and sends information to the output regions of this system by means of either a direct inhibitory pathway to the GPi and/or an indirect pathway that projects first to the GPe, which in turn projects to the STN, and finally, information is send from STN to the GPi (Fig. 1.3.4) (see Parent and Hazrati, 1995 for a view that questions the utility of the indirect pathway). An unbalance in the activity of the direct and indirect pathways results in complex motor deficits, although it is uncertain as to what exactly this unbalance of basal ganglia output causes in terms of overall movement.

1.3.4.1 THE SUBTHALAMIC NUCLEUS

The subthalamic nucleus (STN) has extensive connections with brain structures that are located at both the subcortical (Kita and Kitai, 1987) and cortical level (Afsharpour, 1985; Berendse and Groenewegen, 1991; Maurice *et al.*, 1998). With more recent evidence improving our understanding of the anatomical connectivity of the STN with other structures (Rouzaire-Dubois and Scarnati, 1987; Berendse and Groenewegen, 1991), and the functional specificity of distinct anatomical territories of this structure (Fig. 1.3.4.1), the significance of this nucleus in basal ganglia function has changed.

This nucleus is now reported as an important contributor in processing information to the basal ganglia (Mink and Thach, 1993; Parent and Hazrati, 1995; Hamani *et al.*, 2004), similarly to the striatum, via an excitatory pathway originating in cortical areas and projecting directly to the STN, and coined the 'hyper-direct' pathway (Nambu *et al.*, 2002). The dysfunctional over-activity of this

nucleus in Parkinson's disease and the fact that the nucleus is a target for treatment of Parkinson's disease (Bergman *et al.*, 1990; Limousin *et al.*, 1995) means that it is important to understand the contribution of the subthalamic nucleus to motor control and cognition.

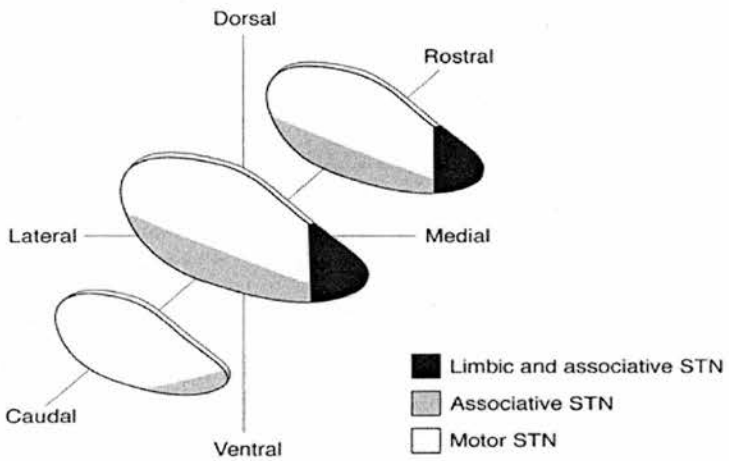

Figure 1.3.4.1. *A schematic representation of the anatomical localization of limbic, associative and motor aspects of the subthalamic nucleus (STN) (figure from Hamani et al., 2004; Brain, 127, pp. 4-20). The STN is divided into three parts according to its anatomical connections. The bulk of this nucleus is linked to motor circuits. One region has connections with both limbic structures and cortical areas and occupies parts of the medial portion. The more ventral region composes the other part of this nucleus that has connections with cortical structures.*

1.3.4.2 THE FUNCTIONAL CONTRIBUTION OF THE SUB-THALAMIC NUCLEUS

There is now accumulating evidence showing that lesions or pharmacological inactivation of the STN in rodents induces motor deficits, for example, in simple and choice reaction time tasks (Phillips and Brown, 1999; Baunez *et al.*, 2001). These deficits are characterised by profound impairments in discriminative accuracy and anticipatory responding, but reaction time itself is generally unimpaired and the nature of these deficits appears to be a difficulty in movement inhibitory control.

For instance, STN-lesioned animals show difficulty in inhibiting prepotent responses towards target locations with high probability (D.M. Thompson, PhD thesis), and recent theorising has suggested a role for the STN in blocking competing responses by exerting an inhibitory influence on motor output (Mink, 1996; Baunez *et al.*, 2001). However, the multiplicity of the behavioural effects seen after STN inactivation makes it difficult to disentangle the precise functional contribution of this brain structure. Not only does this structure appear to play a crucial role in motor behaviour, but a few recent reports indicate that it may also contribute to cognition, specifically attention (Baunez and Robbins, 1997; Schroeder *et al.*, 2002; Chudasama *et al.*, 2003a; Winstanley *et al.*, 2005). In terms of the latter, lesions to this nucleus in the rat impair performance on the 5-choice serial reaction task (Baunez and Robbins, 1997; Chudasama *et al.*, 2003a; Winstanley *et al.*, 2005), which taxes spatial selective and divided attention. Thus, although some behavioural patterns observed after damage to the STN appears to be accounted for by proposing a motor impairment, recent studies indicate that this structure may also contribute to other behavioural operations, particularly those which require the allocation of attentional processes. To further complicate matters, however, some studies have not found any effect after unilateral lesions of this structure on accuracy or reaction time performance in a visual choice reaction time task (Phillips and Brown, 1999), nor in exogenous (Phillips and Brown, 2000) or endogenous (D.M. Thompson, PhD thesis) orienting of attention. It is therefore still unknown whether bilateral STN damage is required to see evidence of attentional impairment and, in addition, in what kind of attentional aspect(s) the STN might play a role.

1.3.5 THE ASCENDING NORADRENERGIC SYSTEM

The locus coeruleus noradrenergic system has been implicated in a variety of cognitive processes (for a review see Berridge and Waterhouse, 2003). Though there are other noradrenergic clusters situated in the brain stem, such as the lateral tegmental area, the locus coeruleus has perhaps been of most interest to researchers that attempt to identify the functional contribution of noradrenaline (NA)••. The noradrenergic neurons of the locus coeruleus ascends in the dorsal noradrenergic bundle (DANB) and innervate major regions, such as the frontal cortex, cingulate cortex, hippocampal formation, amygdala, thalamus and cerebellum (e.g. Foote *et al.*, 1983) (Fig. 1.3.5.1).

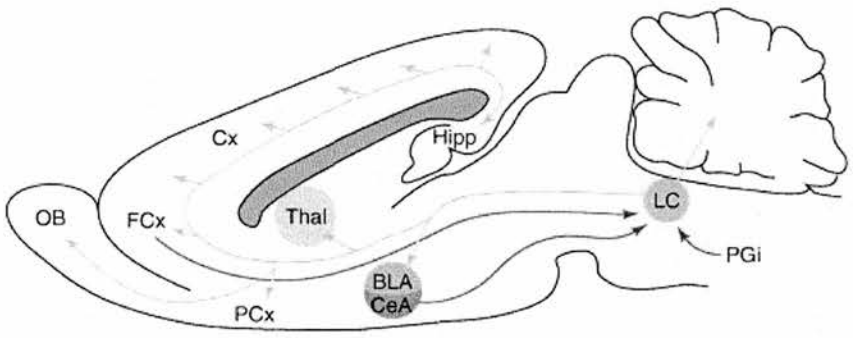

Figure 1.3.5.1 *Major projection sites of the locus coeruleus nucleus (LC) via the dorsal noradrenergic bundle (DNAB) in the rat brain. Areas that receive noradrenergic projections from the LC are the septum, thalamus (Thal), cortex, including the frontal cortex (FC), and hippocampus (HC) (In Bouret and Sara, 2005, Trends in Neurosci 28: 574-582).*

•• Noradrenaline (NA) and dopamine (DA) are both catecholamines, and the early stages in the synthesis of these two neurotransmitters are identical. Some neurons contain the enzyme **dopamine-beta-hydroxylase**, which enables them to convert DA into NA, and these neurons release NA. Conversely, those cells that do not contain this enzyme release DA.

1.3.5.1 THE NORADRENERGIC SYSTEM & BEHAVIOUR

Perhaps the most recent idea of LC function comes from neurophysiological investigations that report LC neurons that show a response to the conditioned stimulus (CS) associated with reinforcement (CS+) (Bouret and Sara, 2004). It has been suggested that these results reflect the behavioural adaptation of the animal to the external environment where LC neurons respond to task relevant stimuli when their timing cannot be fully predicted (Bouret and Sara, 2005), and are in agreement with findings that show increased firing of LC neurons to 'novel' stimuli (Vankov *et al.*, 1995). Together with less recent observations that show that LC neurons are related to the wake-sleep cycle, these findings have, in part, led to the hypothesis of LC playing a role in attentional processes (Aston-Jones *et al.*, 1999, 2000). This hypothesis is further strengthened by studies in humans that report an involvement of the noradrenergic system in some aspects of attention. For instance, clonidine, a α_2 adrenoceptor agonist, has been reported to alter sustained attention (Coull *et al.*, 1995a; Coull *et al.*, 1995b) and alter the validity effect in the Posner task (Clark *et al.*, 1989), while reducing the alerting effect in the monkey (Witte and Marrocco, 1997).

1.3.5.2 THE DORSAL NORADRENERGIC BUNDLE

In the examination of the functional significance of noradrenaline (NA), one approach has been to lesion the dorsal ascending noradrenergic bundle (DNAB) using the neurotoxin 6-hydroxydopamine (6-OHDA)[••]. The DNAB is the major pathway by which NA reaches the forebrain (Ungerstedt, 1971; McNaughton and

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[••] **6-Hydroxydopamine** (6-OHDA) is a neurotoxin used to selectively kill dopaminergic and noradrenergic neurons, and is taken up by neurons by means of dopamine and noradrenaline reuptake transporters.

Mason, 1980). Although projections from the LC do not contribute greatly to NA content in the hypothalamus, which is mainly supplied by the ventral noradrenergic bundle (VNAB), it is often the case that DNAB depletion, to a varying extent, affects NA level in the hypothalamic region (Carli *et al.*, 1983; Selden *et al.*, 1990 see also study described in Chapter V). Nevertheless, a consideration of the available behavioural evidence indicates that the changes seen in performance after lesions to the dorsal ascending noradrenergic system are likely attributed to alterations in neocortical NA level.

There are now accumulating evidence showing that lesions of this structure in rodents induce behavioural alterations in various behavioural settings, such as, reinforcement schedules (Owen *et al.*, 1982), aspects of learning (Everitt *et al.*, 1983; Connor *et al.*, 1992; Langlais *et al.*, 1993; al-Zahrani *et al.*, 1997), fear conditioning (Tsaltas *et al.*, 1984; Selden *et al.*, 1991) and tasks assessing attention (Mason and Fibiger, 1978; Selden *et al.*, 1990; Lapiz and Morilak, 2006). Pharmacological manipulation of this system using idazoxan (a noradrenergic α_2 receptor antagonist) has been shown to alter behaviour to novel objects (Devauges and Sara, 1990), and also lesions of this system has been reported to change behaviour to novelty in a food preference test (Cole *et al.*, 1988). Furthermore, the integrity of brain noradrenaline also appears to play an important role under certain distracting conditions (Carli *et al.*, 1983; Cole and Robbins, 1992), although the effects of distracting stimuli on behaviour after DNAB lesions are equivocal (Mason and Fibiger, 1978), as both an impairment and a lack thereof have been found using both auditory (Carli et al., 1983; Mason and Fibiger, 1978) and visual distractors (Mason and Fibiger, 1978; McGaughy *et al.*, 1997). Despite the seemingly wide nature of the behavioural effects of DNAB lesions, the available

experimental data suggests that the noradrenergic system somehow plays an important role in attentional processes (Mason and Iversen, 1979; Mason and Lin, 1980; Carli *et al.*, 1983; Clark *et al.*, 1989; Cole and Robbins, 1992). The particular attentional conditions under which this system is necessary, however, require further delineation.

1.3.5.3 THE FUNCTIONAL LINK BETWEEN THE NORADRENERGIC & CHOLINERGIC SYSTEMS

The subtle and intricate nature of the functional contribution of the ascending noradrenergic system is further convoluted by studies that suggest an interaction between NA projections from LC and the cholinergic system (Decker and Gallagher, 1987). The precise relationship between these two systems, however, is somewhat unclear but a few experiments indicate contrasting behavioural effects after lesions to the DNAB and NbM on performance in the Morris water maze task (Connor *et al.*, 1992), where DNAB lesions improved and NbM lesions impaired acquisition of this task. In the same study, however, during a water escape delayed conditional discrimination task, all groups exhibited similar performance, suggesting that the effect of DNAB lesions was task specific. Though few, these studies indicate a functional link between the noradrenergic and cholinergic systems in some aspects of behaviour. More research is necessary, comparing these two systems in behaviour, before any firm conclusions can be reached as to the extent of their functional interactions. After reviewing the literature on the behavioural effects of manipulations to the noradrenergic and cholinergic systems, Yu and Dayan (2005) suggested that these two systems probably play a synergistic role in behaviour. In their model, noradrenaline (NA) is predicted to play a role whenever the subject is faced with, what they called, unexpected uncertainty (an

abrupt change in stimulus-reward contingencies, e.g. attentional set-shifting), while acetylcholine (Ach) is considered to play a part in expected uncertainty (the probability of a valid cue, e.g. Posner task). Although the model appears to account for Ach and NA involvement in some tasks, the line between expected and unexpected uncertainty needs conceptual refinement.

1.3.6 THE CHOLINERGIC SYSTEM

Similarly to noradrenaline, the cholinergic system has been implicated in attentional processes (Everitt and Robbins, 1997; Baxter and Chiba, 1999; Sarter *et al.*, 2003; Sarter *et al.*, 2005). The investigation of the function(s) of acetylcholine has mainly focused on cholinergic neurons that project to cortical areas (Muir *et al.*, 1994; Dalley *et al.*, 2001) and hippocampus (Baxter *et al.*, 1997; Baxter *et al.*, 1999), though there is another group further rostral consisting of the laterodorsal tegmental region and the pedunculupontine nucleus projecting to the thalamus, hypothalamus and basal forebrain (Woolf, 1991). The cholinergic neurons of primary interest herein are those that project to cortical regions, in particular the neocortex (consisting of the nucleus basalis magnocellularis; NbM, substantia innominata; SI and the horizontal diagonal band; HDB) (Mesulam *et al.*, 1983; Eckenstein *et al.*, 1988) (Fig. 1.3.6), although the cholinergic projections to the hippocampal formation has also received considerable attention in the literature, receiving input from the medial septum region (consisting of medial septal area; MS and the vertical diagonal band; VDB). Recent improvements in lesioning techniques have provided a better understanding of the functions of the cholinergic basal forebrain system. Experiments have in the past used ibotenic acid to lesion the basal forebrain or the excitotoxin AMPA (alpha-amino-3-hydroxy-5-methyl-4-

isoxazolepropionic acid) to lesion this system. However, AMPA is not completely selective for cholinergic neurons and relatively recently have investigators effectively developed a toxin that is selective for cholinergic neurons, such as the immunotoxin 192 IgG saporin•• (Wiley *et al.*, 1991).

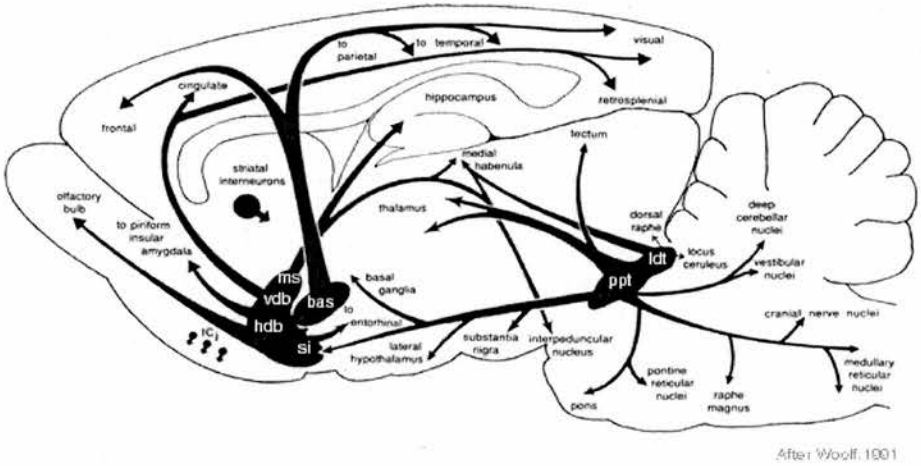


Figure 1.3.6. *Schematic illustration of the major projection sites of the cholinergic system. Major terminating sites of the NbM basal forebrain cholinergic cells are the neocortex, hippocampus (allocortex), amygdala, olfactory bulb, cingulate and visual regions (In Woolf, Prog Neurobiol, 1991, pp. 475-524).*

1.3.6.1 THE FUNCTIONAL CONTRIBUTION OF THE CHOLINERGIC SYSTEM

The subsequent development of 192-IgG saporin has reduced uncertainty as to the neurochemical source of the behavioural alterations seen after lesions to the basal forebrain cholinergic system (see Wrenn and Wiley, 1998 for a brief review).

•• **192-IgG saporin** is an antibody to the p75 nerve growth factor (NGF) receptor coupled to a ribosome inactivating compound saporin. Infusion of 192-IgG saporin into the basal forebrain selectively kills cholinergic neurons, which contain NGF receptors, without damage to noncholinergic neurons.

Previous doubts as to the neurochemical underpinnings of the behavioural alterations seen in performance can now be made with greater scrutiny.

It has been suggested that the memory impairments seen in Alzheimer's disease is partly due to degeneration of the cholinergic neurons of the NbM (Bartus *et al.*, 1982; Coyle *et al.*, 1983). More recently, however, it has been speculated that impairments on tasks considered to assess some aspect of memory may have been caused by an attentional deficit, and thus, also raising an issue as to the relative role of the cortical cholinergic system in memory processes. More recently, studies strongly indicate a significant role for the nucleus basalis cholinergic system in many aspects of attentional processing, such as selective (Chiba *et al.*, 1995), divided (Turchi and Sarter, 1997), in addition to sustained attention and vigilance (Muir *et al.*, 1994, 1995; McGaughy *et al.*, 2002). In particular, lesions of the basal forebrain have been shown to impair exogenously cued covert attention, as evident in an increase of the validity effect in the Posner task (Voytko *et al.*, 1994) and similar results have been found in Alzheimer's disease (Parasuraman *et al.*, 1992), which supports the idea that these attentional impairments seen in this patient group partially result from loss of basal forebrain cholinergic neurons (Lawrence and Sahakian, 1995). Due to the seemingly wide nature of cholinergic function, whether the cholinergic system operates a 'general' attentional mechanism or whether its role in attentional processes is more specific needs additional investigation if its role in behaviour is to be clarified.

1.3.6.2 NICOTINIC ACETYLCHOLINE & BEHAVIOUR

Further evidence supporting a role for the cholinergic system in attentional function is obtained from investigations that examine drug-induced alterations in performance. Observations indicate that both muscarinic and nicotinic cholinergic

receptor subtypes are important for attentional processes (Davidson *et al.*, 1999; Phillips *et al.*, 2000), but that the two receptor subtypes may exert a differential role in cognitive processes (Mirza and Stolerman, 2000). This is, in part, likely explained by differences in receptor density in distinct brain regions. There is a high density of muscarinic receptors in the basal forebrain, while this area has relatively few nicotinic receptors (Monferini, 1992). The behavioural evidence, however, suggests that nicotine still may play a role in the restoration of performance after basal forebrain lesions (Muir *et al.*, 1995), or in Alzheimer's disease (Sahakian *et al.*, 1989; Levin *et al.*, 2006) either directly or indirectly via its interactions with other neurotransmitters. Furthermore, evidence indicates that lesions of the NBM also increase sensitivity to nicotine by cortical neurons (Abdulla *et al.*, 1995).

Similar to lesions of the cholinergic system, nicotine appears to contribute to exogenous covert attention as reported in both humans (Murphy and Klein, 1998) and rats (Phillips *et al.*, 2000). Contrary to lesions of this system, however, nicotine produces a reduction of the validity effect in the Posner task by reducing reaction times to invalid trials. Though nicotine appears to have a general facilatory effect on performance, the understanding of its functional contribution under various attentional circumstances is still rather limited.

1.4 EXPERIMENTAL OUTLINE

The research described in this thesis began with the attempt to develop a working memory task for the rat. The aim was to develop a task that assessed working memory at a species-appropriate level, which compensated for the insufficiencies inherent in working memory tasks employed so far in the rat. Due to the close association between working memory and attention, it became a natural

progression of this thesis to pursue further the neurobiology of attentional processes. There are several successful tasks developed to assess attention in the rat, which allow a fruitful exploration into its neural correlate. Interestingly, the STN, a structure typically viewed in terms of motor behaviour, has been implicated in attentional processes. Study 2, therefore, explored the contribution of the subthalamic nucleus (STN) to attentional processes, and more specifically, divided attention. As the STN has been found to impair response preparation in a task used to assess endogenous covert orienting of attention, without affecting directed attention, it was decided to explore further the possible neural substrate of the attentional aspect of this task. As noradrenaline appears to show subtle and not well-understood effects on tasks used to assess attention, more research is required to further specify the role of noradrenaline in attentional processes. Study 3, therefore, examined the effects of DNAB lesions on endogenous covert orienting of attention. Finally, there appears to be strong evidence that the cholinergic system participates in covert attention, in particular exogenous covert attention. It was subsequently decided to explore the relative contribution of acetylcholine to endogenous covert attentional orienting in the last study described in this thesis.

CHAPTER II:

GENERAL PROCEDURES FOR ALL STUDIES

2.1 PROCEDURES FOR STUDIES 1 TO 4

In this chapter the methodological aspects that were common to all experiments undertaken during the PhD are outlined. The chapter is divided into similar sub-sections as found under the relevant method section for each study described in this thesis. The study described in Chapter III differed from all the other studies, in that it was a non-automated task requiring human interaction and observation. All other studies used automated tasks (computer-controlled). However, the sub-sections of this chapter are also applicable to study 1 (e.g., animals, apparatus, etc.). Those methodological aspects that were specific to an experiment are outlined in the relevant chapter.

The majority of work presented in this thesis was carried out at the School of Psychology at the University of St Andrews. Study 3, which is described in Chapter V, was done in collaboration with T.W. Robbins and J.W. Dalley and D. Theobald at the School of Experimental Psychology at the University of Cambridge, who carried out the biochemical analysis for this experiment.

2.2 ANIMALS

Each study described in this thesis used a set of twenty-four naïve Lister hooded rats (Harlan, UK). A notable exception was Study 4, described in Chapter VI, which recruited the same animals in the examination of the behavioural effects after both neuroanatomical and pharmacological manipulations of the cholinergic system. Not all animals that took part in the experiments presented herein, however, completed the experiment they were assigned to, and the causes for this are described under the relevant section for each study.

Animals were housed in pairs pre-and post-operatively and maintained in 25x45x15 cm cages. In the study described in Chapter IV, all animals were single-housed post-operatively due to antagonistic behaviour that commonly accompanies lesions of the subthalamic nucleus. All animals followed a 12-hr light/dark cycle (lights on 7.00 am and off 7.00 pm) where behavioural training and testing was carried out during the light phase. The animals were given 15-20 g of standard laboratory rat chow (Special Diet Services, Essex, UK) daily, in addition to earned food in the behavioural task, and had free access to water in the home cage. This amount of food was maintained throughout behavioural testing in all the experiments presented herein.

2.3 APPARATUS

This PhD involved both automated (computer-controlled) testing systems and a naturalistic task requiring human interaction and observation in its examination of attentional control in the rat, and these apparatuses are described in Chapter III, sections 3.2.1.2 and 3.3.1.2, respectively.

2.4 BEHAVIOURAL TRAINING

The extent of training necessary for animals to attain good stable performance on the tasks used in the experiments varied according to individual abilities and task requirements. In the operant tasks, animals typically needed extensive training to achieve baseline performance. Animals were trained once a day and mostly seven days a week. The number of training sessions needed to achieve baseline performance and training protocol are detailed under each study. The animals

required training in simple stimulus-reward association, and the details of this are described in the sub-section that follows.

2.4.1 Operant tasks

Each of the operant studies described herein had some common characteristics in the initial training regimen. All animals were initially trained to push the food panel to attain food from the food tray. Whenever the rat pushed the panel a food pellet was delivered. A session lasted for 30 minutes. When animals were able to retrieve and consume approximately 60 food pellets within a 30-minute session, they progressed to the next stage in training. At the next stage, the light in the central aperture was turned on when the animal pushed the access panel to the food tray, and the rat was required to make a nose poke in the lit aperture to receive reward. This repeated until 30 minutes had elapsed or until the animal had earned 120 food pellets, whichever came first. To accustom the animals to sustain nose poke in the central aperture for a variable delay, animals were trained using a 0.25 second delay followed by a 0.5 second delay. If the animal retracted its nose-poke before the end of the delay an anticipatory response was registered and a brief (1sec) offset of the house light occurred and the animal was required to press the food panel to initiate the next trial. When animals performed reliably (i.e. low anticipatory rate; anticipatory responses defined in section 2.5.2, data collection and analysis, of this Chapter) they progressed to the main task.

2.4.2 Naturalistic task

The non-automated study described in Chapter III also required initial training of stimulus-reward association similar to the studies carried out in the operant chamber. The day before testing a bowl filled with sawdust was placed in the home

cage overnight. The bowl contained several Honey Loops that were buried in the sawdust. The next day the animal was placed in the plastic box with two bowls, one in each of the two small compartments. Both bowls were filled with sawdust and ½ Honey Loop, and the animal was allowed to freely explore both bowls. When the animal had retrieved the reward from both bowls the barrier was set in place and the bowls taken out and baited with Honey Loops. The bowls were then placed in the two small compartments, and the barrier removed to start another trial. This continued until the animal had obtained twelve rewards. The animal then underwent a simple discrimination (SD) of odour followed by digging medium. In the odour discrimination, the animal was presented with two bowls, each of which contained a different exemplar. Only one of the two exemplars from each dimension was correct (baited), while the other was incorrect (unbaited). A trial started by removing the barrier that separated the large compartment where the animal was situated, allowing access to the presented bowls. The exemplars used for the odour discrimination were oregano (rewarded) and mint (unrewarded), and shredded paper (rewarded) and polystyrene (unrewarded) as digging mediums.

2.5 DATA COLLECTION AND ANALYSIS

When animals achieved stable performance in the operant tasks, they were pseudorandomly divided into two groups (control and lesion) before surgery. The data for the two experimental groups were then checked to ensure that they were matched, to the extent possible, on the variables of interest and adjusted if necessary. The same behavioural measures were recorded in the operant studies, which were performance accuracy, anticipatory responses, late errors, movement

time and reaction time (statistical factors and levels are specified under each study). In the non-automated task, behavioural measures were recorded differently, and are explained under each of the measures described below.

In the operant tasks, raw data was transferred from the computers attached to the experimental apparatus and collapsed into one data file using AWK (a text based program that extracts particular records in a file). All data were collapsed over sessions to give average values of each variable for each animal. The data files were opened using Microsoft Excel. Repeated measures analysis of variance (ANOVA) was conducted using SPSS version 12.0. All studies reported applied a criterion for statistical significance with a probability level of $p < 0.05$.

2.5.1 PERFORMANCE ACCURACY

This task measure was an indicator of the performance level displayed by the animal in the task. In all operant paradigms described animals were required to make a directional (left versus right) response according to task rules, and performance accuracy was recorded in terms of the percentage of incorrect responses made (i.e. number of incorrect responses divided by the number of correct plus incorrect responses). Percentage of incorrect responses were used rather than percentage of correct responses as the measure of performance accuracy, since the latter changes as a result of any error (including premature responses), whereas percentage incorrect considered only those trials in which a response is made after the target is presented.

For the non-automated task, performance accuracy was recorded as the number of trials required to achieve a criterion of six consecutive trials correct.

2.5.2 ANTICIPATORY RESPONSES

Anticipatory responses (or premature responses) occurred when the nose-poke was withdrawn prior to the onset of target stimulus. The percentage of anticipatory responses made by the animal was calculated as the number of anticipatory responses divided by the total number of completed trials (i.e. correct plus incorrect trials, excluding late errors). This measure was not applicable in the naturalistic task.

2.5.3 LATE ERRORS

This task measure was an indicator of the animal's aptitude to make a response. Late errors were recorded when the animal did not make a response after the onset of target stimulus and before the end of the trial. The percentage of late errors was calculated as the number of late errors divided by the total number of completed trials (i.e. correct plus incorrect trials, excluding anticipatory responses).

For the non-automated task, late errors were recorded if the animal had not made a response within 10 minutes, whereby the next trial would start. If the animal had not made a response (i.e. dug in a bowl) on three consecutive trials (each of which lasted 10 minutes) the session would be terminated.

2.5.4 MOVEMENT TIME

Movement time was an indicator of the time it took the animal to make a response after the target stimulus appeared and was the time from when the rat withdrew nose from central hole and responded in either of the two side apertures (left or right).

2.5.5 REACTION TIME

This measure is typically viewed in terms of the time taken between a stimulus and the response to it. However, not to confound it with movement time, reaction time was measured as the time it took from target onset (triggered by nose poke in central aperture) to withdrawal of the nose from the central aperture (i.e. prior to movement time). Reaction time was assessed in terms of the mean in study 2 and expressed as the mode in study 3 and 4 (the basis for this is explained in the relevant chapters).

For the non-automated task, reaction time was measured as the time it took from the barrier being removed until the animal made a dig. No distinction could be made between movement and reaction time on this task.

2.6 SURGERY

All animals that underwent surgery were anaesthetised by inhalation of Isoflurane (Abbott Laboratories Ltd.; level of anaesthesia was maintained at 2 - 2.5 percent throughout surgery.) and placed in a stereotaxic frame using ear bars (Kopf, Tujunga, CA). The level of skull during surgery was not common to all studies and is therefore described under the relevant section for each experiment. Animals' heads were shaved and skin cut open to access the skull and a drill used to expose the surface of the brain at the specified co-ordinates. Anterior-posterior co-ordinates were taken from bregma, while medial-lateral co-ordinates were taken using the sinus vein. All dorsal-ventral co-ordinates were taken from dura. After surgery, the wound was closed using metal suture clips. The stereotaxic co-ordinates used, type of toxin, together with dose and concentration are specified under each experiment. During early recovery after surgery animals were housed

singly and weight patterns were monitored. The condition of the wounds and general health were also examined and the animals were handled daily.

All procedures followed the requirements of the UK Animals Scientific Procedures Act (1986).

CHAPTER III:

DEVELOPMENT OF A WORKING MEMORY TASK FOR THE RAT

- This chapter describes the attempt to develop a working memory task for the rat. The aim was to develop a task that assessed working memory at a species-appropriate level, which compensated for the insufficiencies inherent in tasks employed so far in the rat.

3.1 INTRODUCTION

In the examination of the neural substrate(s) underlying working memory in the rat, studies exploit a relatively simple notion of this construct in their paradigms that does not correspond to tasks used with humans (see General Introduction, section 1.2). Indeed there are studies that directly question the utility of these tasks. Not only do rats appear to show difficulty in performing some delay tasks in the operant chamber (see Steckler *et al.*, 1998 for a thorough review of tasks used with rats), but problems also exist in terms of understanding how rats perform other types of tasks used to assess this construct. For example, in a study by Herremans and colleagues (1995) rats were tested on an object delayed non-matching to sample task considered to assess working memory. In their task the sample object was presented to the rat, which was presented again along with another object after a delay. The choice objects were first presented in a fixed order and then the order was reversed. They found a considerable drop in performance when the order of object presentation at choice reversed, and animals were unable to learn the task when order of presentation was randomised. They suggested that perhaps performance is based on a discrimination of objects at choice rather than working memory of sample object, although the exact cue(s) used for this discrimination was uncertain. The importance of good tasks, however, is further stressed by the introduction of species-typical behaviour, which has been shown to sometimes elicit alterations in animals' performance. After extensive training on a task or task routines, animals' behaviour can change from specific learned behaviours towards more naturalistic behaviours to obtain reward (refer to Thorpe *et al.*, 2004 for discussion of impacts of species-typical behaviour on memory tasks), and can be falsely interpreted as an inability to do the task. Experimental instances such as the

one described cast doubt as to whether rats exhibit similar cognitive complexity in regard to working memory as found in primates. This is likely to contribute to difficulties in inter-species comparison due to the inherent limitations placed on the inferences that can be drawn from experimental data with rats. The development of a working memory task that tap onto similar cognitive requirements as tasks used with primates would ameliorate this problem. In this respect, species appropriate stimuli are an important consideration in task development, and studies have shown that rats typically learn much quicker using tacile and olfactory stimuli (Birrell and Brown, 2000), than the learning curve typically produced in operant tasks. In the current research, therefore, two tasks were designed to assess working memory capacity in rats; one task was designed for use in the operant chamber and the other was designed to assess working memory in a naturalistic environment, using tactile and olfactory stimuli. The aim was to develop a version of a match-to-sample task that assessed working memory at a species-appropriate level, which compensated for the insufficiencies inherent in tasks employed so far.

3.2 STUDY 1: WORKING MEMORY TASK DESIGNED FOR USE IN THE OPERANT CHAMBER

3.2.1 METHOD

3.2.1.1 Animals

The study began with twenty-four naïve Lister hooded rats (Harlan, UK). Housing and testing conditions were the same as outlined in the General Procedures (Chapter II; section 2.2).

3.2.1.2 Apparatus

The behavioural apparatus used was the nine-hole box. Eight nine-hole boxes (25 x 25 cm; see (Carli et al., 1983), located within sound-attenuating cabinets, were used (Paul Fray Ltd, Cambridge, UK) in each experiment (Fig. 3.2.1.2). Each box had an array of nine apertures (diameter 2.5 cm) that were situated at the curved rear wall of the chamber and 2 cm above the grid floor. An infrared photocell beam that detected responses was located at the entrance of each aperture. Only the three central apertures were used in the experiments described herein, while all others were blocked using transparent caps. Visual stimuli were presented at the rear of each aperture, while auditory stimuli were presented through a loudspeaker positioned in the ceiling of the chamber. A house light (3-W) illuminated the testing chamber.

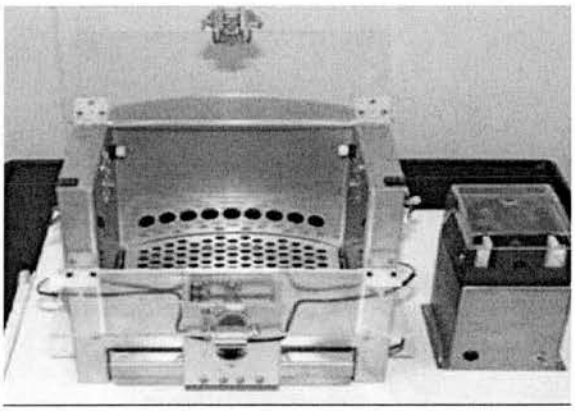


***Figure** 3.2.1.2. A photograph depicting the internal construction of the "9-hole box" used in the computer-controlled experiments.*

Food pellets (Noyes Pellets, 45 mg, Sandown Scientific) were provided using a dispenser that was connected to a food tray. Food could be obtained by pushing an access panel at the front wall, where panel pushes were detected by a micro switch on the hinged panel. Four computers were used to control stimuli input and data output (two boxes per computer) using programming software by BBC Basic (SPIDER version; Paul Fray Ltd, Cambridge, UK).

3.2.1.3 Task design

To compensate for the drawbacks inherent in current examination of working memory in the rat, it was essential that the task featured the following: 1) *the animal had to hold in 'mind' at least two items, each of which required a different response, until presented with a cue that signalled correct response, and* 2) *the task needed to be a delay comparison task.* This would require the animal to compare previous and/or upcoming items for correct response and make the animal unable to prepare its response in advance.

3.2.1.4 Behavioural protocol

All animals underwent training in simple stimulus-reward association before they were introduced to the main task (described in Chapter II, section 2.4.1).

Behavioural task: Animals had to make a response according to the brightness and spatial location of lights. Lights appeared in left and/or right side apertures immediately adjacent to the central aperture. Animals were presented with two consecutive lights at either the same or different spatial location. They were then presented with a choice that consisted of two lights of different brightness (one dim and the other bright), simultaneously occurring in both side holes. Correct responding was according to the following rules: when presented with two consecutive lights of same luminance (both dim or both bright), correct response was to match luminance of lights at choice. If the animal was presented with two consecutive lights of different luminance (one dim and one bright), correct response was to match side of presentation at choice irrespective of spatial location of brightness of lights. Importantly, in match brightness trials, the two consecutive lights appeared at *different* spatial locations. During match side trials, the two

consecutive lights appeared at the *same* spatial location. The presentation of the second sample (cue) indicated which rule was currently in effect (i.e. match side or match light). A trial began when the rat made a nose poke into the illuminated side hole (see figure 3.2.1.4 of current section for a schematic illustration of the behavioural task). Sample cue light was turned off by the withdrawal of the nose from the illuminated side hole. The animal was subsequently required to push the food panel to initiate the onset of the next sample cue light, and again nose poke in the illuminated hole, and finally push the food panel again to be presented with the choice. After a response had been made, the next trial started by pushing the access panel to the food tray.

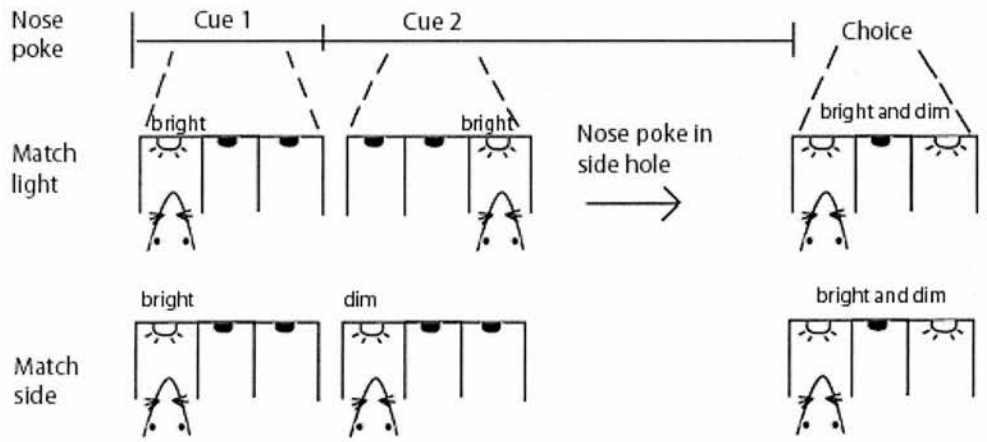


***Figure** 3.2.1.4. An illustration of the working memory task. The animal had to nose poke in the illuminated side hole. The target stimulus was turned off when the animal made a nose poke in hole. In match light condition, sample cue lights were presented in different side holes (both bright or dim), but could occur on either side at choice. In match side condition, sample cue lights were presented in same side hole, and side of brightness was irrelevant.*

3.2.1.5 Behavioural training

1) Animals were first presented with the task without shaped training. Eight sessions were carried out, and performance is shown in Figure 3.2.1.5.

2) As animals completed very few trials, a re-think of training was in order. To encourage animals to make a response, lights at choice remained on until the rat made a nose poke in the correct hole. Additionally, only the match light condition was presented. Both the two consecutive sample cue lights and correct hole at choice were all presented on the same side. Seventeen sessions were carried out. Number of trials completed by the animal increased considerably (Fig. 3.2.1.5), together with an increase in performance accuracy.

3) Modification of version 2 above: in contrast to version 2, each of the two sample cue lights were presented in different side holes instead of on the same side, but could occur on either side at choice. Thirty-one sessions were carried out. Number of trials completed remained similar to previous version, but performance accuracy decreased (Fig. 3.2.1.5).

4) Rats were presented with one sample cue light in central hole instead of in the side holes, followed by a choice (simple match sample to choice). If the animal made an incorrect response, a timeout of 1 sec ensued of which house light was switched off, and the animal had to press the panel to begin the next trial. Animals underwent seven sessions of this shaping task. Performance accuracy did not improve (Fig. 3.2.1.5).

5) Modification of version 4 above: in contrast to version 4, animals were required to match dim light only. The light (dim) in central hole was not turned off at nose poke, but remained on until the rat responded at choice. Eleven sessions were carried out. Number of trials completed remained similar to previous version, and performance accuracy improved.

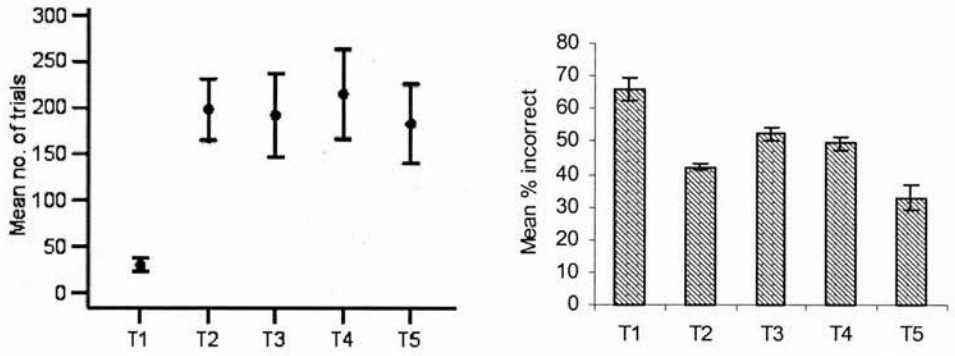


***Figure** 3.2.1.5. Left) Shows mean number of completed trials (± SEM) for all animals for each of the training versions. Right) Shows mean percentage incorrect (± SEM) for all animals for each of the training versions. T1, T2, etc., represent the training order that animals underwent.*

3.2.2 STUDY 1: DISCUSSION

Performance accuracy was poor throughout training. Several attempts to shape training to enhance performance were made and animals' accuracy in the last training version (T5) was promising. However, T5 was a simple match sample to choice and the data indicates that time taken to progress to the working memory task would be lengthy if animals would be able to perform the task. The improvement in performance of animals in the last training version (T5) (i.e. no delay period between sample and choice) compared to T4, show that rats do match sample (dim light) with the corresponding brightness at choice. However, performance at T4 suggests that animals are unable to match sample with corresponding target at choice when there is a delay between sample and target. Thus, data implies that during short delays between sample and choice the rat is unable to remember the sample from the time of its offset until target (choice) onset, which is in line with previous suggestions (Steckler *et al.*, 1998).

It is entirely possible that differences in task requirements evident throughout its development led to performance deterioration at later versions due to interference of previously learned behaviours or elicitation of species-typical behaviour, or a combination of both these factors.

3.3 STUDY 2: WORKING MEMORY TASK DESIGNED FOR USE IN A NATURALISTIC SETTING

3.3.1 METHOD

3.3.1.1 Animals

Two animals that took part in the development of the task in the operant chamber were used.

3.3.1.2 Apparatus

The behavioural apparatus used was a rectangular plastic box (approx. 40x70x18 cm), which was divided into a large open space and one smaller division at one side of the box that was further subdivided into two independent compartments of equal size (approx. 15x15 cm) (Fig.3.3.1.2). A plastic barrier was available to block these two compartments from the larger compartment. Each compartment in the box was covered using a Plexiglas lid that could be opened and closed as appropriate. Ceramic bowls were used for digging purposes and had an internal diameter of approx. 7 cm and a depth of 4 cm. The reward was ½ a Honey Loop (Kellogg, Manchester, UK), and was buried in the digging medium that filled the bowls.

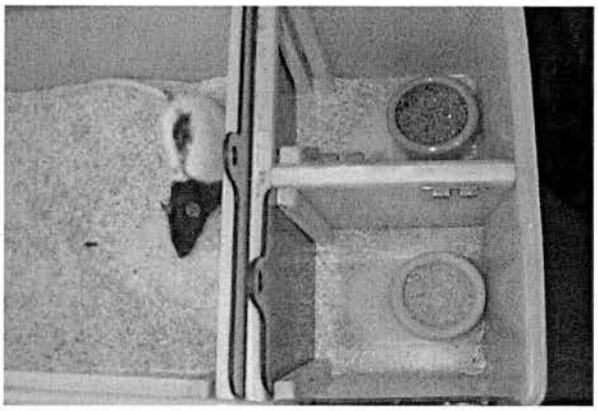


Fig. 3.3.1.2 Depicts the experimental set up (kit) used to assess working memory in a naturalistic setting.

3.3.1.3 Behavioural protocol

Animals underwent training in simple stimulus-reward association before being presented with the main task (described in Chapter II, section 2.4.2).

The task involved presentation of several discriminations. The first four trials of each discrimination were regarded as discovery trials, which meant that the rat was allowed to dig in the correct bowl if it dug in the incorrect bowl first. However, an error was recorded if the rat first dug in the bowl that did not contain the reward. After these initial trials the rat was refused access to the bowl containing the reward if it dug in the incorrect bowl first. The animal was regarded as having made a dig when it was considered possible to retrieve the reward with the dig. If the animal had not made a dig within ten minutes the trial ended and a new trial began.

Behavioural task: the task was a version of the match-to-sample task. Animals were presented with digging bowls that contained a mixture of one exemplar of odour and one exemplar of digging medium. Correct responding was according to the following rules: when presented with two consecutive sample bowls of which

contained the same exemplar of odour but different exemplars of digging medium, correct response was to match odour at choice. If the animal was presented with two consecutive bowls of which contained different exemplars of odours but same exemplar of digging medium, correct response was to match digging medium at choice. Reward was provided in each of the two sample bowls. The correct bowl at choice contained the corresponding exemplar of the relevant dimension (odour or medium) and one exemplar of the other dimension that had not been presented previously in the trial. The incorrect bowl at choice contained an exemplar of the relevant dimension that had not been presented previously in the trial and one exemplar of the irrelevant dimension, which was the same exemplar presented in the sample bowl immediately prior to choice.

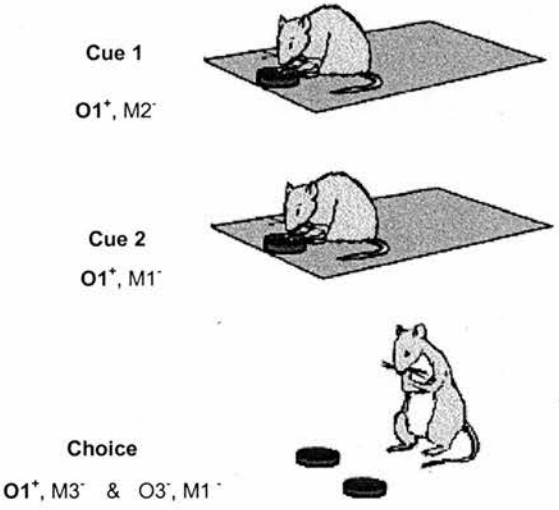


***Figure** 3.3.1.3. Example of a combination of exemplars used for each stage where the relevant exemplar is depicted in bold. Each of the exemplars of one dimension was paired with each exemplar from the other dimension.*

A trial started by placing one sample bowl in the large compartment. When the rat had retrieved the reward from the bowl, the bowl was removed and another sample bowl presented at the same place. After retrieval of the reward, the bowl was taken away and the animal was presented with a choice.

For each rule (match odour or match digging medium) there were 4 trial types comprising 8 trial combinations overall. Order of presentation of the two sample bowls was counterbalanced. Trial combinations were presented in a pseudorandomised order and occurred no more than three times in a row. Each rule appeared in 50 % of the total number of trials. To reduce number of possible combinations, two odours and two mediums were used as matching samples, while the third odour or medium belonged to the irrelevant dimension at choice only.

3.3.1.4 Behavioural training

1) Animals were presented with a match-sample-to-choice that consisted of the presentation of one sample bowl followed by a choice. The exemplars belonging to the irrelevant dimension stayed the same across both bowls at choice (exemplars used: odours - basil, thyme; mediums - course and fine shavings). Performance is shown in Figure 3.3.1.4.1 Animals were able to achieve 6 consecutive trials correct.

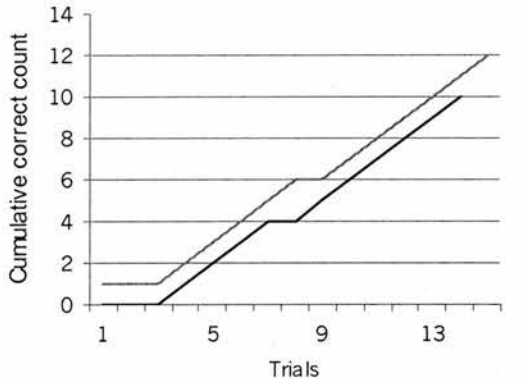


Figure 3.3.1.4.1 *Cumulative correct count to achieve six consecutive trials correct when the exemplars that belong to the irrelevant dimension stayed the same across samples at choice.*

ANIMAL 1

2) Modification of version 1 above: in contrast to version 1, the exemplars that belonged to the irrelevant dimension differed across the two samples at choice (exemplars used: odours - thyme, cumin and mint; mediums - shavings, fine

sawdust and sand). The rat performed poorly (i.e. unable to achieve 6 consecutive trials correct (Fig. 3.3.1.4.2).

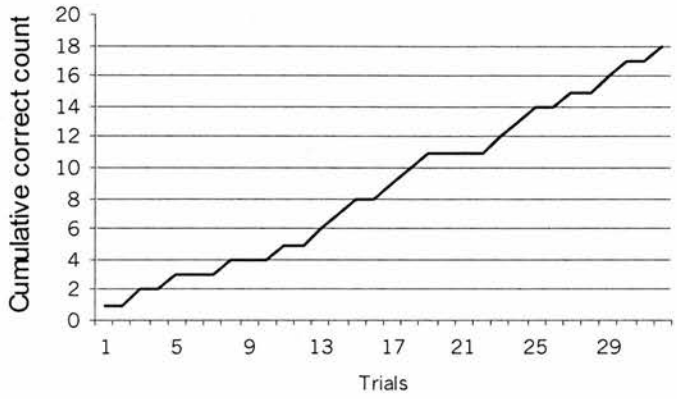


***Figure** 3.3.1.4.2 Cumulative correct counts across 32 trials when the exemplars belonging to the irrelevant dimension differed across bowls at choice.*

3) To facilitate learning of the working memory task, both sample bowls were presented simultaneously in the large compartment. Each dimension was presented separately; odour first and medium second (exemplars used: odours - thyme, basil and rosemary; mediums - shavings, sawdust and sand). The rat performed well (i.e. able to achieve 6 consecutive trials correct) (Fig. 3.3.1.4.3).

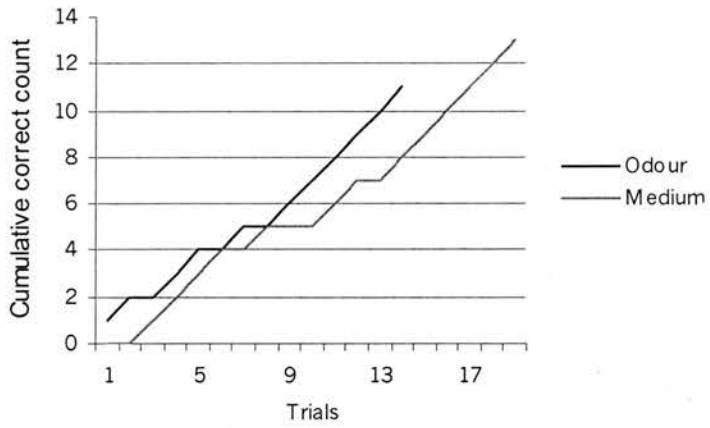


***Figure** 3.3.1.4.3 Cumulative correct counts across 32 trials when presented with both sample bowls simul-taneously followed by the choice.*

4) The animal was presented with the working memory task (Fig. 3.3.1.4.4), and achieved 6 consecutive trials correct within a session (exemplars used: odours - thyme, basil and rosemary; mediums - pebbles, sawdust and tea). However, data

indicated that the rat needed to re-learn the task within each session, and did not carry-over learning from the previous session(s).

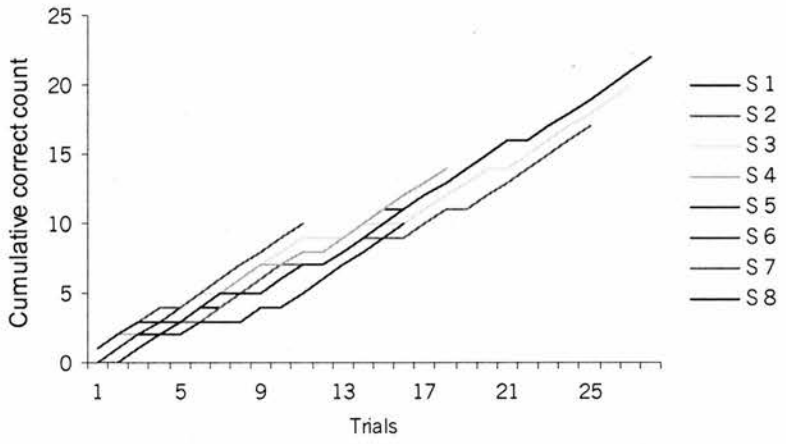


Fig. 3.3.1.4.4 *Number of trials to reach criterion of 6 consecutive trials correct for each individual session in the working memory task (S = session).*

ANIMAL 2

2) To facilitate learning of the working memory task, both sample bowls were presented simultaneously in the large compartment. In contrast to animal 1, each dimension was presented interchangeably (exemplars used: odours - mint, dill, rosemary; mediums - pebbles rubber, tea). The rat performed poorly (i.e. unable to achieve 6 consecutive trials correct) (Fig. 3.3.1.4.5).

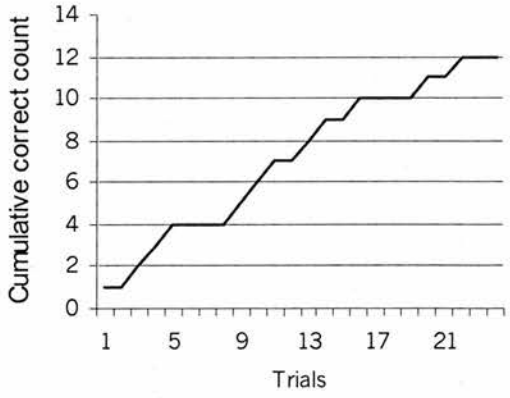


Figure 3.3.1.4.5 *Cumulative correct counts across 32 trials when presented with both sample bowls simul-taneously followed by the choice.*

3) Each dimension was then presented separately; odour first and medium second (exemplars used: odours - mint, dill, rosemary; mediums - pebbles rubber, tea).

The rat performed well (i.e. able to achieve 6 consecutive trials correct) (Fig. 3.3.1.4.6).

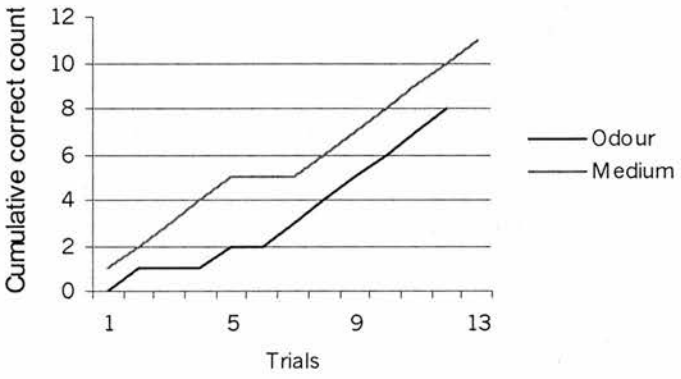


Figure 3.3.1.4.6 *Cumulative correct counts across 32 trials when presented with both sample bowls simultaneously followed by the choice. Each dimension presented separately.*

4) The animal was presented with the working memory task, and achieved 6 consecutive trials correct within a session. Similarly to Animal 1, data indicates that the rat needed to re-learn the task within each session.

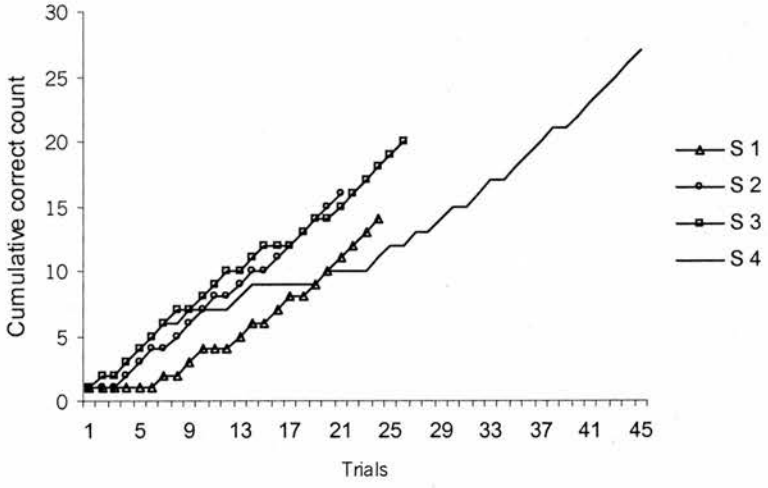


Fig. 3.3.1.4.7 Number of trials to reach criterion of 6 consecutive trials correct for each session in the working memory task.

3.3.2 STUDY 2: DISCUSSION

Several attempts to shape training to enhance performance were made and animals' accuracy on the working memory task was promising. However, the data indicated that animals needed to re-learn the task within successive sessions, which stand in contrast to other naturalistic tasks (Birrell and Brown, 2000). Similarly to

the training received in the operant chamber, it is entirely possible that order of training evident throughout its development led to performance deterioration due to interference of previous learned behaviours or elicitation of species-typical behaviour.

Some conclusions concerning the animals' behaviour on the working memory task can, however, be deduced from the various changes made in task parameters. For instance, one suggestion for poor performance is the inability to remember the first sample. As each of the two exemplars in the second sample bowl is presented in different bowls at choice, the second sample could not be used to infer correct response without reference to the first sample. However, in one animal the second sample cue was removed, while all other aspects inherent in the working memory task remained. The animal performed poorly, which suggests that, in the working memory task, it is not the nature of the second sample that creates difficulty. It appears that the problem is at choice. Support for this idea comes from animals' successful performance when the irrelevant exemplar at choice stayed the same across both bowls at choice. In the working memory task the exemplars belonging to the irrelevant dimension differed between each bowl at choice. These data indicates that it is the irrelevant dimension at choice that poses the difficulty. More importantly, it further implies that the rat does not simply match the corresponding exemplar at choice with the sample(s) prior to choice. It appears, then, that successful performance is dependent on the irrelevant dimension and corresponding exemplar at choice, rather than the relevant dimension and its exemplar. It is speculative as to the generalisability of these findings. It does, however, lend support to the interpretation that performance difficulty appears to be caused by the discrimination required at choice instead of an inability to hold

sample items presented prior to choice in working memory (Herremans *et al.*, 1995).

3.4 GENERAL CONCLUSION

This study reported an attempt at the development of a task to assess working memory capacity in rats. In line with tasks used with humans, animals were required to hold onto items, each of which required different responses, until presented with a cue that indicated which response was to be made. We have briefly discussed the potential difficulties inherent in the development of this task above under each task description. Overall, the data from this study does not provide support for the idea that rats exhibit working memory of similar complexity as that found in humans. Although, we have narrowed down the possibilities of how the rat might perform in tasks that assesses working memory capacity by changing various task parameters, it remains to be decided whether rats can and do use working memory in paradigms considered to assess this construct. One possibility for this negative result, however, concerns the relative similarities of the rat frontal cortex and that of primates. As working memory is commonly attributed to the prefrontal cortex, the extent to which rats have a prefrontal cortex can also affect the question concerning the existence of working memory in this species. However, it still remains a chance that other brain regions or systems may serve this role. Nonetheless, the result from our operant study suggested that rats might have a difficulty in remembering information even across a short delay when various spatial cues cannot be used to enhance performance, as is the case in naturalistic tasks. The findings from the naturalistic task herein, however, suggests that animals use a more 'global' learning strategy (i.e. context

appears to be important), where not only the relevant stimulus is used in determining the correct response, but that the correct response is based on a decision that incorporates both the relevant and irrelevant stimulus. This 'global' learning strategy might have been what has prevented the animals from learning the task. If the animals learn by associating the relevant stimulus with only one irrelevant stimulus, this might prevent acquisition of the task as the relevant stimulus is paired with several possible irrelevant stimuli on different trials across a session.

CHAPTER IV:

ASSESSMENT OF ANIMALS WITH LESIONS OF THE SUBTHALAMIC NUCLEUS ON CROSS-MODAL DIVIDED ATTENTION

- This chapter reports the contribution of the subthalamic nucleus (STN) to selective and divided attention. As experimental evidence suggests a role of the STN in attentional performance, the aim of this study was to further elaborate on what kind of attentional aspect(s) the STN might play a role in.

4.1 INTRODUCTION

The working memory load inherent in the task described in Chapter III seems to place too high demands on attentional control in the rat and a different approach was taken. The anatomical structure under investigation was the subthalamic nucleus (STN), as recent findings imply a role for this structure in attentional processes, and more specifically, sustained attention and vigilance (Baunez and Robbins, 1997; Chudasama *et al.*, 2003a). The idea of a participation of the STN in attention is intriguing, considering that this brain region has commonly been viewed within a framework that relates it to motor behaviour. Nevertheless, it is rather speculative as to whether the impairments seen in these studies can be genuinely attributed to impaired attentional processes. In the study by Baunez and Robbins (1997), STN lesions caused alterations in several behavioural measures, such as discriminative accuracy, anticipatory responses, and perseverative errors, and it is rather difficult to determine whether these deficits were indeed due to impaired allocation of attention or difficulties in action selection. The task used herein was a modified version of the cross-modal divided attention task developed by McGaughy *et al.* (1994), and adapted for use in the 9-hole boxes (J. Birrell, PhD thesis, University of St Andrews), in which attention is modality selective (visual or auditory) or divided between sensory modalities. There is evidence to suggest that an intact cholinergic system is necessary for dividing attention between modalities (Turchi and Sarter, 1997). In the task the behavioural manifestation of impaired attention or action selection should differ from one another. If the STN contributes to divided attention, lesions of this structure should alter the reaction time difference observed between the selective and divided condition without differentially affecting discriminative accuracy.

4.2 METHOD

4.2.1 Animals

The experiment began with twenty-four naïve Lister hooded rats (Harlan, UK). Housing and testing conditions were the same as outlined in general procedures (Chapter II; section 2.2).

4.2.2 Apparatus

The behavioural apparatus used was the nine-hole box. The layout of the box is outlined in Chapter III; section 3.2.1.2).

4.2.3 Behavioural protocol

All animals underwent training in simple stimulus-reward association before being presented with the main task (refer to Chapter II; section 2.4.1).

Behavioural task. Animals had to make a directional (left or right) response according to either the brightness of lights or the continuity of an auditory tone. The visual discrimination consisted of the presentation of either dim or bright lights. Lights appeared simultaneously in both left and right side apertures immediately adjacent to the central aperture. Correct responding in the visual discrimination was according to the following rules: when presented with dim lights correct response was to nose poke on the left side of the central aperture, while for bright lights correct response was to the right side. During the auditory discrimination, the animal was presented with either a continuous or a pulsing tone. For this latter modality, presentation of a continuous tone was associated with a correct response to the left side, while for pulsing tone [state pulses per second] correct response was to the right side.

A trial began when the rat made a nose poke into the illuminated central hole (see figure 4.2.3 of current section for a schematic illustration of the behavioural task). A fixed foreperiod of 0.2 seconds then preceded the onset of the target stimulus. When the target appeared, the light in the central hole was turned off. Presentation of the target stimulus was terminated when the rat withdrew its nose from the central aperture. A response then had to be made to either the left or right side within 5 seconds otherwise a late error was recorded and the house light was turned off for 1 sec. If the rat withdrew the nose before the target was presented, an anticipatory response was recorded and the house light was turned off for 1 sec. No reward was given after late errors or anticipatory responses. After the house light was turned back on, the next trial could be initiated by the rat pushing the access panel to the food tray.

Behavioural training. To optimise the speed of training, of the four stimulus-response associations, the visual and auditory stimuli that prompted the same response were paired and presented simultaneously. Dim lights were paired with continuous tone (i.e. correct response to the left side) and bright lights were paired with pulsing tone (i.e. correct response to the right side). To encourage animals to make a response, incorrect responses were not punished at this early stage of training (i.e. the animal was allowed to 'correct' the response by moving to the other side, so that all trials were rewarded, but only after a correct response). When animals readily made responses in either of the two side holes, task contingencies were changed and animals were not subsequently allowed to correct their response.

After this initial shaping task, the two modalities were presented separately and animals received single modality sessions in an alternating order (i.e. auditory only, then visual only etc.). Good stable performance was attained sooner in the visual compared to the auditory discrimination. Animals were subsequently trained on the auditory discrimination until they acquired stable performance. To begin with, a correction procedure was used where current trial would be repeated until correct response was obtained. Subsequently, for testing, the trial code could change on every trial, irrespective of the response on previous trial.

When animals achieved stable performance on both modality discriminations (i.e., no evidence of continued improvement, but with above chance performance of greater than 60 %), they were tested. There were two main conditions of testing: animals were tested in sessions which either consisted of all trials being of one modality (visual or auditory; unimodal condition; *modality certainty*) or with modality unpredictable from trial to trial (bimodal condition; *modality uncertainty*). Initially, the task comprised two unimodal blocks of trials followed by a bimodal block within one session. The order of modality presentation in the unimodal condition was counterbalanced, so that in one session the visual discrimination was presented first, while in the next session the auditory discrimination came first. The visual and auditory discrimination blocks consisted of 30 trials each (comprising a total of 60 trials for the unimodal condition), while the bimodal block consisted of 60 trials. The bimodal condition always followed the unimodal blocks of trials.

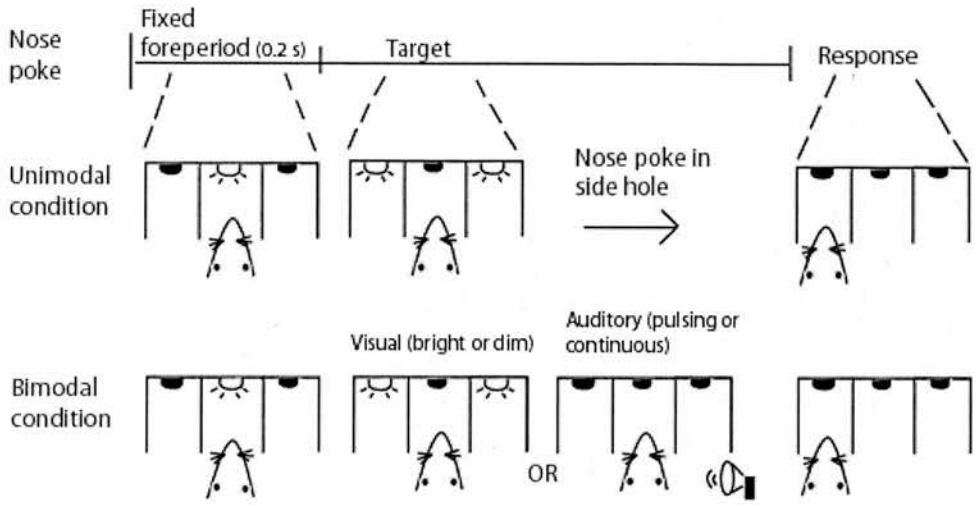


Figure 4.2.3. *An illustration of the divided attention task used in the present experiment. The animal had to nose poke (respond) in the correct side hole as indicated by target stimulus (visual modality – bright lights (go right) or dim lights (go left); auditory modality – pulsing tone (go right) or continuous tone (go left)). The target stimulus was on until the animal retracted the nose from central hole.*

After twenty-five sessions the above order of presentation was changed to a single daily session of either the unimodal or bimodal condition presented alone. In the unimodal condition, half the rats were given the visual discrimination and the other half the auditory discrimination on any given day. On the next day the rats that did the visual discrimination on the previous day would do the auditory discrimination and vice versa. All animals were tested on the same day in the bimodal condition. To mitigate against the development of bias or a behavioural strategy to 'dismiss' certain trials, the first ten trials in the bimodal condition were always in the auditory modality. Furthermore, a modality change only occurred after correct response of the other modality. The number of training sessions was thirty-seven from the beginning of training on the final task until animals underwent surgery (animals underwent 139 sessions during the course of training until surgery).

4.2.4 Surgery

Neurotoxin was delivered by glass micropipettes, prepared in-house. A manually constructed appliance was used to heat the centre of glass tubes to produce two pipettes, approximately 4cm in length, from each tube. Using a microscope, the tip of the pipette was adjusted to approximately 30μm in external diameter. To measure the volume dispensed, scale graduations were made at 1mm intervals on the side of the pipette (internal volume per 1mm equals 200nl). Animals were anaesthetised by inhalation of Isoflurane (Abbott Laboratories Ltd.) and placed in a stereotaxic frame with atraumatic ear bars (Kopf, Tujunga, CA) with the nose bar set at -3.3 mm. The level of anaesthesia was maintained with 2-2.5% Isofluorane delivered in oxygen throughout surgery.

A craniotomy was performed to expose dura using a hand drill. Infusions were made manually, using a 10 ml plastic syringe that was connected to the pipette via a flexible rubber tube. Twelve rats received injections of 400 nl/side of 0.06 M ibotenic acid (Tocris Cookson Avonmounth, UK). The pipette was left in site for 1 minute to allow for diffusion. Lesions were made bilaterally using co-ordinates from the atlas of Paxinos and Watson (1998). Anteroposterior (AP) from bregma -3.8, mediolateral (ML) ± 2.3, dorsoventral (DV) -7.8 (from dura). Another eight rats (controls) underwent the same procedure as the lesion group but were injected with sterile phosphate buffer rather than ibotenic acid. Animals were given a recovery period of approx. 1 week after surgery before testing. To prevent self-biting during the initial recovery from anaesthesia, small wooden sticks were placed in the mouth across the jaw of STN-lesioned animals and the animals were closely monitored. Diazepam (Valium™; 2.5 mg) was given intraperitonealy (i.p.) immediately after surgery to prevent convulsions.

4.2.5 Histology

After completion of behavioural testing, rats were overdosed with 0.8 ml Dolethal (Univet, Bicester, Oxfordshire, UK). Animals were then perfused transcardially with phosphate buffer saline, followed by 4% paraformaldehyde. The brains were removed and placed in a 20% sucrose solution at 4 °C until processed the next day. Using a freezing microtome (Jung Histoslide 2000, Reichert-Jung, Cambridge Instruments GmbH) brains were cut into 50 µm coronal sections and placed in 0.1 M phosphate buffer saline, where every fourth section was stained.

NeuN staining of brain tissue

In order to determine the extent and location of STN lesions, brain tissue was first stained with NeuN (Neuronal Nuclei). At first, sections were washed in phosphate buffer saline (PBS) (2x2 min.). Sections were then immersed in blocking solution (79 % PBS, 20 % goat serum, 1 % Triton) and left on shaker for 30-45 minutes. Brain tissues were then washed again in PBS (2x5 minutes) and incubated in Anti-NeuN (Chemicon) (1:1000) in anti-body diluting solution (ADS; 98 % PBS, 1% goat serum, 1 % Triton) and left overnight on shaker at room temperature. Sections were then washed in PBS (5x3 min.) and immersed in mouse IgG (Vector Laboratories Ltd) in ADS (5µl/ml ADS) and left on shaker for 1.5 hrs. Subsequently, tissues were again washed in PBS and then incubated in ABC solution (Vector Laboratories) for 1 hr with reagents A and B (each reagent 10µl/ml ADS). Finally, sections were washed in PBS and placed in DAB solution (Diaminodenzidine tablets; Sigma Chemicals) in distilled water until adequate colour was developed. Sections were subsequently washed in PBS and mounted onto glass slides.

Cresyl violet staining of brain tissue

After brain sections were stained with NeuN, they were allowed to dry before stained with cresyl violet. Sections were first defatted in Xylene and then rehydrated in graded alcohols (100 %, then 50 %). Following this, sections were washed in running tap water. The sections were then dipped in cresyl violet solution until good colour developed, whereby they were placed in running water for a few minutes before rehydrated in 50 % and then 100 % alcohol. Finally, sections were again dipped in xylene and brain slides were covered with thin glass slides for protection using DPX.

4.2.6 Data collection and analysis

Rats were assigned to surgical (control and lesion) group pseudorandomly before surgery. The preoperative data for the two groups were checked to ensure that they were matched, to the extent possible, on the variables of interest. The behavioural measures assessed were accuracy, anticipatory responses, late errors and reaction time. In this study, no predicted effects of lesion were made for movement time. These measures are explained in detail under General Procedures, section 2.5, Chapter II. Data was analysed using repeated measures analyses of variance (ANOVA). The between-subject factor was group at two levels: controls and STN lesions. The within-subject factors included condition (unimodal and bimodal), modality (visual and auditory), and side (left and right). During the course of training, three rats were dropped from the experiment, having failed to attain performance of greater than 60 % correct. An additional seven rats were excluded after surgery - four animals died and three rats sustained lesion damage

outside the STN (refer to result section 4.3.1). The final group sizes were, therefore, 7 lesions and 7 controls.

4.3 RESULTS

Animals were tested one week after surgery, on the bimodal condition, using an extended maximum trial length of 10 seconds until stable performance was reached whereby the window for late errors was reduced to 5 seconds, as used pre-surgery. The period of data collection comprised seven days of data from the bimodal condition and four days of the unimodal condition.

4.3.1 Anatomical observations

Three lesioned animals were excluded, with extensive damage to the ventroposteromedial thalamic area or with significant portions of the STN remaining in tact. Animals that were included in the data analysis were required to match in lesion size and exhibit symmetry of lesion in both hemispheres. Fig 4.3.1.1 shows the extent of the smallest and largest lesioned areas for those animals included in the behavioural analysis. In all animals, the lesion, to some extent, extended beyond the STN and into the zona incerta and dorsal part of the lateral hypothalamic area. Moreover, some sparing of tissue was evident in the most posterior part of the STN in four out of seven animals (see Fig. 4.3.1.2 for a representative photomicrograph at the level of STN of a sham-operated and STN-lesioned animal).

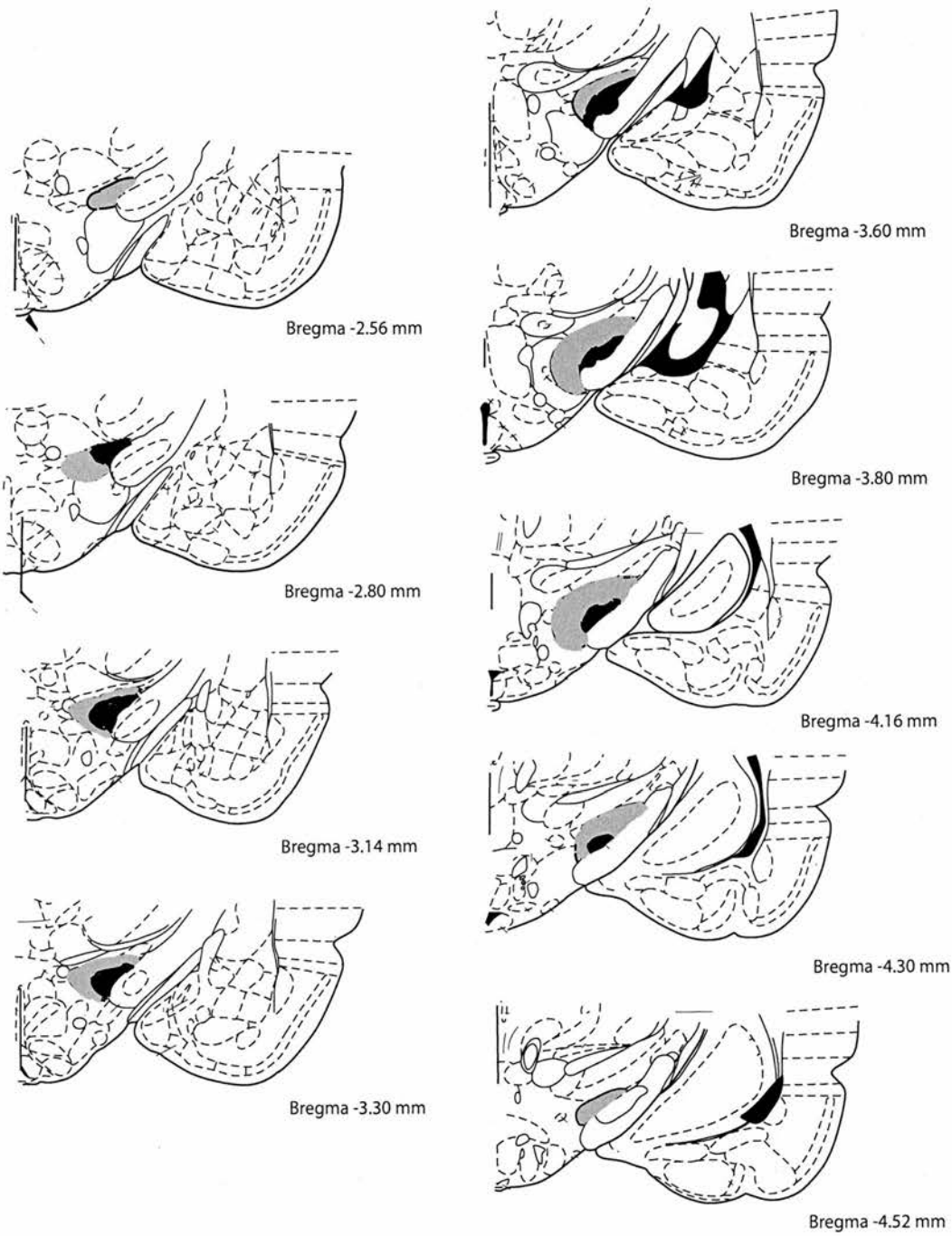


Figure 4.3.1.1. A reconstruction of STN lesions at varios anteroposterior levels (- 2.56 to – 4.52 mm from bregma; adapted from Paxinos and Watson, 1998). Black shading indicates the extent of the smallest lesion, while the largest lesion is depicted in grey.

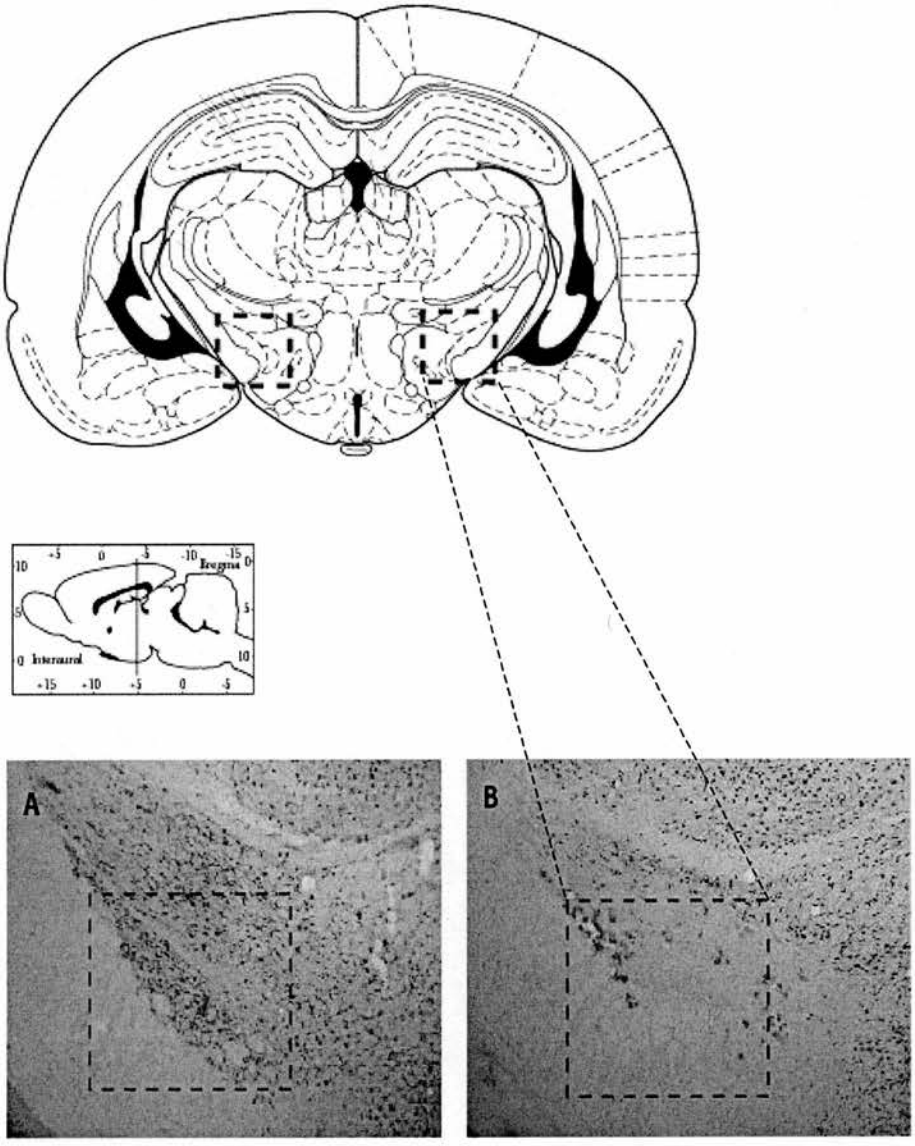


***Figure* 4.3.1.2.** *Brain sections stained with cresyl violet and NeuN, at the level of the STN (ca. – 3.70mm from bregma), are shown using photomicrographs in a sham-control animal (A) and in a lesioned animal (B) (right hemisphere). The location of STN is outlined by a dashed line.*

4.3.2 BEHAVIOURAL RESULTS

Reaction time (RT). Reaction time did not differ significantly between controls and STN animals pre- ($F_{1,12} = 0.60$, *ns*) or post-operatively ($F_{1,12} = 0.55$, *ns*). As shown in Figure 4.3.2.1, reaction times were longer when the modality of the target was uncertain (bimodal condition), than when modality was predictable (unimodal condition) (main effect of condition; $F_{1,12} = 5.93$, $p < 0.05$). Overall, animals performed faster in the visual compared to the auditory modality (main effect of modality; $F_{1,12} = 11.89$, $p < 0.01$).

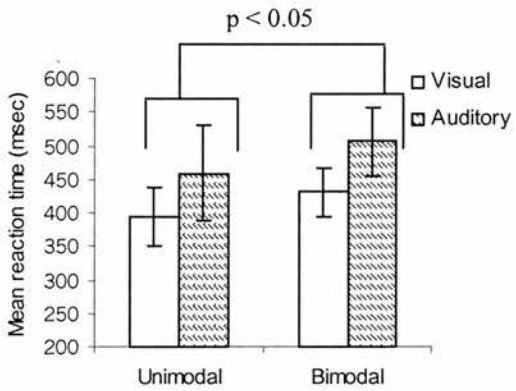


Figure 4.3.2.1. *Shows mean (± SEM) reaction time performance for each modality across condition, post-surgery. Animals were faster in the visual compared to the auditory modality, and also responded faster in the unimodal compared to the bimodal condition.*

Percent incorrect. Relative to controls, STN-lesioned animals showed a greater impairment for the visual compared to the auditory modality (interaction of group x modality; $F_{1,12} = 16.78$, $p < 0.01$; Fig. 4.3.2.2), and overall animals performed better to the visual compared to the auditory modality (main effect of modality; $F_{1,12} = 59.21$, $p < 0.001$). As illustrated in Figure 4.3.2.3, STN lesions impaired overall performance accuracy compared to controls (main effect of group; $F_{1,12} = 22.12$, $p < 0.01$). Importantly, increasing the attentional load did not change the magnitude of

the lesion-induced deficits in performance (interaction group x condition, *ns*; Fig. 4.3.2.3).

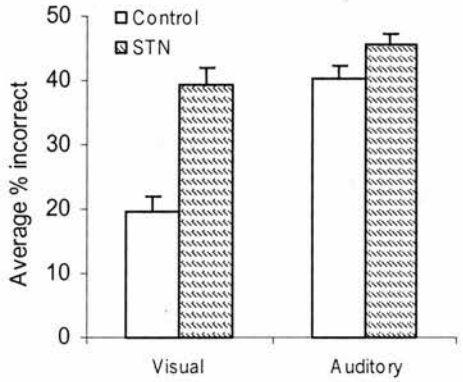


Figure 4.3.2.2. Top; *Effects of bilateral STN lesions on percent incorrect for visual and auditory modalities, post-surgery, collapsed across conditions.*

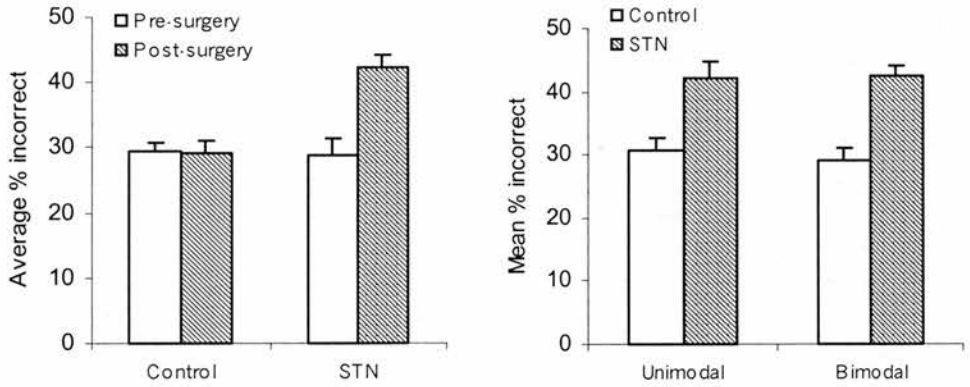


Figure 4.3.2.3. Left*: Effects of bilateral STN lesions on percent incorrect pre- and post-surgery for the bimodal condition. There was an increase in mean (± SEM) percentage incorrect in rats with STN lesions compared to the control group.* ***Right****: Shows mean percentage incorrect for both groups across conditions. Performance accuracy remained the same across conditions for each group.*

Anticipatory responses. As anticipatory responses are made prior to target onset, they are not influenced by spatial location of stimulus or modality. Therefore, anticipatory responses were analysed without respect to side or modality. The two experimental groups were not well matched prior to surgery, as the control group made a greater percentage of anticipatory errors ($F_{1,12} = 5{,}76$, $p < 0.05$). The reliable

effect of STN lesions is an increase in anticipatory responding, and this effect was replicated here. After surgery, the STN lesion group showed an increase in anticipatory responses compared to pre-surgery, while in controls the number of anticipatory responses remained similar pre-and post-surgery (interaction of surgery x group; $F_{1,12} = 9.02$, $p < 0.05$) (Fig. 4.3.2.4). There was no effect of lesion on the number of anticipatory responses made in each condition (interaction of condition x group; $F_{1,12} = 2.33$, *ns*), but overall anticipatory responses increased in the unimodal compared to the bimodal condition (main effect of condition; $F_{1,12} = 8.25$, $p < 0.05$), post-operatively (Fig. 4.3.2.4).

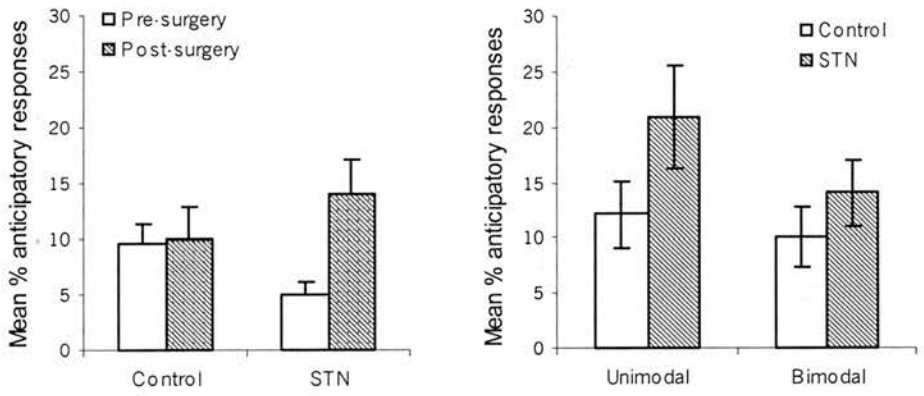


Figure* 4.3.2.4** *Effect of bilateral STN lesions on mean (± SEM) percentage anticipatory responses.* ***Left*: there was an increase in anticipatory responses in rats with STN lesions, post- compared to pre-surgery.* ***Right****: effect of condition (unimodal and bimodal) on number of anticipatory responses made. Animals showed an increase in anticipatory responses in the unimodal compared to the bimodal condition, post-surgery. Anticipatory responses are shown as a percentage of the total number of trials.*

Late errors. Late errors were collapsed across modality and side, as number of late errors was negligible (mean percentage less than 3%). Notwithstanding the small number of late errors, the STN-lesioned animals showed an increase in the percentage of late errors after surgery compared to pre-surgery, while for the

control group, late errors were reduced (surgery by group interaction; $F_{1,12} = 5.56$, $p < 0.05$).

4.4 DISCUSSION

This study reported the effects of bilateral STN lesions on a divided attention task developed for use with the rat. We have shown that STN lesioned rats exhibit deficits on this task in terms of anticipatory responses and discriminative accuracy that were not exacerbated by the attentional demands of the task. Furthermore, STN lesioned animals did not show a significant change in reaction time compared to controls.

Previous studies have reported both slower (Baunez and Robbins, 1997) and faster (Baunez *et al.*, 1995) RTs after bilateral STN lesions, while Phillips and Brown (1999) did not find any change in reaction time performance in unilaterally STN-lesioned rats compared to controls on a choice RT task. These differences are possibly due to both differences in the lesions and in the nature of the specific tasks and in the profile of other aspects of performance. For example, it is known that reaction times on correct trials which follow error trials, is frequently slowed - therefore there will be an impact of increased errors on the reaction times for correct trials. Conversely, if a greater proportion of anticipatory errors are made, some trials designated as 'correct' (post-target trials) will in fact belong to the distribution of anticipatory responses: that is to say, it is not possible to distinguish 'slow anticipations' from 'fast correct responses'.

In the 5-choice serial reaction task (5-SCRT; see Carli *et al.*, 1983), it was proposed that longer latencies and decreased accuracy following STN lesions could reflect an inability of the STN-lesioned rats to scan and monitor the spatial locations where

the target stimulus could be presented, due to an attentional impairment (Baunez and Robbins, 1997; Chudasama *et al.*, 2003a). Nevertheless, this particular observation could also reflect a motor impairment. Here we report that decreasing the attentional load did not alleviate the deficit in accuracy, suggesting that a reduction in attentional load did not change the magnitude of the lesion-induced deficits in performance. Additionally, the discriminative stimuli used in the present study were not spatial and, thus, the requirement to 'scan' space cannot account for the impaired discriminative accuracy seen here. It is more parsimonious to assume a single deficit accounts for impaired accuracy in both tasks and that this deficit concerns inhibitory motor control rather than attention.

The STN group exhibited an increase in anticipatory responding, which is in agreement with previous findings after both unilateral and bilateral STN lesions (Baunez *et al.*, 1995; Phillips and Brown, 2000). As suggested by others (Baunez and Robbins, 1997), the impairment is not necessarily related to the impaired discriminative behaviour observed following STN lesions, as anticipatory responses and discriminative accuracy are dissociated in sub-regions of the rat frontal cortex (Muir *et al.*, 1996; Chudasama *et al.*, 2003b). Rats with STN lesions made significantly more anticipatory errors overall and this was particularly the case in the unimodal compared to the bimodal condition. Discriminative accuracy was not differentially impaired in either condition, further suggesting that these variables are dissociable. However, it is unlikely that the increase in anticipatory errors seen in lesioned animals in the unimodal condition can be explained in terms of a possible differential effect of condition on attentional resources in STN lesioned animals compared to controls. Both groups showed elevated anticipatory errors in the unimodal condition, and it is therefore more plausible that the

additional increase in errors in STN animals occurred simply because lesioned animals made more anticipatory responses overall (a so-called 'scaling effect'). It could be expected that more effortful processing (the bimodal condition requiring increased attentional load) would, if anything, lead to greater performance deterioration and therefore more anticipatory errors. The result is likely explained, however, in terms of the differentially required allocation of attentional resources between the two conditions, where task demands that tax more effortful processing lead to additional allocation of attentional processes, which in effect reduces the number of anticipatory responses in the bimodal compared to the unimodal condition, perhaps by slowing response preparatory processes.

In conclusion, the pattern of behaviour exhibited by animals with STN lesions, which can largely be dissociated in other structures, underlines the intricate nature of the seemingly multiple effects induced by STN lesions. The findings presented here suggest that the deficits caused by lesions of this structure reflect a motor impairment and consequently a role of the STN in complex response control processes, rather than in a neural system that participates in attentional operations.

CHAPTER V:

ASSESSMENT OF ANIMALS WITH NORADRENERGIC DEPLETIONS ON ENDOGENOUSLY CUED COVERT ATTENTION

- Previous findings show rather broad and subtle behavioural effects after lesions to the dorsal ascending noradrenergic bundle (DNAB) on tasks assessing attentional processes. The aim of this experiment was to further explore the particular attentional conditions under which this system is necessary.

5.1 INTRODUCTION

In the rat, covert orienting of attention has been demonstrated using exogenous cues (Posner task) (Phillips and Brown, 2000), but not endogenous cues. The task used here was a modification of the standard Posner 'target detection task', where the animals were required to make a directional response according to the spatial location of target stimulus. However, rather than a spatial cue preceding the target in the target location (valid cue) or the opposite location (invalid cue), the target location was cued by its relative probability. That is to say, the probable location of target varied as a function time such that early in the foreperiod, the target was most likely to occur on the left, while later in the foreperiod it was more likely to occur on the right. Previous work has shown that lesions of the STN impair the response preparatory aspect of this task, while endogenous covert orienting of attention remains unaffected by the lesion (D.M. Thompson, PhD thesis, University of St Andrews). Together with the results presented in the previous Chapter IV, this suggests that the STN more likely participates in action selection and inhibition rather than attentional processes. Similarly to the task used to assess divided attention in Chapter IV, the behavioural manifestation of action selection and attention can be dissociated within the paradigm. While the neural correlate of the response preparatory aspect of the task has been targeted, the neurobiology of the attentional aspect remains uncertain. This study, therefore, decided to explore the neural basis of endogenous covert orienting of attention.

Evidence has implicated the noradrenergic system (NA) in attentional processes, (Carli *et al.*, 1983; Aston-Jones *et al.*, 1994; Devauges and Sara, 1990; Lapiz and Morilak, 2006; see Chapter I, section 1.2.5.2), and a recurrent supposition of its relative functional contribution concerns the ability to focus and selectively attend

to stimuli of environmental significance, while, at the same time, ignore irrelevant and distracting stimuli. This idea is illustrated by findings that show impaired performance on tasks when background noise is presented after noradrenergic lesions (e.g. Carli *et al.*, 1983; Cole and Robbins, 1992). An attentional hypothesis of this system, however, is not straightforward. Though 'broadening' of attention and a lack of selective attention seem to characterise some of the behavioural alterations seen in NA depleted animals, NA depleted animals do not appear to show any alterations during baseline conditions on some tasks used to assess attention (Carli *et al.*, 1983; McGaughy *et al.*, 1997). Thus, alterations in this system's sensitivity to environmental stimuli is often subtle, and it is consequently difficult to pin down the exact attentional contribution of NA. It was, thus, decided to investigate the possible role of NA in endogenous covert attention. In the task, the animals readily learned the probabilities associated with target locations and directed their attention to the more probable location of the target such that reaction time was more rapid when the target was presented in the more probable location ('valid' cue) compared to the less probable location ('invalid' cue). The primary question addressed herein was whether lesions of the dorsal noradrenergic bundle (DNAB) alter the pattern of costs and benefits of spatial probability of target location.

5.2 METHOD

5.2.1 Animals

Twenty-four Lister hooded rats took part in the experiment. Housing and testing conditions were the same as outlined in General Procedures (Chapter II, section 2.2).

5.2.2 Apparatus

The behavioural apparatus used was the nine-hole box. The layout of the box is outlined in Chapter III, section 3.2.1.2.

5.2.3 Behavioural procedures

Animals underwent training in simple stimulus-reward association before being presented with the main task (refer to Chapter II; section 2.4.1).

Behavioural training. Animals were initially trained on a simple two choice discrimination task. The rat made a nose poke into a lit central aperture, and was required to 'hold' this for an unpredictable duration (the so-called 'variable foreperiod') of 0.2, 0.3, 0.4, 0.5, or 0.6 secs. The end of the foreperiod was marked by the offset of the central light, the onset of an alerting continuous tone and the onset of the target light in either the right or left response aperture. The tone ended when the animal withdrew the nose from the central aperture. A response (nose poke) then had to be made at the same location as the target. The target light remained on until the animal made a response.

During task acquisition, there was an equal chance of target appearing at either location for all foreperiods. The session ended after 30 min or when the animal had performed 120 correct responses, whichever occurred first. To facilitate responses, during initial training there was no maximum response time, but after four sessions, a maximum response interval was set at 10 seconds (that is to say, response times of greater than 10 seconds were deemed aborted trials - 'late errors'). As animals became more proficient in performing the task, the maximum response time was reduced to 5 seconds and the duration of target was gradually reduced to 3, then 2 and finally 1 second. If an animal ceased responding, the maximum response time was increased again until responding was re-established.

This training period lasted for thirty-two daily sessions (see Fig. 5.2.3.1 for a schematic illustration of the task). In the next stage of training, the session was reduced to 100 correct trials (by this time, all rats were completing 100 trials within 30 minutes), and spatial probability of the target was varied as a function of the length of foreperiod maximum response time of 5 seconds was reduced to 3 seconds, and the duration of the target light was reduced to 0.3. If a response was made in the hole that did not contain the target, the response was recorded as 'incorrect' and the house light was turned off for 1 sec and all responses were ineffective ("Time Out").

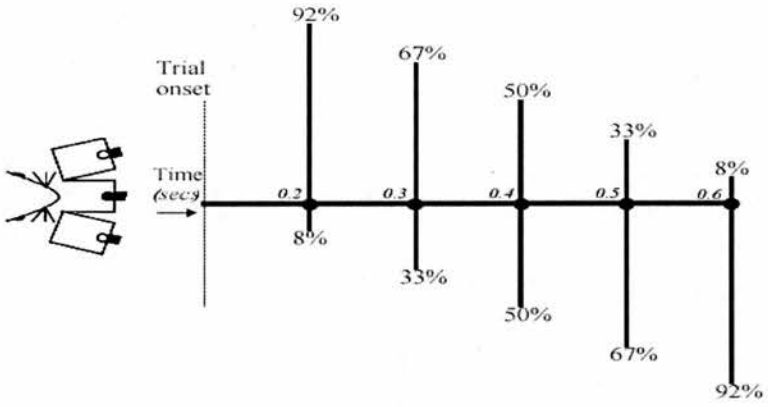
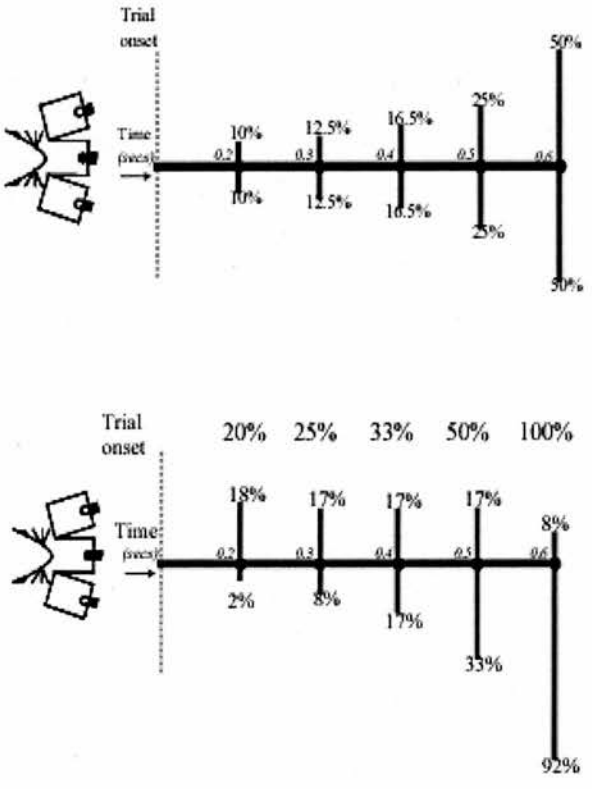


***Figure** 5.2.3.1. Illustration of the task used in the present experiment to assess endogenous covert attentional orienting depicting a priori spatial probability of target location as determined by length of foreperiod. The animal had to maintain nose poke in the illuminated central hole for a variable foreperiod until target light appeared in one of the two side holes. A response (nose poke) then had to be made at the spatial location where target occurred.*

To prevent side bias developing, a correction procedure was used whereby incorrect trials were repeated until correct response was obtained. If the rat withdrew the nose before the foreperiod was over, an 'anticipatory' response was recorded and the animal was punished with a 1 sec 'time out'. No reward was given after late, incorrect or anticipatory responses. After a Time Out, the house

light was turned back on. The trial could be initiated again by pushing the access panel to the food tray. The total number of sessions that animals underwent during the course of training until surgery was ninety-eight.

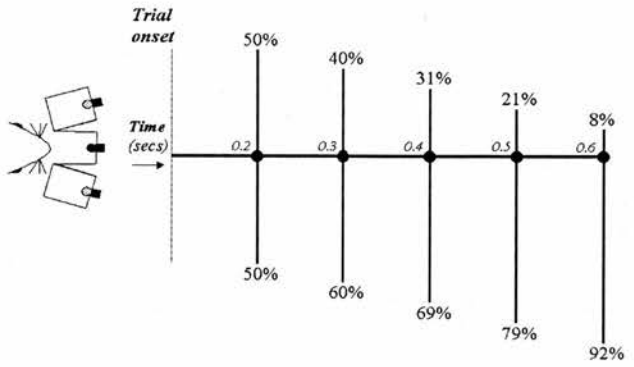
Task design: There are two factors that can influence performance in the task. One factor is the spatial probability of target location, and the other is time (foreperiod). The relative contribution of each of these two factors to overall performance can be modelled to depict the information available to the rat at any given point in time, and is illustrated in Fig. 5.2.3.2 and Fig. 5.2.3.3 below.



Top: ***Conditional temporal probability:*** The likelihood of target occurrence increases with increasing foreperiod (time) considering that target has not appeared yet.

Bottom: ***Conditional spatio-temporal probability:*** The likelihood of target occurrence as determined by both spatial likelihood and foreperiod. A priori there is a higher chance of target occurring on the left side at shorter foreperiods, and information concerning target location accumulates with time that results in greater certainty concerning target location at longer compared to shorter foreperiods.

Fig. *5.2.3.2. Shows the information available to the rat and the likelihood of target location in terms of both conditional temporal probability (top) and conditional spatio-temporal probability (bottom).*



Top: ***Conditional spatial probability:*** The likelihood of target occurring on the right relative to the left side increases with increasing foreperiod (time).

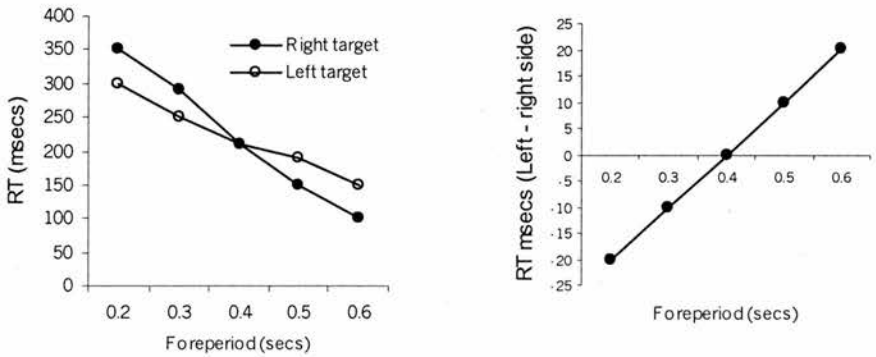


Fig. *5.2.3.3. A model to illustrate the predicted effect of spatio-temporal probability on reaction time performance (RT) (reaction times to each side is hypothetical merely to show RT difference between each side according to spatial probability of target).* Left: *RT is predicted to be slower for right than left targets at shorter foreperiods (unexpected location), while faster than left targets at longer foreperiods (expected location).* Right: *Depicts the same information by side subtraction, which reflects the cost and benefit of spatial probability. At shorter foreperiods the subtracted value is negative, as RT is slower on the right compared to left side, while at longer foreperiods, the subtracted value is positive, as RT is faster on the right compared to left side.*

Experimental manipulations After baseline data collection was completed post-operatively (total of twelve sessions), additional task parameters were manipulated.

1) Distractors. Irrelevant distractor stimuli were added to the task to evaluate the effect of 'background noise' on performance. The distractor stimulus

was a flashing house light, which was triggered by the nose poke in the central aperture and terminated at the end of the foreperiod (i.e. lasted throughout the entire foreperiod). The distractors were present on 20 percent of the total number of trials within a session. Seventeen sessions were carried out.

2) Reversal of spatial probability. After data collection from the distractor condition was obtained, the spatial probability was reversed to examine the rate of learning the new probability distribution. Thirty sessions were carried out.

5.2.4 Surgery

Animals were given an intraperitoneal (i.p) injection of 50 mg/kg Pargyline-hydrochloride (Sigma Chemicals) 30 min prior to surgery (this injection was given to "potentiate" the neurotoxicity of 6-OHDA). The 6-OHDA HBr (Sigma Chemicals) was dissolved in 0.1 % ascorbic acid in 0.9 % saline and kept on ice and in the dark until time of injection. Animals were anaesthetised by inhalation of Isoflurane (Abbott Laboratories Ltd.) and placed in a stereotaxic frame using ear bars (Kopf, Tujunga, CA) with skull level set at -2.4 mm. Level of anaesthesic gas was maintained at 2 - 2.5 percent throughout surgery. Using a 5 µl Hamilton syringe, twelve rats were subjected to injections of 2 µl/side at 2µg/µl of 6-OHDA (free-base). The injection extended over 4 min and the syringe was left in site for 2 minutes to allow for diffusion. Lesions were made bilaterally using co-ordinates from the atlas of Paxinos and Watson (1986). Anteroposterior (AP) from bregma -6.0, mediolateral (ML) ± 1.0, dorsoventral (DV) -5.0 (from dura). Another twelve rats (controls) underwent the same procedure as the lesion group but were given sterile phosphate buffer. Animals were given a recovery period of approx. 5 days after surgery before retesting on the task. Diazepam (Valium™; 1.5 mg) was given intraperitonealy (i.p.) immediately after surgery to prevent convulsions.

5.2.5 Neurochemical analysis

The animals were killed by inhalation of the gas carbon dioxide followed by cervical dislocation. The brains were rapidly removed and placed in a small beaker of isopentane buried in dry ice. The brains were removed from the beaker with forceps after 2-3 minutes and placed in a universal container buried in the dry ice. The brains were immediately sent to Cambridge for assessment of NA concentration. Tissues were stored at -80 °C until NA concentration was analysed. Whole brains were cut on a cryostat at -10°C. Tissue samples from neocortex, hippocampus, amygdala, nucleus accumbens, striatum, primary somatosensory cortex, secondary visual cortex and hypothalamus were analysed using standard high-performance liquid chromatography (HPLC) with electrochemical detection.

5.2.6 Data collection and analysis

The behavioural measures examined were accuracy, premature responses, and reaction time (refer to Chapter II, section 2.5 for an explanation of these measures). In this study, no predicted effects of lesion were made for movement time. Number of late errors was negligible with mean percentage less than 1% for both DNAB-lesioned and sham-operated animals throughout behavioural testing and was therefore not subject to further analysis. The results were expressed as means across the multiple sessions for each rat for each variable. Both mean and modal reaction times were analysed. The modal reaction time was used as a more accurate measure of central tendency of the reaction time distribution because mean reaction time is more sensitive to extreme values at the high ends of the probability distribution, which contained fewer trials. To reflect the cost/benefit of the spatial probability distribution, the reaction time to correct responses on the left side was subtracted from the reaction time on the right side for each

foreperiod. Repeated measures analyses of variance (ANOVA) was conducted using SPSS version 12.0. The criterion for statistical significance was a probability level of $p < 0.05$. Neurochemical data were compared using independent samples t-tests. Analysis of pre-and post-surgery baseline performance was carried out. The between-subject factor was group at two levels; controls and DNAB lesioned animals. The within-subject factors included surgery at two levels (pre vs. post-surgery), foreperiod at five levels (200, 300, 400, 500, 600 msec), and side at two levels (left and right). To analyse the effect of distractors on performance, data from non-distractor trials were compared to distractor trials. The within-subject factors were condition at two levels (with and without distractors), foreperiod, and side. Finally, examination of the rate of learning the reversal of spatial probability was divided into six blocks, each consisting of five daily sessions. The within-subject factors were block at six levels, foreperiod, and side. One control animal had damage to brain tissue and NA concentration could therefore not be performed, and the animal was excluded from the analysis. An additional lesioned rat died shortly after surgery.

5.3 RESULTS

5.3.1 Neurochemical observations

Neurochemical data for all lesioned and control animals are shown in Table 5.3.1. Injection of 6-OHDA in the dorsal noradrenergic bundle significantly reduced NA levels in 5 of 8 areas assayed. Compared to controls, lesioned animals showed the greatest reduction in NA levels in primary somatosensory cortex and secondary visual area. Neocortex and hippocampus were noticeably reduced, while NA content in hypothalamus and striatum were relatively unaffected by the lesion. In

three lesioned animals the NA level in neocortex was meagre. One animal showed a depletion of 44 %, while another two exhibited more unilateral depletions with good depletions of the left hemisphere, while the right hemisphere remained almost undamaged (< 50 % hemispheric difference in NA level). There were no overall significant effects of the lesion on the levels of dopamine (overall mean: lesion, 13.898 ± 2.460 pmoles/mg; sham, 13.171 ± 2.189 pmoles/mg; ns).

Table 5.3.1. Shows mean ± SEM of NA concentration in brain structures assayed of DNAB lesioned and control animals.

	NA concentration		Percent
Tissue region	DNAB	Sham	depletion %
Neocortex	0.922 ± 0.178	2.769 ± 0.266	66
NAcc	4.851 ± 1.888	9.440 ± 2.933	49
Str	0.678 ± 0.224	0.944 ± 0.406	28
S1	0.487 ± 0.114	2.218 ± 0.271	78
Amyg	2.330 ± 0.361	4.195 ± 0.478	44
Hypo	10.324 ± 1.170	11.606 ± 1.583	11
V2	0.875 ± 0.224	3.497 ± 0.913	75
Hipp	1.147 ± 0.320	4.110 ± 0.323	73

Abbreviations: Neocortex includes infra- and prelimbic region, cingulate cortex, primary motor cortex and orbitofrontal region; NAcc, nucleus accumbens; Str, striatum; S1, primary somatosensory cortex; Amyg, amygdala; Hypo, hypothalamus; V2, secondary visual cortex; Hipp, hippocampus. Values are expressed as pmoles/mg of wet tissue weight and are an average across both left and right hemispheres. NA content in NAcc, Str and Hypo was not significantly reduced compared to controls. The lesion resulted in a significant depletion in secondary visual cortex ($p < 0.05$) and highly significant depletions ($p < 0.01$) in all other structures assayed.

5.3.2 BEHAVIOURAL PERFORMANCE

5.3.2.1 Baseline performance

Reaction time (RT). There was a decrease in modal reaction time as a function of foreperiod (main effect of foreperiod; $F_{4,80} = 77.96$, $p < 0.001$). There was no effect of lesion on any of the variables. Reaction time data was also analysed using the mean. No effect of lesion was evident on any of the behavioural variables assessed.

Incorrect responses. Incorrect responses were related to the a priori target probability, reflecting directed attention, as revealed by a significant interaction between delay x side ($F_{4,80} = 13.97$, $p < 0.001$; Fig. 5.3.2.1.1). Incorrect responses were greater at longer compared to shorter delays; main effect of delay ($F_{4,80} = 10.89$, $p < 0.001$. Furthermore, animals made more incorrect responses when target appeared on the left than on the right side; main effect of side ($F_{1,20} = 4.66$, $p < 0.05$). There was no effect of lesion on performance.

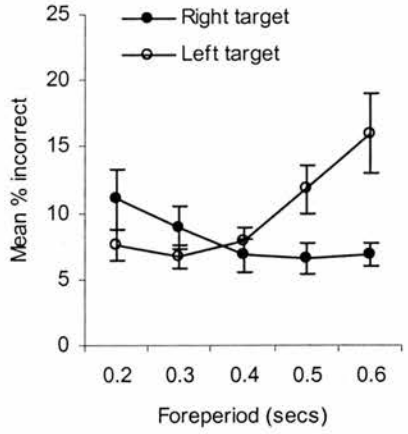


***Figure** 5.3.2.1.1 Shows mean (± SEM) percentage incorrect responses to left and right targets across foreperiods, and reflect the effect of a priori spatial likelihood on performance accuracy.*

Anticipatory responses. As anticipatory responses are made prior to target onset, they are not influenced by spatial location of the subsequent target. Therefore, anticipatory responses were analysed without respect to side. Figure 5.3.2.1.2 illustrates the mean percentage of anticipatory responses made pre-and

postoperatively on baseline performance for each group. Anticipatory responses increased as a function of foreperiod (main effect of foreperiod; $F_{4,80} = 134.24$, $p < 0.001$). Post-operatively, there was a slight decrease in anticipatory responses in sham-operated animals and a slight increase in lesioned animals compared to pre-surgery (interaction between surgery x group; $F_{1,20} = 8.21$, $p < 0.01$).

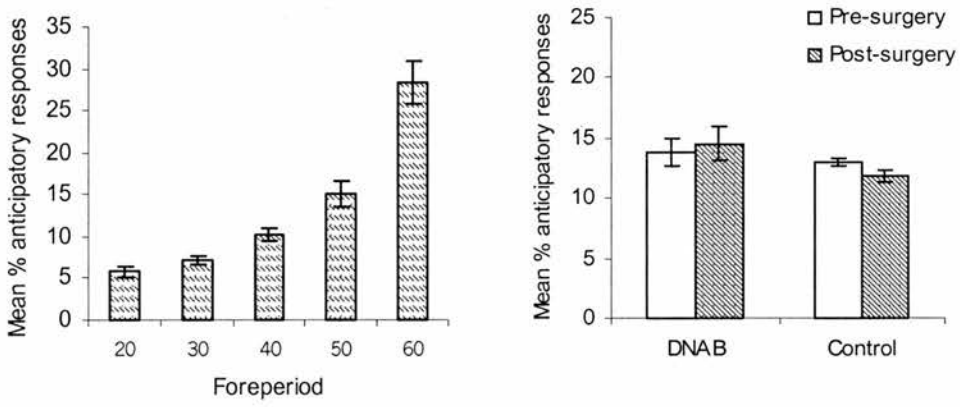


Figure 5.3.2.1.2. *Left) There was a gradual increase in mean (± SEM) percentage anticipatory responses as a function of foreperiod. Right) Shows mean (± SEM) percentage anticipatory responses pre- and post-surgery for DNAB-lesioned and sham-operated animals. Anticipatory responses are shown as a percentage of the total number of trials.*

5.3.2.2 Distractor condition

Reaction time (RT). RT was related to the a priori target probability, reflecting directed attention (interaction of delay x side; $F_{4,80} = 3.20$, $p < 0.05$; Fig. 5.3.2.2.1). RT decreased as a function of foreperiod, and this was true for both groups ($F_{4,80} = 71.23$, $p < 0.001$). Animals showed slower reaction times during distractors compared to non-distractors trials (main effect of condition; $F_{1,20} = 28.46$, $p < 0.001$), but there was no effect of lesion on modal RT. Mean RT was also analysed. In both experimental groups mean RT was related to the a priori spatial probability during non-distractors trials. During distractors trials RT no longer appeared to reflect a

priori spatial probability in controls, while in lesioned animals distractors did not appear to affect RT as related to the a priori probability (3-way interaction of condition x side x group; $F_{1,20} = 5.74$, $p < 0.05$) (Fig. 5.3.2.2.2).

Incorrect responses. Similarly to baseline performance, there was a significant interaction between delay and side ($F_{4,80} = 15.51$, $p < 0.001$). Animals made more incorrect responses at longer foreperiods (main effect of delay; $F_{4,80} = 13.53$, $p < 0.001$), and more incorrects on the left compared to the right side (main effect of side; $F_{1,20} = 15.75$, $p < 0.01$). Finally, there was an overall increase in number of incorrect responses made during distractors compared to non-distractors trials (main effect of condition; $F_{1,20} = 7.32$, $p < 0.05$) (Fig. 5.3.2.2.1). There was no effect of lesion on any of the variables.

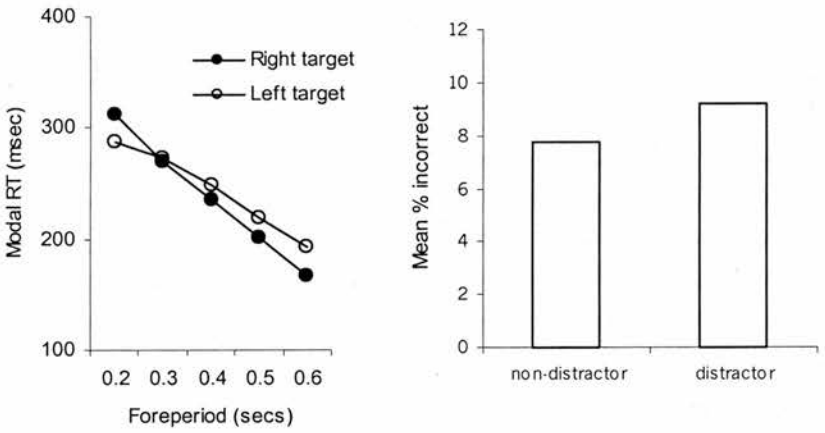


Figure* 5.3.2.2.1.** ***Left: *Shows modal reaction time for right and left target across foreperiods in the distractor condition. Reaction time was related to the a priori target likelihood, reflecting directed attention.* ***Right****: Depicts overall incorrect responses for non-distractor compared to distractor trials.*

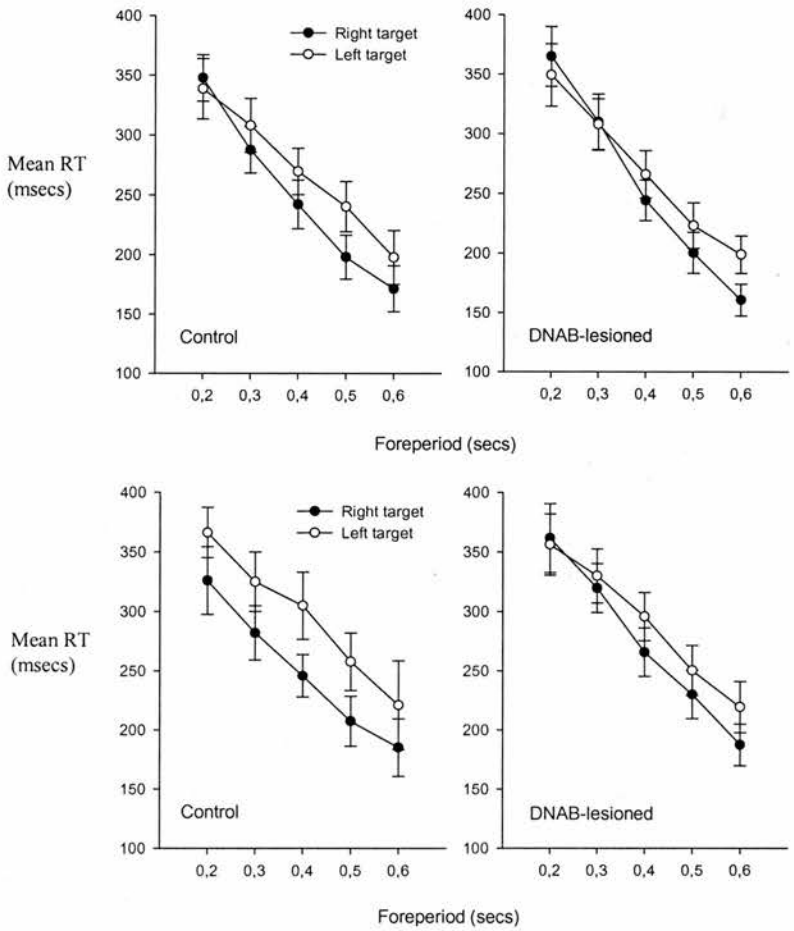


Fig. **5.3.2.2.2.** Top. *Mean reaction time (± SEM) for right and left targets across foreperiods in controls and DNAB lesioned animals for non-distractor trials.* Bottom: *Mean reaction time (± SEM) for right and left targets across foreperiods in lesioned and control animals for distractor trials.*

Anticipatory responses. Similarly to baseline performance, anticipatory responses increased as a function of foreperiod (main effect of foreperiod; $F_{4,80} = 85.86$, $p < 0.001$). The number of anticipatory responses made across foreperiods remained similar during both distractors and non-distractors trials in controls, while lesioned animals made fewer anticipatory responses during distractors compared to non-distractors trials at the longest foreperiod (3-way interaction of group x foreperiod x condition $F_{4,80} = 2.67$, $p < 0.05$) .

5.3.2.3 Reversal of a priori spatial probability

Reaction time (RT). There was a change in modal RT to each side as a function of foreperiod across reversal blocks (3-way interaction between block x foreperiod x

side; $F_{20,400} = 2.13$, $p < 0.05$), and RT was no longer related to the a priori target probability (interaction between delay x side; $F_{4,80} = 1.21$, *ns*) (Fig.5.3.2.3). There was no effect of lesion on any of the variables. Analysis of mean RT did also not show any effect of lesion on performance.

Incorrect responses. There was a change in incorrect responses to each side as a function of foreperiod across reversal blocks (3-way interaction between block x foreperiod x side; $F_{20,400} = 2.86$, $p < 0.001$). Incorrect responses were no longer related to the a priori target probability (interaction between delay x side; $F_{4,80} = 1.24$, *ns*). There was no effect of lesion compared to controls on reversal (Fig.5.3.2.3).

Anticipatory responses. Animals made fewer anticipatory responses as a function of foreperiod in the second reversal block compared to the first, but then made more anticipatory responses in subsequent blocks with a decrease in the last block (interaction of block x foreperiod; $F_{20,400} = 4.51$, $p < 0.001$). There was no effect of lesion on number of anticipatory responses made during reversal learning.

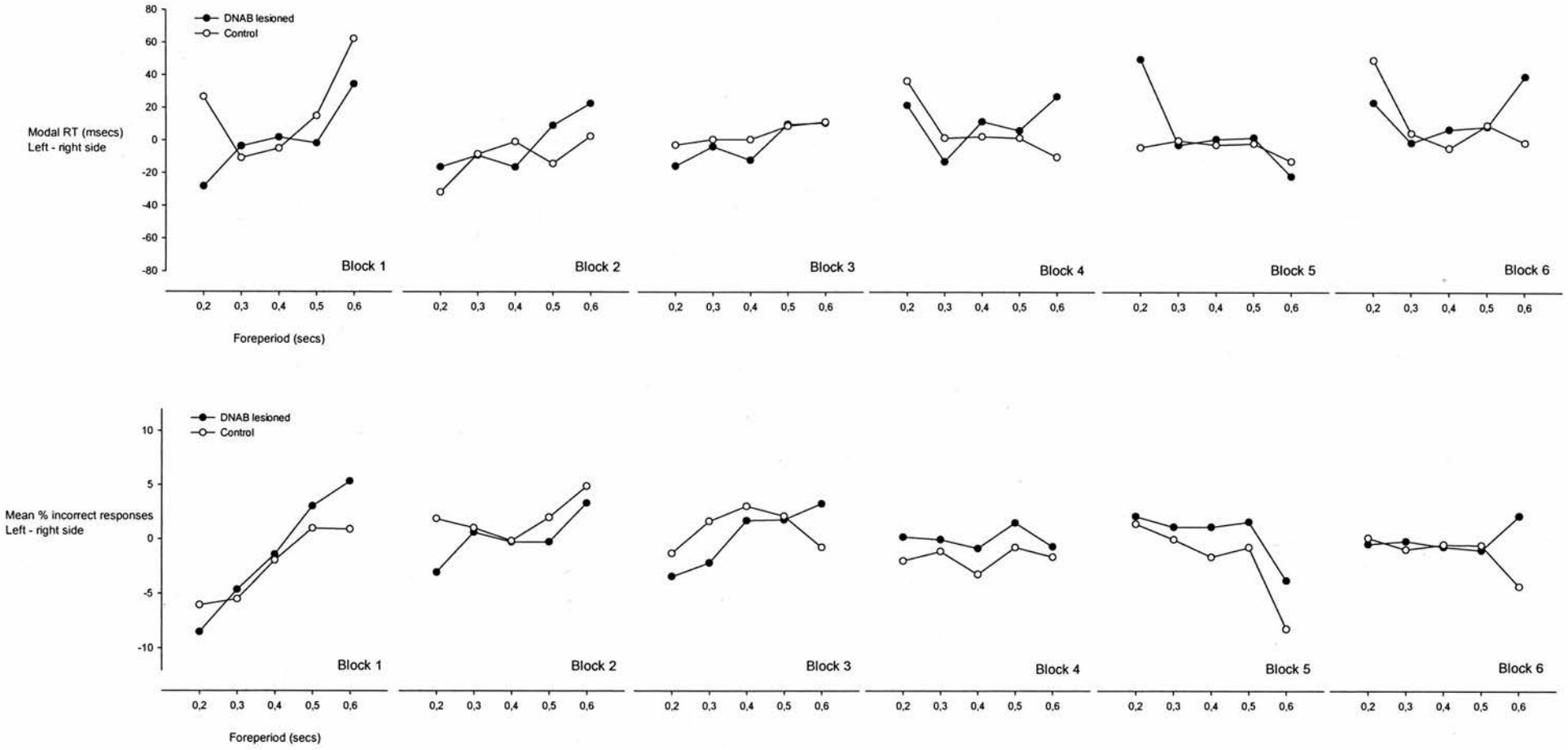


***Figure** 5.3.2.3. Shows RT (top) and percent incorrect responses (bottom) as side subtraction values across foreperiods for DNAB-lesioned and sham-operated animals for each reversal block (1-6). The interaction between foreperiod and side diminished across reversal blocks, but the lesion did not have an effect on rate of reversal learning.*

5.3.3 Data analysis based on lesion extent

In case an effect was concealed by the inclusion of poor lesions in the analysis, all behavioural data were also analysed when the three animals showing poor lesions of the neocortex were excluded. No effect of lesion was evident on any of the variables assessed.

5.3.4 Correlations between NA level and behavioural performance

Behavioural data from post-surgery baseline and distractor condition was subject to correlational analysis (Pearson) with level of NA in neocortex. Measures that were subject to correlational analysis with NA were reaction time (mode and mean), accuracy and anticipatory responses. None of the variables correlated with neocortical NA level.

5.4 DISCUSSION

This study reported the behavioural performance of animals after lesions to the dorsal noradrenergic bundle (DNAB) on an endogenous covert attentional orienting task in the rat. We have shown that DNAB lesioned rats do not exhibit deficits on any examined behavioural aspect of this task. In particular, DNAB lesioned animals still showed an ability to direct attention according to probability of target as determined by length of foreperiod, which was reflected in faster reaction times and less errors when target occurred at its most likely spatial location.

No effect of lesions was found on accuracy suggesting that response preparation is still intact after noradrenergic depletions. Interestingly, lesions appeared to alter RT to target locations under distracting conditions compared to control animals. In

both experimental groups reaction time was related to the spatial probability during non-distractors trials. During distractor trials RT no longer appeared to reflect the spatial probability in controls, and this seems to be largely due to faster RTs to right compared to left targets at shorter foreperiods. In other words, the effect of distractors appeared greater at shorter relative to longer foreperiods. An effect of time (foreperiod) on distractor interference might indeed be expected considering that information concerning target location accumulates with time, while the occurrence of distractors remains constant. Interestingly, RT in lesioned animals appeared unaffected by the distractors. These results imply that 'background noise' impair attentional orienting in controls, while lack of noradrenaline seem to facilitate selective attention in this task.

This seemingly 'facilatory' result of noradrenergic depletion on orienting of attention differs from those of previous studies reporting an impairment after DNAB lesions compared to controls on performance in the 5-choice serial reaction time task, using auditory distractors (Carli *et al.*, 1983). Furthermore, in a task designed to assess selective attention and vigilance, using visual distractors (flashing houselights) similar to the present study, no change in performance was found in DNAB animals compared to controls (McGaughy *et al.*, 1997). This suggests that the effects of noradrenaline depletion on performance under distracting conditions are likely specified by task requirements. Importantly, the results stand in contrast to previous conceptions suggesting that NA depletion impair selective attention. It is therefore somewhat uncertain what the contributing factors are in terms of altered behaviour after DNAB lesions under distracting conditions.

The lesions in our task did not effect reversal of spatial probability on any of the examined behavioural measures. There are some likely explanations for this lack of effect. Not only is the time course of reversal learning longer and more gradual in our operant task compared to many other paradigms, which could require fundamentally different cognitive processes, but also directed attention is not necessary or sufficient for reinforcement. If the noradrenergic system is sensitive to attentional shifts of stimulus-reward associations only, then lesions of this system would not affect a reversal of probability of target location, and this may account for the ineffectiveness of lesions on reversal learning.

The neocortical NA depletion was relatively low (average 66 %) compared to other studies showing > 80 % (e.g. Carli *et al.* 1983; Selden *et al.*, 1990; Cole and Robbins, 1992). This low value was largely due to a few animals showing hemispheric asymmetry in NA level. However, there was still no change in behaviour of DNAB animals compared to controls when these animals were excluded from analysis. Moreover, correlational analysis of NA content with different task measures did not suggest that level of NA was related to performance on this task. It remains a possibility, however, that a more severe depletion of NA in neocortex and hippocampus could alter behavioural performance on this task. In addition, rats typically performed very well (ca. 90 % correct) and it is a possibility, although unlikely, that ceiling effects could have obscured behavioural alterations in DNAB compared to sham-operated animals.

In conclusion, the pattern of behaviour exhibited by animals with DNAB lesions indicate that the dorsal noradrenergic bundle and the coeruleo-noradrenergic

system is unlikely to participate in endogenous covert orienting of attention. DNAB animals did, however, differ in their performance compared to controls under distracting conditions by still showing directed attention, a feature that diminished in sham-operated animals, and adds support for a participation of NA in selective attention.

CHAPTER VI:

ASSESSMENT OF ANIMALS WITH ALTERATIONS OF CHOLINERGIC TRANSMISSION ON ENDOGENOUS COVERT ORIENTING OF ATTENTION

- Acetylcholine has been strongly implicated in attentional processes, and more specifically, exogenous covert orienting of attention. The aim of this final study was to further our understanding of this system's role in covert attentional processes by assessing the effect of NbM lesions and nicotine administration on endogenous covert orienting of attention.

6.1 INTRODUCTION

The present study examined the ability of rats with selective immunotoxic 192-IgG saporin lesions of the basal forebrain (NbM/SI region) and systemic administration of nicotinic acetylcholine on the task designed to assess endogenous covert orienting of attention described in Chapter V. Additional manipulations of task parameters in this task suggested that NA depleted animals appeared insensitive to 'background noise', while directed attention remained unaffected by the lesion. This observation supports the idea of a role of the noradrenergic system in selective attention, but, at the same time, leaves open the neuroanatomical source of endogenous covert attention. Both NA and Ach are neurotransmitters that are closely associated with attentional processes, and it is a possibility that Ach mediates this attentional aspect, while NA participates in other types of attention. Indeed, lesions of the basal forebrain have been shown to modulate exogenously cued covert attention, as evident in an increase of the validity effect in the 'Posner task' (a cued target detection task) (Voytko *et al.*, 1994), and stand in contrast to the effects of lesions of the thalamic reticular nucleus (TRN) which seem to abolish this validity effect (Weese *et al.*, 1999). In contrast to the effect of basal forebrain lesions, nicotinic acetylcholine has been shown to modulate attention in the 'Posner task' in humans (Murphy and Klein, 1998), monkeys (Witte *et al.*, 1997) and rats (Phillips *et al*, 2000) by reducing the validity effect. In addition, evidence indicates that lesions of the NbM increase sensitivity to nicotine by cortical neurons (Abdulla *et al.*, 1995), and improved attentional processing in animals with basal forebrain cholinergic lesions has been reported after nicotine administration (Muir *et al.* 1995). The relative contribution of the NbM cholinergic system to *endogenously* cued covert attention, however, is unknown. Therefore, to further our understanding of the role

played by this system in covert attentional processing, one aim was to assess rats with cholinergic lesions of the NbM on endogenous covert attention. In accordance with the effect of basal forebrain lesions seen in exogenous covert attention, it was predicted that NbM lesions would increase the cost of re-directing attention as reflected in a greater reaction time difference between expected and unexpected spatial locations (validity effect). Furthermore, the aim was to further specify the role played by nicotine in this attentional aspect, and explore the possible effects of nicotine on performance in acetylcholine deprived NbM-lesioned animals compared to controls.

6.2 METHOD

6.2.1 Animals

Twenty-four Lister hooded rats took part in the experiment. Housing and testing conditions were the same as outlined in the General Procedures, section 2.2, Chapter II.

6.2.2 Apparatus

The behavioural apparatus used was the nine-hole box. The layout of the box is outlined in section 3.2.1.2, Chapter III.

6.2.3 Behavioural procedures

All animals underwent training in simple stimulus-reward association before being presented with the main task (refer to Chapter II; section 2.4.1).

Behavioural task. Animals were trained on the same task used to assess endogenous covert orienting of attention in study three (refer to Chapter V, section 5.2.3 for task description). Briefly, the rat made a nose poke into a lit central aperture, and was required to 'hold' this for a variable foreperiod of 0.2, 0.3, 0.4, 0.5, or 0.6 secs. The

session ended after 30 min or when the animal had performed 100 correct responses, whichever occurred first. The end of the foreperiod was marked by the offset of the central light, the onset of an alerting continuous tone and the onset of the target light in either the right or left response aperture. The tone ended when the animal withdrew the nose from the central aperture, and a response (nose poke) then had to be made at the same location as the target. If a response was made in the hole that did not contain the target, the response was recorded as 'incorrect' and the house light was turned off for 1 sec and all responses were ineffective ("Time Out"). To prevent side bias developing, a correction procedure was used whereby incorrect trials were repeated until correct response was obtained. If the rat withdrew the nose before the foreperiod was over, an 'anticipatory' response was recorded and the house light was turned off for 1 sec. No reward was given after late, incorrect or anticipatory responses. After a time Out, the house light was turned back on. The trial could be initiated again by pushing the access panel to the food tray. During task acquisition, there was an equal chance of target appearing at either location for all foreperiods. To facilitate responses, a maximum response interval was set at 10 seconds (that is to say, response times of greater than 10 seconds were deemed aborted trials - 'late errors') and the target light remained on until the rat responded or until 5 secs had elapsed. This training period lasted for fifteen daily sessions. In the next stage of training spatial probability of the target was varied as a function of the length of foreperiod and maximum response time of 5 seconds was reduced to 3 seconds, and the duration of the target light was reduced to 0.3 secs. This training period lasted for sixteen daily sessions. One animal produced very slow reaction times, and the maximum response time was therefore 'tightened' and set to last 1 sec and target

remained on until animals made a response. As animals' reaction time performance was unaffected by the tightened max. response time, continued training and testing used this latter response 'window'. The animal with very slow reaction times was excluded from analysis. The total number of sessions that animals underwent during the course of training until surgery was fifty-two.

6.2.4 Surgery

Injections were made into the substantia innominata/nucleus basalis region (SI/NbM), which contain the majority of ascending cortical cholinergic neurons (Alheid and Heimer, 1988). Using a 5 µl Hamilton syringe, twelve rats were subjected to injections of 0.5 µl/side at 0.25 µg/µl of 192-IgG saporin. The needle was left in situ for 3 minutes before the bolus infusion and left in site for a further 3 minutes after administration to allow for diffusion. Lesions were made bilaterally using co-ordinates from the atlas of Paxinos and Watson (1986). Anteroposterior (AP) from bregma - 0.7, mediolateral (ML) ± 2.9, dorsoventral (DV) - 6.7 (from dura). Another twelve rats (controls) underwent the same procedure as the lesion group but were given sterile phosphate buffer.

6.2.5 Immunocytochemical analysis

After completion of behavioural testing, rats were overdosed and perfused as described in section 4.2.5, Chapter IV.

In order to determine the extent and location of NbM lesions, brain tissue was stained with ChAT (choline acetyltransferase). Sections were washed in phosphate buffer saline (PBS) (2x2 min.) and immersed in blocking solution (94 % PBS, 5 % rabbit serum, 1 % Triton), and left on shaker for 30-45 minutes. Brain tissues were

then washed again in PBS (2x5 minutes) and incubated in Anti-ChAT (Chemicon) (1:800) in anti-body diluting solution (ADS; 98 % PBS, 1 % rabbit serum, 1 % Triton) and left overnight on shaker at room temperature. Sections were then washed in PBS (5x3 min.) and immersed in goat IgG (Vector Laboratories Ltd) in ADS (5µl/ml ADS) and left on shaker for 1.5 hrs. Subsequently, tissues were again washed in PBS and then incubated in ABC solution (Vector Laboratories) for 1 hr with reagents A and B (each reagent 10µl/ml ADS). Finally, sections were washed in PBS and placed in DAB solution (Diaminodenzidine tablets; Sigma Chemicals) in distilled water until adequate colour was developed. Sections were subsequently washed in PBS and mounted onto glass slides. The following day, sections were defatted in xylene and brain slide were covered with protection glass slides using DPX.

6.2.6 Data collection and analysis

Behavioural measures recorded were as described in general procedures, Chapter II; section 2.5. Briefly, the measures recorded were performance accuracy, reaction time, anticipatory responses, later errors and movement time. Similarly to study three (refer to Chapter V) animals produced very few late errors and no predicted effects were made for movement time, and therefore these two measures were not assessed further.

6.3 RESULTS

Retesting of animals began approx. 10 days after surgery. All data collected post-operatively used the same light duration and intra-trial interval as pre-surgery testing, and comprised of eighteen sessions.

6.3.1 Histological observations

An assessment was made of the extent of 192-IgG saporin lesion of basal forebrain cholinergic neurons. Every forth section (50 μm) was stained for choline acetyltransferase (ChAT)-positive cells. As shown in Figure 6.3.1.1, infusions of 192-IgG saporin into the basal forebrain resulted in an overall reduction in ChAT-positive neurons of 58 % in the NbM (sham-operated, mean ± 32,87; NbM-lesioned, mean ± 13,83). A decrease in ChAT neurons was also found throughout the horizontal limb of the diagonal band (HDB) to a varying extent in lesioned subjects (Fig. 6.3.1.2), but was not as substantial as the reduction found in nucleus basalis of meynert. From the HDB cell count, two lesioned animals showed greater loss of cholinergic cells in one hemisphere relative to the other at more caudal coordinates. One animal showed greater loss in the left hemisphere, while the other animal exhibited a greater reduction in cholinergic cells in the right hemisphere. One lesioned animal stopped performing post-surgery. This animal was excluded from further statistical analysis, and thus, data was extracted from eleven animals in each experimental group.

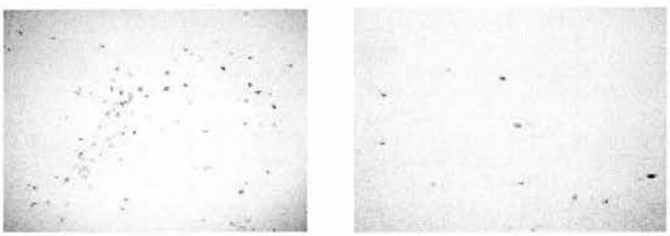


Figure 6.3.1.1. *Shows representative brain sections stained with ChAT, at the level of the NbM (ca. -0.9 mm from bregma), are shown using photomicrographs in a sham (Left) and in a lesioned animal (Right). Number of cholinergic neurons is reduced in lesioned compared to controls.*

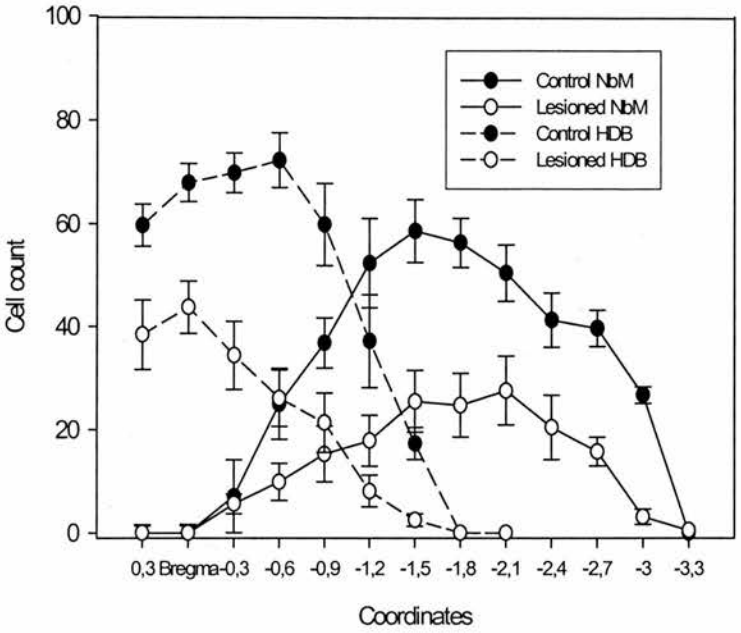


Fig. 6.3.1.2 Shows the number of cholinergic cells counted at different stereotaxic co-ordinates for NbM-lesioned and sham-operated animals. Cholinergic neurons in NbM (nucleus basalis of meynert) and SI (substantia innominata) were counted together due to the difficulty of anatomically dissociating these regions. The NbM lesion also resulted in a reduction of cholinergic cells in the horizontal limb of the diagonal band (HDB).

BEHAVIOURAL PERFORMANCE

6.3.2 Effects of NbM lesion on performance

Incorrect responses. Incorrect responses were related to the a priori target probability, reflecting directed attention, as revealed by a significant interaction between delay x side ($F_{4,80} = 13.97$, $p < 0.001$). However, there was no effect of lesion on performance (Fig. 6.3.2.1).

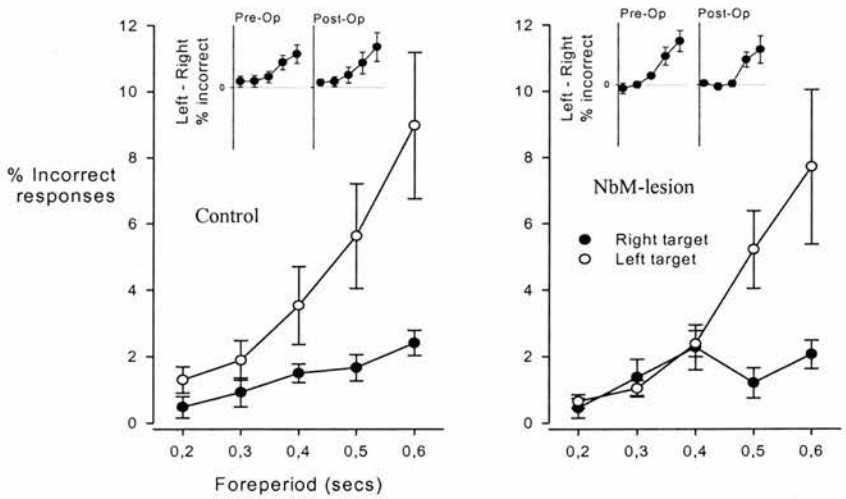


Fig. 6.3.2.1 Shows incorrect responses to left and right targets across foreperiods for controls and NbM-lesioned animals. The effect of a priori spatial likelihood on performance accuracy was unaffected by the lesion.

Modal reaction time (RT). RT decreased as function of foreperiod, and this was true for both groups even after surgery; main effect of foreperiod ($F_{4,80} = 91.69$, $p < 0.001$, (Fig. 6.3.2.3); interaction of group x surgery x foreperiod ($F_{4,80} = 0.81$, ns;). The decrease in RT by foreperiod was greater for targets on the right side, such that overall, reaction time to right targets was faster than reaction time to left targets. However, this pattern seen clearly in both groups pre-surgery was not seen in the lesion group post-surgery; significant interaction of surgery x side x group ($F_{1,20} = 5.09$, $p < 0.05$; see Fig. 6.3.2.2). Fig. 6.3.2.2 also shows the same data plotted as side subtracted values to illustrate the effect of a priori target probability on RT. Mean RT was also analysed, and although the pattern was similar to Modal RT, the interaction of surgery x side x group only approached significance ($F_{1,20} = 3.32$, $p = 0.08$).

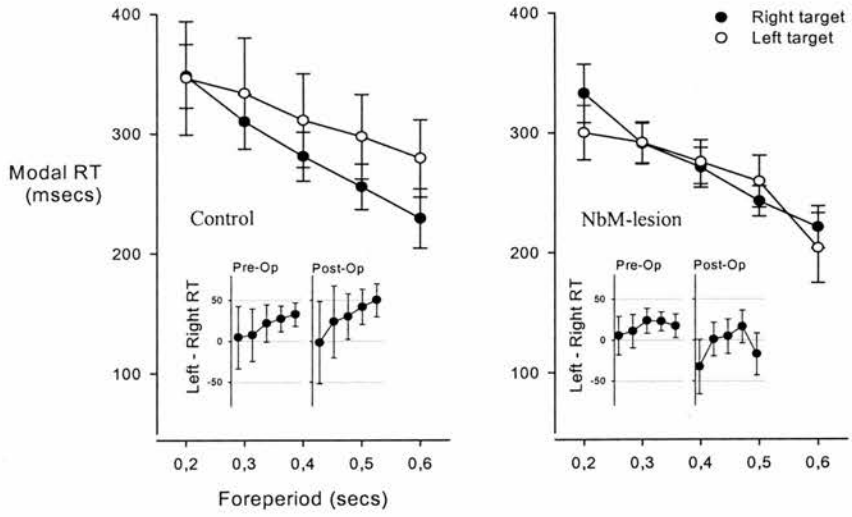


Fig. 6.3.2.2. *Shows modal reaction time (± SEM) to left and right targets across foreperiods for controls and NbM-lesioned animals. The effect of a priori spatial likelihood on reaction time was abolished in NbM-lesioned animals post-operatively.*

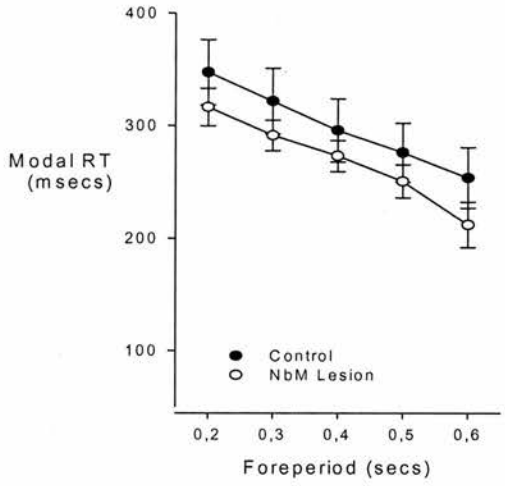


Fig. 6.3.2.3. Shows modal reaction time (± SEM) decreasing as function of foreperiod, and this was true for both groups even after surgery.

Anticipatory responses. As anticipatory responses are made prior to target onset, they are not influenced by spatial location of the subsequent target. Therefore, anticipatory responses were analysed without respect to side. Anticipatory responses increased as a function of foreperiod ($F_{4,80} = 134.24$, $p < 0.001$), but there was no effect of group on this measure ($F_{4,80} = 1.78$, ns).

Summary of study 1

NbM lesioned animals did no longer show attentional orienting as reflected in an abolishment of the effect of a priori spatial likelihood on reaction time to expected and unexpected target locations. Moreover, NbM animals continued to show delay-dependent speeding indicating that the change in performance was not due to a deficit in motor readiness. Response accuracy also remained unaffected by the lesion, suggesting that response preparation is intact in lesioned animals.

6.3.3 Assessment of performance of control and NbM-lesioned animals after nicotine administration

To further specify the role of the cholinergic system in attentional processing, sham-operated and NbM-lesioned animals were administered nicotine and performance on the task examined. Nicotine was predicted to re-establish the spatial probability effect on reaction times in NbM-lesioned animals, while reducing this effect in controls.

6.3.3.1 Nicotine administration

Subsequent to post-surgery testing, NbM-lesioned and sham-operated animals were injected with nicotine tartrate (Sigma), and performance on the task recorded and analysed.

Nicotine tartrate (Sigma) was dissolved in 0.9 % saline and administered subcutaneously (s.c.) 15 min prior to testing. Testing was carried out in three blocks, each block consisting of four consecutive days. The first block of nicotine administration was considered as priming, as the behavioural effects of nicotine are inconsistent before sensitisation (see Balfour et al., 1998), and therefore only the two last blocks of data are presented. Animals received one of four doses (0.0, 0.2, 0.4, 0.8 mg/kg) on each testing day. Drug administration was according to a Latin square procedure, where the experimental and control group were each divided into four groups with rats within each group receiving the following dose order: one group received, 0.0, 0.2, 0.4, 0.8; second group, 0.2, 0.4, 0.8, 0.0; third group, 0.4, 0.8, 0.0, 0.2 and fourth group, 0.8, 0.0, 0.2, 0.4.

6.3.3.2 Results of nicotine administration

Incorrect responses. Performance accuracy was related to the a priori target probability (significant interaction delay x side; $F_{4,80} = 9.83$, $p < 0.001$; main effect of Group, ns)). This measure, unlike reaction time, reflects response preparatory processes rather than directed attention. Thus, it is noteworthy that there was no effect of nicotine on this measure (interaction of group x drug; $F_{3,60} = 0.92$, *ns*; main effect of dose; $F_{3,60} = 2.19$, *ns*).

Modal reaction time (RT). Two control animals had missing values for left targets at the longest foreperiods at 0.4-mg/kg dose of nicotine. Rather than exclude them from analysis, the missing data points for these two animals were replaced with the average value of all other control animals. Overall RT was similar for both groups when saline was administered. Both groups became faster after administration of nicotine, however, this effect was greater in the lesioned compared to sham-operated controls; interaction of drug x group ($F_{3,60} = 3.37$, $p < 0.05$; see Fig. 6.3.3.2.). In controls, the decrease in RT by foreperiod was greater for targets on the right side, such that overall, reaction time to right targets was faster than reaction time to left targets. However, this pattern was still not seen in the lesion group after nicotine administration, though this only approached significance; interaction drug x side x group ($F_{3,60} = 2.70$, $p = 0.054$). There was also no effect of nicotine on delay-dependent speeding (interaction of foreperiod x drug; $F_{12,240} = 1.15$, *ns*) or group (interaction of foreperiod x group; $F_{4,80} = 0.94$, *ns*).

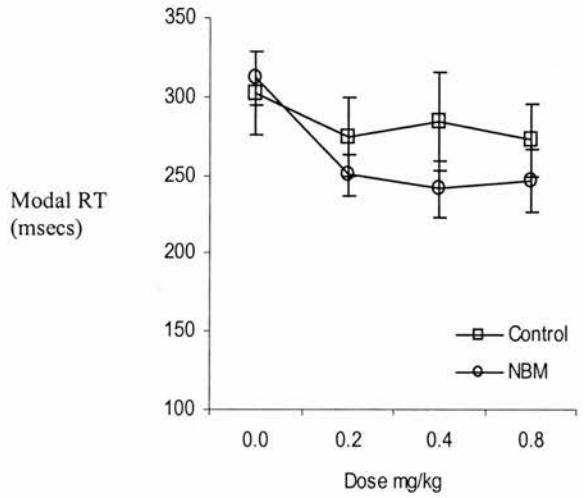


Fig. 6.3.3.2. Shows modal reaction time across nicotine doses for controls and NbM-lesioned animals. Both groups showed similar RT performance at dose 0.0, while lesioned animals were faster than controls at all other doses.

Anticipatory responses. As seen in baseline data, anticipatory responses increased as a function of foreperiod. This effect was enhanced by nicotine in a dose-dependent manner; interaction foreperiod x drug ($F_{12,240} = 2.10$, $p < 0.05$) and main effects of foreperiod ($F_{4,80} = 63.45$, $p < 0.001$) and drug ($F_{3,60} = 22.15$, $p < 0.001$). However, there was no effect of the lesion on this measure.

Summary of study 2

Nicotine administration did not re-establish the effect of probability on RT in NbM lesioned compared to control animals. Moreover, NbM animals continued to show delay-dependent speeding similarly to controls and response accuracy remained intact. There was no overall effect of nicotine on motor readiness or response preparation, suggesting that these two aspects are not reliant on nicotinic acetylcholine.

6.4 DISCUSSION

This study reported the behavioural performance of animals after lesions to the basal forebrain (NbM) on an endogenous covert attentional orienting paradigm in the rat. The lesion did not impair accuracy of performance, however, the reaction

times no longer reflected attentional orienting in lesioned animals. Lesioned animals continued to show delay-dependent speeding prior to the target similar to controls, suggesting that changes in reaction times were not due to effects on motor readiness. This result confirms conclusions reached previously in human studies suggesting that, although temporal orienting of attention and motor readiness may be interlinked, their underlying neural mechanisms can be dissociated (Coull *et al.*, 2000).

The finding that NbM lesions abolish the effect of spatial probability on RT to expected and unexpected target locations could be interpreted as evidence for enhanced ability to disengage and move attention to a different spatial location after lesions to this system. However, such a conclusion stands in contrast to the effects of basal forebrain lesions seen in exogenous covert attention (Voytko *et al.*, 1994), which appear to increase the cost of re-directing attention. It, thus, appears that the basal forebrain cholinergic system alters the pattern of costs and benefits of re-directing attention differently during endogenous compared to exogenous covert attention, although this remains to be confirmed by the use of a paradigm where task parameters can be manipulated to assess both exogenous and endogenous covert attention.

In contrast to the beneficial effects seen after nicotine administration in basal forebrain lesioned animals in the 5-choice serial RT task (Muir *et al.*, 1995), nicotine did not appear to re-establish the effect of probability on reaction time performance in lesioned compared to control animals in the task described herein. These observations are in agreement with earlier suggestions that nicotine might not be required in endogenous spatial expectancy (Murphy and Klein, 1998), indicating that nicotine plays distinct roles in endogenous and exogenous processing. The

lack of any consistent behavioural effect after administration of nicotine in the present study does raise the possibility that the alteration seen in NbM animals under these conditions may indeed be attributed to disruption of the muscarinic cholinergic receptor system, by which nicotine may exert limited or different influence on during endogenous orienting of attention. In line with recent reports of a possible differential role of nicotinic and muscarinic cholinoceptors in cognitive processes (Mirza and Stolerman, 2000), this idea could perhaps explain the contrasting behavioural effects of NbM lesions seen between current task and the Posner task, but remains to be explored further.

That performance accuracy still reflected a priori probability in lesioned animals similar to controls also suggest that the basal forebrain cholinergic system is only involved in the attentional aspect of the task, while the motor inhibition of prepotent responses remains intact. It remains possible, though, that NbM lesion affect some, but not all, aspects of behavioural inhibition. However, no change in the number of anticipatory responses were found in lesioned compared to sham-operated animals, which adds confidence to the idea that the present observations are caused by an alteration in attentional processes.

In conclusion, these findings support the idea that endogenous attentional orienting reflects a different, and independent, process from that of response preparation and that normal cholinergic function is required for the former but not the latter. The nicotinic cholinoceptor does not appear to contribute, however, to endogenously cued covert attention, suggesting a possible role for the muscarinic cholinoceptor system in this attentional aspect. In light of previous research, the present data indicate some common neural substrate underlying both endogenous and exogenous covert orienting of attention.

CHAPTER VII:

DISCUSSION AND CONCLUSIONS

7.1 INTRODUCTION

In this thesis we have examined the behavioural manifestation of some attentional processes in the rat and their neuroanatomical and pharmacological underpinnings. The thesis has emphasised the wide nature of attention and its biological correlates, and contributed to our understanding of the neural basis of some of these attentional aspects. It has also generated questions for future experiments in the field. This chapter will discuss the broader implications of these results.

7.2 GENERAL OVERVIEW OF MAIN FINDINGS

The initial aim was to develop a task that assessed working memory at a species appropriate level, which compensated for the insufficiencies inherent in tasks employed so far. Several manipulations of task parameters were carried out, but rats showed difficulty in task acquisition and were unable to perform successfully on this task. Satisfactory paradigms for the assessment of attention have, however, been generated and findings herein confirm inter-species similarities in the behavioural expression of attention. The aim of the experiment in chapter IV was to examine the effects of lesions of the STN on selective and divided attention. It was found that increasing the attentional load in the divided attention condition did not change the magnitude of the lesion-induced deficits in performance, suggesting that the STN does not contribute to divided attentional allocation on this task and is more likely involved in motor aspects of behaviour.

Chapters V and VI reported the role played by the neurotransmitters noradrenaline and acetylcholine on attentional performance in a task used to assess endogenous

covert attentional orienting. Lesions to the dorsal noradrenergic bundle (DNAB) did not produce any effects on baseline performance, suggesting that NA does not play a role in endogenous covert attention. However, when 'background noise' was introduced to the task NA depletion appeared to 'facilitate' performance. That is to say, distractors impaired attentional orienting in sham-operated control animals, while performance remained unaffected by distractors in lesioned animals.

The final experiment in this thesis examined endogenous covert attention after lesions of the basal forebrain (NbM). Basal forebrain lesions produced behavioural alterations in performance suggestive of a contribution of acetylcholine in this attentional aspect. Performance remained similar after systemic injection of nicotine in NbM-lesioned animals compared to controls, suggesting that endogenous covert orienting of attention is not controlled by nicotinic receptors. Within the framework of Yu and Dayan's model (2005), disruption of noradrenergic transmission should not affect performance on our task, while reduction in cholinergic transmission should. The reason for this is that, according to the model, the task used herein is an example of a paradigm that assesses expected and not unexpected uncertainty. The animal gradually learns the uncertainty associated with the spatial location of target, and therefore expects this uncertainty. In contrast, in the attentional set-shifting task described in the general introduction, the animal is faced with a sudden shift in the relevant dimension and therefore is considered unexpected to the animal. Lesion to the DNAB impairs performance at the extradimensional stage of the task (D. Tait, V.J. Brown, A. Farovik, D. Theobald, J.W. Dalley and T.W Robbins, unpublished). The exact

defining criteria that should be used for understanding the conceptual difference between expected and unexpected uncertainty is somewhat blurred, and requires further elaboration. However, despite this limitation, the model is in accordance with our results.

7.3 IMPLICATIONS FOR THEORY

7.3.1 The neural basis of working memory

It is suggested that the current theoretical framework used to examine the biological correlates of working memory needs to be reconsidered, where awareness of the importance of using a single definition that comprise specified criteria is emphasised. Only then can we make clear conclusions concerning the neural basis of this construct, and is of the very essence if we are to develop good treatments for cognitive impairments in working memory. There is an additional issue that needs to be addressed, however, in terms of the assessment of cognitive function in animals, and relates to the extent to which animals exhibit similar anatomy. As already mentioned in chapter three in relation to working memory, this issue becomes crucial, as there is considerable debate as to the relative similarities of the rat frontal cortex and that of primates. The extent to which rats have a prefrontal cortex does not directly affect the question concerning the existence of working memory in this species, since other brain regions or systems may serve this role. However, we need good animal models if we are to develop satisfactory treatments for cognitive impairment in humans. Taken together with the data presented herein, the fact that there is an issue regarding the existence of a rat prefrontal region do question the idea that they exhibit similar working memory capacity as found in primates. This issue, of course, indicates that

differences in neural connectivity across species do, to some extent, place limitations on understanding the neural basis of cognition and its 'dysfunctions' in humans. However, if the species used is carefully considered from an anatomical perspective and there is a strong emphasis on task validity inherent in the research process, these limitations can be considerably reduced. Indeed, the information concerning the neural basis of cognition already obtained from animal research and the benefits that this has had for medical developments far outweighs the already mentioned potential difficulties in homology and task development.

7.3.2 The subthalamic nucleus and attention

The involvement of the subthalamic nucleus in attention is speculative. Deficits in performance accuracy have been found on the 5-choice serial RT task by Baunez and colleagues (2001). However, in the divided attention task used in this thesis no difference between lesioned and sham-operated animals was found. This leaves open the possibility that the nucleus contributes to attentional processes. In view of its extensive anatomical connections with other motor structures together with its major functional contribution to processes related to motor behaviour, it is a likely possibility that deficits seen in tasks commonly used to assess attention indeed reflect an alteration at the motor output stage, and is in accordance with major theories of basal ganglia function that suggests a role of the STN in motor behaviour.

7.3.3 The noradrenergic system and attention

In accordance with the idea that LC neurons respond to task relevant stimuli when their timing cannot be fully predicted (Bouret and Sara, 2005), it would be plausible

to hypothesise that lesions to the NA system would somehow alter performance in our task, as target is temporally unpredictable. That DNAB lesioned animals still showed directed attention based on the relative uncertainty of target location suggests that the particular involvement of the coeruleus-cortical system in stimulus and temporal unpredictability requires further investigation.

The observed changes seen after depletion of brain noradrenaline compared to controls under distracting conditions could be explained as a reduction in sensitivity to irrelevant stimuli. However, this idea is not in accordance with the explanations provided after impaired behaviour in noradrenergic depleted animals, which suggest that NA depleted animals show increased sensitivity to irrelevant stimuli and a 'broadening' of attentional resources, which consequently causes a lack of focus and impaired performance. More work is necessary to identify the mechanism and conditions by which attention to irrelevant stimuli occurs. As our experimental data indicate an impairment in directed attention in controls when faced with background noise and an apparent lack of effect of noise on directed attention in DNAB-lesioned animals, it is becoming clear that the role of the DNAB in attention is subtle. Taken together with reports that indicate a role of the DNAB in several cognitive functions, other, more specific, hypotheses as to the functional contribution of the DNAB and the noradrenergic system is needed.

7.3.4 The cholinergic system and attention

Previous studies, together with the results in this thesis, indicate that regulation of attention during covert orienting may be mediated by the basal forebrain cholinergic system. To date, experimental data indicate that enhanced cholinergic

transmission facilitates, while reduced cholinergic transmission impairs, the disengagement of attention. However, the seemingly facilatory nature of re-directing attention after NbM lesions discussed in Chapter VI together with the non-apparent effect of nicotine on performance in lesioned compared to control animals, imply that the behavioural effect(s) after enhancement or reduction of cholinergic transmission is task specific, which support previous reports after nicotine administration (Mirza and Stolerman, 1998). It is important to note, however, that the abolishment of spatial probability found after NbM lesions is not necessarily related to an improvement but rather can be viewed a deficit in the disengagement of attention. Additional time is required to re-direct attention after an invalid cue in normal behaviour, and thus, enhanced responses can be viewed as an illustration of an impairment. Importantly, the finding that the basal forebrain cholinergic system appears to also be involved in endogenously cued covert attention does not support the idea that exogenously and endogenously controlled attentional allocation requires distinct neural mechanisms, as suggested by others (Klein, 1994) and indicate that some form of common neural substrates is necessary in terms of these aspects of attention.

That nicotine did not seem to be beneficial in NbM animals in our task, while showing improved effects after NbM lesions in other attentional processes (Muir *et al.*, 1994), implies that nicotine may serve different functions at the level of the basal forebrain-cortical cholinergic system under different behavioural conditions, likely by its interaction with other neurotransmitters in the basal forebrain or other brain regions.

7.4 RECOMMENDATIONS FOR CURRENT RESEARCH

In the examination of the neural basis of cognition in experimental animals, it should be of the very essence to carefully consider the validity of the tasks used. Not only will this allow for a constant framework by which various cognitive processes can be investigated, but also allows for better comparison of experimental results of independent studies. This is particularly needed in the investigation of the neural correlates of working memory, which at present, appears to consist of many methodological pitfalls.

From the experiments presented herein of the role of NA and Ach in covert orienting of attention, several possible future experiments become clear. In terms of the DNAB and the noradrenergic system there are some open possibilities as to studies that would further our understanding of its contribution in attentional processes. Past findings indicate that systemic administration of clonidine affect the alerting but not the validity effect during exogenous covert attention in the Posner task (Witte and Marrocco, 1997), while administration of nicotine, which act on the cholinergic system, alter the validity effect on the same task (Phillips *et al.*, 2000). The site of action of systemic injection of clonidine, however, is uncertain, and lesions of the ascending locus coeruleus pathway via DNAB to cortical areas remains to be carried out, and would allow us to further specify the anatomical basis of the alerting effect. Furthermore, as NA appears to be important in some learning situations (e.g. Connor *et al.*, 1992), an idea is to examine lesions of this system on acquisition of the Posner task. As proposed by others (Yu and Dayan, 2005), NA appears to be more involved in learning rather than performance after acquisition and vice versa for Ach. Thus, an additional study could explore lesions of the NA and Ach systems on acquisition of the endogenous covert attention

paradigm used herein. Of course, in terms of neurotransmitter systems it is difficult to specify the anatomical location(s) that may be involved as transmitters may serve distinct functions in separate regions. Further experiments are needed to specify these areas in terms of both the noradrenergic and cholinergic systems in exogenous and endogenous covert orienting of attention.

The data in our task indicate that nicotine does not affect performance in NbM-lesioned compared to control animals and it would be interesting to follow up on a possible muscarinic basis of the effects seen on performance by administration of pharmacological agents or selective lesions that act on muscarinic receptors only. This, of course, presents us with the juicy possibility of an involvement of nicotinic receptors in exogenous covert attention and muscarinic receptors during endogenous covert attention. Further studies are needed to extend our understanding of the part played by different cholinergic receptors in attention. It is possible that performance in various tasks is caused by a combination of cholinergic receptors. To date, no studies have clarified the extent to which separate aspects of attention can be mediated by a single cholinergic receptor system.

7.5 CONCLUSIONS

The purpose of this thesis was to explore how attention exerts influence over responding and its neural basis. The use of common rodent working memory tasks as assays in the examination of the underlying neural processes of this construct has provided theoretical as well as experimental challenges. The analysis provided herein has only scratched the surface of the complexity involved in the development of valid tasks in experimental animals. To enhance our

understanding of the neural basis of attention, this thesis has approached the subject from both a neuroanatomical and neuropharmacological perspective. The data presented herein suggests that the subthalamic nucleus, a structure considered to be crucial for motor behaviour, is unlikely to participate in attention. It still remains an open possibility, however, that this structure also contributes to cognition. Interestingly, the data presented herein indicate separable roles of the noradrenergic (NA) and cholinergic (Ach) systems in some attentional processes, which are in line with some previous reports that suggest a functional interaction between these two neurotransmitters. In terms of NA, the behavioural manifestations of lesions to this system appear complex. There was no evidence of a contribution of the noradrenergic system to endogenous covert orienting of attention, but the data still implies a role in selective attention. The results observed herein indicate an apparent 'facilatory' effect of NA depletion under some attentional conditions, and encourage future experiments to further specify the exact contribution of NA to selective attention. Finally, the result support previous hypotheses of an involvement of acetylcholine in covert attention, and is the first study to show the neural basis of endogenous covert orienting of attention. Taken together with previous work that reports a role of acetylcholine in exogenous covert attention, this suggests that exogenous and endogenous covert attentional orienting might share a common neural substrate. Further analysis is necessary to specify the biology of covert orienting of attention.

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