— Executive function and prefrontal cortex in rats —

Anaïs M. Duffaud

MSc (Bordeaux 1)

A thesis submitted for the degree of

Doctor of Philosophy

School of Psychology

Cardiff University

December 2008

ProQuest LLC
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106-1346

DECLARATION

This work has not previously been accepted in substance for any degree and is not concurrently submitted in candidature for any degree.

Signed  (candidate) Date 9/12/08

STATEMENT 1
This thesis is being submitted in partial fulfillment of the requirements for the degree of PhD.

Signed  (candidate) Date 9/12/08

STATEMENT 2

This thesis is the result of my own independent work/investigation, except where otherwise stated. Other sources are acknowledged by explicit references.

Signed  (candidate) Date 9/12/08

STATEMENT 3

I hereby give consent for my thesis, if accepted, to be available for photocopying and for inter-library loan, and for the title and summary to be made available to outside organisations.

Signed  (candidate) Date 9/12/08

STATEMENT 4

I hereby give consent for my thesis, if accepted, to be available for photocopying and for inter-library loans, after expiry of a bar on access previously approved by the Graduate Developpment Committee.

Signed (candidate) Date

Contents

Acknowledgements

The present dissertation was conducted at the Behavioural Neuroscience Laboratory (BNL) of the School of Psychology in Cardiff. Some experiments were also realised at Eli Lilly, Erl Wood Manor Surrey. This work strongly benefited from a "CASE" studentship provided by the School of Psychology Cardiff and by Eli Lilly and from their excellent research facilities.

It is a pleasure to thank the many people who made this thesis possible.
First and foremost, my sincerest thanks to my supervisors Pr Simon Killcross and Dr Dave George for their endless enthusiasm, patience and guidance over the past three years. I would also like to thank Dr Sophie Dix (from Eli Lilly) for her support and help during my research time at Eli Lilly.
A thank you is also due to all the members of the BNL and in particular Jeff Lewis, Valerie Pearce, Lynn Hill, Kerry Housler and Dennis Simmonds for their excellent work and relief of a lot of worries.

In addition, I would like to thank some of my fellow PhD students, Christina Howard and Joni Karanka, with who have shared a lot of time (not only working) over the past three years. Special thanks are also due to Jo Haddon and Helen Pothuizen for sharing their experience and knowledge (about stereotaxie and MedPC programming among others) but also for all these Friday evenings.
I also want to thanks all the French people I met in UK for bringing a bit of "Frenchness" in my British life. A more special thank you to Elsa Pioli and Mathieu Albasser for

standing by me in good and bad times (I bet they had some hard times listening to my moaning!), and to Anne-Helene Clugery for her unconditional support and our marathon shopping days in London;

Finally, I would like to express my deepest gratitude to my mum, Marie-Josée Lada. She raised me, bore me, supported me, taught me and loved me.
" Maman, merci. Merci de m'avoir élevée, supportée, soutenue et surtout aimée. "
And last but not least, I would like to give my special thanks to Benjamin Lopez, not only for who he is but also because without him this "adventure" would not have been possible. "Merci de m'avoir conduite dans cette aventure"

Summary

The term executive function describes a set of high-level abilities that influence more basic motor, sensory and mnemonic processes. These functions include working memory, behavioural flexibility, inhibitory control, attentional processes and decision making. A large number of evidence, from human studies, non-human primates, rats and mice studies, has demonstrated a role for the prefrontal cortex in these higher cognitive processes.

The central aim of this thesis was to investigate two important aspects of the cognitive executive control: working memory and behavioural flexibility. The experiments described in the first two empirical chapters present the design of new operant paradigms to study these processes. Two further empirical chapters consider the neurobiological basis of behavioural flexibility, with a particular emphasize on the infralimbic (IL) and prelimbic (PL) regions of the rat medial prefrontal cortex (mPFC).

Although, the IL and PL regions have generally been considered as a single functional unit, empirical findings presented in this thesis provide evidence suggesting that the IL and PL mPFC can be viewed as independent but interactive regions with complementary roles in the control of behaviour. That is, the PL brings simple cue-outcome associations and more complex behavioural patterns under the modulatory influence of contextual, or other task-relevant, information and in contrast, the IL exerts an inhibitory influence over the PL biasing the animal towards simple, prepotent, learned or innate behavioural patterns.

List of abbreviations

5-CSRTT: five choice serial reaction time

ACC: anterior cingulate cortex

AID: dorsal agranular insular cortex

AIV: ventral agranular insular cortex

ANOVA: analysis of variance

AOM: medial anterior olfactory nucleus

AOV: ventral anterior olfactory nucleus

BC: buzz/clicker

BF: behavioural flexibility

CC: corpus callum

Cg2: cingulate cortex area 2

CR: conditioned response

DC task: delayed-comparison task

DNMTP: delay non matching to position

DNMTS: delay non matching to sample

DM: dark/magazine

DMTP: delay matching to position

DMTS: delay matching to sample

DR task: delayed-response task

EDS or ED shift: extradimensional shift

Gcc: genu of corpus callum

HPC: hippocampus

IDS or ID shift: intradimensional shift

ID/ED effect: intradimensional-extradimensional effect

IL: infralimbic cortex

ITI : intertrial interval

LTHT: low tone/high tne

LI: latent inhibition

LP: lever pressing

LO: lateral orbital cortex

M1: primary motor area

MA: magazine approach

MD: mediodorsal thalamic nucleus

MO: medial orbital cortex

mPFC: medial prefrontal cortex

MTP: matching to position

NAC: nucleus accumbens

NMTP: non-matching to position

NPE: non-preexposed

OB: olfactory bulb

O_{cc}: Occasion setter

OS: optional shift

PE: preexposed

PFC: prefrontal cortex

PL: prelimbic cortex

PL/IL: prelimbic-infralimbic

PrC: precentral cortex

SART: sustained attention to response task

S_D: discriminative stimulus

SF: steady/flashing

SST: Stop Signal Task

THT: tone/high tone

VLO: ventral lateral orbital cortex

VO: ventral orbital cortex

WCST: Wisconsin Card Sorting Test

WM: working memory

1 General introduction

1.1 Executive functions

The term "executive function" refers to a complex cognitive construct encompassing the whole set of processes underlying controlled goal-directed responses to novel or difficult situations. It is also referred to as the central executive function or cognitive control. As their name suggests, these executive functions are hypothesised to direct many other mental processes such as sensory, motor or mnemonic processes. These processes are generally named as lower-level processes and refer to the mechanisms that are automatically executed, without effortful control (e.g. driving a car). In everyday life, many situations are controlled by these lower-level processes (e.g. answering a ringing phone, putting the kettle on when one wants to have tea); however other situations that do not correspond to a routine or that elicit conflicting responses require a higher-level cognitive control. This cognitive control is vital in situations where a routine process is inadequate, for example overcoming habitual responses, during planning, decision making, or error correction when a response is novel, or a situation uncertain or difficult. This allows us to be flexible and adaptive rather than stimulus-bound slaves to the current sensory input. Here is an explicit example of the importance of these executive functions in everyday life. You are home, the phone starts ringing and the doorbell is chiming. So, you are trying to decide which of two aspects of a situation you should respond to (a ringing phone or a chiming bell). This situation requires high-level cognitive mechanisms: conflict detection and resolution (realising both events are occurring and making the decision to answer the door first), inhibition (ignoring the 'phone to answer

the door), working memory (holding in mind that the 'phone was ringing as you answer the door, so you can check for an answer later), attention (attending to what the person at the door is saying even though the 'phone is still ringing), and behavioural flexibility (answering the phone or checking for an answer once you closed the door).

A large number of dissociable executive functions have been proposed, and evidence for all of them exists not only from human studies but from non-human primates and rats and mice as well. These functions include such abilities as *working memory* (WM) which is the ability to maintain a goal, or task-relevant information, over a period of time, *behavioural flexibility* (BF) which encompasses the ability to switch between different tasks according to the current goal, *inhibitory control* which invokes abilities to suppress dominant response tendencies in favour of more goal-appropriate behaviour, *attention* which refers to the ability to efficiently allocate processing resources to different aspects of the environment based on current situational demands, and *decision making* which refers to the ability to make a decision based on the situation demand. In this thesis, I will mainly focus on working memory, inhibitory control, behavioural flexibility and attention.

In the next section, I will briefly outline the different paradigms and constructs used to assess executive functions in humans. A discussion of designs investigating executive functions in rats will follow in the subsequent section (section1.1.2). These reviews are by no means exhaustive as I intend to focus on paradigms that are the mostly commonly used in clinical neurosciences and in experimental psychology. It is also important to note

that the terminology used to describe concepts show considerable overlap. For instance, Wisconsin Card Sorting Test (WCST) is a paradigm that can be viewed as a procedure to assess behavioural flexibility but it also involves aspects of inhibitory control.

1.1.1 Aspects of executive functions in humans

Working memory

Working memory is the short-term retention of information that is no longer accessible in the environment, and the manipulation of this information for subsequent use in guiding behaviour (Baddeley, 1992). Classically, paradigms used to assess working memory comprise three phases: a sample phase where information is presented to the subject, a delay phase when that information is withdrawn for a period of time and a choice phase where that information, along with some relatively novel comparison information, are presented and where subject has to identify the previously presented information. Paule and Bushnell (1998) have identified at least 3 sub-processes for successful completion of a working memory task: "*discrimination* - the process by which perceptual identification of the information being presented during the sample phase of a trial occurs, *encoding* - the process by which the perceptual information available in the presence of the sample stimulus is converted into an internal representation of that information, and *retention* - the process by which the veracity of that information is maintained".

Most frequently, "N-back" tasks have been used to assess working memory in humans (D'Esposito et al., 1997; Gevins et al., 1997; Owen et al., 2005). In the continuous

matching version, subjects were asked to monitor a series of stimuli and to respond whenever a currently presented stimulus was the same as the one presented "n" trials previously, where "n" was a pre-specified number (usually 1, 2, or 3). The task required on-line monitoring, updating, and manipulation of remembered information and was therefore assumed to place substantial demands on the key processes within working memory (Owen et al., 2005). N-back tasks can be divided along two dimensions: the nature of the stimulus material used (verbal or non verbal) and the nature of the stimulus feature to be remembered (spatial or non spatial). Normal participants performed accurately when "n" was increased (up to 3) in all type of tasks (Owen et al., 2005) but their reaction time increased. Another paradigm widely used to asses working memory is the "self-ordered" task. Subjects are required to monitor self-generated responses (i.e. subjects themselves generated the sequence of responses to be remembered). The self-ordered task was originally reported by Petrides and Milner (1982). Subjects were presented with a pile of sheets of paper on each of which a list of stimuli was printed; on each sheet the list positions of the stimuli were different and randomly determined. Subjects were required to touch all the stimuli, one at the time and in any order they wished, but without touching any given stimulus more than once. They were told to touch only one stimulus per sheet of paper and that after each response they were to turn the next sheet, touch another stimulus, and continue in this manner. The experiment consisted of repetitions of 3 successive trials of a given list. Order of responding, errors made and time to complete each trial were recorded. This task could differ in the type of stimulus material used (e.g. verbal or non verbal). According to Petrides and Milner, this self-ordered task placed considerable demands on an active, working memory compared

to the externally ordered task, as it constantly required the subjects to compare the responses that they had made to those that still remained to be carried out. In 1990, Owen et al. developed a computerized spatial version of this task. In this version, subjects were required to search tokens that were hidden in boxes in order to fill an empty column located in a corner of the screen. The aim of the task was to find the hidden token without re-opening a previously opened box. The key instruction was that once the token was found within a particular box, then that box would never be used again to hide a token. Order of responding and type of response made (within-search errors corresponding to the re-opening of an empty box in the same search sequence – between-search errors corresponding to re-opening of a box where a token was previously found) were recorded. In such tests, normal participants performed accurately with few items but when the number of items in the list increased (therefore the memory load increased) they tended to make more errors.

Cognitive/Behavioural flexibility

In humans, the construct of cognitive flexibility generally refers to the ability to shift between avenues of thought and action, enabling us to adapt to changing environmental contingencies. According to Eslinger and Grattan (1993) the concept of cognitive flexibility is not unitary as it can be dichotomized into spontaneous flexibility and reactive flexibility.

Spontaneous flexibility describes the ready flow of ideas and answers in response to a question, and it can typically be measured by tasks such as word fluency (e.g. Verbal Associative Fluency Test).

Reactive flexibility refers to the ability to shift attention, to shift cognitive set, to respond to different stimulus configurations or conditions in different ways as particular tasks demand, or to adapt to changing response rules. Within reactive flexibility, a distinction between "lower" and "higher" order flexibility has to be made. Lower order flexibility is less demanding on cognitive processes than higher-order flexibility. Paradigms used to assess lower order flexibility are typically reversal learning tasks (tasks where changes in stimulus-reward contingencies occur). As for higher order reactive flexibility the most commonly used test is the WCST (Milner, 1963). This paradigm was a dynamic categorization task in which participants were instructed to sort cards according to a certain feature of the images on the cards (shape or colour or number), receiving feedback only about success or failure of their sorting. Participants started by learning a particular rule (i.e. sort according to shape) and once this had been acquired, the experimenter changed the rule (i.e. to colour) without informing the participant. The participant then had to adjust their sorting on the basis of feedback from the experimenter, in order to learn the new rule. Rules were acquired and changed until all cards had been sorted according to all possible rules. Any given card could be associated with a number of possible responses, and so no single stimulus-response pairing would always lead to the correct response, as the relevant stimulus element for the rule changed periodically (Milner, 1963). The intradimensional-extradimensional shift (ID/ED shift) is

another test that has become popular as mean to assess higher order reactive flexibility (Owen et al., 1993). This task, adapted from the WCST, assesses the subject's ability to form an attentional set and to shift it from one perceptual dimension to another. Subjects were initially trained to discriminate between pairs of stimuli, each of which is composed of two distinct perceptual dimensions, but only one of these dimensions predicted reinforcement. Then subjects performed a series of shifts: intradimensional shift (IDS or ID shift), extradimensional shift (EDS or ED shift) and reversal learning shifts. In IDS, subjects trained to respond to a particular stimulus dimension were required to transfer that rule to a novel set of exemplars of that same stimulus dimension; therefore the relevant dimension remained relevant and the irrelevant dimension remained irrelevant. In EDS, they were required to shift response set to the alternative, previously irrelevant dimension; in other word the previously relevant dimension became irrelevant and the previously irrelevant dimension became relevant. The learning of novel discriminations was relatively rapid when the relevant stimulus dimension remained constant (IDS) allowing the use of an established attentional set, whereas learning was slower when attention had to be shifted to a previously irrelevant dimension (EDS).

Inhibitory control

The process of inhibitory control is viewed as "the ability to suppress undesirable response tendencies" (Lansbergen et al., 2007) and it is generally accepted that it comprises several different behavioural processes (Barkley, 1999; Nigg, 2000; Eagle and Robbins, 2003). Among others, Barkley (1999) suggested a taxonomy of the behavioural or response inhibition construct that distinguishes three interrelated processes: (i)

inhibiting the initial prepotent (automatic) response to an event, (ii) stopping an ongoing response or response pattern, thereby permitting a delay in the decision to respond or continue responding and (iii) protecting this period of delay and the self-directed responses that occur within it from disruption by competing events and responses (interference control). Failure to exhibit an adequate inhibitory control can lead, for instance, to impulsivity (responding before it is time to) and perseveration (continuation of a response that is no longer relevant).

The stop signal task (SST) is a laboratory test commonly used to asses inhibition of prepotent responses (Barkley, 1999). This motor inhibition task required the subject to withhold a planned movement in response to an infrequent countermanding signal. Subjects were instructed to make a response as quickly as possible to a go signal (no-stop-signal trial) but on a minority of trials, a stop signal was presented (e.g. auditory signal) and subjects had to inhibit the previously planned response (stop-signal trial). Stopping an ongoing response or response pattern refers to the subject's ability to stop an initiated response and to switch to a more effective one when feedback indicates that the ongoing response pattern is no longer correct. The WCST provides just such an evaluation of response shifting or flexibility. Indeed, in this dynamic categorisation task subject had to learn sorting rules that periodically changed (for details see section 1.1.1 Cognitive/Behavioural flexibility). Failure to stop and switch to ongoing response pattern often induced preservative behaviour in WCST, with subjects still responding on the previously relevant but currently irrelevant dimension. One of the most widely used tasks for assessing interference control is the Stroop Colour- Word test. In the Stroop task

(Stroop, 1935), the participants were presented with stimulus compounds of colour words written in coloured ink and were required to either name the colour or to read the word. Compounds were composed of either matching (the word "blue" written in blue ink, the word "red" written in red ink) or mismatching (the word "blue" written in red ink, the word "red" written in blue ink) word and colour ink combinations. This task involved the selective attention to the task-relevant attribute, whilst ignoring the distracting stimulus element. This process became vital when the aim of the task was to name the colour of a mismatched stimulus (e.g. the word "blue" written in red), as participants had to ignore and/or inhibit the interfering response (the colour word), resulting in a greater number of errors and longer latencies to respond. The increase in the time for reacting to colours caused by the presence of conflicting word stimuli was taken as the measure of the interference of word stimuli upon naming colours.

Inhibiting prepotent responses, stopping ongoing response patterns and interference control are interrelated processes, and therefore there are no sharp boundaries between these processes. Thus, tasks used to assess inhibitory control (such as the ones described above) can require one or more of these processes at the time. For instance, the Stroop task that Barkley (1999) categorised as a task to assess interference inhibitory control can also be taxed to tap into inhibiting prepotent response processes.

Attention

Attention refers to the mechanisms by which one is able to efficiently allocate processing resources to different aspects of the environment based on current situational demands.

As with many other constructs in cognitive psychology, attention is not a unitary construct and involves aspects of selective (focussed) attention, divided attention and sustained attention (Coull, 1998; Perry and Hodges, 1999).

Selective attention is the ability to focus attention on one relevant stimulus or location, ignoring any non-relevant stimuli or locations (Perry and Hodges, 1999). To some extent, the Stroop task can be viewed as tapping into selective attention processes (Coull, 1998). When a subject was presented with mismatching compound stimulus (the name of a colour written with a different ink colour; e.g.: "blue" written in red ink) and asked to either name the colour or read the word, selective attention was required as the subject had to ignore one attribute of the stimulus compound in favour of the other. Divided attention refers to the ability to distribute attentional resources between two or more competing inputs at the same time and is usually assessed with a dual-task paradigm (Perry and Hodges, 1999). A dual task procedure requires the subject to perform two tasks (A and B) separately before performing both tasks simultaneously. Normal subjects show a deterioration in performance (dual-task decrement) on task A and/or B when they are performed together compared with when they are performed on their own. A typical example is the combination of tracking and digit span repetition used by Baddeley et al. (1986). In this paradigm, task A required the use of a light sensitive pen to follow a white square as it moved randomly about the screen and task B required the repetition of strings of digits. Both tasks were attempted on their own for 2 min before being performed together for a further 2 min. A decline in performance was observed when the tasks were performed concurrently compared to when they were performed separately; therefore this

decline could be seen as a deficit in the ability to divide or share attention when the demand was for attention to be in more than one place at a time. Sustained attention or vigilance refers the ability to maintain attention to a particular target (e.g. stimulus, location, response) for prolonged periods of time (Perry and Hodges, 1999). One task assessing sustained attention has received more investigations: the sustained attention to response task – SART – (Robertson et al., 1997; Whyte et al., 2006). This task, developed by Robertson and colleagues (1997), was a continuous performance paradigm involving key presses to frequently presented non-targets but with the requirement to withhold motor responses to occasional targets. The authors argued that this task required high level of continuous attention to response set and is sensitive to transitory reductions in attention. Rosvold et al. (1956) proposed a paradigm assessing sustained attention to stimulus that has also been widely used. This task consisted of a series of letters presented on a computer screen. The subject had to respond to one particular letter or a combination of letters (the target) and refrain from responding to all other stimuli. The number of missed targets (omissions) is considered a measure of inattention, and the number of erroneous responses (commissions) a measure of impulsivity. Classic results observed in this task consisted in an increase in reaction time and omissions errors during the course of the test.

1.1.2 Assessing executive functions in rodents

Working memory tasks

For rodents, the concept of working memory originates in the experiment of Olton and Samuelson (1976). They designed a classic task for assessing working memory in rodents

using a radial arm maze (comprised of eight arms radiating from a central platform). In this task the rat was placed on the central platform and learned to retrieve food available at the ends of each arm. Animals rapidly learned to visit all arms without re-entering a previously visited arm. This suggested that rats were able, in a single session, to remember which arms they had visited without using intramaze cues or responses chains to solve the task. This, for Olton and Samuelson, was working memory: memory that allowed animals to remember which arms had been visited within a session and was "reset" between sessions. Furthermore, they argued that working memory used flexible stimulus-response associations and was sensitive to interference. Since this historical experiment, working memory has been well studied and parameters influencing performance are well characterized: delay, retention interval, proactive and retroactive interferences and interfering stimulus (Dunnett and Martel, 1990; Herremans et al, 1993). An increase of retention interval, induced a deterioration in response accuracy; but to be related to the animals' working memory capacity, this deterioration must be delay dependent (Dunnett and Martel, 1990). However, such delay-dependent impairments are not sufficient proof for the involvement of WM processes. Another key is to assess the effect of proactive interference. There is substantial evidence that stimuli and response on the previous trial can interfere with the choice accuracy on the current trial. This occurs when the sample from the previous trial differs from the sample of the current trial and such interferences (Dunnett and Martel, 1990, Steckler et al, 1998).

There is a broad range of paradigms used to assess working memory in rodents, and therefore a classification exists based on the nature of the information to be remembered

across the delay. This classification varies along two categories: delayed-response (DR) and delayed-comparison (DC) tasks. In delayed-response tasks, all information necessary to determine the correct response is given before the onset of the delay phase, so the correct response is defined before the retention interval and the remembered information is about the response to be made. In delayed-comparison tasks, information presented prior to and after the delay has to be compared in order to determine the correct response; the correct response can only be determined after the delay and the remembered information is about the sample stimulus.

Among the wide range of DR tasks, the most popular is the delayed (non) matching to position (DMTP or DNMTP). A maze version has been described by Olton and Markowska (1993). In this version (using T or Y maze), a trial started with a forced sample phase where animal had to visit an open arm (with the alternative arm being blocked). After a delay both arms were made accessible. In the DMTP version, the animal had to visit the previously visited arm; in the DNMTP version, the animal had to visit the alternative arm. Comparable tasks can also be scheduled in more complex mazes, for example in the 8-arm radial maze. Operant versions of the DMTP/DNMTP that are also based on comparison of spatial stimuli have also been developed. Operant chambers may be equipped with two retractable levers that served both as spatial stimuli and operanda. Alternative stimuli such as stimulus lights and operanda such as holes for nose poking could also be used to schedule operant DMTP/DNMTP (Steckler et al., 1998). During the sample phase, either the left or the right lever was presented, the animal had to respond to the lever and the lever was retracted. Following a delay, both

levers emerged. In DMTP the animal was required to press the lever that had been presented before. In DNMTP the animal had to press the alternative lever.

The corresponding delayed-comparison tasks are the delayed matching to sample and delayed non-matching to sample (DMTS/DNMTS). Theses tasks have been widely used to study working memory and are characterized by the pairing of events. In these tasks a sample stimulus was presented, withdrawn and following a delay, comparison stimuli were displayed. The subject had to discriminate between these comparison stimuli and to respond according to a matching or non-matching rule (Steckler et al., 1998). DMTS/DNMTS tasks in rodents have been predominantly limited to semi-automated, maze-based tests (Pontecorvo et al., 1996). After a review of literature, it seems that there are no fully automated, operant DMTS/DNMTS tasks using delays of more than 12 sec. For instance, in an attempt to develop such a task Ennaceur et al. (1997) failed to show a delay-dependant deficit. In this task, although animals were able to perform above chance level at various delays (0 – 12sec), they did not show a consistent decline across delays. Non-operant versions of DMTS/DNMTS are commonly based on the use of objects. Mumby et al. (1990) developed a DNMTS task using a straight runway and trial unique stimuli. Rats were restricted to a central position on the runway by two guillotine doors. During the sample phase, one of the two doors was opened to provide access to a sample object. To gain reward, rats had to displace this object. Following a delay, the other door was opened, and rats could find the sample object and a novel object at the opposite end of the runway. To gain reward, rats had to displace the novel object. They tested the rats

with delays up to 600-sec and observed a delay-dependant deficit in choice accuracy (as there was a significant interaction delay x response).

Based on the brief review of literature presented above, it appears evident that there is a broad range of tasks that have been used or are currently used to assess working memory in rodents. However, different challenges are associated with each of these paradigms. The major confound when using delayed response tasks is that animals can and do use overt mediating strategies such as holding a position, body orientation (Chudasama and Muir, 1997). As for the delayed-comparison tasks which are mostly maze based, the major confound is that they provide poor control over stimulus and response parameters. Pro and cons of delayed-response and delayed-comparison tasks will be further discussed in the Working Memory experimental chapter (Chapter 2).

Behavioural flexibility tasks

In rodents, the intradimensional-extradimensional shift effect was first demonstrated by Lawrence (1949). Lawrence trained rats on a successive discrimination and then on a simultaneous discrimination. In the simultaneous discrimination stimuli drawn from one stimulus dimension (e.g., brightness: black vs. white) signalled the location of a food reward, whereas stimuli from a second dimension (e.g., floor texture: rough vs. smooth) were present but irrelevant. The rats were then transferred to a novel successive discrimination. For some animals, the solution to the successive discrimination was dependent upon stimuli that belonged to the same dimension that had been relevant during the simultaneous discrimination. Rats that received this intradimensional shift

learned the task more rapidly than those that received an extradimensional shift where the dimension that had been irrelevant to the simultaneous discrimination was relevant to the solution of the successive discrimination. Since Lawrence's (1949) demonstration of the ID/ED shift effect, another procedure using the ID/ED shift design has become popular. This task, developed by Birrell and Brown (2000), was formally the same task as one used in New World primates and humans. In human and non-human primates visual stimuli were used, whereas in rodents olfactory and tactile stimuli were used. Rats were trained to dig in bowls for food reward. The bowls were presented in pairs and rats had to select the single rewarded bowl in which to dig by its odour or the medium that filled the bowl. In a single session, animals performed a series of discriminations, starting with a simple discrimination (SD) in which bowls differed along one of the two dimensions (e.g. odour). In the compound discrimination (CD), a variation on the second dimension was introduced (e.g. digging medium) but was irrelevant for the solution of the discrimination. Animals then received a series of discrimination each involving a different type of shift (see Table 1.1). It started with a reversal learning shift (learning of a new stimulus-reward association while maintaining attentional set) followed by an intradimensional shift where new exemplars of each dimension were used but in which the relevant dimension was unchanged (e.g. odour). This phase was followed by a second reversal learning shift. In the last two shifts, animals first had to perform an extradimensional shift where exemplars of each dimension were renewed but the relevance of the dimensions was reversed – the previously relevant dimension became irrelevant and the previously irrelevant dimension became relevant – followed by a third reversal learning shift.

	Dimensions	
Discriminations	Relevant	Irrelevant
Simple (SD)	odour	medium
Compound (CD)	odour	medium
Reversal (Rev1)	odour	medium
Intradimensional shift (IDS)	odour	medium
Reversal (Rev3)	odour	medium
Extradimensional shift (EDS)	medium	odour
Reversal (Rev3)	medium	odour

Table 1.1: experimental design used by Birrell and Brown (2000).

Typical observations from this ID/ED shift paradigm showed a discrepancy in rats' readiness to learn the different shift discriminations (IDS *vs.* EDS), with more rapid learning when the discrimination was based on the previously relevant perceptual dimension (IDS) compared with the EDS when attention had to be shifted to the previously irrelevant dimension. Such results support this idea that animals formed a perceptual attentional set where they attended more to the stimuli that had been relevant in the past.

Assessing the ability of rats to shift "behaviour guiding a strategy" (as is seen during an ED shift) can also be realized using other paradigms. These paradigms include task switching from a spatial (place) strategy to a visual cue strategy in a water maze or a

cheeseboard apparatus (Ragozzino et al., 1999b), or from a spatial to a response rule and vice-versa in the cross maze (Ragozzino et al., 1999a), or between more abstract rules such as matching to position (MTP) to non-matching to position (NMTP) rules in a T-maze or operant chamber (Joel et al., 1997a).

Inhibitory control tasks

The stop signal task measures an important aspect of behavioural inhibition: the ability to inhibit a response that has already be initiated. The SST as described by Eagle and Robbins (2003) used an operant procedure. Trials were initiated with a nose-poke to the central food well, after which the left lever was presented. A press on the left lever resulted in the right lever being presented. On a "go" trial rats were required to respond as fast as possible on the right lever and response speed was maintained by limiting the time for which the right lever was presented (limited hold). On a "stop" trial, a tone was presented between presses on the left and right levers and rats were required to refrain from pressing the right lever during the limited hold. Stop trials were randomly presented in order to discourage the rats from anticipating their presentation.

Extinction paradigms require an animal to stop (inhibit) conditioned responding to a stimulus that previously signalled the occurrence of reinforcement but no longer does. Therefore extinction involves some aspects of flexibility and inhibition of prepotent learned responses. Extinction paradigms can examine development of extinction *per se*, as well as some aspects of the transfer of extinction from one session to the next. Paradigms studying extinction typically employ Pavlovian procedures (Bouton, 2004).

Attentional tasks

Numerous paradigms have been used to study sustained, selective and divided attention in rodents. To date, the 5-choice serial reaction time (5-CSRTT) remains the most popular paradigm (Carli et al., 1983; Muir et al., 1996; Chudasama et al., 2003). This task required rats to detect brief flashes of light presented randomly in one of five locations. Attentional load in this task could be manipulated by increasing the rate of stimuli presentation, by making the stimuli unpredictable or by presenting an interfering stimulus. Depending on the parameters used, this task involved aspects of sustained (maintaining attention across trials), selective (ignoring distracter) and divided (maintaining attention on five different locations) attention.

Other paradigms examining individual attentional function have also been developed. For instance, Sarter and McGaughy (1995; 1998) have proposed an operant paradigm investigating sustained attention or vigilance in rodents. This task required the animals to respond to visual signals by operating one lever and to the absence of signal by operating the opposite lever. They showed that performance on this task is a function of the signal length, the shorter the signal the higher the number of incorrect response. Recently Haddon and Killcross (2006) developed a Stroop-like task measuring aspects of cue and response competition in rodents. Rats were trained on two conditional discriminations (one auditory and one visual), in two different contexts. At test, audiovisual compounds of the training stimuli were presented. These compounds comprised stimuli that had required either the same instrumental response (congruent) or different responses (incongruent) during training. Haddon and Killcross showed that responding to

Incongruent trials was dictated by the context present during the test. This showed that animals were able to respond according to the task relevant (context appropriate) information while ignoring irrelevant (context inappropriate) information.

1.1.3 Conclusion

In this first section, I have summarised several of the tasks used most commonly to assess executive functions in humans and rodents. More specifically, I emphasised paradigms used to investigate working memory, cognitive flexibility, inhibitory control and attention. From this outline, it is evident that there is a variety of tasks which are well suited for use with rats, with several demonstrating a specific effort to adapt the task to rodents (cf. the evident concern to use salient stimuli) and to use designs that are formally similar to the ones used in humans. However, it is also the case that some of these tasks can be challenged in term of underpinning processes. For instance, delayed-response paradigms used to assess working memory can be challenged in term of cognitive (or otherwise) processes supporting performance; that is, mediating strategies may seem to be more involved in performance than working memory *per se*. In the specific introductions to the experimental chapters, I will further discuss challenges associated with working memory and behavioural flexibility constructs.

1.2 Executive functions and prefrontal cortex

The cognitive importance of the frontal region of the brain first became apparent through studies of First World War veterans, which demonstrated that soldiers with frontal lobe

injuries were unimpaired on routine tasks, but had difficulty mastering new tasks or grasping the entirety of a complicated task. Since then, there has been a notable increase in research activity focused on the on prefrontal cortex (PFC) and converging evidence has demonstrated a role for the PFC in higher cognitive processes. Furthermore, the PFC has the ideal infrastructure for processing the diverse range of input necessary for top-down control of such complex operations, receiving multiple polymodal inputs, and having reciprocal connections with many cortical and sub-cortical brain regions.

In this section, I will present findings providing evidence for the involvement of the prefrontal cortex in executive functions, in humans as well as in rats. I will focus on working memory and behavioural flexibility as they are processes that require complex cognitive control typically associated with the dorsolateral prefrontal cortex functioning in humans (Fuster, 2000; Brown and Bowman, 2002).

1.2.1 Evidence of PFC involvement in executive functions in humans

Working memory

Ample evidence from both neuropsychological patients and human imaging studies indicate that the lateral prefrontal cortex plays a critical role in working memory processes. For that reason a number of researchers have put forward theories of prefrontal cortical function in terms of working memory processes and two major competing models that differ with respect to how different subregions of the PFC are thought to contribute to working memory have emerged. According to Petrides (1982; 1996)

organization of the working memory within the PFC is *process-specific* in the sense that the dichotomy between different subregions of the PFC is based on the level of involvement in WM. Thus, the dorsolateral PFC appears to be a specialized area in which information can be held online for monitoring and manipulation of stimuli whereas the ventrolateral PFC is involved in active selection, comparison and judgement of stimuli held in memory. In contrast, Goldman-Rakic (1987) proposed a *domain-specific* organisation of the working memory processes, suggesting that different subregions contribute to working memory processes based on the sensory nature of the information being processed. Goldman-Rakic (1987) proposed a dichotomy between the mnemonic processing of spatial information in the dorsolateral PFC and non-spatial mnemonic processing in the ventrolateral PFC.

Several fMRI studies appear to support Petrides' hypothesis. In a PET scan experiment, Petrides et al. (1993) observed a clear activation of the mid-dorsolateral frontal cortex during the performance of a task requiring monitoring of self-generated responses and a task requiring externally generated responses. Owen et al. (1996) further examined the activation of the different subregions of the prefrontal cortex in a spatial working memory task where manipulation and monitoring demands varied. Imaging data provided by PET indicated a role for the dorsolateral prefrontal cortex in both monitoring and manipulation processes, whereas the ventrolateral prefrontal cortex appeared to be involved in the maintenance and organization of information held in working memory. In support of the Goldman-Rakic's model, functional imaging studies of human cognition have indicated a role for the dissociable regions of the lateral prefrontal cortex in spatial

and non spatial working memory tasks. Using fMRI, McCarty et al. (1996) showed increases in activity within the dorsolateral prefrontal cortex during spatial memory tasks and increases in activity of the more ventral prefrontal regions during non-spatial tasks. In another fMRI experiment examining the pattern of activity of the prefrontal cortex during performance of subjects in a non-spatial working memory task, Cohen et al. (1994) also reported activation extending into more ventral prefrontal regions. These findings support the idea of a functional dorsal/ventral dissociation of the lateral frontal cortex depending on the material held on working memory, "where" and "what" respectively.

Behavioural flexibility

The ability to shift cognitive set from one perceptual attribute of a complex stimulus to another has been widely studied using WCST and ID/ED shift paradigms. Cumulative evidence from patients with frontal lobe damage and functional neuroimaging studies in humans indicates a critical role of the PFC in cognitive flexibility. Milner's (1963) study was one of the first demonstrations of the involvement of the PFC in cognitive flexibility. Using the WCST, she demonstrated that both normal, healthy participants and patients with prefrontal impairments were able to acquire the initial rule, but only the normal healthy participants were able to alter their responding – shifting their cognitive set from one attribute (e.g. shape) to another (e.g. colour) – in accordance with the changes in rule. Thus, patients with damage to the PFC were able to learn the correct rule initially, but could not switch sorting rule, leading them to perseverate on the incorrect response. Human imaging studies have also found evidence for PFC activation during rule changes

in the WCST (Wang et al., 2001). In 2001, Nagahama et al. provided another piece of evidence regarding the involvement of the PFC in performance of the WCST; their study supplied findings about a functional dissociation within the PFC when two levels of response selection were required (attentional set shift and reversal shift). According to their findings, the posterior PFC is involved in the reorganisation of stimulus-response associations (reversal shift) whereas the rostro-dorsal PFC is associated with higher order shift of attention. Further evidence of the humans PFC involvement in behavioural flexibility comes from the use of the ID/ED shift task, an extension of the WCST. Owen et al. (1991) showed that a group with frontal lobe damage, but not temporal lobe-damaged group or amygdalo-hippocampectomy patients, was selectively impaired in shifting response set to a previously irrelevant dimension (ED shift); all patient groups were able to shift attention to new exemplars of a previously relevant dimension (ID shift). More recently, Hampshire and Owen (2006) with an event related fMRI demonstrated that ventrolateral prefrontal cortex was involved in switching attention between dimensions whereas posterior parietal cortex mediated changes in stimulus-response mapping.

It is now generally accepted that perseveration (use of a currently irrelevant, but previously relevant, rule) on the WCST (or ID/ED paradigm) is a relatively specific indicator of frontal lobe dysfunction and is usually interpreted as the deficit of attentional set-shifting ability (Owen et al., 1993).

1.2.2 Evidence of PFC involvement in executive functions in rodents

Working memory

Effects of prefrontal damage on working memory processes in the rat have been extensively studied with contradictory findings: some studies have shown impairments on working memory tasks following prefrontal cortex lesions - implicating the rat prefrontal cortex in working memory processes (Dunnett, 1990; Granon et al., 1994; Sloan et al., 2006) - whereas others have shown preserved working memory abilities in rats with prefrontal damage (Kolb et al., 1994; Aggleton et al., 1995; Chudasama and Muir, 1997). In an early study by Dunnett (1990) the medial prefrontal cortex lesion was found to impair an operant DMTP task, with some rats showing delay-dependent deficits and others performing at chance across all delays, mainly because of strong response biases. Based on these behavioural results and histology, Dunnett concluded that rostral medial prefrontal damage was sufficient to induce delay-dependent deficits, but that lesions involving the caudal prelimbic cortex could induce a more severe, delay-independent impairment in performance. A more recent study (Sloan et al., 2006), using a similar DMTP task, found a similar pattern of impairment. Rats with lesions of the medial prefrontal cortex (mPFC) exhibited a significant delay-dependent impairment on the DMTP task. However, others studies also using delayed-response paradigms failed to find an effect of prefrontal lesions on working memory. Chudasama and Muir (1997) showed that NMDA-induced lesions centred on the prelimbic cortex (PL) resulted in delay-independent deficits in DMTP. Using the same type of lesion technique but a DNMTP task, Aggleton et al. (1995) also showed that NMDA lesions of the PFC induced delay-independent deficits on a DNMTP task.

Contradictory findings have also been reported using delayed-comparison tasks. In their study of the effect of prefrontal lesion on DMTS and DNMTS paradigms, Granon et al. (1994) showed that PFC lesion restricted to prelimbic cortex induced a consistent working memory impairment in both type of tasks. However, in the same year Kolb et al. (1994) published a study in which medial prefrontal lesions appeared to spare performance on DNMTS in rats.

The heterogeneity of behavioural paradigms (delayed-response/delayed-comparison, spatial/non-spatial) and neuroscience methodology (lesion technique) employed have led to ambiguous results. Therefore, despite some evidence of medial prefrontal cortex involvement in working memory processes, the discrepancy in results limits interpretation of this research and indicates some uncertainty about a role of the rat prefrontal cortex in working memory.

Behavioural flexibility

Using an ID/ED task which is formally the same as the one used in humans and non-humans primates, some evidence about the involvement of the rat's PFC in behavioural flexibility has been found. Birrell and Brown (2000), showed that bilateral lesions of the medial prefrontal cortex induced a selective impairment in shifting attentional set between stimulus dimensions (ED shift), but spared performance on initial acquisition and reversal learning. Conversely, orbital prefrontal cortex lesions resulted in a selective impairment of reversal of stimulus-reward contingencies, leaving attentional set-shifting capacities intact (Brown and Bowman, 2002). Together, theses results support the view

that different cognitive functions are distributed across specific regions of prefrontal cortex; the orbital prefrontal cortex is involved in lower-order cognitive flexibility and the medial prefrontal cortex is involved in higher-order cognitive flexibility. Furthermore, these findings provide evidence for homologies between species. Thus, the pattern of impairments observed in rats with either mPFC or orbital lesions mirrors that reported in marmosets, with either lesions of the lateral or orbital prefrontal. This extends to the rat the dissociation between reversal attentional set and reversal of "affective associations" (Dias et al., 1996). Other studies employing different paradigms also provide evidence of the role of the mPFC in rule learning and the ability to use and switch between strategies. With a WCST analogue (reversal from non matching to sample to matching to sample), Joel et al. (1997a) demonstrated that mPFC lesioned rats were slower at shifting an abstract rule (from matching to non-matching rule) in a Skinner box as compared with Shams-operated animals. In tasks examining behavioural flexibility of place and response learning or spatial to visual-cue learning, Ragozzino et al. (1999a; 1999b) showed that prelimbic-infralimbic (PL/IL) inactivation did not impair acquisition of place and response rule or spatial and visual-cue version but impaired the switch from a place to a response discrimination or from spatial to visual-cue discrimination and vice-versa. In one of these studies (Ragozzino et al., 1999a), they further examined the role of the prelimbic-infralimbic areas in reversal learning and showed that prelimbic-infralimbic inactivation did not disrupt animals' capacities to perform a reversal learning task employing either a place or a response discrimination. These findings are consistent with a role for the mPFC in cross-modal shifts (reversal of learning set) but not in intra-modal shifts (simple reversal learning).

1.2.3 Conclusion

In humans, there is ample evidence highlighting the role of the prefrontal cortex in higher cognitive processes (specifically, for the purposes of this thesis, working memory and cognitive flexibility). Moreover, a substantial amount of evidence supports the hypothesis that there is a functional dissociation within the prefrontal cortex; that is, distinct regions of the human PFC carry out independent, but complementary processes (Dias et al., 1996). The clearest functional distinction appears to be between the dorsolateral PFC and the ventral PFC (orbital PFC) which can be broadly divided into cognitive and affective roles respectively. Similarly in working memory, a dissociation between dorsolateral and ventrolateral PFC has been suggested; however debate continues about the nature of the dissociation: domain-specific *vs* process-specific.

In the case of rodents, findings are less clear regarding the contribution of the prefrontal cortex in executive functioning. On the one hand, there are a number of controversial results that lead to some uncertainty regarding the role of the rat PFC in working memory processes. On the other hand, there is little doubt that the PFC is involved in behavioural flexibility. As in humans, there is a dissociation between the medial and the orbital prefrontal cortex in behavioural flexibility. The former seems to be involved in attentional set shifting whereas the latter seems to be involved in reversal learning. It is less clear however to what extent each subregion (specifically prelimbic and infralimbic) contributes to higher order cognitive flexibility. Recent research has not provided many data to fractionate the role of the different mPFC subregions (prelimbic, infralimbic and anterior cingulate) in these aspects of cognitive function (working memory and

behavioural flexibility). That is, most studies claim to report an effect (or absence of effect) of medial prefrontal lesions but the extent and location of lesions vary across studies but to the best of my knowledge there are very few studies (Chudasama et al., 2003) that have systematically investigated the contribution of each subregion.

Since the main concern of the thesis is to study the role of the rat prefrontal cortex in executive functions, the following discussion of prefrontal cortex anatomy will be focused on the rat prefrontal cortex rather than on human or non-human primate anatomy.

1.3 Anatomy of the prefrontal cortex in rats

1.3.1 Do rats have a prefrontal cortex?

The lack of a single anatomical or functional definition of the prefrontal cortex has led to different, and in some respects controversial, views on the existence of a prefrontal cortex in non-primates, and in particular in rats (Uylings et al., 2003).

Until Rose and Woolsey's paper (1948), the general idea was that a prefrontal cortex was unique to primate - human and non-human – species (Uylings and van Eden, 1990; Dalley et al., 2004). In their paper they defined the prefrontal cortex as the cortical projection area of the mediodorsal thalamic nucleus (MD). Although this definition was only based on a single anatomical criterion (and therefore not sufficient to give a complete definition of the prefrontal cortex), it was the first time that the existence of PFC in all mammals was suggested. Since this classic paper, anatomical, physiological and behavioural findings have greatly extended our knowledge. From an anatomical point

of view, some debate is still ongoing. In an early study, Leonard (1969) showed that the MD of the rat projected to two distinct areas of the PFC in a manner analogous to projections seen in primates. More recently, it has been shown, however, that the primate MD sends projections to the dorsal, medial and orbital surfaces of the frontal lobe (Giguere and Goldman-Rakic, 1988), whereas the rat MD projects to the medial and orbital surfaces but not to the dorsolateral regions (Hohl-Abrahao and Creutzfeldt, 1991). Thus, in the rat, there appears to not be a topological equivalent of the primate dorsolateral prefrontal cortex. In rodents, findings from cognitive paradigms that are based on neuropsychological tests used in human (and also those used in non-human primates) allow a better investigation of possible functional homologies between species. There is growing evidence to suggest that rats demonstrate some of the complex behaviours that in primates are dependent upon dorsolateral prefrontal cortex. It is recognized that mPFC lesions in rats lead to deficits in behavioural flexibility, working memory and attentional tasks similar to those reported in primates (Kolb and Tees, 1990; Brown and Bowman, 2002), therefore it seems sensible to assume that medial prefrontal cortex in rats is functional, if not neuroanatomically, related to the dorsolateral PFC of marmosets.

In summary, based on anatomical and behavioural findings, it is now generally accepted that rats have a region which is equivalent to humans prefrontal cortex in enough ways to be labelled prefrontal cortex.

1.3.2 Rats prefrontal cortex anatomy

The rodent PFC is not a single functional region but, rather, a group of anatomically (in term of connections and cytoarchitecture) and functionally heterogeneous areas (Krettek and Price, 1977). Although some differences remain in precise terminology, most investigators agree on the boundaries of the prefrontal cortex area (Öngür and Price, 2000; Dalley et al., 2004). The rodent PFC is divided into three topologically distinct regions: medial, lateral and ventral regions (see figure 1.1). The medial cortical region, named the *medial prefrontal cortex*, constitutes the major portion of the medial wall of the hemisphere anterior and dorsal to the genu of the corpus callosum. The laterally located cortical region, the *lateral prefrontal cortex*, is located in the anterior part of the rhinal sulcus. And, the ventrally located cortical regions that is termed the *orbital prefrontal cortex*, lies in part dorsal to the caudal end of the olfactory bulb in the dorsal bank of the rhinal sinus (Heidbreder and Groenewegen, 2003). Within each region, several areas can be distinguished. The medial prefrontal region can be sub-divided into a dorsal area which includes precentral (PrC) and anterior cingulate (ACC) cortices and a ventral area which includes the prelimbic (PL), infralimbic (IL) and medial orbital (MO) cortices. The lateral region includes the dorsal and ventral agranular insular (AID, AIV) and lateral orbital (LO) cortices. And the ventral region encompasses the ventral orbital (VO) and ventral lateral orbital (VLO) cortices.

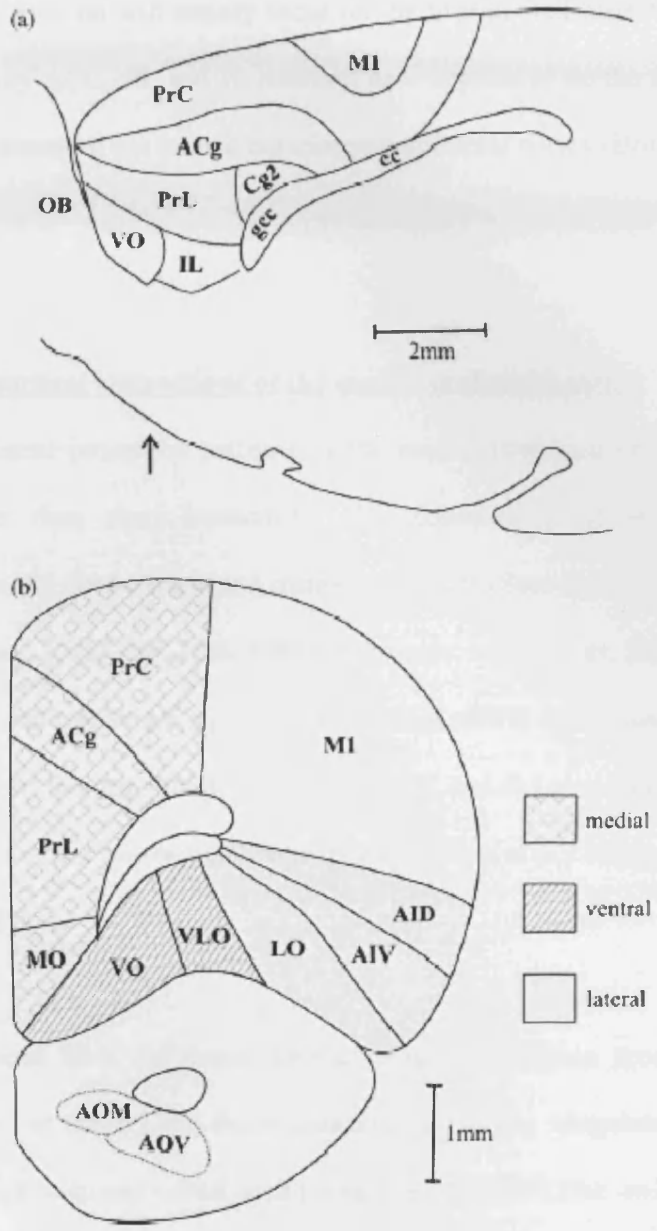

Figure 1.1: Illustrative diagram of the rat prefrontal cortex (Dalley et al., 2004)
(a) Lateral view, 0.9mm from the midline. (b) Unilateral coronal section, approximately 3.5 mm forward of bregma (as shown by the arrow). The different shadings represent the tree main subdivisions of the prefrontal cortex (medial, ventral and dorsal). ACg or ACC: anterior cingulate cortex; AID: dorsal agranular insular cortex; AIV: ventral agranular insular cortex; AOM: medial anterior olfactory nucleus; AOV: ventral anterior olfactory nucleus; cc: corpus callosum; Cg2: cingulate cortex area 2; gcc: genu of corpus callosum; IL: infralimbic cortex; LO: lateral orbital cortex; M1: primary motor area; MO: medial orbital cortex; OB: olfactory bulb ; PL: prelimbic cortex; PrC: precentral cortex; VLO: ventrolateral orbital cortex; VO: ventral orbital cortex.

The following discussion will mainly focus on the medial wall aspect of the prefrontal region (specifically ACC, PL and IL cortices) as it appears to be the rat brain area that can reflect functioning of the human dorsolateral prefrontal cortex (Brown and Bowman, 2002).

1.3.3 Cortico-cortical connections of the medial prefrontal cortex

Efferent and afferent projection patterns of the medial prefrontal cortex show gradual transitions rather than sharp boundaries. The connectivity of the mPFC changes progressively over its dorsoventral and rostrocaudal axes (Sesack et al., 1989; Heidbreder and Groenewegen, 2003). For each medial prefrontal cortex area, the main sources of cortico-cortical connections are inputs from the other mPFC areas rostrally and caudally adjacent. Moreover, the three subdivisions (PL, ACC and IL) also receive afferents from the matching area in the contralateral hemisphere (Hurley et al., 1991; Reep et al., 1984; Sesack et al., 1989).

Efferent projections from the dorsal anterior cingulate division produce a pattern of anterograde labelling that reflects dense connections with the cingulate and retrosplenial areas, and in venterolateral orbital area (Sesack et al., 1989).The main targets of these projections are certain neocortical fields including the motor, somatosensory and visual cortices (Sesack et al., 1989). Projections from the prelimbic cortex reach the infralimbic, anterior cingulate, premotor, agranular insular, perirhinal and entorhinal areas. A clear difference appears between the dorsal and ventral prelimbic areas; only the ventral part projects to the piriform cortex whereas the dorsal part possesses more specific projections

to the sensorimotor areas in the frontal and parietal areas (Heidbreder and Groenewegen, 2003). From the infralimbic cortex emerge three efferent pathways (Hurley et al., 1991): a dorsal pathway projects to the prelimbic and anterior cingulate cortices; a lateral pathway innervates the insular and perirhinal cortices; and axons from a ventral pathway terminate in the piriform cortex.

It is now recognized that the distribution of cortical afferents of the medial prefrontal cortex differs not only along the dorsoventral coordinates but also in a rostrocaudal direction, ignoring to a certain degree the boundaries between cytoarchitectonically distinct cortical fields (Uylings and van Eden, 1990; Condé et al., 1995; Heidbreder and Groenewegen, 2003). A ventro-medial part (including PL and IL) receives cortical input mainly from the perirhinal and the ventral agranular insular areas. A more dorsal part (encompassing dorsal PL, ACC and PrC cortices) appear to be mostly innervated by secondary visual, posterior agranular and retrospenial cortices whereas a rostral part (including rostral ACC and PrC) receive projections from the fronto-parietal motor and somatosensory areas.

1.3.4 Sub-cortical connections of the medial prefrontal cortex

Study of the PFC thalamic projections have received considerable attention, as the PFC has been defined as the MD-projection cortex (Rose and Woolsey, 1948; Öngür and Price, 2000). Vertes (2002) gave a remarkably detailed examination of projections from the mPFC to the thalamus. The infralimbic, prelimbic, anterior cingulate cortices distribute to midline/medial structures of the thalamus, including the paratenial,

paraventricular, interanteromedial, anteromedial, intermediodorsal, mediodorsal, reuniens. Moreover, all three divisions of the mPFC project densely to the nucleus reuniens (RE) of the thalamus. Retrograde labelling studies have found that several thalamic nuclei (including the lateral part of the mediodorsal nucleus, the nucleus reuniens, centrolateral and centromedial nuclei) have connections with multiple areas of the medial prefrontal cortex (Vertes, 2002).

The mPFC also has extensive brain stem projections, including those to the superior colliculus, periaqueductal grey, parabrachial nuclei, nucleus of the solitary tract, motor nucleus of the vagus, nucleus ambiguous and various other brain stem (Hurley et al., 1991). However, there appears to be a dorsoventral distinction of these connections: with stronger efferent projections originating from the ventral PL and IL areas compared with the ACC area (Heidbreder and Groenewegen, 2003). In return, the mPFC receives inputs from the raphe nuclei and the locus coerulus, as well as projections from the ventral tegmental area.

"Core" limbic structures such as the hippocampus (HPC) and amygdala are predominantly connected with the ventral located medial prefrontal areas. Connections with the hippocampal formation are virtually unidirectional: the projections from the mPFC to the HPC are indirect rather than direct (via entorhinal areas and subcortical diencephalic structures) whereas the inputs from the HPC are direct (Heidbreder and Groenewegen, 2003). By contrast, the connections between the PFC and the amygdaloid complex are reciprocal and more extensive. Each sub-regions of the mPFC possesses a

distinctive innervation with the amygdaloid complex. The IL area projects to the majority of the amygdaloid complex (Hurley et al., 1991; McDonald et al., 1996; Vertes, 2004) whereas the PL area innervates more restricted regions such as the magnocellular basal, basolateral, and lateral nuclei (Sesack et al., 1989; McDonald et al., 1996; Vertes, 2004). As for the ACC area, it only reaches the magnocellular basal nucleus (Sesack et al., 1989). Amygdaloid projections to the medial prefrontal cortex arise predominantly from the caudal parts of the basal amygdaloid complex and to a lesser degree from the lateral amygdaloid nucleus and the periamygdaloid cortex (Krettek and Price, 1977; McDonald et al., 1996).

Studies of the cortico-striatal projections (Öngür and Price, 2000; Heidbreder and Groenewegen, 2003) show that mPFC, as a whole projects to the ventromedial part of the striatum; however different areas project to different regions of the striatum. Thus, the efferent projections of the ACC terminate more medially and dorsally into the core of the nucleus accumbens (Heidbreder and Groenewegen, 2003). Within the PL, dorsal and ventral parts project differentially to the basal ganglia structures: whereas the dorsal part projects preferentially to the medial region of the striatum and the core of the nucleus accumbens, the ventral part sends fibers to ventro-medial parts of the striatum, the core of the nucleus accumbens as well as the ventral and medial parts of the shell (Ding et al., 2001). The IL cortex reaches almost exclusively the medial shell of the nucleus accumbens. Based on this description it is clear that the mPFC targets the basal ganglia nuclei in a topographical manner: axons from the ventral part (IL region) reach the shell of the nucleus accumbens whereas axons from more dorsal prefrontal regions (PL and

ACC regions) terminate in the core of the nucleus accumbens. Reciprocal connections from striatal regions to the prefrontal cortex have rarely been reported, and few appear specific to any particular sub-regions. However, the ventral pallidum appears to project to all divisions of the medial prefrontal cortex (Condé et al., 1995) and the PL area has also been reported to receive afferents from the ventral tegmental area. Thus it may be that striatal regions exert an influence on the rat prefrontal cortex via the reciprocal connections (striato-pallido-thalamus circuits) with thalamic structures connected to prefrontal sub-regions (Condé et al., 1995; Heidbreder and Groenewegen, 2003).

From the summary of the cortico-cortical and sub-cortical connections of the medial prefrontal cortex above, it is clear that PL and IL cortices distribute very differently throughout the brain (despite some common projections, such as projections to the midline thalamus). The main projections sites of PL are: the basolateral nucleus of amygdala; the dorsal and median raphe nuclei of the brainstem; the core of the nucleus accumbens. By contrast, main projections of IL are: the medial, basomedial, central and cortical nuclei of amygdala; the parabrachial and solitary nuclei of the brainstem; the shell of the nucleus accumbens. These differential patterns of projections reflect functional differences between these two subregions. It is often assumed that IL modulates visceral/automatic activity whereas PL is involved in limbic and cognitive functions (Vertes, 2004).

1.4 Overview of the thesis

Based on the studies reviewed in this introductory chapter, it is clear that there is a wide range of paradigms available to investigate executive functions in rodents. However, some of the paradigms (and often the most popular) can be challenged in term of the cognitive processes likely to underpin them. Furthermore, although the involvement in cognitive functioning of the rat prefrontal cortex has been extensively investigated few studies have investigated systematically the contribution of the each medial prefrontal subregion in working memory or behavioural flexibility, despite evidence that infralimbic and prelimbic regions have subtly different roles. Killcross and Coutureau (2003) proposed that PL cortex is involved in the execution of goal-directed behaviours whereas the IL cortex may act to override goal-directed behaviours and permit habit-based behaviour; and the balance between the two behaviours may possibly be maintained via mutual inhibition between these two regions.

Chapter 2 introduces a novel behavioural paradigm for rats that will be utilised to investigate working memory. The primary concern of this chapter is to set up an operant task which is a delayed-comparison task that is not open to interpretation in terms of mediating strategies, but is unambiguously dependent on memorial processes. Further experiments investigate explicitly the possible use of mediating strategies as well as the impact of manipulation of several task parameters. Chapter 3 proposes new designs to assess behavioural flexibility in rats. Two designs are investigated: the first one is based on the ID/ED shift paradigm and the second one on an optional shift design (Kendler et al., 1964) that also examines attentional set. Chapter 4 aims to investigate the underlying

neurobiological basis of behavioural flexibility using one of the paradigms described in Chapter 3, and in particular investigating the role of different subregions of the rat medial prefrontal cortex (PL, ACC and IL). Finally, Chapter 5 investigates further the attentional processes involved in behavioural flexibility, using an on-baseline aversive latent inhibition paradigm. Further experiments investigate the effect of large and discrete lesions of the medial prefrontal cortex (mPFC, PL and IL). Finally, in the General Discussion (Chapter 6), the results presented in this thesis are summarized and discussed. Grounds for a functional dissociation within the medial prefrontal cortex in cognitive functioning are discussed with respect to the data presented here and previous findings. And finally, a hypothesis of the functioning of the PFC subregions is presented.

2 Working memory

2.1 Introduction

Working memory is the short-term retention of information that is no longer accessible in the environment, and the manipulation of this information for subsequent use in guiding behaviour. Classically, paradigms used to assess working memory comprise three phases: a sample phase where information is presented to the subject, a delay phase when that information is withdrawn for a period of time and a choice phase where that information, along with some relatively novel comparison information, are presented and where a subject has to identify the previously presented information. Although it appears simple to set a working memory paradigm, in a true memory task, the correct answer should not be signalled by the sample, but should require memory of the sample and then become apparent only at the time of the choice.

As discussed in the general introduction (section 1.1.2), there is a broad range of paradigms used to assess working memory in rodents. Most of the tasks are non-automated procedures (e.g. maze-based) and therefore are very labour intensive and provide relatively poor control over stimulus and response parameters (Steckler et al., 1998). Among operant procedures a distinction between delayed-response and delayed-comparison paradigms is made. The main difference between these two types of task is the nature of information held in memory during the delay; in the former type of task this information concerns the response (as the correct response is defined before the delay)

whereas in the latter procedure this information concerns the sample (as information before and after the delay have to be compared in order to determine the correct response). This distinction is of importance because it is source of discussion about the validity of tasks. Delayed-response tasks have often been challenged by suggestions that animals use motor mediating strategies (such as holding a position or particular body orientation) which facilitate correct responding (Pontecorvo et al., 1996; Chudasama and Muir, 1997; Ennaceur and Aggleton, 1998). Motor mediation refers to a class of responses strategies adopted by rats to overcome the cognitive demands imposed by the introduction of longer and unpredictable retention delays (Ennaceur and Aggleton, 1998). The opportunity of using motor mediation arises because in delayed-response tasks the response is predictable from the onset of the retention delay. Several attempts to overcome the problem of mediating strategies in DMTP/DNTMP tasks have been made (Dunnett, 1993; Herremans and Hijzen, 1997), but to date none of them have proved to be entirely successful. Delayed-comparison tasks do not have this weakness as information presented before and after the delay has to be compared. To the best of my knowledge, however, there are no fully automated, operant DMTS/DNMTS tasks using delays greater than 12s. For instance, Ennaceur et al. (1997) attempted to set up a delay-comparison working memory paradigm in operant chamber. In this task, although animals were able to perform above chance level at various delays (0 – 12s), they did not show a consistent decline across delays.

Here, I propose an alternative operant paradigm, which is not based on pairing of events rule (such as matching or non-matching rule). Instead, it relies on the use of a cue that is

not directly involved in the discrimination as the to-be-remembered stimulus (occasion setter). This cue then allows the subject to disambiguate conflicting information in a subsequent test discrimination. Since the correct response cannot be predicted before the onset of the retention delay, this task involves a delayed-comparison. This task has been adapted from the Stroop-like task in rodent developed by Haddon and Killcross (2006).

2.2 Experiment WM 1: effect of different delays

The aim of Experiment WM 1 was to demonstrate that animals could learn to use, and remember across various delays, a task-setting cue (occasion setter - O_{cc}) to disambiguate conflicting responses. Initially rats were trained on two operant conditional discriminations, one visual and one auditory. Following the presentation of one occasion setting cue, $O_{cc}1$, lever presses LP1 and LP2 led to reinforcement if they were produced, respectively, during the presentation of auditory cues A1 and A2. Similarly, following the presentation of the occasion setter $O_{cc}2$, lever presses LP1 and LP2 led to reinforcement if they were produced, respectively, during the presentation of visual cues V1 and V2. This type of trial was referred to as a Simple trial. After initial training, during which subjects were only exposed to Simple trials, animals were then trained to respond during the presentation of audiovisual compounds stimuli, termed Incongruent trials (A1V2 and A2V1 preceding by either $O_{cc}1$ or $O_{cc}2$). Incongruent stimulus compounds were composed of elements that elicited different lever press responses during initial training (for instance compound stimulus A1V2 is composed of A1 that elicited LP1 and V2 that elicited LP2). Correct responses were defined with respect to the occasion setter ($O_{cc}1$ or $O_{cc}2$) that was presented prior to the compound stimuli (for instance LP1 responses

would lead to reward if A1V2 was presented after $O_{cc}1$ but if the same audiovisual compound was presented after $O_{cc}2$, LP2 would be reinforced). Thus, the occasion setters were cues that rats had to remember and use to disambiguate conflicting response information provided by audiovisual compounds. In the test session, the ability of rats to remember the occasion setters across various delays was assessed. Delays (0 – 180s) were introduced between the end of the occasion setter presentation and the presentation of the audiovisual stimulus compound (Incongruent trial).

2.2.1 Method

Subjects

The subjects were twenty four naïve male Lister Hooded rats (supplied by Harlan OLAC, UK) with a mean *ad libitum* weight of 263g (range 255-270g). Prior to the start of the experiment they were reduced to 85% of their *ad libitum* weights and were maintained at this level throughout the experiment by being fed a limited quantity of food following each day's training. The rats had free access to water in their home cages. They were housed in pairs in a light-proof holding room maintained on a 12h light/dark cycle (7 am to 7 pm). The subjects were tested on successive days, at the same time of day, during the period that the lights were on in their holding room. One animal has been excluded from all data analyses because some data from the training phase were not recorded.

All procedures complied with the UK Animals Scientific Procedures Act 1986 and were subject to Home office approval (Project Licence PPL 30/2158).

Apparatus and Stimuli

Eight identical operant chambers were used (30cm wide x 24cm deep x 21cm high; supplied by Med Associates Inc., St Albans, VT) each housed in sound attenuating, ventilated enclosure. Each chamber consisted of two aluminium walls and three clear Perspex walls, one of them serving as ceiling. Each chamber had a floor constructed of 19 stainless steel rods (4.8mm in diameter, spaced 1.6cm apart). The chambers were illuminated by a houselight (2.8 W; 1.25 cm diameter) located at the top centre of the left-hand wall. A recessed 5.1 cm x 5.1 cm food magazine, into which 45-mg food pellets (traditional formula, P. J. Noyes, Lancaster, NH) could be delivered, was located in the middle of the right-hand wall, with its base 0.5 cm above the grid floor. Access to the magazine was detected by means of infra-red detectors mounted across the mouth of the recess. Two flat-panel retractable levers could be inserted to the left and right of the magazine. Two panel lights (2W, 2.5 cm diameter) were located in the right hand wall of the chamber and a magazine light (2W) was sited in the top of the magazine. Auditory stimuli consisted of a 2kHz tone, a 100Hz buzzer and a 10Hz train of clicks delivered from speakers located in the left-hand wall of the chamber. Visual stimuli were flashing panel lights, steady panel lights plus the magazine light and a dark period of time in which the houselight was not illuminated. A computer equipped with MED-PC software (Med Associates Inc.) controlled the operant chambers and recorded the data.

Behavioural procedure

Pretraining: magazine and lever press training

On each of the first two days of training, animals received one session of magazine training during which they learnt to retrieve food pellets from the magazine. Each session lasted 30min and pellets were delivered on average every 120s. Following this there were at least three sessions of lever press training. During each of these sessions the rats received 24 lever presentations, 12 each of the right and the left levers. Each lever presentation lasted for 60s and the inter trial interval (ITI) was 60s long. Total session duration was 48min. On the first day of lever press training rats were rewarded on a continuous reinforcement schedule; this was altered on the second day to a RI 15s schedule (RI: random interval – in each second there was a one in fifteen chance that reward became available whereupon the next lever press response led to delivery of a reward). The houselight was on for the whole duration of each session. Rats moved on to conditional training once they had produced at least 100 lever presses on each lever during a single session.

Conditional discrimination training

Table 2.1 shows the experimental design for all animals. Rats were initially trained on two conditional discriminations, one auditory and one visual. One of the two occasion setters was presented for the entire duration of the ITI period, terminating with the presentation of the auditory or visual discriminative stimuli (S_Ds). One of the occasion setters, $O_{cc}1$ was presented prior to auditory S_Ds (A1 or A2), and the other, $O_{cc}2$, was presented prior to the visual S_Ds (V1 or V2). The same reinforcer (pellet) was delivered

following correct responses made during both auditory and visual stimuli. That is, $O_{cc}1$:A1→LP1:R; $O_{cc}1$:A2→LP2:R; $O_{cc}2$:V1→LP1:R; $O_{cc}2$:V2→LP2:R. Where $O_{cc}1$/ $O_{cc}2$ were buzz and dark, A1/A2 were clicker and tone, V1/V2 were flashing and steady panel lights, LP1/LP2 were left and right levers, counterbalanced across animals, and R was pellet delivery. Each day, rats received training with 48 S_D presentations, 24 visual cues and 24 auditory cues in a pseudo-random order such that each stimulus was presented once in each successive block of four trials. Both levers were presented during each trial (S_D presentation) and were retracted during the ITI (variable interval: 20-80s, mean: 60s). Discriminative stimulus presentation was 60s long and, for the purpose of data analysis, split into two periods: S_D1 and S_D2. The S_D1 period was the portion of the stimulus presentation prior to the delivery of the first reward, and was always at least 10s. The S_D2 period corresponded to the remaining portion of the 60s trial. Only responses made during the S_D1 period were recorded. These data provided a measure of discrimination performance uncontaminated by reinforcement. The houselight was on for the whole session except during periods of 'dark' O_{cc} presentation.

There were three stages in the training phase: The first two phases were as described above, the only difference was that in Phase 1, training on the two discriminations (auditory and visual) occurred on different sessions: one in the morning and one in the afternoon; in Phase 2, rats were trained only once a day and received training on the auditory and visual discriminations within the same session. In Phase 3, animals were trained on both simple and compound S_Ds. Simple stimuli comprised a single auditory or visual cue (A1, A2, V1 or V2), whereas the compounds were incongruent audiovisual

combinations of these simple stimuli (A1V2 and A2V1). These compound cues were termed incongruent because they were composed of elements that elicited different lever press responses during the initial training ($O_{cc}1$:A1→LP1; $O_{cc}1$:A2→LP2; $O_{cc}2$:V1→LP1; $O_{cc}2$:V2→LP2). To produce the correct response during an incongruent compound, animals had to recall which occasion setter had been presented immediately prior to the presentation of that compound. With reference to the example presented in the previous paragraph, if the incongruent stimulus compound A1V2 was presented following $O_{cc}1$ (which was associated with the auditory discrimination) then the correct response would be LP1, as this behaviour had previously been associated with the stimulus element A1. If the same compound was presented following $O_{cc}2$, the occasion setter associated with the visual discrimination, the correct response would be LP2 as this had previously been reinforced in the presence of stimulus element V2. Thus, the O_{cc} had to be used to disambiguate the conflicting responses associated with the individual elements of the incongruent compounds. In the Phase 1 and Phase 2, rats were rewarded according to a RI 15s reinforcement schedule; this was altered in final phase of the training (Phase 3) to a RI 7s schedule. The RI schedule was changed because the animals initially performed at chance level on Incongruent trials.

	Phase 1	Phase 2	Phase 3	Test
			Simple Trials	*Simple Trials*
			A1→LP1	A1→LP1
			A2→LP2	A2→LP2
$O_{cc}1$:	A1→LP1	A1→LP1		
	A2→LP2	A2→LP2	*Incongruent Trials*	*Incongruent Trials*
			A1V2→LP1	.....A1V2→LP1
			A2V1→LP2	.....A2V1→LP2
	AM session			
			Simple Trials	*Simple Trials*
			V1→LP1	V1→LP1
			V2→LP2	V2→LP2
$O_{cc}2$:	V1→LP1	V1→LP1		
	V2→LP2	V2→LP2	*Incongruent Trials*	*Incongruent Trials*
			A1V2→LP2	.....A1V2→LP2
			A2V1→LP1	.....A2V1→LP1
	PM session			

Table 2.1: Experimental design for all animals

$O_{cc}1$/ $O_{cc}2$ were buzz and dark, A1/A2 were clicker or tone, V1/V2 were flashing or steady panel lights, LP1/LP2 were left and right levers, ... was the delay (either 0, 10, 30, 60 or 180s). Design counterbalanced across animals.

Test session

In the test session, the design was the same as in the last phase of training (Phase 3) with the exception that, for half of the Incongruent trials, a delay (10, 30, 60 and 180s) was introduced between the end of the presentation of the occasion setter and the beginning of the S_D presentation. There were 48 trials per session: 16 Simple trials and 32 Incongruent trials (16 with delay and 16 without delay) presented in a pseudo-random order. Occasion setters were on during the ITI interval. Both levers were present during each trial (during S_D presentations) and were retracted during the ITI. Discriminative stimulus presentations lasted 60s; reinforcement was unavailable during the first 10s (S_D1), and was available during the final 50s (S_D2). The houselight was on during the whole session.

2.2.2 Behavioural results

Throughout this thesis, responses made on the reinforced lever shall be referred to as correct responses, and those made on the non-reinforced lever shall be referred to as incorrect. Responses (correct and incorrect) during the S_D1 period were assessed.

All statistical tests are evaluated with respect to an alpha level of 0.05.

Pretraining: magazine and lever press training

All rats learnt successfully to retrieve pellets from the magazine and to produce lever press responses for reward.

Training on two conditional discriminations : Phase 1 and Phase 2

The mean rates of responding on correct and incorrect levers during the initial training (Phase 1 – left panel and Phase 2 – right panel) for both auditory and visual discriminations are shown in Figure 2.1. In Phase 1, rats acquired both discriminations, producing more correct than incorrect responses for both the auditory and the visual discriminations. Moreover, training on both auditory and visual discriminations within the same session (Phase 2, right panel) did not affect animals' performance: they continued to respond more on the correct than on the incorrect lever.

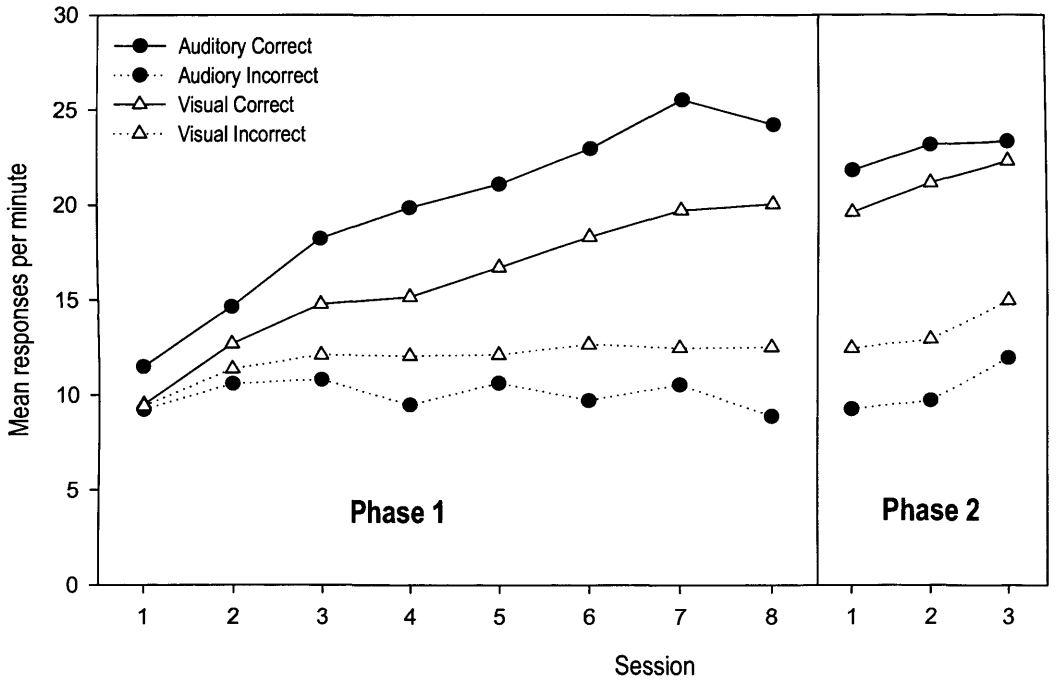

Figure 2.1: The mean rates of correct and incorrect lever-press responding per minute during the first 10s of the S_D period. Response rates are shown for Phase 1 and Phase 2 of the training for both auditory dimension and visual dimension groups.

A within-subject ANOVA with the factors of discrimination (auditory, visual), day (1-8) and lever (correct, incorrect) revealed main effects of day ($F(7, 154) = 34.74$, $p < 0.0001$, MSE = 13.25) and lever ($F(1, 22) = 107.78$, $p < 0.0001$, MSE = 74.90) but no effect of discrimination ($F < 1$). Significant two-way and three-way interactions of discrimination x lever ($F(1, 22) = 21.71$, $p < 0.0001$, MSE = 53.64), day x lever ($F(7, 154) = 43.82$, $p < 0.0001$, MSE = 6.57) and discrimination x day x lever ($F(7, 154) = 3.01$, $p < 0.01$, MSE = 6.86) were also observed. Analysis of the simple effects for each discrimination revealed a significant effect of lever on both auditory ($F(1, 44) = 119.58$, $p < 0.0001$, MSE = 64.27) and visual discriminations ($F(1, 44) = 24.16$, $p < 0.0001$, MSE = 64.27). Thus, despite the significant interaction of discrimination x lever, animals performed well on both auditory and visual discrimination tasks.

The presentation of the different discriminations within the same session (Phase 2) did not affect animals' performance, as all rats produced more responses on correct than incorrect lever on both discriminations. This observation was confirmed by the results of an ANOVA with the within-subject factors of session type (Phase 1, Phase 2), discrimination (auditory, visual) and lever (correct, incorrect). This analysis did not reveal a significant effect of the session type ($F(1, 22) = 1.73$, $p > 0.1$, MSE = 6.31) or significant interactions of session type x lever ($F(1, 22) = 2.03$, $p > 0.1$, MSE = 10.19) or session type x discrimination x lever ($F(1, 22) = 1.34$, $p > 0.1$, MSE = 7.02) but indicated a significant effect of lever ($F(1, 22) = 218.25$, $p < 0.0001$, MSE = 23.59). Once again, analysis revealed a significant two-way interaction of discrimination x lever ($F(1, 22) = 27.54$, $p < 0.0001$, MSE = 16.47); no other effects and interactions were significant (max

$F(1, 22) = 2.04$, MSE = 10.19). Simple effect analysis for each discrimination revealed a significant effect of lever on both auditory ($F(1, 44) = 216.09$, $p < 0.0001$, MSE = 20.03) and visual discriminations ($F(1, 44) = 63.52$, $p < 0.0001$, MSE = 20.03). This analysis also revealed a significant effect of discrimination on both correct ($F(1, 44) = 11.97$, $p < 0.001$, MSE = 15.56) and incorrect ($F(1, 44) = 15.56$, $p < 0.0001$, MSE = 16.56) levers.

Training on two conditional discriminations: Phase 3

In this phase animals received 15 sessions of training. The RI schedule was changed because the animals initially performed at chance level on Incongruent trials. Hence, during the last 5 sessions a RI 7s schedule was used. Only the data from these five sessions were analysed. The mean rates of responding on correct and incorrect levers for both auditory and visual discriminations during Simple trials (top panel) and Incongruent trials (bottom panel) are presented in Figure 2.2.

A within-subject ANOVA with session (1-5), discrimination (auditory, visual), trial type (Simple, Incongruent) and lever (correct, incorrect) as factors, showed a significant effect of session ($F(4, 88) = 5.09$, $p < 0.001$, MSE = 139.74) and lever ($F(1, 22) = 72.09$, $p < 0.0001$, MSE = 163.33), no other effects or interactions were significant (max $F(1, 22) = 1.58$, MSE = 364.54). This analysis indicated that animals had acquired the conditional stimulus-response parings and were able to use the O_{cc}s to perform the correct response on Incongruent trials.

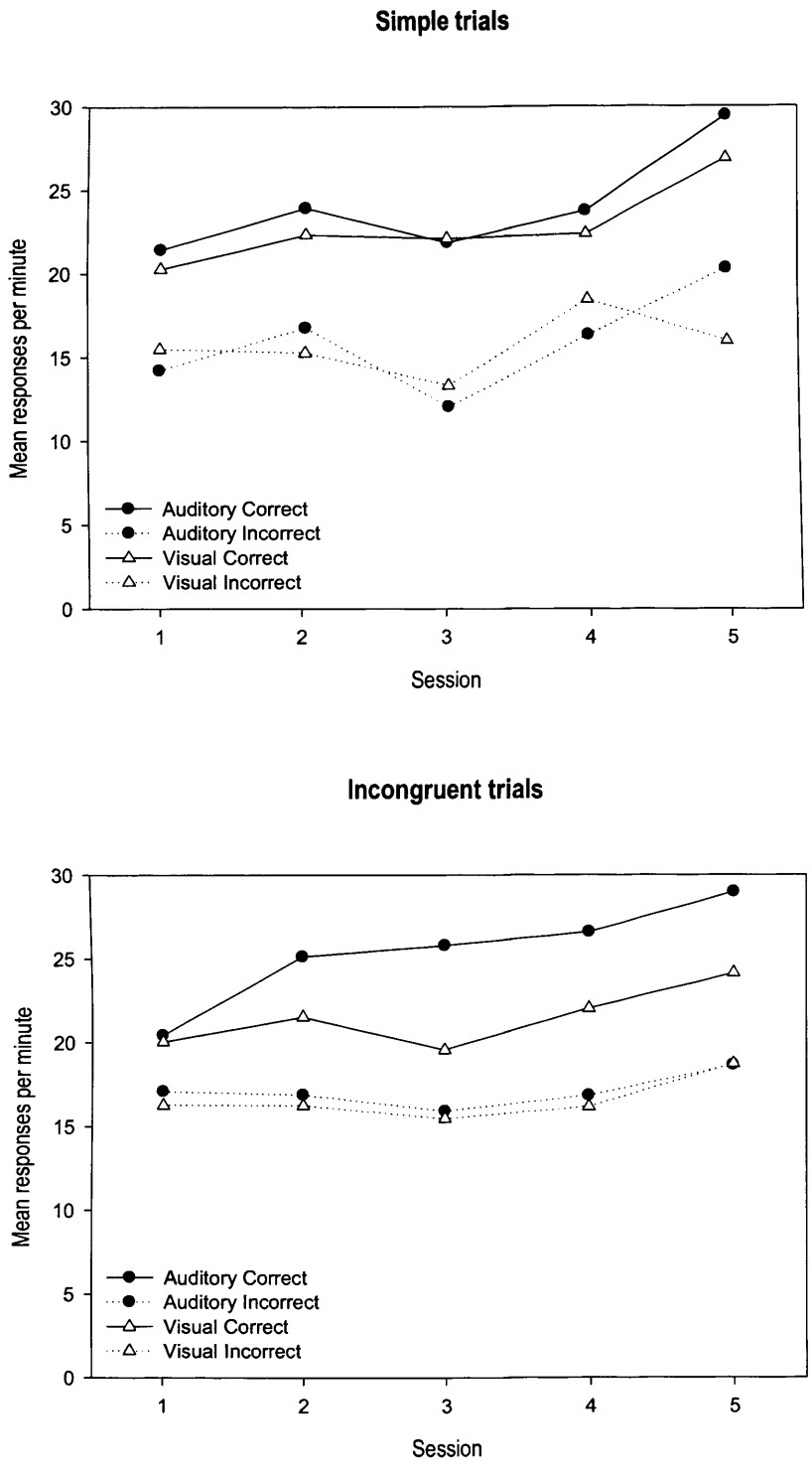

Figure 2.2: The group mean rates of correct and incorrect lever-press responding per minute during Phase 3 of the training. Response rates are shown for the Simple (top panel) and Incongruent trials (bottom panel).

Test session

Since all stimuli and occasion setters were fully counterbalanced and performance was equivalent for both discriminations during Phase 3 training, data for the following analyses will be collapsed across discriminations.

The mean rates of correct and incorrect lever-press responding for Incongruent trials for the five different delays (0, 10, 30 60 and 180s) are shown in Figure 2.3.

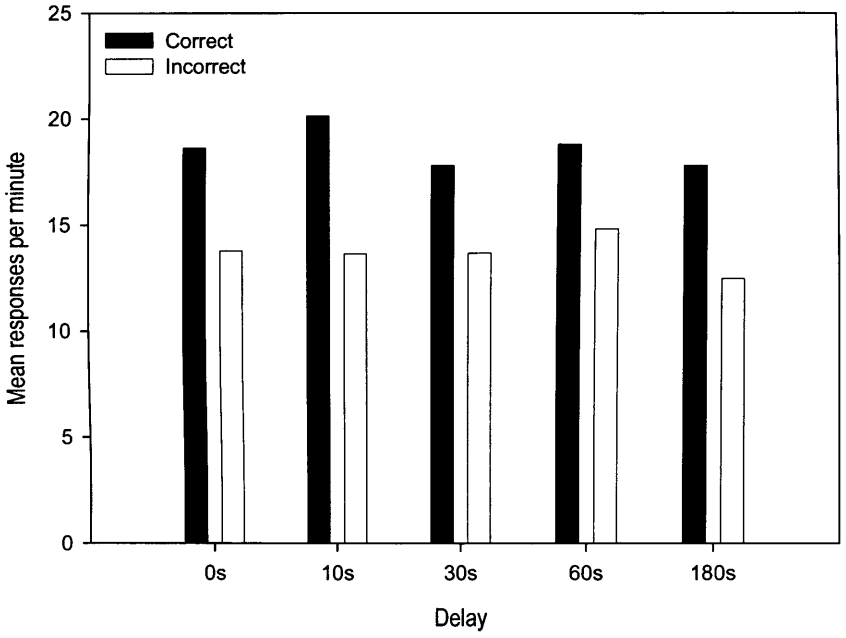

Figure 2.3 The mean rates of correct and incorrect lever-press responding during S_D1 for Incongruent trials for different delays (0, 10, 30 60 and 180s). Regardless of the length of the delay, animals showed good performance.

Rats demonstrated good discrimination performance to the incongruent stimulus compounds with no delay, responding more on the correct than the incorrect lever. This is consistent with the animals having successfully acquired the conditional discriminations

during the initial training. Moreover, animals displayed the same pattern of responding (making more correct than incorrect responses) during Incongruent trials with a delay, regardless of the duration of the delay (delay ranging from 10 to 180s). Hence, the introduction of a delay between the end of the presentation of the O_{cc} and the beginning of the presentation of the incongruent compounds did not affect animals' performance. Animals were able to remember the identity of the O_{cc} across the delay and to use it to disambiguate conflicting responses (Incongruent trials). Statistical analysis confirmed this description of the data. A within-subject ANOVA with delays (0, 10, 30, 60 and 180s) and lever (correct, incorrect) as factors revealed a significant effect of lever ($F(1, 22) = 51.95$, $p < 0.0001$, $MSE = 26.93$) but no other effect or interaction was significant (all $Fs < 1$).

Here is a brief summary of the results from the experiment WM 1. Rats were trained on two conditional discrimination tasks, one auditory and one visual, with each discrimination associated with a different occasion setter ($O_{cc}1$ and $O_{cc}2$) serving as task-setting stimuli. Following completion of initial conditional discrimination training (Phase 1 and Phase 2), rats were presented with audiovisual compounds composed of training stimulus elements that required different responses during the initial training (termed Incongruent trial). Accurate responding in the presence of incongruent stimulus compounds suggests that the occasion setting cues came to control behaviour, such that rats responded according to the stimulus element previously trained in combination with the specific occasion setting cues. Introduction of a delay between the presentation of the occasion setters and the presentation of the incongruent stimulus compounds did not

affect rats' performance. Indeed, at test, animals displayed the same pattern of responding on Incongruent trials with delay as they did on Incongruent trials without delay, making more correct than incorrect response. The absence of a delay dependent deficit is not consistent with most previous findings. Indeed, with classic paradigms such as DMTP/DNMTP, a delay dependent deficit is observed for rather short delays (frequently inferior to 1 min). Delay dependant deficits observed in delayed-response tasks have often been interpreted as reflecting a failure in the maintenance of an effective mediating strategy rather than a mnemonic failure. As such mediating behaviour can act as confounding variable and affects the validity of traditional delayed-response tasks. Contrary to delayed-response tasks, the design used here is a delayed-comparison paradigm. Prior to the start of the delay, animals do not have knowledge about the correct response that they will have to produce, and for that reason they can not use a mediating strategy (such as holding a position towards the correct lever) that would help them to respond accurately without using mnemonic processes. To give an explicit example, if animals are presented with $O_{cc}1$ the subsequent stimulus compound could be A1, A2, A1V2 or A2V1, with A1 and A1V2 requiring LP1 response and A2 and A2V1 requiring LP2 response. Animals can not predict the nature of the S_D stimulus that will be presented and therefore they cannot predict the nature of the correct response. Thus they cannot use a mediating strategy based on the expected response.

Examination of the experimental design might, however, lead one to argue that animals could use a non-mnemonic strategy that would involve the task setting cue (O_{cc}) rather than the response. This strategy could facilitate correct responding without requiring the

animal to maintain on line information about the task setting cue presented prior to the delay. A possible strategy to adopt would be a "stay/shift" tactic. Firstly, and depending on the O_{cc} presented, rats could stand in one corner of the box. Then, according to the audiovisual compounds presented they would either "stay" on the same side of the box and hit for the lever in front of them or "shift" to the other side of the box and press on the other lever. An example of this strategy is presented in Table 2.

a)

	Incongruent Trial	
	A1V2	**A2V1**
$O_{cc}1$: stand left	***"Stay"***→ LP1	***"Shift"***→ LP2
$O_{cc}2$: stand right	***"Stay"***→ LP2	***"Shift"***→ LP1

b)

	Incongruent Trial	
	A1V2	**A2V1**
$O_{cc}1$	LP1	LP2
$O_{cc}2$	LP2	LP1

Table 2.2: a) Pattern of responding on Incongruent Trials if a "Stay/Shift" strategy was used
b) Pattern of responding expected on Incongruent Trials according to the experimental design.
$O_{cc}1$/ $O_{cc}2$ were buzz and dark, A1/A2 were clicker or tone, V1/V2 were flashing or steady panel lights, LP1/LP2 were left and right levers

If $O_{cc}1$ is presented, animals could stand on the left hand side of the box. Then if A1V2 is presented, rats could "stay" in the side of the box where they have been standing and press the lever located in front of them (e.g. LP1 or the left lever); but if A2V1 is presented, they could "shift" to the other side of the box and respond on the alternative

lever (e.g. LP2 or the right lever). A similar strategy could be employed when $O_{cc}2$ is presented but the starting position would be to stand in the right hand side of the box. In all these circumstances, the animals would produce the response expected (as shown in Table 2.2 b). If animals were to use such a strategy they would be likely apply it to all trial types including the Simple trials (after all, during the O_{cc} presentations, animals do not know whether the following S_D presentation will be a simple or incongruent compound cue). Hence, if A1 was presented following the presentation of $O_{cc}1$ (which would lead animals to stand on the left hand side of the box) animals would "stay" and press the lever located in front of them (e.g. LP1 or the left lever) whereas if A2 was presented, they would "shift" to the other side of the box and press the alternative lever (e.g. LP2 or the right lever). Similarly, when $O_{cc}2$ is presented, they would stand in the right hand side of the box and "stay" in this side to respond on LP2 (e.g. the right lever) if V2 was the subsequent stimulus but they would "shift" to the other side of the box and press the alternative lever (e.g. LP1 or the left lever) if V1 was presented.

The "stay/shift" strategy would be a way for the animals to reduce the memory load while performing the task successfully. As described previously, this strategy is based on adopting a specific body position dependent on the occasion setter presented and adjusting this position (either "stay" or "shift") when the discriminative cues are presented. Therefore, if the presentation of a given occasion setter is followed by the presentation of discriminative cues that are normally associated with the alternative occasion setter (e.g. $O_{cc}1$ followed by visual cues that are normally associated with $O_{cc}2$) and the animals were using this "stay/shift" strategy then their responding would be

incorrect. For instance (see table 2.3a, top panel), if $O_{cc}1$ was presented, animals would stand on the left hand side of the box and when the visual discriminative cue V1 was presented the animals would "shift" to the other side of the box and press the lever in front of them (e.g. LP2 or the right lever).

a)

	Simple Trial	
	V1	**V2**
$O_{cc}1$: stand left	***"Shift"***→ LP2	***"Stay"***→ LP1

b)

	Simple Trial	
	V1	**V2**
$O_{cc}2$	LP1	LP2

	Simple Trial	
	A1	**A2**
$O_{cc}2$: stand left	***"Stay"***→ LP2	***"Shift"***→ LP1

	Simple Trial	
	A1	**A2**
$O_{cc}1$	LP1	LP2

Table 2.3: a) Pattern of responding on Simple Probe trials if a "Stay/Shift" strategy is used
b) Pattern of responding on Simple Probe expected according to the experimental design.
$O_{cc}1$/$O_{cc}2$ were buzz and dark, A1/A2 were clicker or tone, V1/V2 were flashing or steady panel lights, LP1/LP2 were left and right levers

If the discriminative cue V2 was presented following Occ1, animals would stay on the same side of the box and respond on the lever in front of them (e.g. LP1 or the left lever). In these two trial types responding would be inaccurate (see table 2.3b, top panel), since

the expected responses are LP1 and LP2 when V1 and V2 were presented with the correct occasion setter ($O_{cc}2$), respectively. A similar pattern of incorrect responding would be observed if $O_{cc}2$ was presented prior the auditory discriminative cues (see table 2.3a and b, bottom panels)

2.3 Experiment WM 2: "stay/shift" strategy: probe test

The aim of experiment WM 2 was to demonstrate that animals were making correct use of the task setting cues (occasion setter) rather than using a "stay/shift" strategy. Therefore, in this experiment, animals were presented with Simple Probe trials. These trials consisted of the presentation of a simple discriminative stimulus following the presentation of the incorrect occasion setter (in other words, the occasion setter presented was the one that was associated with the other simple discriminative stimulus type). For instance, if $O_{cc}1$ was associated with auditory cues, during Simple Probe trials these auditory cues were presented following the presentation of $O_{cc}2$. Similarly with the visual cues that were normally associated with $O_{cc}2$, during Simple Probe trials they were presented after the presentation of $O_{cc}1$. If rats were using the "stay/shift" strategy previously described, responding to these Simple Probe trial type would be inaccurate, however if they did not use this strategy their responding should be similar to that observed in normal Simple trials.

2.3.1 Method

Subjects, apparatus and stimuli

The subjects, apparatus, and stimuli, were the same as those describe for experiment WM 1.

Behavioural procedure

Pre-test baseline performance

Experiment WM 2 directly followed the end of experiment WM 1. Performance on Incongruent trials (without delay) during the final phase of experiment WM 1 was used as an index of baseline performance against which behaviour during the test session was assessed.

Test session

Rats received a single test session. This test session was identical to that in experiment WM 1, with the exceptions that four Simple Probe trials (one for each simple cue: A1, A2, V1 and V2) were presented in addition to Simple and Incongruent (with and without delay) trials, and that the ITI duration, and therefore the duration of O_{cc} presentation, was fixed at 60s rather than being variable. The Simple Probe Trials were the trials where one simple discriminative stimulus type was presented following the presentation of the occasion setter that had been associated with the other simple discriminative stimulus type. In other words the simple discriminative stimuli V1 and V2 (that were normally associated with $O_{cc}2$) were presented following $O_{cc}1$ and simple discriminative stimuli A1 and A2 (that were normally associated with $O_{cc}1$) were presented following $O_{cc}2$.

In this section, I will only present results from Simple and Simple Probe trials, results from Incongruent trials will be presented in the next section.

2.3.2 Behavioural results

Pre-test baseline performance

Performance on Incongruent trials without delay during experiment WM 1 was used as an index of the pre-test baseline performance. An ANOVA with the within-subject factor of lever (correct and incorrect) on Incongruent trials without delay performance in experiment WM 1 revealed a main effect of lever ($F(1, 22) = 49.29$, $p < 0.0001$, MSE = 5.90).

Test session

The mean rates of responding on correct and incorrect levers during Simple and Simple Probe trials are presented in Figure 2.4. Rats demonstrated good discrimination performance on both Simple and Simple Probe trials, responding more on the correct than the incorrect lever. This shows that animals did not use the "stay/shift" strategy, as previously described. This description of the data was confirmed by the results of a within-subject ANOVA with the factors of trial type (Simple, Simple Probe) and lever (correct, incorrect). This analysis indicated a significant effect of lever ($F(1, 22) = 44.94$, $p < 0.0001$, MSE = 22.80) but no significant effect of trial type or a significant interaction trial type x lever (both $Fs < 1$).

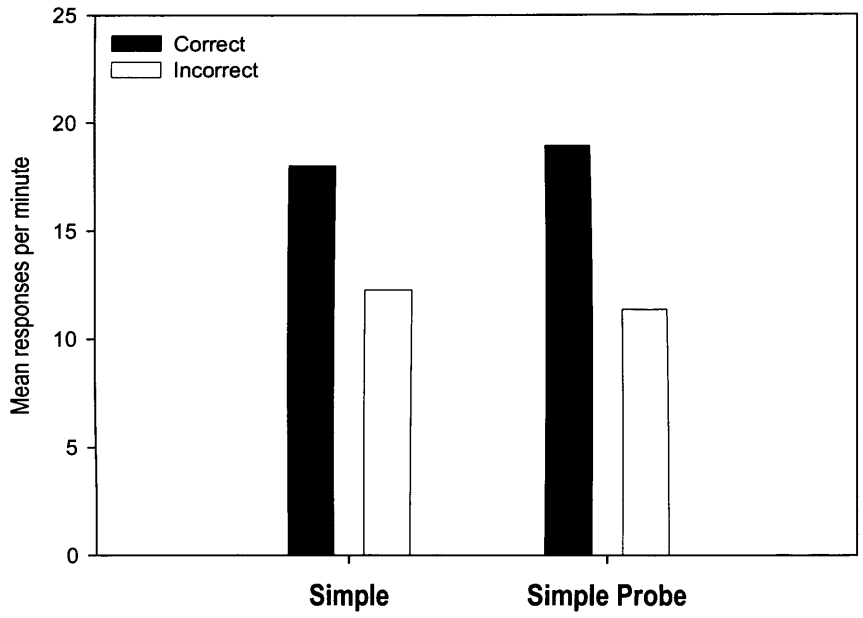

Figure 2.4: The mean rates of correct and incorrect lever-press responding during S_D1 for 2 types of trials: Simple and Simple probe trials. Good performance on Simple Probe trials indicated that animals did not use a mediating strategy such as a "stay/shift" strategy.

2.4 Experiment WM 3: effect of reducing the occasion setter duration

In experiment WM 1, a novel paradigm was introduced which can be used to investigate working memory capacity in rats. However, long delays (up to 180s) did not produce a deficit in responding to Incongruent trials. This shows that animals were able to remember, over long delays, information about the task setting cues. Experiment WM 2 was conducted to further examine if animals were using a strategy in order to facilitate their performance. The results of experiments WM 1 and WM 2 provide evidence that (i) the occasion setting cues were accurately used to disambiguate information about conflicting responses (following presentation of incongruent cues) and (ii) information

about occasion setters can be remembered across a long period of time without the use of a mediating strategy. The current experiment examined the impact of reducing the duration of the inter trial interval (ITI) that corresponded to the period where the occasion setter was presented. Reducing the length of the ITI duration was intended to increase task difficulty in two ways. First, shortening the presentation of the O_{cc} (down to 5s) might give the animals less opportunity to encode information about the to-be remembered cue. Second, it has been shown that reducing the ITI duration induced a decrease in performance (Van Hest and Steckler, 1996; Herremans and Hijzen, 1997) that may be attributed to proactive interference. Two different forms of proactive interference have been distinguished (Roitblat and Harley, 1988; Steckler et al., 1998). The first type relates to the degree of intrusions from one trial to another (e.g. due to the similarity of cues) and the second type depends on the duration of the ITI (e.g. trace decay model) and manifests as an overall improvement of performance with longer ITIs. Although, the former type of interference may result from the influence of the information acquired on a previous trial on the information of the ongoing trial and the latter type may result from altered information processing or attention to the sample, the effect of both interference types is decrease when the ITI is increased.

2.4.1 Method

Subjects & apparatus

The subjects, apparatus, and stimuli, were the same as those describe for experiment WM 1.

Behavioural procedure

Pre-test baseline performance

Animals did not receive retraining between each of the four test sessions in this experiment. However, performance on Incongruent trials without delay during the preceding test session was used as an index of the baseline performance.

Test sessions

Rats received four test sessions in total (Test 1, Test 2, Test 3 and Test 4), one per day. These test sessions were identical to that in experiment WM 1, with the exception that the duration of the occasion setting cues presentation was fixed within each session but changed between the four test sessions: for Test 1, the duration was 60s; Test 2, the duration was 25s; Test 3, the duration was 15s and Test 4, the duration was 5s. For this experiment S_D duration remained constant (60s). Changes in the duration of the delays between O_{cc} and S_D presentations were also made: 30, 60, 180 and 300s were the four delays used.

2.4.2 Behavioural results

Pre-tests baseline performance

Performance on Incongruent trials without delay during the preceding test session was used as an index of the pre-test baseline performance for each of the four tests. An ANOVA with the within-subject factor of lever (correct and incorrect) on performance during the Incongruent trials without delay preceding each test session revealed a main effect of lever for each test: Pre-test 1 baseline performance: ($F(1, 22) = 75.31$, $p <$

0.0001, MSE = 2.68); Pre-test 2 baseline performance: ($F(1, 22) = 49.40$, $p < 0.0001$, MSE = 1.63); Pre-test 3 baseline performance: ($F(1, 22) = 13.01$, $p < 0.0001$, MSE = 4.22) and Pre-test 4 baseline performance: ($F(1, 22) = 9.87$, $p < 0.0001$, MSE = 4.76).

Test sessions

The mean rates of responding on correct and incorrect levers for the four tests using various durations of occasion setter are shown in Figure 2.5. Good discrimination performance for incongruent stimulus compounds was evident for all durations of O_{cc} regardless of the length of O_{cc}-S_D delay. These results showed that reducing the length of the occasion setter presentation did not affect performance on Incongruent trials with or without delay. A within-subject ANOVA with the factors of duration of O_{cc} presentation (60, 25, 15 and 5s), delay duration (0, 30, 60, 180 and 300s) and lever (correct, incorrect) confirmed this observation. This analysis revealed a significant effect of lever only ($F(1, 22) = 188.58$, $p < 0.0001$, MSE = 8.15). No other effects or interactions were significant (max $F(4, 88) = 2.11$, MSE = 11.05).

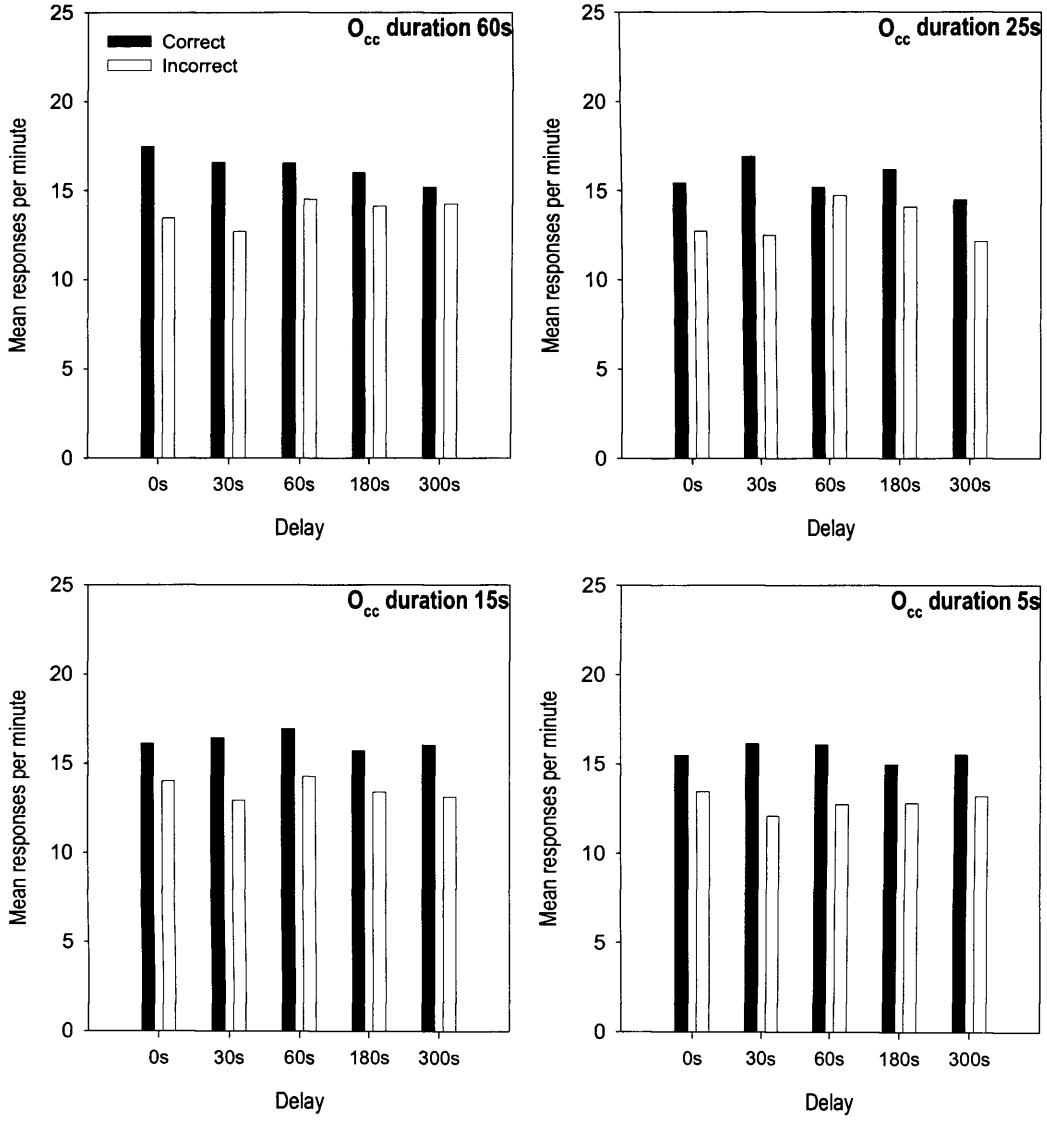

Figure 2.5: The mean rates of correct and incorrect lever-press responding for incongruent trials. Response rates are shown for each of the four tests where occasion setter duration was altered: O_{cc} = 60s – top left panel; O_{cc} = 25s – top right panel; O_{cc} = 15s – bottom left panel and O_{cc} = 5s – bottom right panel.

2.5 Experiment WM 4: effect of reducing the S_D duration

In the previous experiment (WM 3), task difficulty was increased by reducing the occasion setter presentation duration. Results presented in the previous section showed that changing this parameter did not influence performance at any delay. In this section, S_D duration – a parameter also known to be critical for accurate performance (Van Hest and Steckler, 1996; Givens and McMahon, 1997) - was manipulated (60, 15 and 5s). The S_D period was the period of time during which animals have to produce responding to a given cue (either visual, auditory or audiovisual cue). Reducing the opportunity to make responses during the choice phase should impact on accuracy, since animals have little chance to adjust their responding over a short period of time. When the shortest duration (e.g. 5s) was used, the procedure could be regarded as akin to a discrete-trial procedure.

2.5.1 Method

Subjects & apparatus

All details were as described for experiment WM 1.

Behavioural procedure

Pre-tests baseline performance

Similar to experiment WM 3, animals received no retraining sessions between the test sessions. Again, performance on Incongruent trials without delay during the preceding test session was used as an index of baseline performance.

Test sessions

Rats received three test sessions in total (Test 1, Test 2 and Test 3), one per day. These test sessions were identical to those in experiment WM 3, with two exceptions: the duration of the S_D presentation decreased across test sessions: for Test 1, the duration was 60s; for Test 2, the duration was 15s and for Test 3, the duration was 5s, and for all these tests, the occasion setting cue duration was fixed and lasted 60s.

2.5.2 Behavioural results

Pre-tests baseline performance

Performance on Incongruent trials without delay during the preceding test was used as an index of the pre-test baseline performance. Separate analyses were conducted prior to each test session. ANOVAs with the within-subject factor of lever (correct and incorrect) on these Incongruent trials without delay revealed a main effect of lever for each test: Pre-test1 baseline performance: ($F(1, 22) = 75.31$, $p < 0.0001$, $MSE = 2.68$); Pre-test2 baseline performance: ($F(1, 22) = 30.48$, $p < 0.0001$, $MSE = 8.43$) and Pre-test3 baseline performance: ($F(1, 22) = 7.27$, $p < 0.05$, $MSE = 8.13$).

Test sessions

The mean rates of responding on correct and incorrect levers for the three tests using various durations of S_D are shown in Figure 2.6. At test, animals demonstrated comparable pattern of responding, making more correct than incorrect responses, for all S_D durations, regardless of the length of O_{cc}-S_D delay. Moreover, the reduction of the

duration of S_D presentation resulted in an overall reduction of the rate of responding but not of the level of performance.

A within-subject ANOVA with the factors of duration of S_D presentation (60, 15 and 5s), delay duration (0, 30, 60, 180 and 300s) and lever (correct, incorrect) revealed a significant effect of lever ($F(1, 22) = 57.29$, $p < 0.0001$, $MSE = 8.15$) and a significant interaction of S_D duration x lever ($F(2, 44) = 4.00$, $p < 0.05$, $MSE = 14.07$). No other effects or interactions were significant (max $F(4, 88) = 1.16$, $MSE = 15.45$). Simple effect analysis of the S_D duration x lever interaction revealed a significant effect of lever for all S_D durations: Test 1, S_D duration 60s, ($F(1, 66) = 22.50$, $p < 0.0001$, $MSE = 16.68$); Test 2, S_D duration 15s, ($F(1, 66) = 48.50$, $p < 0.0001$, $MSE = 16.68$) and Test 3, S_D duration 5s, ($F(1, 66) = 11.00$, $p < 0.001$, $MSE = 16.68$). There was also, a significant effect of S_D duration on responding on correct ($F(2, 88) = 60.07$, $p < 0.0001$, $MSE = 29.94$) and incorrect ($F(2, 88) = 41.27$, $p < 0.0001$, $MSE = 29.94$) levers. Post hoc Newman-Keuls analyses of the interaction of S_D duration x lever indicated that there was a significant difference for responding on both correct and incorrect lever between S_D60/S_D5 ($q(3,88) = 6.24$ and $q(3,88) = 5.57$, respectively) and between S_D15/S_D5 ($q(3,88) = 5.73$ and $q(3,88) = 4.01$, respectively) but not between S_D60/S_D15 ($q(3,88) = 0.51$ and $q(3,88) = 1.56$, respectively). This analysis confirmed that although the reduction of the discriminative stimulus presentation resulted in an overall reduction in rate of responding (specifically when $S_D = 5s$), accuracy of responding during Incongruent trials with or without delay was not affected.

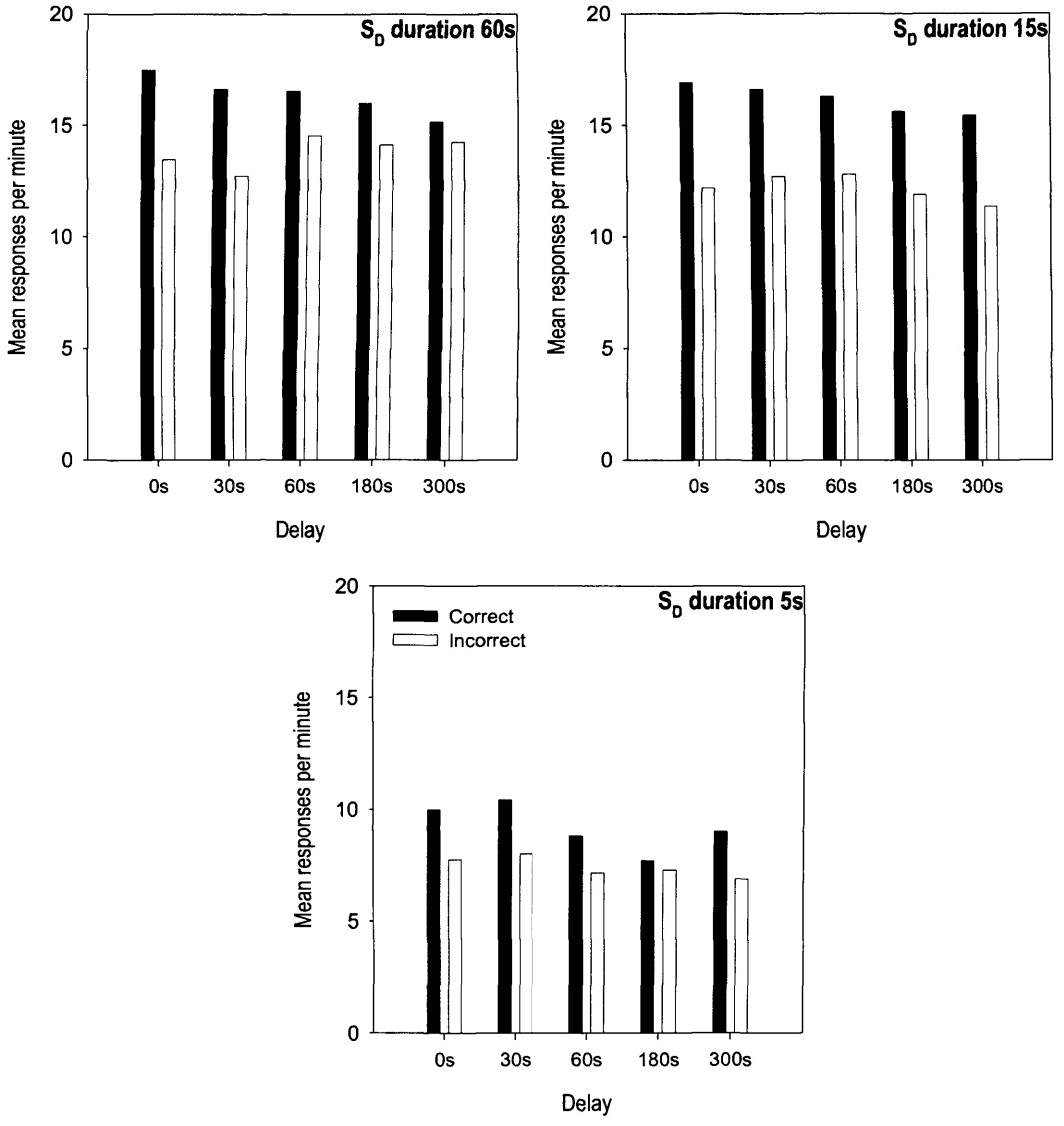

Figure 2.6: The mean rates of correct and incorrect lever-press responding for Incongruent trials. Response rates are shown for each of the three tests where S_D duration was altered: S_D = 60s – top left panel; S_D = 5s – top right panel and S_D = 5s – bottom panel.

2.6 Discussion

The four experiments presented in this chapter were designed to develop a new paradigm to assess working memory in rodents. In this within-subject design rats were trained on two conditional discrimination tasks, one auditory and one visual, with each discrimination associated with a different occasion setter ($O_{cc}1$ or $O_{cc}2$) serving as a task-setting stimulus. Following learning of the initial pairing (one stimulus dimension associated with one occasion setter), rats were presented with audiovisual compounds composed of training stimulus elements that required different responses during the initial training (termed Incongruent trials). Correct responses to Incongruent trials were then defined according to the occasion setter that was presented prior to the audiovisual stimulus compound. At test, delays were introduced between the end of the presentation of the occasion setting cue and the presentation of the audiovisual compound.

Experiment WM 1 demonstrated that rats were able to perform accurately when presented with simple stimulus or with incongruent stimulus compound. This shows that (1) rats had learnt the relationship between a particular discrimination (stimulus-response pairings) and the particular test context defined by the occasion setter, and (2) occasion setters influenced responding during presentation of conflicting compounds. At test, introduction of a delay between the end of the occasion setter presentation and the incongruent S_D presentation did not impair memory performance; instead rats showed significant more correct than incorrect responding for all delays used (0 – 180s) and did not show a delay dependent decline.

Experiment WM 2 was designed to demonstrate that animals were making correct use of the task setting cues rather than using a strategy that would facilitate their responding without having to hold on line the information about the task setting cue (reducing the cognitive task demand). A possible strategy to adopt would be a "stay/shift" tactic. This strategy would involve two steps. Firstly, animals would need to hold a position in one side of the Skinner box, depending on the occasion setter presented; secondly they would produce a "shift" (i.e. movement to the other side of the box) or "stay" (i.e. remain in the same side of the box) movement to target the correct lever, depending on the discriminative cues presented. If animals had used such a strategy they would need to employ it for all type of trials (Simple and Incongruent trials). A way to assess the possible use of a "stay/shift" strategy was to study animals responding during the presentation of a given simple discriminative stimulus that followed the presentation of the occasion setter that was normally associated with the alternative simple discriminative stimulus type; this type of trial was referred to as Simple Probe trial. Results from experiment WM 2 showed that animals displayed the same pattern of performance on Simple and Simple Probe trials, making more correct than incorrect responses. This finding is inconsistent with the use of a "stay/shift" strategy; indeed if animals were using that strategy, performance during Simple Probe trials would have been below chance.

Experiments WM 3 and WM 4 were designed to further investigate the design presented in experiment WM 1 by examining the impact of the manipulation of two central parameters of a working memory task: the intertrial interval (ITI) and the duration of

presentation of the comparison cue. Manipulation of the ITI aimed to increase the task difficulty in two ways: firstly, proactive interference was likely to occur when the ITI was decreased; secondly, a short ITI duration / short O_{cc} presentation decreased animals' opportunity to encode information about the O_{cc}. Results from experiment WM 3 showed that reducing the ITI to as little as 5s did not affect animals' performance. Indeed, good performance on Incongruent trials was observed at all delays used (0 – 300s) independently of the duration of the ITI / O_{cc} presentation (5 – 60s). This absence of proactive interference is not surprising. Proactive interference occurs usually when the recall of stimuli presented during the previous trial interferes with the recall of the sample stimulus during the choice phase of the current trial (Van Hest and Steckler, 1996). Proactive interference is therefore more likely to occur when ITI is short, and when similar stimuli are used in the sample and choice phases (as in delay (non)matching tasks and continuous working memory tasks). However, in the design presented here, the stimuli used as O_{cc} differed from the stimuli used as S_Ds and this may be why proactive interference was not observed. In experiment WM 4, the duration of the response period (S_D duration) was manipulated. Reducing the S_D duration was attempted in order to increase the task difficulty by reducing the period of time available to generate a response. Manipulating this parameter did not affect animals' performance, as they were accurately responding at all delay used (0 – 300s), regardless the length of the S_D duration. The level of training might explain the absence of effect of the manipulation of the S_D duration. Indeed, the experiment WM 4 was conducted at the end of a series of experiment, as a result animals were well trained and could easily perform a discrete trial like procedure (e.g. when S_D duration was 5s).

To summarize, this chapter presented a new design to assess working memory in rats. With this delayed-comparison design, no delay dependant deficit was observed when long delays were used. Moreover, increasing the task difficulty by manipulating the O_{cc} and S_D presentation did not affect animals' performance. This paradigm, although perfectly designed to assess working memory functioning in rats did not provide evidence for failure in the retention of specific information during a delay period in normal animals. As discussed previously, delay dependant deficits observed in delayed-response tasks have often been interpreted as reflecting a failure in the maintenance of an effective mediating strategy rather than a mnemonic failure (Chudasama and Muir, 1997; Ennaceur and Aggleton, 1998). Therefore, with a delayed-comparison paradigm (such as the one used here), there may be no reason to expect a delay dependant deficit in rats as no mediating strategy can be employed.

Although the paradigm presented here overcomes potential drawbacks of classic working memory paradigms (e.g. mediating strategy), it may nevertheless not be adequate to study the involvement of the prefrontal cortex in working memory. Using a paradigm similar to this one, Haddon and Killcross (2006) showed that medial prefrontal lesions (encompassing ACC, PL and IL cortices) disrupted responding to conflicting information provided by incongruent stimulus compounds. Therefore, it would not be possible to assess the effect of prefrontal lesions on the online retention of information using this paradigm as performance would already be at chance with 0" delay. It might, however, be utilised to assess hippocampal function. To the best of my knowledge, there is no fully automated delayed-comparison paradigms assessing working memory in rodents that

have shown delay dependant deficit and that can be used to study the involvement of the PFC in such processes.

In a recent study, Gisquet-Verrier and Delatour (2006) presented an interesting set of data providing evidence that the rat prefrontal cortex may be involved in more global processes than the capacity of short term retention of information. They showed that PL/IL lesions only had a disruptive effect when the delay was progressively increased but not when all delays were inter-mixed from the start of acquisition. Moreover, the same lesion also induced a transient deficit of performance when sets of interfering events were presented. The authors concluded that the medial prefrontal cortex is transiently brought into action when significant changes occur within the experimental situation (change in the delay retention period, interfering events) rather than in the temporary storage of information. Therefore, the rat prefrontal cortex plays a role in monitoring/processing functions related to the regulation of working memory that can be seen as taxing behavioural flexibility.

3 Behavioural flexibility

3.1 Introduction

In the general introduction (section 1.1.2), the most commonly used procedure to assess behavioural flexibility in rats was described in detail. This ID/ED shift task, developed by Birrell and Brown (2000), provides a powerful tool to study behavioural flexibility. A few interesting features of the task might be pointed out: (i) the task is formally the same as the one used in monkeys and humans, therefore it provides a useful tool to make across species comparison; and (ii) the ID/ED effect (a "facilitation" of ID shift learning compared with ED shift learning) has been proved to be a reliable effect, with several demonstrations published by different laboratories. However, some drawbacks must also to be highlighted: (i) this is a hand-run experiment which is labour intensive and does not provide a great degree of control over stimulus presentations (especially given that one of the dimensions used is olfactory); (ii) the key comparison is made between performance on two discriminations (ID and ED shifts) learned at different times (4^{th} and 6^{th} phase of the single session, respectively); and (iii) because of the absence of within-subject counterbalancing and the simple discrimination given at the start of training, stimuli belonging to the perceptual dimension used during this phase will always be more familiar than stimuli belonging to the dimension that is introduced later in the training.

In this chapter, two novel, automated procedures to assess attentional changes that result from discrimination learning in rats are described. Both of these procedures are designed

to overcome the drawbacks described above. The first procedure is based on a between-subjects design and intends to compare readiness to learn new discriminations involving either an ID or an ED shift. The second paradigm uses a within-subject design and is based on an optional shift design (Kendler et al., 1964).

3.2 Intradimensional, extradimensional shift – IDS/EDS 1

In this experiment I employed a novel automated procedure that was designed to assess the attentional changes that result from discrimination learning in rats. First, all animals were trained on an audiovisual conditional discrimination in which the presentation of components from one (relevant) dimension indicated which response would lead to reward, and accompanying components from a second (irrelevant) dimension provide no information regarding the response to produce. Subsequently, one group of animals was given a new audiovisual conditional discrimination involving an intradimensional shift (IDS) wherein novel components from the previously relevant dimension were relevant and novel components from the formerly irrelevant dimension were irrelevant. A second group was given a new audiovisual conditional discrimination involving an extradimensional shift (EDS) wherein novel components from the previously relevant dimension were then irrelevant and novel components from the formerly irrelevant dimension were relevant. On the basis of previous findings (Lawrence, 1949; Mackintosh and Little, 1970), one might expect that by the end of the initial training, animals would attend more to the relevant than to the irrelevant dimension. Furthermore, one might expect animals to acquire a discrimination task more readily if they are attending to the

task-relevant cues than if they are not. Therefore, animals should learn a new discrimination task more rapidly following an ID shift than following an ED shift.

3.2.1 Method

Subjects

The subjects were thirty two naïve male Lister Hooded rats (supplied by Harlan OLAC, UK) with a mean *ad libitum* weight of 294g (range 264-330g). Prior to the start of the experiment they were reduced to 85% of their *ad libitum* weights and were maintained at this level throughout the experiment by being fed a limited quantity of food following each day's training. The rats had free access to water in their home cages. They were housed in pairs in a light-proof holding room maintained on a 12h light/dark cycle (7 am to 7 pm). The subjects were tested on successive days, at the same time, during the period that the lights were on in their holding room. All procedures complied with the UK Animals Scientific Procedures Act 1986 and were subject to Home office approval (Project Licence PPL 30/2158).

Apparatus & stimuli

Eight identical standard operant chambers were used; they were as described in detail in experiment WM 1. The stimuli used in the following experiments were two panel lights (2 W; diameter, 2.5 cm), a houselight (2.8 W; diameter, 1.25 cm), a bright white LED (positioned in the magazine dispenser) for visual cues and a100Hz buzzer, a 10Hz train of clicks and 500Hz, 1kHz, 2kHz and 8kHz tones for the auditory cues.

Behavioural procedure

Pretraining: magazine and lever press training

On the first two days of training, animals received one session of magazine training, learning to retrieve pellets from the magazine. Each session lasted 48min and pellets were delivered on average every 120s. Following this, they were moved on to lever press training (at least two sessions). During each of these sessions the rats received 24 lever presentations (12 of each the right and the left levers), each lasting for 60s. The ITI was 60s long, and therefore session duration was 48min. On the first day of lever press training rats were rewarded on a continuous reinforcement schedule; this was altered on the second day to a RI 15s schedule. Rats moved on to the acquisition phase of training when they had produced at least 100 lever presses on each lever during a session.

Acquisition phase

The design of the experiment is shown in Table 3.1 Animals were trained for 12 consecutive days and received one session per day. Rats were trained on a conditional instrumental discrimination using the audiovisual stimulus compounds A1V1, A1V2, A2V1, and A2V2 and the responses LP1 and LP2. For each animal either the auditory or the visual component of each compound was relevant to the solution of the discrimination. For instance, for those animals for which the auditory dimension was relevant, lever-press LP1 was reinforced during presentations of compounds A1V1 and A1V2, whereas lever-press LP2 was reinforced during presentations of compounds A2V1 and A2V2. During the first 10s of each trial (periods: S_D1), reinforcement was unavailable but after this period (period: S_D2) correct responses were reinforced by the

delivery of a single food pellet according to a RI 30s schedule. Each session consisted of 24, 1-min trials – six with each of the four stimulus compounds: A1V1, A1V2, A2V1 and A2V2. Stimulus compounds were presented in a pseudo-random order such that each compound was presented once in each successive block of four trials. Both levers were retracted during the ITI, which had an average duration of 60s.

In this experiment, four combinations of stimuli were used: dark/magazine (DM) where the stimuli were darkness and the magazine light; steady/flash (SF) where the stimuli were steady panel lights and flashing panel lights; buzzer/click (BC) where stimuli were a 100Hz buzzer and a 10Hz train of clicks; and tone/high tone (THT) where stimuli were 1kHz and 2kHz tones.

Transfer phase

In the transfer phase, animals received twelve consecutive days of training, one session per day. Rats were trained on a further conditional instrumental discrimination using new audiovisual stimulus compounds A3V3, A3V4, A4V4, and A4V3 and involving either an intradimensional (IDS) shift or an extradimensional shift (EDS). For half of the animals that had the auditory dimension as relevant in the acquisition phase, the auditory dimension remained relevant (ID group) in the transfer phase and for the other half, the auditory dimension became irrelevant in favour of the visual dimension (ED group). Similarly, for half of the animals that had the visual dimension relevant in the acquisition phase, the visual dimension remained relevant in the transfer phase (ID group) and for the

Acquisition Phase	Transfer Phase		
	IDS		**EDS**
A1V1→LP1 **A2**V1→LP2 **A1**V2→LP1 **A2**V2→LP2	**A3**V3→LP1 **A4**V3→LP2 **A3**V4→LP1 **A4**V4→LP2	or	A3**V3**→LP1 A4**V3**→LP1 A3**V4**→LP2 A4**V4**→LP2
auditory relevant			
A1**V1**→LP1 A2**V1**→LP1 A1**V2**→LP2 A2**V2**→LP2	A3**V3**→LP1 A4**V3**→LP1 A3**V4**→LP2 A4**V4**→LP2	or	**A3**V3→LP1 **A4**V3→LP2 **A3**V4→LP1 **A4**V4→LP2
visual relevant			

Table 3.1: Experimental design for all animals

A1,A2, A3 and A4 were a 100Hz buzzer, a 10Hz train of clicks, 1kHz and 2kHz tones - V1, V2, V3 and V4 were periods of dark, magazine light, flashing or steady panel lights - LP1/LP2 were left and right levers. Design fully counterbalanced across animals. The stimuli that were relevant for the solution of the discrimination are presented in bold.

other half, the visual dimension became irrelevant in favour of the auditory dimension (ED group). For instance, for those animals for which the auditory dimension remained relevant, lever-press LP1 was reinforced during presentations of compounds **A3**V3 and **A3**V4, whereas lever-press LP2 was reinforced during presentations of compounds **A4**V3 and **A4**V4. And, for those animals for which the auditory dimension became irrelevant and the visual became relevant, lever-press LP1 was reinforced during presentations of compounds A3**V4** and A4**V4**, whereas lever-press LP2 was reinforced during presentations of compounds A3**V3** and A4**V3**. All other details were the same as for the acquisition phase.

3.2.2 Behavioural results

For the purpose of data analysis, each trial was divided into two fixed-length periods. The first, S_D1, was the part of the trial where reinforcement was not available and was 10s long. The second period, S_D2 was 50s long. To obtain an uncontaminated measure of performance, only data from S_D1 period were analysed for this experiment.

All statistical tests are evaluated with respect to an alpha level of 0.05.

Pretraining: magazine and lever press training

All rats learnt successfully to retrieve pellets from the magazine and to produce lever press responses to reward.

Acquisition phase

The mean rates of responding on correct and incorrect levers during the initial acquisition phase for both auditory and visual groups are presented in Figure 3.1. The pattern of

performance demonstrates that all groups acquired the initial conditional discrimination task, with all rats producing more correct than incorrect lever press responses by the end of training.

A mixed ANOVA with the between-subjects factors of discrimination (BC, DM, SF and THT) and transfer group (ID, ED) and the within-subject factors of session (1-12) and lever (correct, incorrect) revealed main effects of session ($F(11, 264) = 16.52$, $p < 0.0001$, MSE = 7.46) and lever ($F(1, 24) = 354.32$, $p < 0.0001$, MSE = 12.59) as well as significant two-way interactions of session x lever ($F(11, 264) = 30.53$, $p < 0.0001$, MSE = 4.09) and discrimination x lever ($F(3, 24) = 22.30$, $p < 0.0001$, MSE = 12.59) and a significant three-way interaction of discrimination x session x lever ($F(33, 264) = 2.06$, $p < 0.001$, MSE = 4.09). No other effects or interactions were significant (max $F(3,24) = 2.96$, MSE = 12.59). Analysis of simple effects produced by the discrimination x session x lever interaction revealed a significant effect of lever from session 3 to session 12 for BC group (min $F(1, 288) = 14.17$, MSE = 4.08) and DM group (min $F(1, 288) = 13.74$, MSE = 4.08), from session 5 to 12 for THT group (min $F(1, 288) = 5.88$, MSE = 4.08) and from session 7 to 12 for SF group (min $F(1, 288) = 6.01$, MSE = 4.08).

Animals in all groups successfully acquired the conditional discrimination producing more correct than incorrect responses by the end of training, and prior to the transfer phase both groups (ID and ED) presented the same pattern of performance. However, acquisition had been somewhat slower in animals of the groups THT and SF.

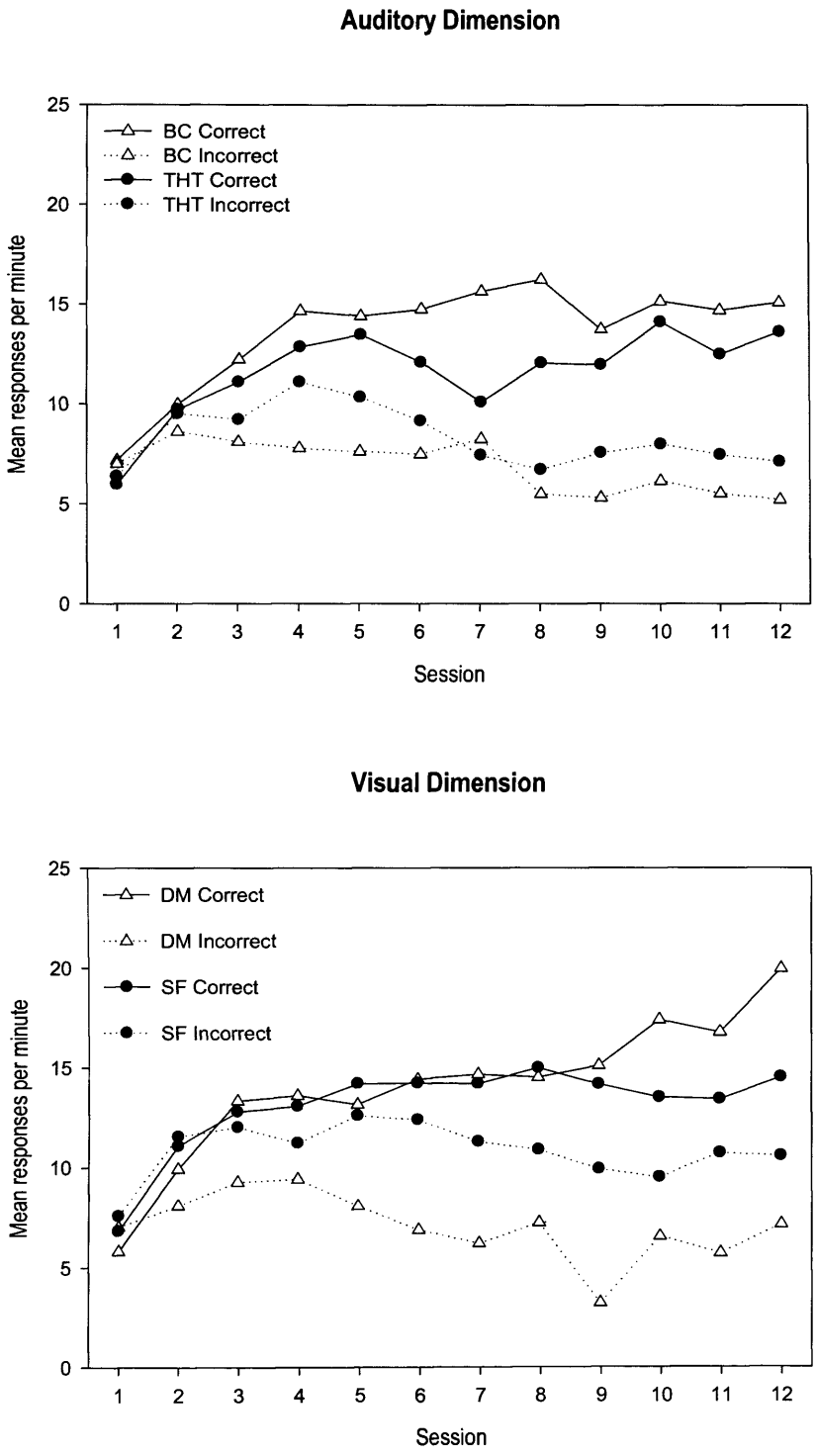

Figure 3.1: The group mean rates of correct and incorrect lever-press responding for the twelve sessions of Acquisition phase for group auditory dimension (top panel) and group visual dimension (bottom panel).

Transfer phase: IDS or EDS

The mean rates of correct and incorrect lever-press responding during the transfer phase for animals receiving an ID or ED shift are presented in Figure 3.2. At test, animals demonstrated comparable pattern of responding for both ID and ED groups regardless of the identity of the relevant dimension (auditory or visual dimension) during the acquisition phase, all animals making more correct than incorrect responses.

A mixed ANOVA with the between-subjects factors of discrimination (BC, DM, SF and THT) and transfer group (ID, ED) and the within-subject factors of session (1-12) and lever (correct, incorrect) revealed main effects of session ($F(11, 264) = 5.31$, $p < 0.0001$, MSE = 6.67) and lever ($F(1, 24) = 143.29$, $p < 0.0001$, MSE = 61.12) and a significant two-way interaction of session x lever ($F(11, 264) = 20.68$, $p < 0.0001$, MSE = 6.54); no other effects or interactions were significant (max $F(3, 24) = 2.31$, MSE = 126.01). Simple effects analysis produced by the interaction session x lever indicated a significant effect of lever from session 2 to 12 (min $F(1, 288) = 12.91$, MSE = 11.09). The retardation in acquiring the initial discrimination observed for the groups THT and SF did not seem to have any impact on the transfer phase as the statistical analyses did not reveal any significant effects or interactions involving the factor of discrimination. Moreover, the nature of the shift (intradimensional or extradimensional) did not seem to affect learning of the new acquisition during the transfer phase. Acquiring the new discrimination with either an ID or an ED shift appeared to be straightforward as from

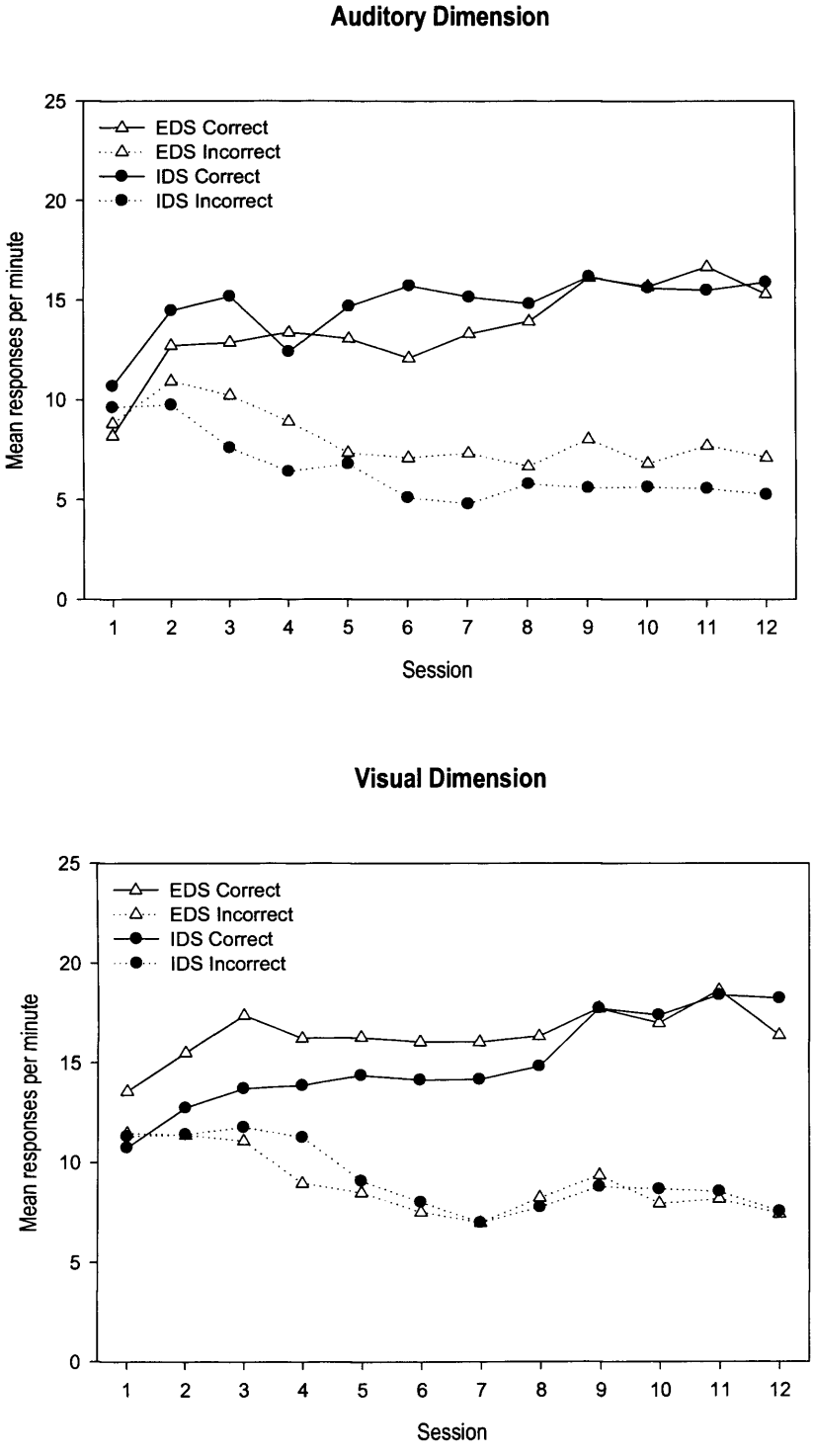

Figure 3.2: The mean rates of correct and incorrect lever-press responding during Transfer phase for animals receiving an ID or ED shift. Response rates are shown for each of the 12 sessions of training received by group auditory dimension (top panel) and for group visual dimension (bottom panel).

session 2 all animals were responding more on the correct than on the incorrect lever. It is possible that any effects of attentional processes on learning during the transfer phase may have been masked by the rapid learning observed in all animals.

3.3 Intradimensional extradimensional shift – IDS/EDS 2

Although the experiment IDS/EDS 1 was designed to investigate the ID/ED effect using a fully automated procedure, it failed to demonstrate such an effect. One reason for this absence of an ID/ED effect may be that the 12 sessions for the acquisition phase were not sufficient for animals to form a perceptual attentional set; in other words following the 12 sessions of acquisition phase animals did not pay more attention to the relevant dimension than to the irrelevant dimension. Another reason could be that attentional changes could occur more slowly than learning and, as a consequence, attentional changes could have been masked by the readiness of the learning of the new discrimination in the transfer phase. To test these two explanations, two main changes were made in experiment IDS/EDS 2. First, the length of the training during the acquisition phase was doubled; animals received 24 sessions of training rather than 12. Second, animals were trained during the acquisition phase on the two discriminations that had been shown to be slightly easier to learn (BC or DM) and during the transfer phase the two slightly harder discriminations (SF and THT) were used. This last change was made in order to slow down learning during the transfer phase and therefore to try to give more opportunity to see an effect during this stage.

3.3.1 Method

Subjects

Twenty eight, naïve, adult, male hooded Lister rats (supplied by Harlan OLAC, UK) served in this experiment. The rats were maintained at 85% of their *ad lib* weights (mean 273g; range: 254-290 g) and had free access to water. Conditions of housing and feeding were the same as for experiment IDS/EDS 1.

Apparatus & stimuli

All details were as described for experiment IDS/EDS 1.

Behavioural procedure

Pretraining: magazine and lever press training

All details were as described in experiment IDS/EDS 1.

Acquisition phase

Experimental details of the acquisition phase were identical to those in experiment IDS/EDS 1 with the exception that animals received 24 sessions of training, 2 sessions per day. There was a minimum of four hours between the end of the first session and the start of the second. Moreover, in this experiment only the discriminations DM and BC were used for the acquisition phase.

Transfer phase: ID or ED shift

Experimental details of the transfer phase were identical to those in experiment IDS/EDS 1 with the exception that only THT and SF discriminations were used.

3.3.2 Behavioural results

Responses during the first 10s of S_D presentation (S_D1) were assessed. Reinforcement was unavailable during this period, so that the behavioural measure used to assess discrimination performance was uncontaminated by presentation of reward.

All statistical tests are evaluated with respect to an alpha level of 0.05.

Pretraining: magazine and lever press training

All rats learnt successfully to retrieve pellets from the magazine and to produce lever press responses to reward.

Acquisition phase

The mean rates of correct and incorrect lever-press responding during acquisition phase for animals learning discrimination with either the visual or the auditory dimension as relevant are presented in Figure 3.3. The observed pattern of performance, greater responding on the correct than the incorrect lever, demonstrates that both groups (auditory and visual) acquired the initial conditional discrimination task.

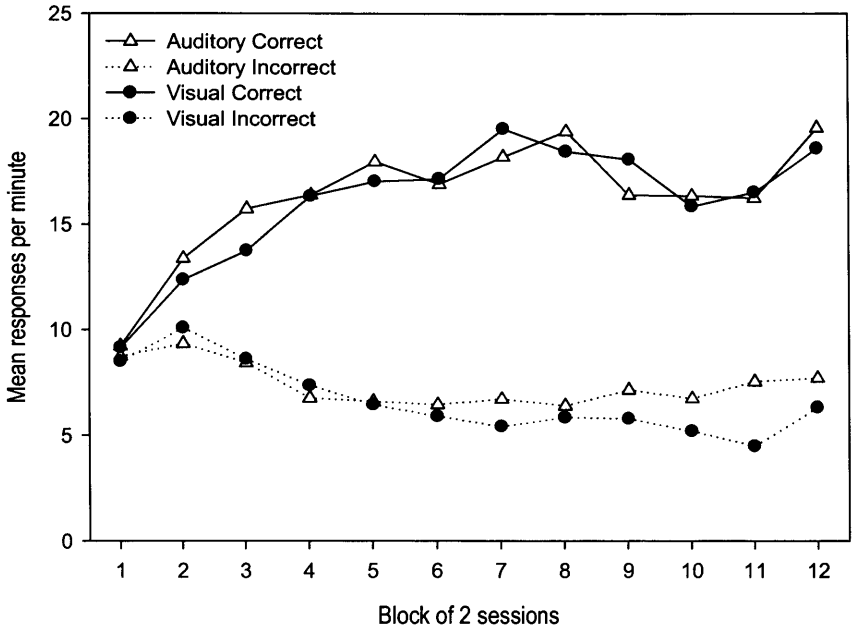

Figure 3.3: The mean rates of correct and incorrect lever-press responding for each of the 12 blocks of 2 sessions of Acquisition phase for both auditory dimension and visual dimension groups.

A mixed ANOVA with the between-subjects factors of discrimination (BC, DM) and transfer group (ID, ED) and the within-subject factors of session (1-24) and lever (correct, incorrect) revealed main effects of session ($F(23, 552) = 5.94$, $p < 0.0001$, MSE = 12.28) and lever ($F(1, 24) = 269.88$, $p < 0.0001$, MSE = 102.68) as well as a significant two-way interaction of session x lever ($F(23, 552) = 22.11$, $p < 0.0001$, MSE = 8.702) and a significant three-way interaction of discrimination x session x lever ($F(23, 552) = 1.59$, $p < 0.05$, MSE = 8.72). No other effects or interactions were significant (max $F(1, 24) = 1.96$, MSE =102.68). Analysis of simple effects produced by discrimination x session x lever indicated a significant effect of lever from session 3 to 12

for auditory group (min F(1, 576) = 13.03, MSE = 12.62) and from session 4 to 12 for visual group (min F(1, 576) = 4.75, MSE = 12.62).

Transfer phase

The mean rates of responding on the correct and incorrect levers during the S_D1 period of each session are shown in Figure 3.4 for animals in group ID and group ED. Acquisition of the new discriminations progressed rapidly, and by the end of transfer phase all animals in both groups were producing a similar pattern of responding, making more correct than incorrect responses. Although there was a difference in the absolute rate of responding between IDS and EDS groups, the ratio of correct/incorrect responses was similar for IDS and EDS animals.

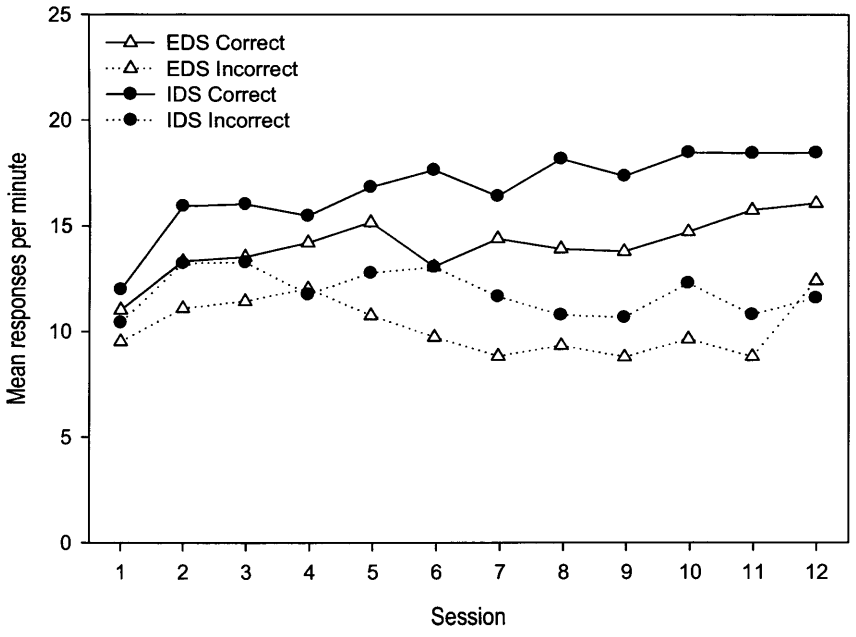

Figure 3.4: The mean rates of correct and incorrect lever-press responding during Transfer phase for animals receiving an ID or ED shift. Response rates are shown for each of the 12 sessions of training for both auditory dimension and visual dimension groups.

A mixed ANOVA with the between-subjects factors of discrimination (BC, DM) and transfer group (ID, ED) and the within-subject factors of session (1-12) and lever (correct, incorrect) revealed main effects of session ($F(11, 264) = 4.14$, $p < 0.0001$, MSE = 11.76) and lever ($F(1, 24) = 117.81$, $p < 0.0001$, MSE = 27.59) as well as a significant two-way interaction of session x lever ($F(11, 264) = 5.69$, $p < 0.0001$, MSE = 7.27). No other effects or interactions were significant (max $F(1, 24) = 1.84$, MSE = 27.56). Analysis of simple effect produced by the session x lever interaction revealed a significant effect of lever from session 2 to 12 (min $F(1, 288) = 9.72$, MSE = 8.96).

3.4 Optional shift design – OS 1

The two previous experiments, using an IDS/EDS design, failed to demonstrate the formation of an attentional set; hence, I adopted an alternate design to investigate attentional processes. The design described here is a novel, automated procedure assessing the attentional changes that result from discrimination learning based on an optional shift design (Kendler et al., 1964; Schwartz et al., 1971; Sirois and Shultz, 2006). The initial phase of training was strictly similar to that acquisition phase of the experiment IDS/EDS 2; rats were trained on a conditional instrumental discrimination using audiovisual compound (**A1**V1→LP1, **A1**V2→LP1, **A2**V1→LP2 and **A2**V2→LP2). In a second phase, the rats received training with audiovisual compounds comprising novel auditory and visual components. During this training, they experienced only two of the possible combinations of these cues. All animals learned that LP1 was reinforced in the presence of compound A3V3 and LP2 was reinforced in the presence of A4V4. Even though both the auditory and the visual components of each compound were

equally diagnostic during this phase of training, it was predicted that animals would learn more about the relationship between the reinforced (correct) responses and the cues belonging to the previously relevant dimension rather than the previously irrelevant dimension (as shown in Table 3.2).

Phase 1	Phase 2	Optional Shift Test
A1V1: LP1 A1V2: LP1	A3V3: LP1	A3V4: LP1 > LP2
A2V1: LP2 A2V2: LP2	A4V4: LP2	A4V3: LP2 > LP1

Table 3.2: Putative associative structure in experiment OS
Stimuli A1 to A4 were 500Hz, 8kHz, and 2kHz tones and a 10Hz train of clicks; V1 to V4 were a houselight, two panel lights, a magazine light and darkness. LP1 and LP2 were left and right lever. Both levers were extended during trials, but only responses on the indicated lever were reinforced by the delivery of food. Solid lines indicate strong stimulus-responses associations and dotted lines weak stimulus-response weak associations.

For example, those animals for which auditory cues were relevant during phase 1 might learn the A3→LP1 and A4→LP2 relationships better than the V3→LP1 and V4→LP2 relationships. In order to test this prediction, probe test trials with the compounds A3V4 and A4V3 were conducted in extinction. For each of these compounds, their components had been associated with difference correct responses in the training compounds. Considering the compound A3V3, LP1 had been reinforced in the presence of the auditory component, A3, whereas LP2 had been reinforced in the presence of the visual component A4. If Phase 1 training had been successful in increasing the attention paid to

the relevant (auditory) cues and/or decreasing attention to the irrelevant (visual) cues, then one would expect the rats to make more LP1 than LP2 responses in the presence of A3V4, but more LP2 responses than LP1 responses in the presence on A4V3. If the discrimination training did not influence the attention paid the relevant and irrelevant cues, then there would be no reason to expect the animals to make different numbers of each response on these test trials.

3.4.1 Method

Subjects

The subjects were thirty two naïve male Lister Hooded rats (supplied by Harlan OLAC, UK) with a mean *ad libitum* weight of 230g (range 270-318g). Prior to the start of the experiment they were reduced to 80% of their *ad libitum* weights and were maintained at this level throughout the experiment by being fed a limited quantity of food following each day's training. The rats had free access to water in their home cages. They were housed in pairs in a light-proof holding room maintained on a 12h light/dark cycle (7 am to 7 pm). The subjects were tested on successive days, at the same time, during the period that the lights were on in their holding room. Prior to the start of the experiment the rats were randomly divided into two groups of equal size: Short or Long. One animal in group Short stopped responding during Phase 1 training and data from this animal have been excluded from all data analyses. Consequently, there were 15 subjects in group Short and 16 in group Long. All procedures complied with the UK Animals Scientific Procedures Act 1986 and were subject to Home office approval (Project Licence PPL 30/2158).

Apparatus & stimuli

All details were as described for experiment IDS/EDS 1.

Behavioural procedure

Pretraining: magazine and lever press training

On the first day of training, animals received one session of magazine training, learning to retrieve pellets from the magazine. The session lasted 48min and pellets were delivered on average every 120s. Following this, there were moved directly to training in Phase 1 (below). Rats did not receive preliminary (non-discrimination) lever press training, because a large number of stimuli were required for this experiment and it was not possible to generate an extra cue that could be used during initial lever press training.

Phase 1 training

The design of the experiment is shown in Table 3.3 Animals were trained for 12 consecutive days; rats in Group Short received one training session per day whereas animals in Group Long received two sessions per day. When animals received two sessions on the same day, there was a minimum of four hours between the end of the first session and the start of the second. Rats were trained on a conditional instrumental discrimination using the audiovisual stimulus compounds A1V1, A1V2, A2V1, and A2V2 and the responses LP1 and LP2. For each animal either the auditory or the visual component of each compound was relevant to the solution of the discrimination. For instance, for those animals for which the auditory dimension was relevant, lever-press LP1 was reinforced during presentations of compounds **A1**V1 and **A1**V2, whereas lever-

press LP2 was reinforced during presentations of compounds **A2**V1 and **A2**V2. Correct responses were reinforced by the delivery of a single food pellet according to a random interval schedule. Each session consisted of 12 4-min trials – three with each of the four stimulus compounds: A1V1, A1V2, A2V1 and A2V2. Stimulus compounds were presented in a pseudo-random order such that each compound was presented once in each successive block of four trials. Between trials both levers were briefly retracted. During the first 6 sessions, responses were reinforced, where appropriate, according to an RI 15-s schedule. On all subsequent sessions, an RI 30-s schedule was used.

In this experiment, four combinations of stimuli were used: dark/magazine (DM) where the stimuli were a dark period of time in which the houselight was not illuminated and magazine light; steady/flash (SF) where the stimuli were steady panel lights and flashing panel lights; Tone/click (TC) where stimuli were a 2kHz tone and a 10Hz train of clicks; and low tone/high tone (LTHT) where stimuli were 500Hz and 8kHz tones.

Phase 2 training

On each of the next three sessions, all rats received a single session of training with the novel compounds A3V3 and A4V4. During presentations of A3V3, LP1 was reinforced and during presentation of A4V4, LP2 was reinforced according to an RI 30s schedule. Trials were ordered in such a way that no more than two trials of the same type could occur in succession. All other details were the same as for Phase 1.

Phase 1	Phase 2	Optional Shift Test
A1V1→LP1 **A2**V1→LP2 **A1**V2→LP1 **A2**V2→LP2		
or	A3V3→LP1 A4V4→LP2	A3V3→LP1 A3V4→ ? A4V4→LP2 A4V3→ ?
A1**V1**→LP1 A2**V1**→LP1 A1**V2**→LP2 A2**V2**→LP2		

Table 3.3: Experimental design

Stimuli A1 to A4 were 500Hz, 8kHz, and 2kHz tones and a 10Hz train of clicks; V1 to V4 were houselight, two panel lights, a magazine light and darkness. LP1 and LP2 were left and right lever. Both levers were extended during trials, but only responses on the indicated lever were reinforced by the delivery of food. "?" denotes that responding on neither lever was not reinforced for these trials. Stimuli in bold in Phase 1 indicate the dimension that was relevant during this phase.

Optional shift test

In the final session of the Phase 2, rats received two probe test trials, one for each of the audiovisual compounds A3V4 and A4V3. The first of these test trials was presented following the sixth trial of normal training, and the second was presented following the final trial of normal training. The test trials were 4 min long and were the same as the training trials except that responses on neither lever were reinforced. As a consequence of these additional trials, this final session of training lasted for 56 min.

3.4.2 Behavioural results

For the purpose of data analysis, each trial in Phase 1 and 2 was divided into two variable-length periods. The first, S_D1, was the part of the trial prior to the delivery of the first reward and was always at least 10s long. The second period, S_D2, constituted the remainder of the trial. To obtain an uncontaminated measure of performance, only data from S_D1 period were analysed for these two stages. For the two probe trials, responding over the 4-min trial was evaluated. For the probe test trials, correct responses were defined as those made on the lever that was reinforced in the presence of the component of the audiovisual compound that belonged to the dimension that had been relevant during Phase 1 (see table 3.3). Consider, for example, an animal that was trained with the auditory cues relevant in Phase 1. In Phase 2, that animal would have learned that LP1 was reinforced in the presence of the compound A3V3. Hence, on a probe test trial with the compound A3V4, lever-press LP1 would have been considered correct since that response was reinforced in the presence of the auditory cue A3. Similarly, for that animal, lever-press LP2 would have been considered incorrect on the test trial with

compound A3V4, but correct on the test trial with compound A4V3.

All statistical tests are evaluated with respect to an alpha level of 0.05.

Pretraining: magazine and lever press training

All rats learnt successfully to approach and retrieve pellet from the magazine.

Phase 1 training

The mean rates of responding on the correct and incorrect levers during the S_D1 period of each trial are shown in Figure 3.5 for animals in group Short (top panel) and group Long (bottom panel). Acquisition of the discrimination progressed rapidly, and by the end of training all animals in both groups were producing more correct than incorrect responses. There did not appear to be any difference in the rate at which the discrimination was learned by animals for which the auditory cues were relevant and those for which the visual cues were relevant. These observations were confirmed by the results of a mixed ANOVA with the between-subjects factors of training (short, long) and acquisition group (DM, HP, TC, LTHT) and the within-subject factor of lever (correct, incorrect) that was conducted on the data collected on the final day of training for each group. This analysis revealed a significant effect of lever only, ($F(1, 23) = 179.38$, $p < 0.001$, $MSE = 7.71$). No other effects or interactions were significant (max $F(1, 23) = 2.53$).

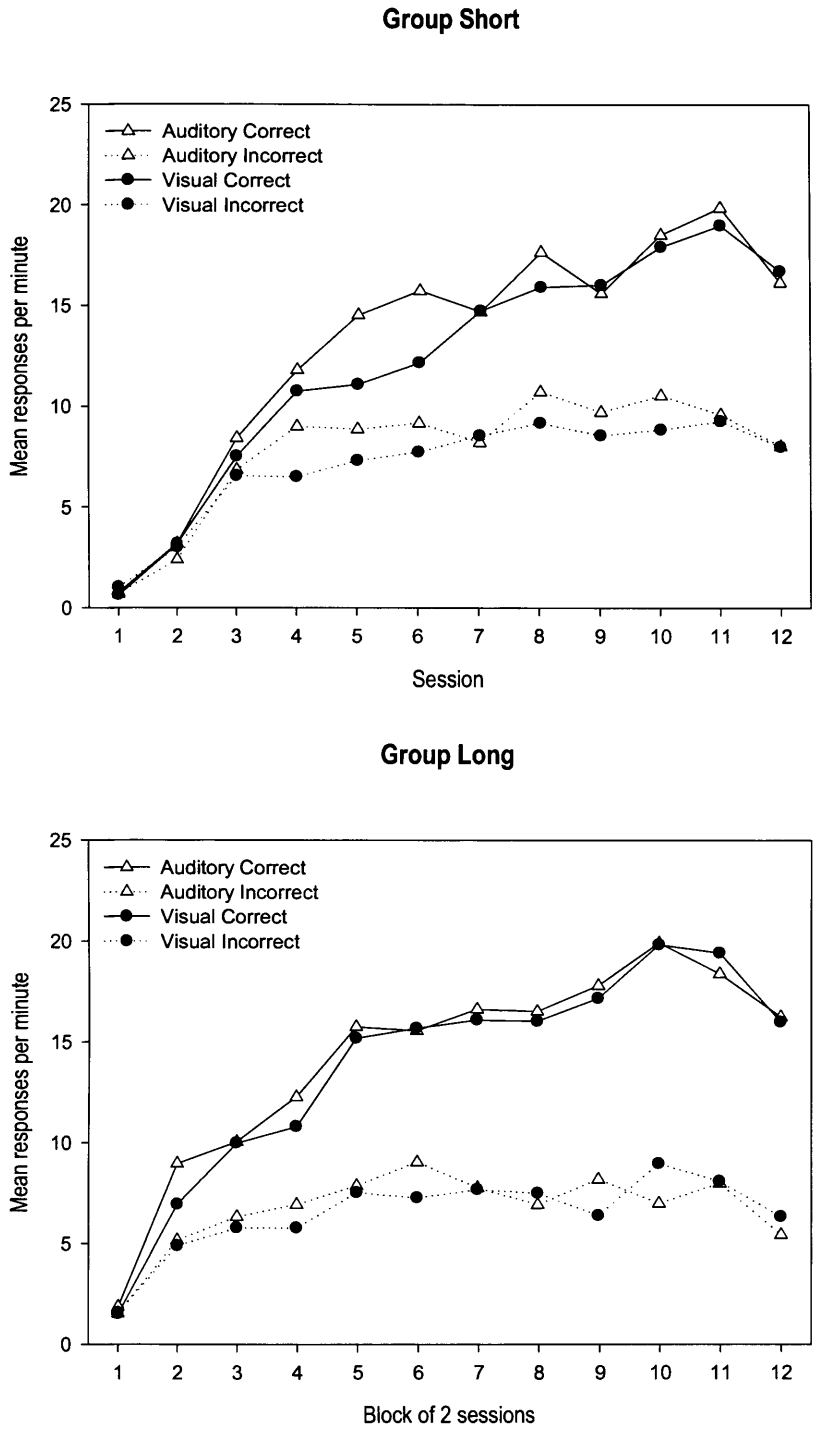

Figure 3.5: The mean rates of correct and incorrect lever-press responding during Phase 1 for animals learning a conditional discrimination where either the auditory or visual components of a compound stimulus were relevant. Response rates are shown for each of the 12 sessions of training received by Group Short in the top panel, and for each of 12 blocks of 2 sessions of training for Group Long in the bottom panel.

Phase 2 training

The mean rates of responding during the training trials presented on each of the three sessions of Phase 2 are presented in Figure 3.6 for animals in group Short (top panel) and group Long (bottom panel). Because only two types of trial (A3V3→LP1 and A4V4→LP2) were presented during Phase 2, cues from both stimulus dimensions were relevant and acquisition of the discrimination was very rapid. A mixed ANOVA with the between-subjects factors of Phase 1 training (short, long) and Phase 1 group acquisition (DM, HP, LTHT, TC) and the within-subject factors of lever (correct, incorrect) and session (1-3) revealed significant effects of lever ($F(1, 23) = 223.29$, $p < 0.001$, MSE = 2428.19) and of session ($F(2, 46) = 11.01$, $p < 0.0001$, MSE = 180.45), but not of training ($F(1, 23) = 2.29$, MSE = 54.167) or acquisition group ($F(3, 23) = 1.15$, MSE = 27.21). There was also a significant two-way interaction of session x lever ($F(2, 46) = 18.39$, $p < 0.0001$, MSE = 157.28). Subsequent simple effect analyses revealed a significant effect of lever on session 1 ($F(1, 69) = 25.412$, $p < 0.0001$, MSE = 9.02), session 2 ($F(1, 69) = 112.61$, $p < 0.0001$, MSE = 9.02) and session 3 ($F(1, 69) = 165.88$, $p < 0.0001$, MSE = 9.02), as well as a significant effect of session on responding on both the correct lever ($F(2, 92) = 19.01$, $p < 0.001$, MSE = 10.80) and the incorrect lever, ($F(2 ,92) = 10.98$, $p < 0.001$, MSE = 10.80). No other interactions were significant (all $Fs < 1$).

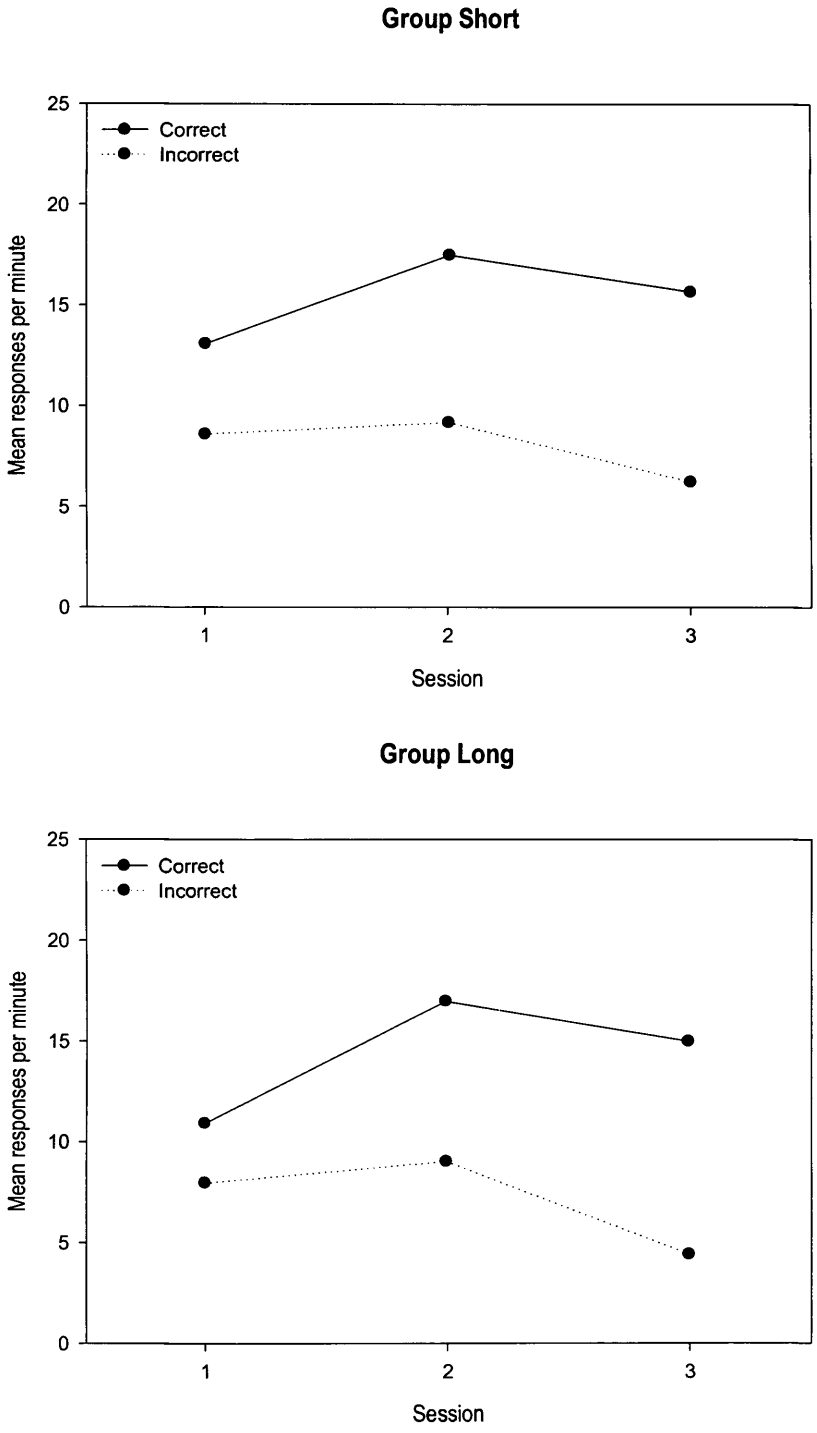

Figure 3.6: The group mean rates of correct and incorrect lever-press responding for the three sessions of Phase 2 training for Group Short (top panel) and Group Long (bottom panel). Response rates for session 3 were derived from the six training trials that preceded the first probe test trial.

Optional shift test

Responding was recorded over the entire course of each 4-min probe test trial. For the purpose of analysis, correct responses were defined as those made on the lever that was reinforced in the presence of the component of the audiovisual compound that belonged to the dimension that had been relevant during Phase 1. Consider, for example, an animal that was trained with the auditory cues relevant in Phase 1. In Phase 2, that animal would have learned that LP1 was reinforced in the presence of the compound A3V3. Hence, on a probe test trial with the compound A3V4, lever-press LP1 would have been considered correct since that response was reinforced in the presence of the auditory cue A3. Similarly, for that animal, lever-press LP2 would have been considered incorrect on the test trial with compound A3V4, but correct on the test trial with compound A4V3.

The mean rates of correct and incorrect responding over each of the test trials are shown in Figure 3.7 for animals in group Short (top panel) and group Long (bottom panel). Inspection of this figure reveals that subjects in both groups responded at a higher rate on the correct lever than on the incorrect lever, although this difference was much greater during the first trial. A mixed ANOVA with the between-subjects factors of Phase 1 training (short, long) and Phase 1 group acquisition (DM, HP, LTHT, TC), and the within-subject factors of lever (correct, incorrect) and test (Test 1, Test 2) confirmed this observation. Overall, there was a significant effect of lever ($F(1, 23) = 5.45$, $p < 0.05$, MSE = 15.21) and test ($F(1, 23) = 16.99$, $p < 0.0001$, MSE = 12.06), but not of training or acquisition group (both $Fs < 1$). None of the interactions involving training or acquisition group reached significance (all $Fs < 1$) and nor did the lever x test interaction ($F(1, 23) = 3.19$, MSE = 20.17).

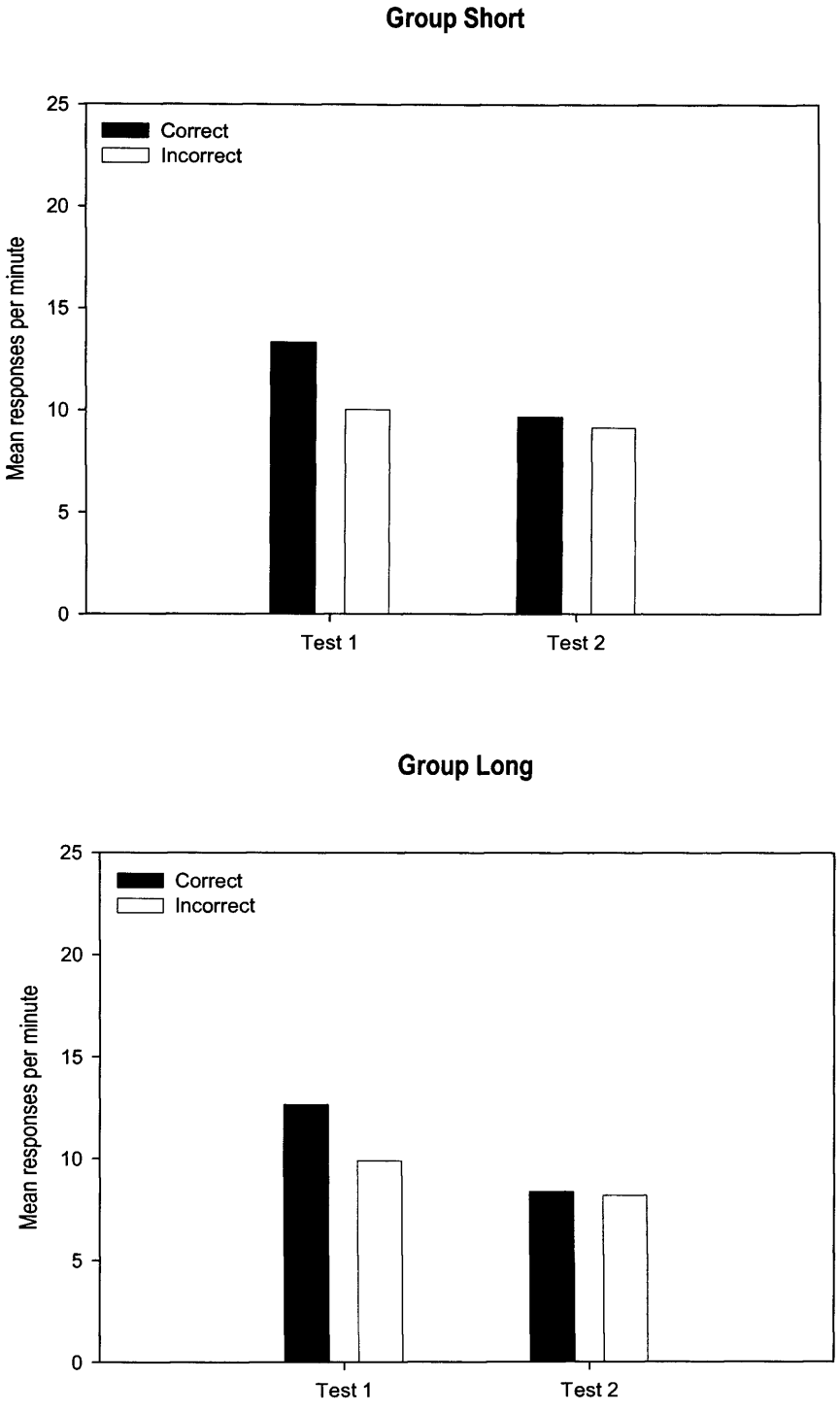

Figure 3.7: The mean rates of responding during each of the probe test trials for animals in Group Short (top panel) and Group Long (bottom panel). See text for a definition of correct and incorrect responses.

3.5 Discussion

The three experiments presented in this chapter were designed to investigate two novel, automated procedures to assess attentional changes that result from discrimination learning in rats. Both of these procedures aimed to overcome potential drawbacks observed in the ID/ED shift procedure described by Birrell and Brown (2000). In their task, rats are first trained on a simple discrimination where the irrelevant dimension is not used. In the second phase of the training, irrelevant stimuli are then used and the subjects are given a series of intradimensional shift tasks and reversals before finally receiving an extradimensional shift. Typically, performance on the final intradimensional shift is compared to that on the single extradimensional shift task. To briefly recap, there are three main problems with this procedure. First, a comparison is made between performance on two discriminations learned at different times, second – because of the simple discrimination given at the start of training – stimuli belonging to one dimension will always be more familiar than stimuli belonging to the other and third, because it is a hand run experiment, it does not allow control over stimulus exposure (especially given that one of the dimensions used is olfactory).

The first procedure explored in this chapter was a between-subjects version of the ID/ED shift procedure. In this task, rats were first trained on a conditional instrumental discrimination where one stimulus dimension (auditory or visual) was relevant and the component from the other dimension was irrelevant. Animals were then transferred to a second phase of learning where they were trained on a new instrumental discrimination involving audiovisual compounds comprising new cues. For half of the animals the

relevant dimension remained the same (ID group), whereas for the other half, the previously irrelevant dimension became relevant and the relevant dimension became irrelevant (ED group). The results from experiment IDS/EDS 1 did not reveal an ID/ED effect. Animals receiving an IDS did not show faster learning of the new conditional discrimination in the transfer phase compared to the ED group. According to associative models of attention (Mackintosh, 1975) animals should learn to selectively attend to the best predictor of outcome and should, therefore, attend more to the components of the relevant dimension than to those of the irrelevant dimension by the end of the initial training. Possible reasons why an ID/ED effect was not observed are that the acquisition phase might not have been long enough for animals to form a perceptual set or that attentional changes could occur more slowly than learning, masking attentional changes. Consequently, the number of training sessions in experiment IDS/EDS 2 was doubled and the discriminations that were found to be slightly more difficult to learn in experiment IDS/EDS 1 were used during the transfer phase. The results from experiment IDS/EDS 2 also failed to demonstrate faster learning following an ID shift compared to the ED shift.

From these results it can be concluded that the attempts to study traditional ID/ED shift effect using auditory and visual cues in operant chamber was unsuccessful; there are a number of potential reasons for this failure. First, the learning rate in the transfer phase may have been too rapid to observe any effect of differential attention to auditory and visual cues, possibly because of the initially high salience of the discriminative cues used. Second, changes in attention to these cues may have occurred rather slowly, minimising

changes in attention and resulting in the formation of a rather weak attentional set. Alternatively, it could be argued that, although the cues used in the first and second phases of the experiments belonged to the same perceptual dimensions (auditory or visual), similarity between the exemplars might not have been sufficient for the attentional set to transfer fully from one set to the other, resulting in a relatively small difference in the attention paid to members of the previously relevant and irrelevant perceptual dimensions in Phase 2.

The second paradigm used a within-subject procedure that was based on an optional shift design. In Phase 1, rats learned a conditional instrumental discrimination where one stimulus dimension (auditory or visual) was relevant and the component from the other dimension was irrelevant. All animals were then transferred to a second discrimination involving audiovisual compounds comprising novel cues. During this phase of the experiment, cues from both stimulus dimensions were equally relevant to the solution of the discrimination. On probe test trials, animals were presented with compounds comprising cues that were associated with different responses. The results from these test trials showed that cues that belonged to the stimulus dimension that was relevant during Phase 1 had a greater influence on responding than cues from the dimension that was irrelevant during Phase 1. These results suggest that, at the end of Phase 1, animals were attending more to the relevant than to the irrelevant dimension. In other words, they suggest that by the end of Phase 1, animals had formed a perceptual attentional set that transferred in Phase 2 resulting in the formation of stronger associations between the correct response and the previously relevant dimension than between the correct response

and the previously irrelevant dimension. Thus, this is a demonstration that although it is hard to detect changes using an instrumental conditioning in Skinner box (because everything changes very quickly and the different dimensions used were both very salient), a perceptual attentional set can be formed. Furthermore, the optional shift design provides a test sensitive enough to magnify any attentional changes, even though attentional set appears to be short-lived with these dimensions and cues and therefore does not confer great advantage to the perceptual dimension. And finally, the design provides a tool to measure the formation and/or the maintenance of an attentional set rather than a tool to measure the ability to shift attention like the Birrell and Brown's procedure.

It is, perhaps surprising that the amount of training given in Phase 1 (12 *vs.* 24 sessions of training) had no effect on performance during Phase 2 or at test. It is possible, however, that changes in attention are most rapid during the acquisition of a discrimination, and that training that extends beyond the point at which asymptotic performance is observed has a relatively small impact on attention. Or alternatively, it is possible that the benefit conferred by an attentional is short-lived and does not extend when the training is increased.

In summary, the results of these experiments show that the changes in the attention paid to a stimulus over the course of discrimination training may be assessed using a within-subject design and a fully automated procedure in rats. This optional shift design overcomes some of the potential problems detected in Birrell and Brown task (i.e.

laborious hand-running, key comparison made between performance on two discrimination and familiarity discrepancy between the two dimensions used (see Chapter One for more details). Moreover, this design does not explicitly require animals to shift their attention (either to the same perceptual dimension or to the alternative perceptual dimension) but aims to assess animals' ability to form a perceptual set when trained on a conditional discrimination where one perceptual dimension is relevant for the solution of the discrimination and the other is irrelevant.

4 Neurobiological assessment of the optional shift effect

4.1 Introduction

There is little doubt that the medial prefrontal cortex (mPFC) is involved in aspects of behavioural flexibility. It is, however, unclear exactly to what extent the mPFC is involved in different types of flexibility. While there is a fair amount of evidence to suggest that the mPFC is involved in flexibility relating to changes in behaviour-guiding strategies, it is far less clear to what extent this region is involved in more basic forms of flexibility such as that required in reversal learning tasks.

Joel et al. (1997a) investigated the effect of medial prefrontal lesions in rats on a WCST analogue and found that mPFC rats were slower at shifting between abstract rules (from a matching to a non-matching rule) compared to sham-operated controls. In a task of behavioural flexibility for spatial and response learning, Ragozzino et al. (1999a) showed that prelimbic-infralimbic inactivation impaired the switch from a spatial to response discrimination and vice-versa. In the same study (Ragozzino et al., 1999a), they further examined the role of the prelimbic-infralimbic areas in reversal learning and showed that prelimbic-infralimbic inactivation did not disrupt animals' capacities to perform a reversal learning of either the spatial or response discrimination. In a more recent study using a similar paradigm, it has been shown that inactivation of the orbitofrontal cortex did not impair initial visual discrimination learning, nor performance on the set-shift but impaired reversal learning (Ghods-Sharifi et al., 2007).

In the case of Birrell and Brown (2000) ID/ED task, formally the same as the one used in humans and non-humans primates, it has been shown that bilateral lesions of the mPFC induced a selective impairment in shifting of attentional set between stimulus dimensions (ED shift), but spared performance on initial acquisition, the intradimensional shift and reversal learning. Conversely, orbital prefrontal cortex lesions resulted in a selective impairment of reversal of stimulus-reward contingencies, leaving attentional set-shifting capacities intact (Brown and Bowman, 2002). Together, these results support one view of the distribution of different cognitive functions within specific regions of prefrontal cortex; with the orbital prefrontal cortex involved in basic forms of cognitive flexibility (reversal learning) and the medial prefrontal cortex involved in higher order cognitive flexibility (reversal learning set).

While a functional dissociation has been made within the prefrontal cortex, the involvement of the different subregions of the medial prefrontal cortex in flexibility relating to changes in behaviour-guiding strategies has received little investigation. However, it is becoming apparent that the mPFC can be fractionated into PL, ACC and IL subcomponents. There is a growing body of evidence that the subregions serve separate and distinct (and possibly complementary) functions (Ragozzino et al., 1999b; Chudasama et al., 2003; Coutureau and Killcross, 2003); this is paralleled by neuroanatomical evidence that these regions can be differentiated on the basis of distinct connectivity with different brain regions (see General Introduction section 1.3.3). The experiments presented in this chapter were intended to compare the effect of discrete lesions of the PL, ACC and IL cortices on optional shift paradigm designed to assess

attentional changes that result from discrimination learning. The aim was to tease apart the involvement of these different subregions of mPFC in behavioural flexibility requiring instrumental responding.

4.2 Experiment OS 2: effect of PL lesions on optional shift behaviour

Although the rat medial prefrontal cortex has been shown to play a critical role in behavioural flexibility for rule shifting, effects of selective lesions of the prelimbic cortex have received little or no investigation. The aim of experiment OS 2 was to determine whether selective lesions of the prelimbic cortex would affect performance on the optional shift design described in experiment OS 1.

4.2.1 Method

Subjects

The subjects were 32 naïve male Lister Hooded rats with a mean a*d-libitum* weight of 353g (range 275-395g). Prior to the start of the experiment sixteen of the rats received bilateral excitotoxic lesions of the prelimbic cortex and the remaining sixteen served as sham-operated controls. Following recovery, the rats were reduced to and maintained at 85% of their age matched ad lib weights and had free access to water. Rats were fed and housed as described for experiment OS 1.

Surgery

Rats were first anaesthetized with Isoflurane, their heads shaved, and they were then placed in a stereotaxic frame (Kopf Instruments, Tujunga, CA). An incision was made in the scalp and then a skull flap overlying the prefrontal cortex was drilled out. Injections of ibotenic acid (63m M, Sigma-Aldrich Co. Ltd., UK.) were made using a 2 µl Hamilton syringe (Reno, NV) mounted on an injection pump. Automated injections of 0.2 µl at a rate of 0.1 µl /min were made at two sites within the prelimbic cortex (AP: +3.2; ML: ± 0.6; DV: −4.0, as previously used in Cardiff BNL). After each injection, the needle was left in position for 5 min to allow absorption of the bolus and to minimize spread of the toxin along the needle tract. Sham-operated controls received an identical procedure with the exception that no toxin was infused.

Histology

Following completion of testing, rats were given a lethal overdose of sodium-pentobarbitone (Euthatal) and perfused with saline (0.9%) and formal-saline (10%, w/v). Brains were taken out and postfixed in formal saline and before cutting were transferred to a 25% sucrose solution in which they remained for 24 hr. Slices (40 µm thick) were made using a cryostat (Leica Instruments) and were mounted onto gelatin-coated slides. These were dried at room temperature for 24h before staining with Cresyl violet, followed by the addition of a coverslip in DPX. The extent and location of cell loss were verified with a light microscope and the brain atlas of Paxinos and Watson (1998).

Apparatus & procedure

Eight standard operant chambers were used; they are described in detail in experiment OS 1. All details about the behavioural training were as described for experiment OS 1, group Long.

4.2.2 Histological results

Since the aim of this experiment was to investigate the impact of selective lesions of the prelimbic cortex, any animals that showed damage to surrounding regions (anterior cingulate and infralimbic cortices) were removed from this experiment. Therefore, three animals were removed from the PL group due to damage to the anterior cingulate cortex. Two sham-operated controls were also removed because they presented cortical damage due to an infection. Post-histology, the final number of rats of each lesion type was 13 PL-lesioned and 14 sham-operated rats. Figure 4.1 illustrates the maximum (striped region) and minimum (grey region) extent and location of the damage in the prelimbic cortex for the remaining animals. Photomicrographs of the lesions and diagrams labelled with the region of interest are presented in the annexes.

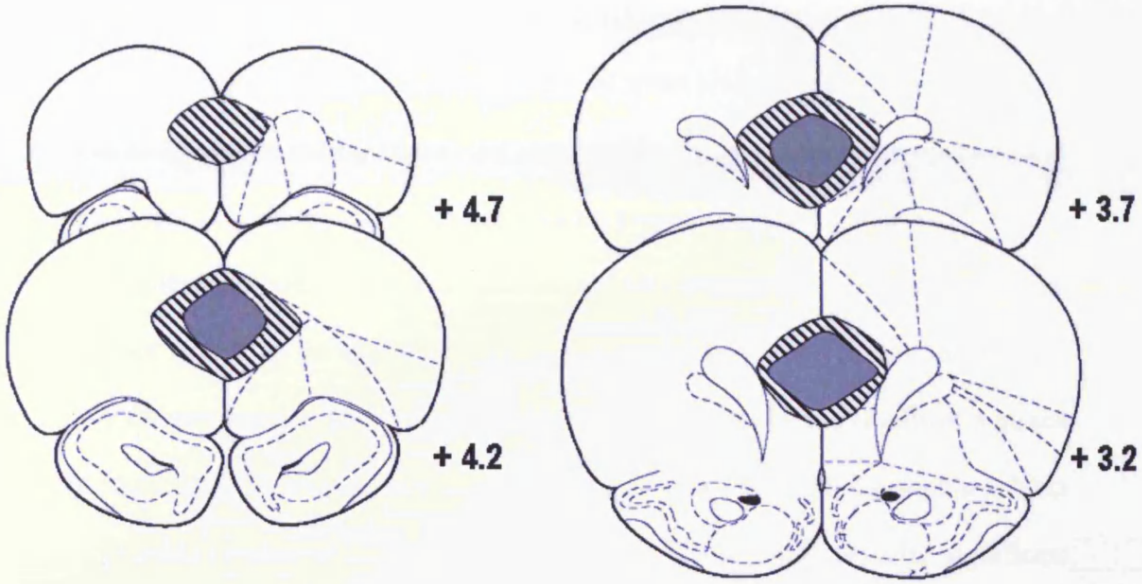

Figure 4.1: Representation of the minimum (grey) and maximum (striped) extent and location[1] of damage within the prelimbic cortex. Outlines are reproduced from Paxinos and Watson (1998) and represent sections ranging from 2.7 to 4.7 anterior to bregma.

4.2.3 Behavioural results

All statistical tests are evaluated with respect to an alpha level of 0.05.

Pretraining: magazine and lever press training

All rats learnt successfully to approach and retrieve pellets from the magazine.

[1] The minimum and maximum extent and location of lesions were calculated as following: for each animal, the extent and location of lesion were determined for a given coordinate; then on any given coordinate were reported the extend and location of the lesions corresponding to the animals with the smallest and the largest lesions.

Phase 1 training

The mean rates of responding on the correct and incorrect levers during the S_D1 period of each block of 2 sessions during Phase1 are presented in Figure 4.2 for sham-operated and PL-lesioned rats. Acquisition of the discrimination progressed rapidly, and by the end of training all animals in both groups were producing more correct than incorrect responses. There did not appear to be any difference in the rate at which the discrimination was learned by the two groups. These observations were confirmed by the results of a mixed ANOVA with the between-subjects factor of lesion (sham, PL) and the within-subject factors of session (1-24) and lever (correct, incorrect). This analysis revealed significant effects of session ($F(23, 575) = 35.56$, $p < 0.0001$, $MSE = 7.05$) and of lever ($F(1, 25) = 394.63$, $p < 0.0001$, $MSE = 34.13$), as well as a significant interaction of session x lever ($F(23, 525) = 31.06$, $p < 0.0001$, $MSE = 4.19$). No other effects or interactions were significant (max $F(1, 25) = 2.52$, $MSE = 110.47$). Analysis of simple effects produced by session x lever interaction indicated a significant effect of lever from session 3 to 24 (min $F(1, 600) = 10.29$, $MSE = 5.43$).

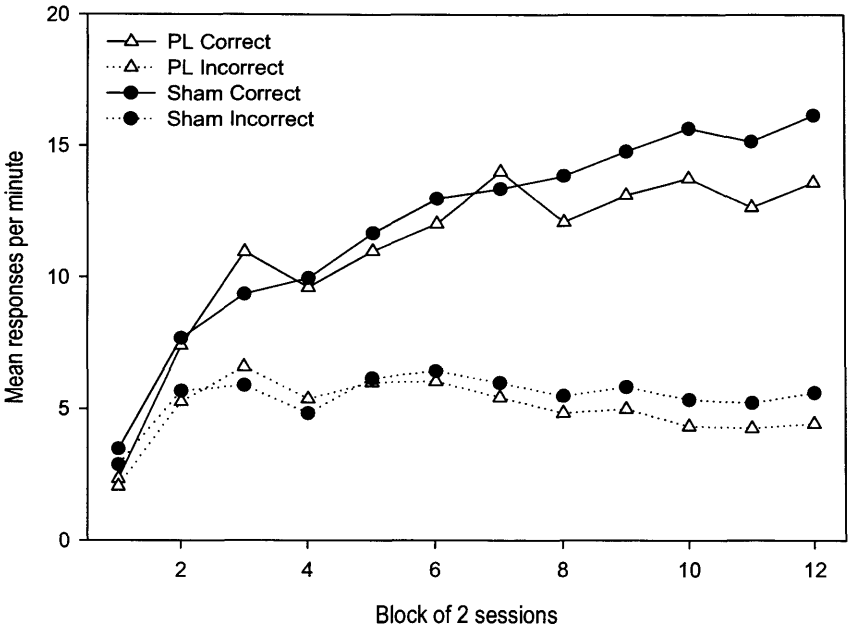

Figure 4.2: The mean rates of correct and incorrect lever-press responding during Phase 1 training for both PL-lesioned and sham-operated animals. Response rates are shown for each of 12 blocks of 2 sessions.

Phase 2 training

The mean rates of responding during the training trials presented on each of the three sessions of Phase 2 are shown in Figure 4.3 for both sham-operated and PL-lesioned animals. Because only two types of trial (A3V3→LP1 and A4V4→LP2) were presented during Phase 2, cues from both stimulus dimensions were relevant and acquisition of the discrimination was very rapid. However, on the final day, sham-operated animals were performing better than PL-lesioned animals; showing a higher rate of responding on the correct lever.

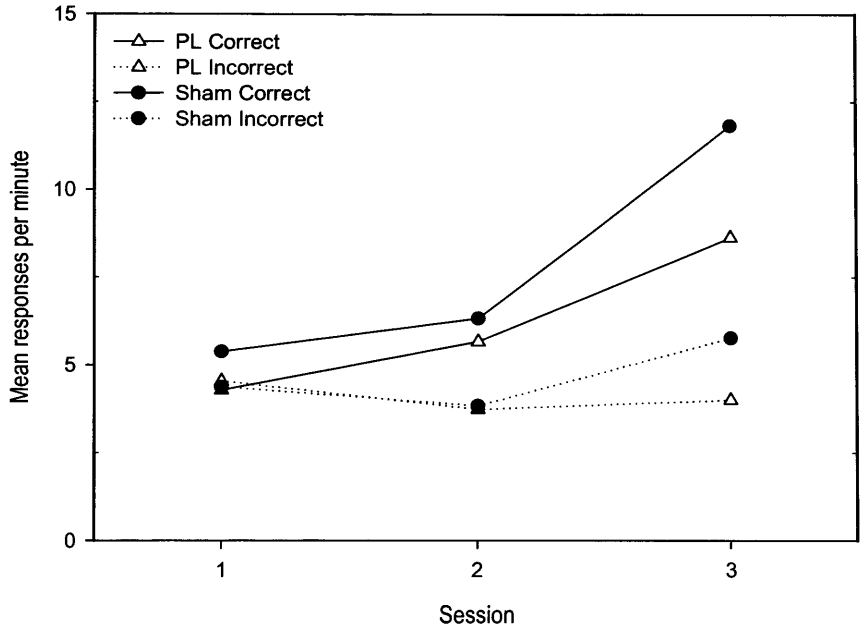

Figure 4.3: The group mean rates of correct and incorrect lever-press responding for the three sessions of Phase 2 training for both PL-lesioned and sham-operated animals. Response rates for session 3 were derived from the six training trials that preceded the first probe test trial.

A mixed ANOVA with the between-subjects factor of lesion (sham, PL) and the within-subject factors of session (1-3) and lever (correct, incorrect) revealed significant effects of session ($F(2, 50) = 8.28$, $p < 0.0001$, $MSE = 21.04$) and lever ($F(1, 25) = 29.83$, $p < 0.001$, $MSE = 2428.19$). There were also significant two-way interactions of lesion x lever ($F(1, 25) = 7.02$, $p < 0.0001$, $MSE = 10.24$) and of session x lever ($F(2, 50) = 8.84$, $p < 0.0001$, $MSE = 9.71$). No other effects or interactions were significant, (min $F(2, 50) = 2.55$, $MSE = 9.71$). Subsequent simple effect analyses produced by lesion x lever interaction revealed a significant effect of lever for both sham-operated ($F(1, 25) = 32.90$, $p < 0.0001$, $MSE = 10.24$) and PL-lesioned animals ($F(1, 25) = 4.32$, $p < 0.05$, $MSE = 10.24$) as well as a significant effect of lesion on responding on the correct lever (F(1,

50)= 9.33, $p < 0.01$, MSE = 15.64) but not on the incorrect lever ($F(1, 50)= 0.000$, $p > 0.05$, MSE = 15.64). Although, sham-operated performed better than PL-lesioned animals (with more marked difference in the 3rd session), both groups were presented a good level of discrimination in the Phase 2 training.

Optional shift test

Responding was recorded over the entire course of each 4-min probe test trial. For the purpose of analysis, correct responses were defined as those made on the lever that was reinforced in the presence of the component of the audiovisual compound that belonged to the dimension that had been relevant during Phase 1.

The mean rates of correct and incorrect responding over each of the two test trials are presented in Figure 4.4 for sham-operated animals (top panel) and PL-lesioned (bottom panel) animals. Inspection of this figure reveals that in Test 1, both PL and sham animals were responding at chance whereas in Test 2, both groups responded at a higher rate on

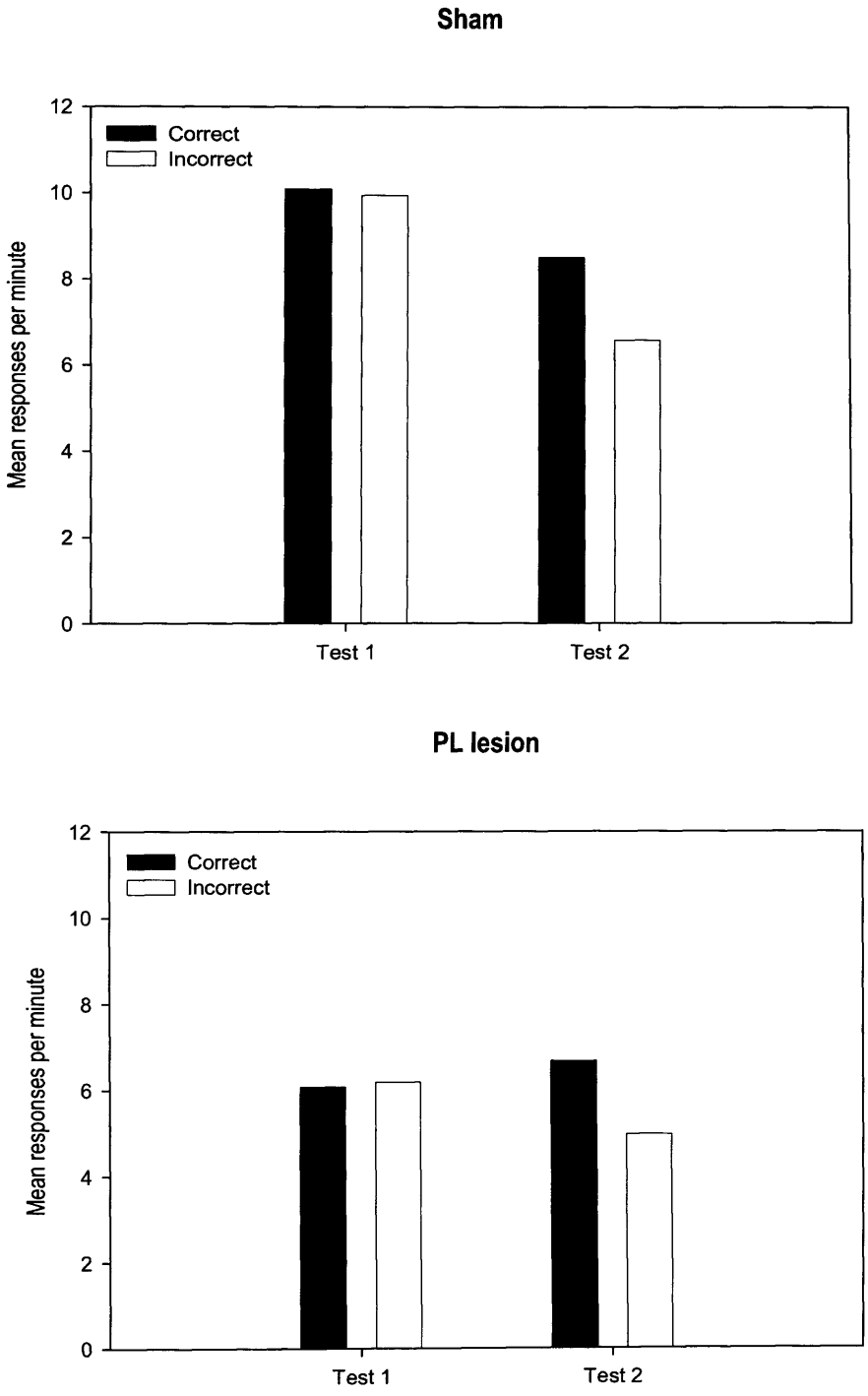

Figure 4.4: The mean rates of responding during each of the two probe test trials for animals in sham (top panel) and PL (bottom panel) groups.

the correct lever than on the incorrect lever. A mixed ANOVA with the between-subjects factor of lesion (PL, sham) and the within-subject factor of lever (correct, incorrect) on Test 2 confirmed this observation. Overall, there was a significant effect of lever ($F(1, 25) = 6.21$, $p < 0.05$, $MSE = 7.12$) but no effects or interactions involving the factor of lesion were significant (all $Fs < 1$).

4.3 Experiment OS 3: effect of ACC lesions on optional shift behaviour

Few studies have investigated the role of anterior cingulate cortex in behavioural flexibility for rule shifting; however they provide some evidence for the ACC involvement in behavioural flexibility depending on the nature of the strategy employed (learned or innate). For instance, in a study by Ragozzino et al. (1999b), it was demonstrated that ACC inactivation produced minimal effects on cross-modal strategy switching in cheeseboard apparatus in which animals were required to shift responding between spatial and visual cues. Similarly, in a study by Joel et al. (1997a), lesions of the dorsal ACC were not found to disrupt either delayed non matching to sample or its reversal (delayed matching to sample). However, Dias and Aggleton (2000) showed that ACC excitotoxic lesions initially impaired matching-to-place acquisition in a T-maze due to the innate tendency to non-matching-to-place, but eventually ACC-lesioned animals learned the matching rule. One plausible explanation forward by the authors was that the ACC may be critical only when an innate preference, rather than a learned strategy, needs to be overcome.

The aim of experiment OS 3 was to further study the effect of the anterior cingulate cortex in behavioural flexibility, using on optional shift design.

4.3.1 Method

Subjects

The subjects were 30 naïve male Lister Hooded rats with a mean a*d-libitum* weight of 396g (range 357-442g). Before the onset of the experiment sixteen of the rats received bilateral excitotoxic lesions of the anterior cingulate cortex and the remaining fourteen served as sham-operated controls. After recovery, the rats were maintained and housed as described for experiment OS 2.

Surgery & histology

Injections of quinolinic acid (0.09 M, Tocris, UK) were made using a 2 µl Hamilton syringe mounted on an injection pump. Quinolinic acid was chosen because this excitotoxin was found in pilot studies to be appropriate for producing selective damage to the anterior cingulate cortex (data from BNL Cardiff). Automated injections of 0.2 µl at a rate of 0.1 µl /min were made at six sites within the anterior cingulate cortex (AP: + 2.2, +2.7 and +3.2, ML: ± 0.5, DV: - 2.4, -2.6, and -2.4, respectively, Haddon and Killcross, 2006). All other details about surgical and histological procedures were as described for experiment OS 2.

Apparatus & behavioural procedure

This experiment – as well as the next one – was conducted at Eli Lilly (Erl Wood Manor, UK), and for that reason pilot experiments were conducted prior to the lesion experiments in order to ensure that the experimental findings were reproducible. Following these pilot

experiments, the experimental design was modified in the following ways: (i) in Phase 2 of the training, animals received five sessions of training (rather than three sessions), (ii) sucrose pellets were used rather than Formula A/I. All other details were as described in experiment OS 2. Sixteen standard operant chambers were used; they were as described in OS 2 experiment.

4.3.2 Histological results

Figure 4.5 depicts the maximum (striped region) and minimum (grey region) extent and location of the damage in the anterior cingulate cortex for the animals kept after histological examination. As the aim of this experiment was to investigate the impact of selective lesions of the anterior cingulate cortex, any animals that did show damage to surrounding regions (mostly prelimbic cortex) or only a small amount of damage to the ACC were removed from this experiment. Consequently, three animals were removed from the ACC group due to insufficient damage to the ACC. Some animals exhibited minor cell loss within the secondary motor cortex, however analysis of the behavioural data did not reveal a systematic relationship between extent of damage and behavioural measures therefore these animals were still included. Post-histology, the final number of rats of each lesion type was 13 ACC-lesioned and 14 sham-operated rats. Photomicrographs of the lesions and diagrams labelled with the region of interest are presented in the annexes.

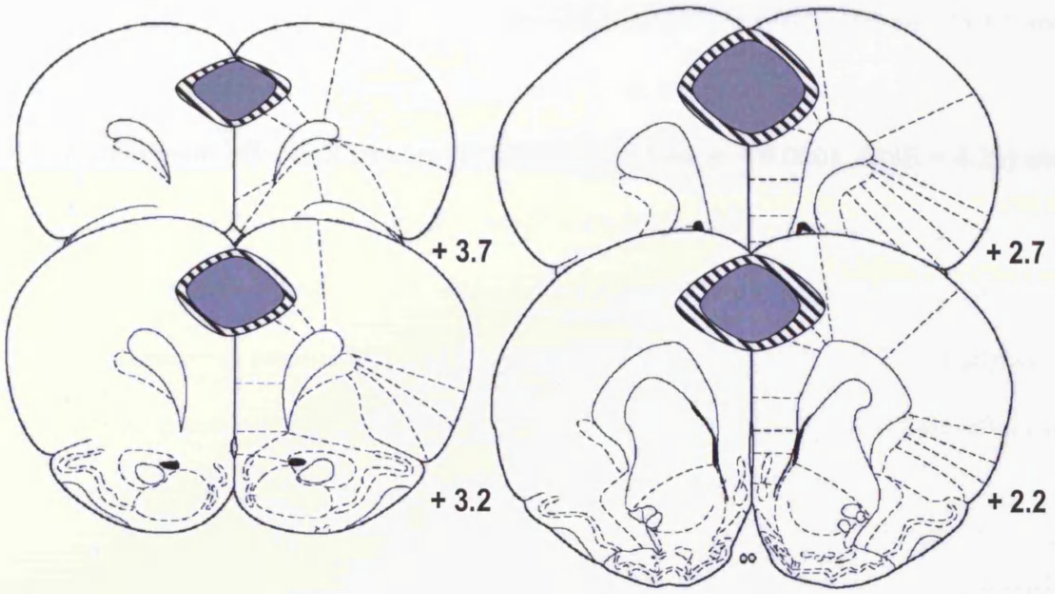

Figure 4.5: Representation of the minimum (grey) and maximum (striped) extent and location of damage within the anterior cingulate cortex. Outlines are reproduced from Paxinos and Watson (1998) and represent sections ranging from 2.2 to 3.7 anterior to bregma.

4.3.3 Behavioural results

Pretraining: magazine and lever press training

All rats learnt successfully to approach and retrieve pellets from the magazine.

Phase 1 training

The mean rates of responding on the correct and incorrect levers during the S_D1 period of each block of 2 sessions during Phase1 are presented in Figure 4.6 for sham-operated and ACC-lesioned animals. Acquisition of the discrimination progressed rapidly and, by the end of training, all animals in both groups were producing more correct than incorrect responses. There did not appear to be any difference in the rate at which the discrimination was learned by the two groups. These observations were confirmed by the

results of a mixed ANOVA with the between-subjects factor of lesion (sham, ACC) and the within-subject factors of session (1-24) and lever (correct, incorrect). This analysis revealed significant effects of session ($F(23, 575) = 52.46$, $p < 0.0001$, $MSE = 4.21$) and of lever ($F(1, 25) = 263.74$, $p < 0.0001$, $MSE = 40.55$), as well as a significant two-way interaction of session x lever ($F(23, 525) = 29.17$, $p < 0.0001$, $MSE = 3.79$). No other effects or interactions were significant (max $F(23, 575) = 1.05$, $MSE = 4.21$). Analysis of simple effects produced by session x lever interaction indicated a significant effect of lever from session 4 to 24 (min $F(1, 600) = 6.02$, $MSE = 5.33$).

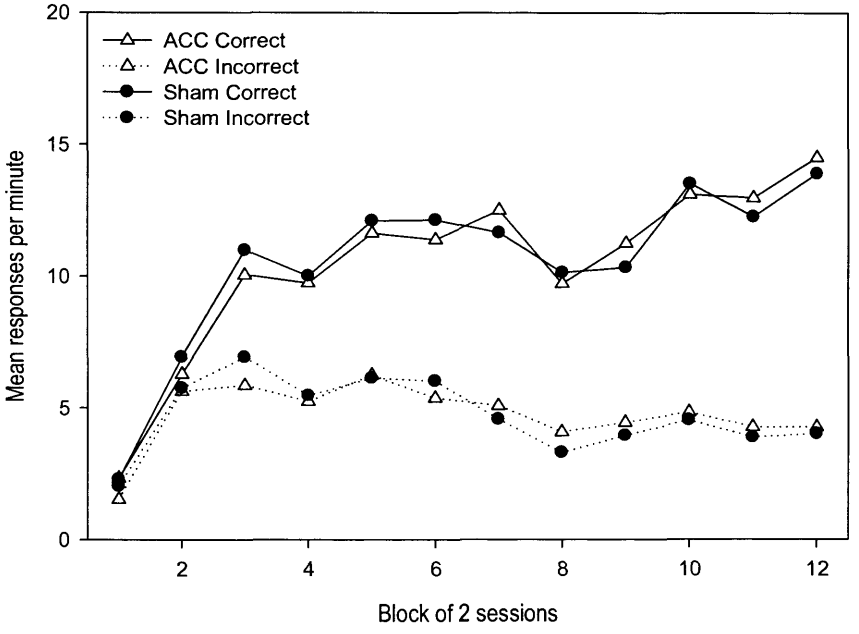

Figure 4.6: The mean rates of correct and incorrect lever-press responding during Phase 1 training for both ACC and sham animals. Response rates are shown for each of 12 blocks of 2 sessions.

Phase 2 training

The mean rates of responding during the training trials presented on each of the five sessions of Phase 2 are shown in Figure 4.7 for both sham-operated and ACC-lesioned animals. Acquisition of the discrimination was very rapid for both groups, with more correct than incorrect responding, and even in session 1.

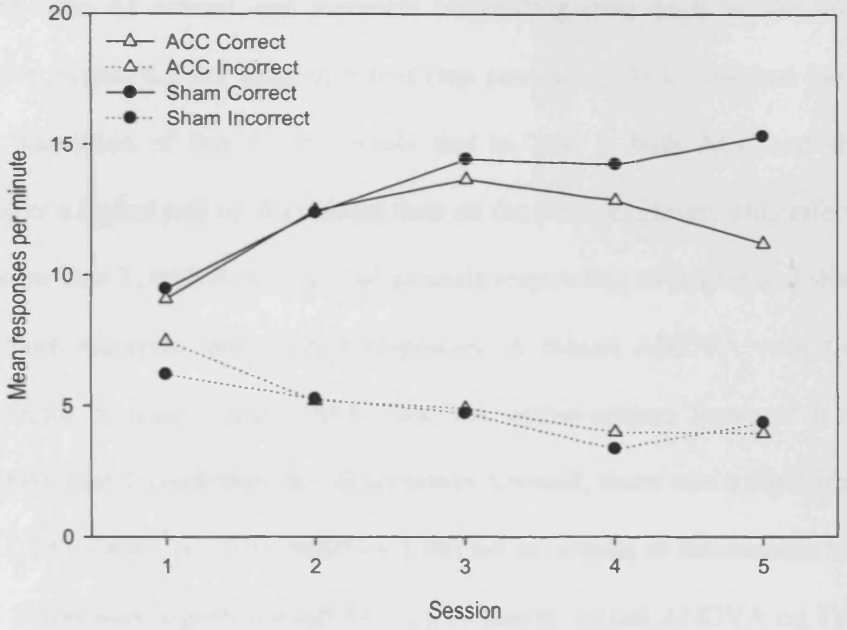

Figure 4.7: The group mean rates of correct and incorrect lever-press responding for the five sessions of Phase 2 training for both ACC and sham animals. Response rates for session 5 were derived from the six training trials that preceded the first probe test trial.

A mixed ANOVA with the between-subjects factor of lesion (sham, ACC) and the within-subject factors of session (1-5) and lever (correct, incorrect) revealed significant effect of lever ($F(1, 25) = 183.37$, $p < 0.0001$, $MSE = 3874.36$) and a significant two-way interaction of session x lever ($F(4, 100) = 22.93$, $p < 0.0001$, $MSE = 5.45$). No other effects or interactions were significant (max $F(4, 100) = 2.32$, $MSE = 6.28$). Subsequent

simple effect analysis produced by session x lever interaction revealed a significant effect of lever from session 1 to 5 (min $F(1, 125) = 9.34$, $MSE = 8.56$), as well as a significant effect of session on responding on both correct ($F(4, 200) = 16.73$, $p < 0.0001$, $MSE = 5.86$) and incorrect ($F(4, 200) = 6.82$, $p < 0.0001$, $MSE = 5.86$) lever.

Optional shift test

The mean rates of correct and incorrect responding over each of the test trials are presented in Figure 4.8 for sham-operated (top panel) and ACC-lesioned (bottom panel) animals. Inspection of this figure reveals that in Test 1, both ACC and sham groups responded at a higher rate on the correct than on the incorrect lever. This effect seemed to disappear on Test 2, with ACC-lesioned animals responding at chance and sham-operated making more incorrect than correct responses. A mixed ANOVA with the between-subjects factor of lesion (sham, ACC) and the within-subject factor of lever (correct, incorrect) on Test 1 confirmed this observation. Overall, there was a significant effect of lever ($F(1, 25) = 4.41$, $p < 0.05$, $MSE = 11.84$) but no effects or interactions involving the factor of lesion were significant (all $Fs < 1$). A similar mixed ANOVA on Test 2 did not reveal any significant effect or interaction involving the factor of lesion (max $F(1, 25) = 1.25$).

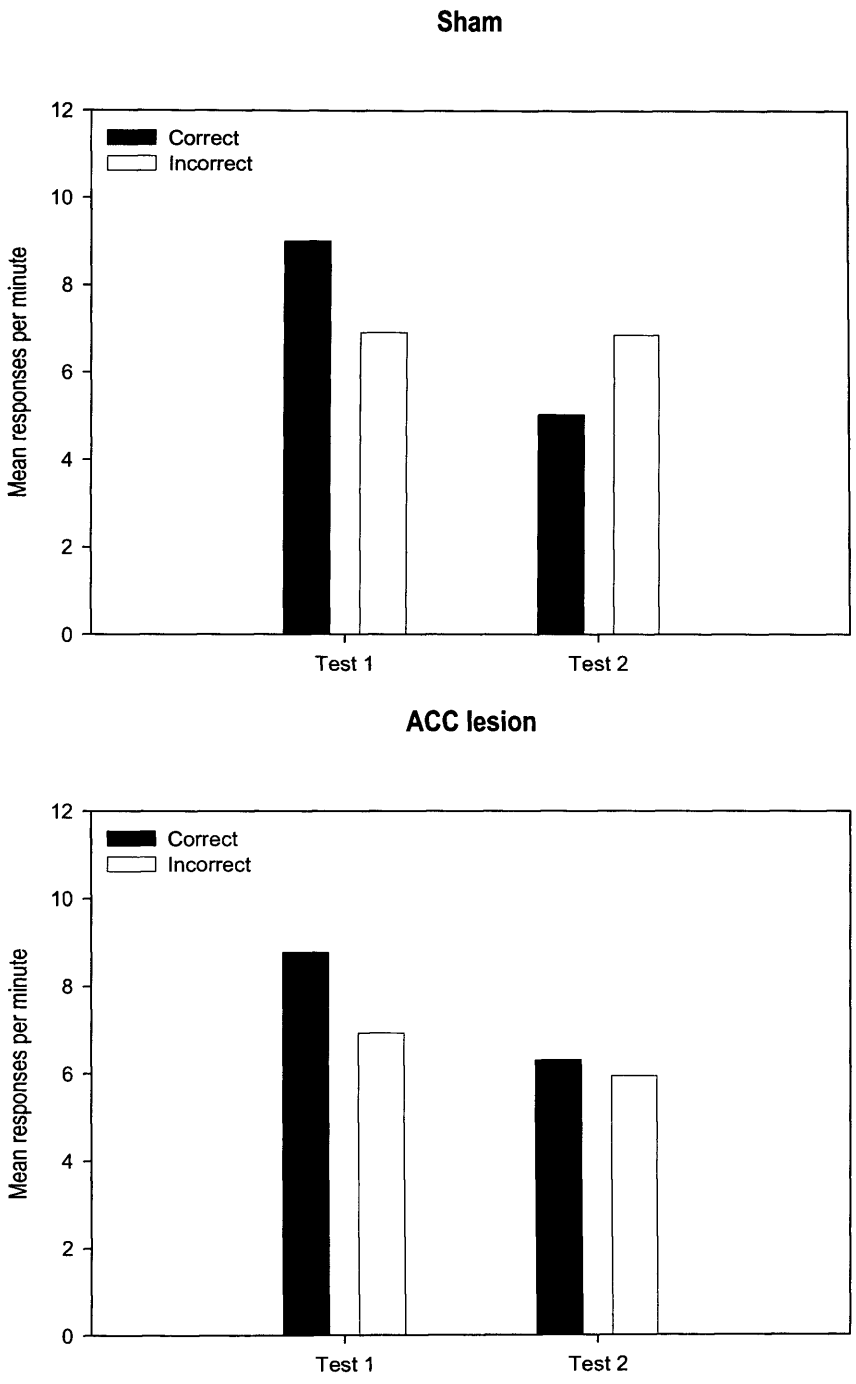

Figure 4.8: The mean rates of responding during each of the 2 probe test trials for animals in sham (top panel) and ACC (bottom panel) groups.

4.4 Experiment OS 4: effect of IL lesions on optional shift behaviour

As for the PL region, the IL region has received little investigation regarding its role in flexibility relating to changes in attention during the course of instrumental training. The aim of experiment OS 4 was to further characterize the involvement of the infralimbic cortex in behavioural flexibility for attentional set formation, using an optional shift design.

4.4.1 Method

Subjects

The subjects were 32 naïve male Lister Hooded rats with a mean *ad-libitum* weight of 353g (range 275-393g). Before the onset of the experiment sixteen of the rats received bilateral excitotoxic lesions of the infralimbic cortex and the remaining animals served as sham-operated controls. After recovery, the rats were maintained and housed as described for experiment OS 2.

Surgery & histology

Injections of ibotenic acid (63m M, Tocris UK.) were made using a 2 µl Hamilton syringe. Automated injections of 0.15 µl at a rate of 0.1 µl /min were made at two sites within the infralimbic cortex (AP: +2.6; ML: ± 0.6; DV: −5.4; Rhodes and Killcross, 2007a). All other details about surgical and histological procedure were as described in experiment OS 3.

Apparatus & behavioural procedure

Details about apparatus used and behavioural procedure were exactly as described for experiment OS 3.

4.4.2 Histological results

Since the aim of this experiment was to investigate the impact of selective lesions of the infralimbic cortex, any animals that did show damage to surrounding regions (prelimbic cortex) or small damage to the IL were removed from this experiment; consequently, five animals were removed from the IL group.

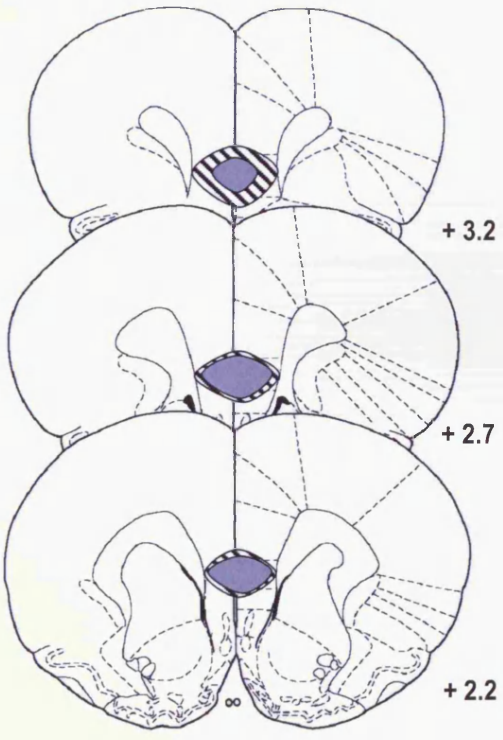

Figure 4.9: Representation of the minimum (grey) and maximum (striped) extent and location of damage within the infralimbic cortex. Outlines are reproduced from Paxinos and Watson (1998) and represent sections ranging from 2.2 to 3.2 anterior to bregma.

Post-histology, the final number of rats of each lesion type was 11 IL-lesioned and 16 sham-operated rats. Figure 4.9 depicts the maximum (striped region) and minimum (grey region) extent and location of the damage in the infralimbic cortex for the remaining animals. Photomicrographs of the lesions and diagrams labelled with the region of interest are presented in the annexes.

4.4.3 Behavioural results

Pretraining: magazine and lever press training

All rats learnt successfully to approach and retrieve pellets from the magazine.

Phase 1 training

The mean rates of responding on the correct and incorrect levers during the S_D1 period of each block of 2 sessions during Phase 1 are shown in Figure 4.10 for sham-operated and IL-lesioned animals. Acquisition of the discrimination progressed rapidly, and by the end of training all animals in both groups were producing more correct than incorrect responses. There did not appear to be any difference in the rate at which the discrimination was learned. These observations were confirmed by the results of a mixed ANOVA with the between-subjects factor of lesion (sham, IL) and the within-subject factors of session (1-24) and lever (correct, incorrect). This analysis revealed significant effects of session ($F(23, 575) = 56.16$, $p < 0.0001$, $MSE = 4.19$) and of lever ($F(1, 25) = 190.98$, $p < 0.0001$, $MSE = 52.915$), as well as a significant two-way interaction of session x lever ($F(23, 575) = 29.53$, $p < 0.0001$, $MSE = 3.76$). No other effects or interactions were significant (max $F(23, 575) = 0.96$, $MSE = 3.763$). Analysis of simple

effects produced by session x lever interaction indicated a significant effect of lever from session 4 to 24 (Max F(1, 600) = 5.13, MSE = 5.81).

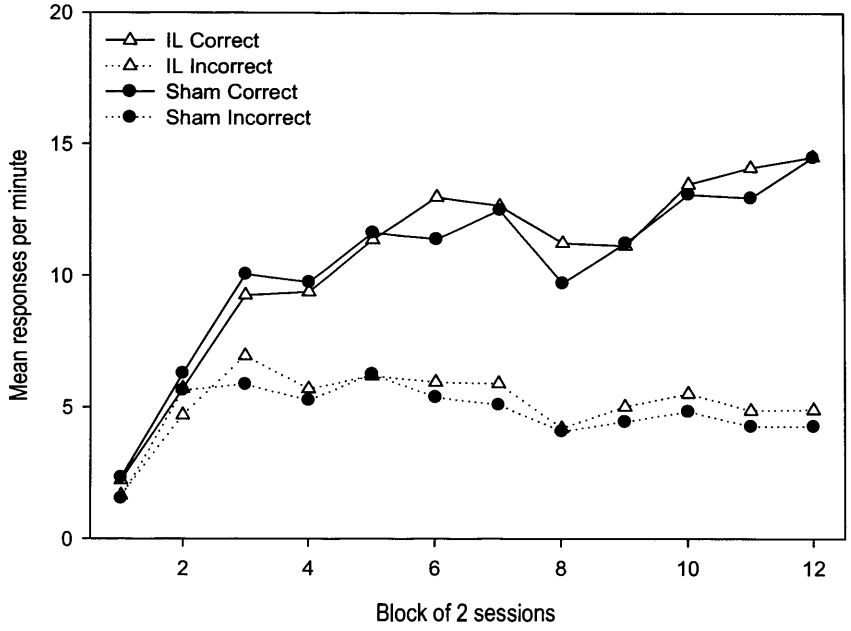

Figure 4.10: The mean rates of correct and incorrect lever-press responding during Phase 1 training for both IL-lesioned and sham-operated animals. Response rates are shown for each of 12 blocks of 2 sessions.

Phase 2 training

The mean rates of responding during the training trials presented on each of the five sessions of Phase 2 are presented in Figure 4.11 for both sham-operated and IL-lesioned animals. Acquisition of the discrimination was very rapid for both groups, with more correct than incorrect responding from session 1. A mixed ANOVA with the between-subjects factor of lesion (sham, IL) and the within-subject factors of session (1-5) and lever (correct, incorrect) revealed significant effects of session ($F(4, 100) = 6.13$, $p < 0.001$, $MSE = 5.59$) and of lever ($F(1, 25) = 206.68$, $p < 0.0001$, $MSE = 20.20$). There

were also significant two-way interactions of session x lever ($F(4, 100) = 22.60$, $p < 0.0001$, $MSE = 4.65$) and lesion x session ($F(4, 100) = 7.67$, $p < 0.0001$, $MSE = 5.59$) . No other effects or interactions were significant, (max $F(1, 25) = 0.943$, $MSE = 20.20$). Subsequent simple effect analyses produced by session x lever interaction revealed a significant effect of lever from session 1 to 5 (min $F(1, 125) = 18.74$, $MSE = 7.76$), as well as a significant effect of session on responding on both correct ($F(4, 200) = 21.24$, $p < 0.0001$, $MSE = 5.12$) and incorrect ($F(4, 200) = 5.97$, $p < 0.001$, $MSE = 5.12$) levers.

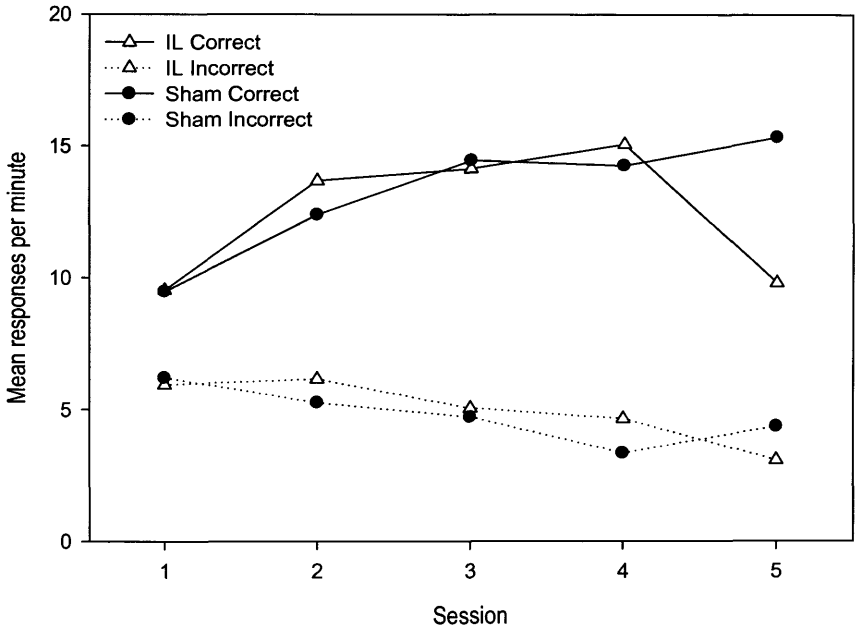

Figure 4.11: The group mean rates of correct and incorrect lever-press responding for the five sessions of Phase 2 training for both IL-lesioned and sham-operated animals. Response rates for session 5 were derived from the six training trials that preceded the first probe test trial.

Furthermore, subsequent effect analyses produced by lesion x session interaction revealed a significant effect of session for both sham-operated ($F(4, 100) = 11.26$, $p < 0.0001$, $MSE = 5.59$) and IL-lesioned animals ($F(4, 100) = 2.54$, $p < 0.05$, $MSE = 5.59$),

as well as a significant effect of lesion for the 5th session of Phase 2 ($F(1, 125) = 20.36$, $p < 0.0001$, MSE = 7.54). Although, there was a significant interaction of lesion x session (due to a decrease in the rate of responding on both the correct and the incorrect lever for IL-lesioned animals in the 5th session of Phase 2), there was no interaction involving both lesion and lever factors, therefore it can be concluded that the level of discrimination in Phase 2 was similar for both sham-operated and IL-lesioned animals.

Optional shift test

The mean rates of correct and incorrect responding over each of the test trials are shown in Figure 4.12 for animals in sham (top panel) and IL lesion (bottom panel) groups. Inspection of this figure shows that in Test 1, sham-operated animals responded at a higher rate on the correct lever than on the incorrect lever whereas IL-lesioned animals were producing the exact reversed pattern of responding. In Test 2, IL-lesioned animals were at chance and sham-operated animals responded more on the incorrect than on the correct lever. A mixed ANOVA with the between-subjects factor of lesion (sham, IL) and the within-subject factor of lever (correct, incorrect) on Test 1 confirmed this observation. Only the two-way interaction of lesion x lever was significant ($F(1, 25) = 6.52$, $p < 0.05$, MSE = 16.50). Subsequent simple effect analyses produced by lesion x lever interaction revealed a significant effect of lever for sham-operated animals ($F(1,25) = 7.60$, $p < 0.01$, MSE = 14.15) but not for IL-lesioned animals ($F(1,25) = 3.96$, $p > 0.1$, MSE = 14.15). It also revealed a significant effect of lesion on responding on correct lever ($F(1, 50) = 14.16$, $p < 0.001$, MSE = 10.87).

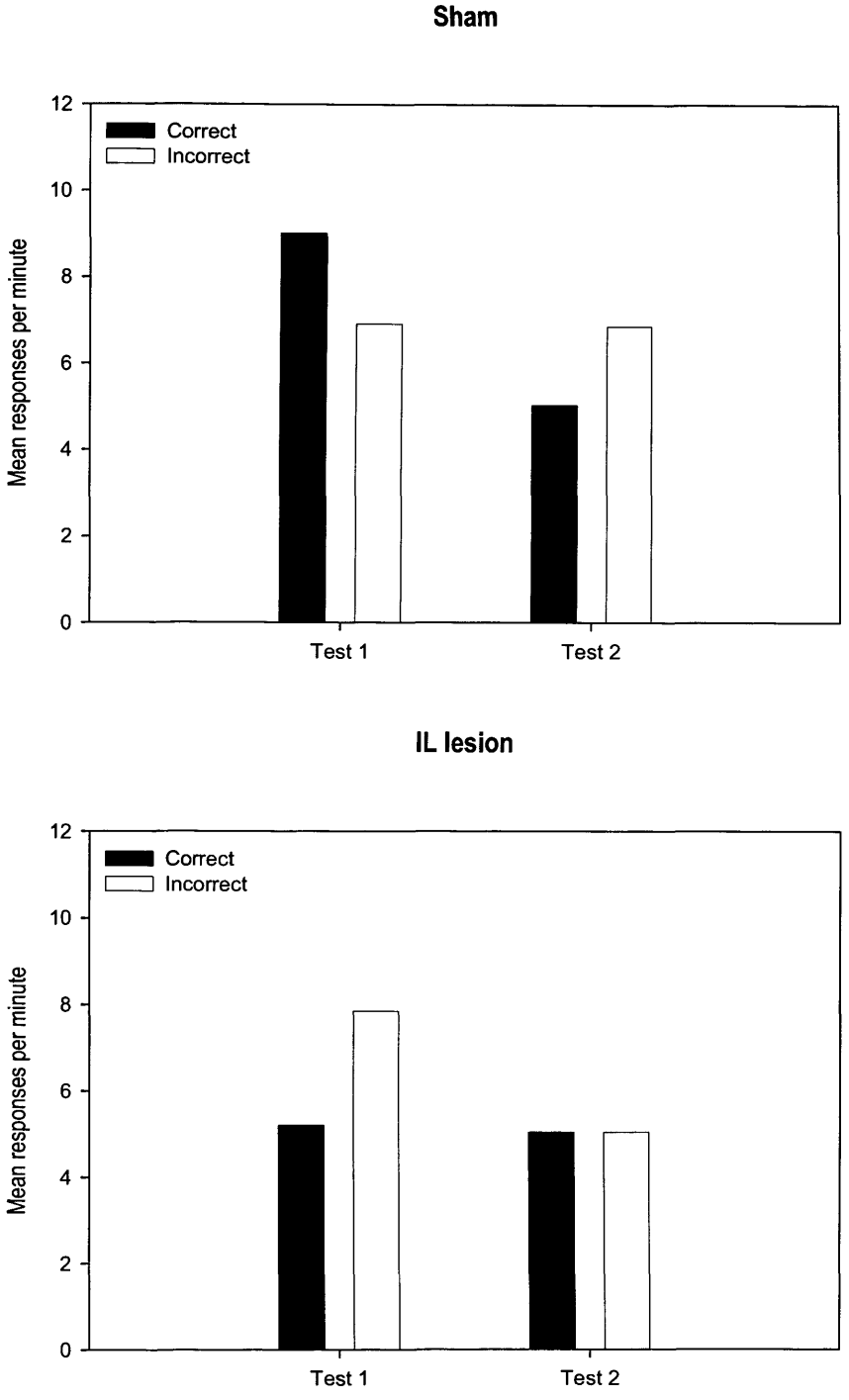

Figure 4.12: The mean rates of responding during each of the 2 probe test trials for animals in sham (top panel) and IL (bottom panel) groups.

4.5 Discussion

The experiments presented in this chapter compared the effect of selective lesions of subregions of the medial prefrontal cortex (prelimbic, anterior cingulate and infralimbic cortices) in behavioural flexibility using an optional shift paradigm and to attempt to tease apart a functional dissociation between these subregions.

The findings of experiment OS 3 provided no evidence to suggest that ACC lesions disrupted acquisition of the conditional discrimination (Phase 1) or acquisition of the Phase 2 discrimination. The results from the test trials showed that ACC lesions did not induce any deficits compared with sham animals. In Test 1, both groups responded more on the correct than on the incorrect lever, whereas in Test 2, both groups were at chance. These results indicate that although both stimulus dimensions were equally relevant to the solution of the discrimination in Phase 2, animals from both groups learned more about the cue that belonged to the stimulus dimension that was relevant in Phase 1. Therefore, ACC lesions did not disrupt changes in attention occurring during the course of discrimination learning. These results are in line with previous findings (Joel et al., 1997a; Ragozzino et al., 1999b; Dias and Aggleton, 2000) and provide further evidence for the selective involvement of the ACC region in rule switching of innate strategy rather than learned strategy.

The results of experiment OS 2 indicate that damage to the PL cortex had no disruptive effect on the acquisition of the conditional discrimination (Phase 1) but slowed down the acquisition in Phase 2 (although the level of performance was significantly reduced in

PL-lesioned animals, they nevertheless showed good discrimination performance). The results from the probe test trials did not reveal any differences between sham-operated and PL-lesioned animals despite the difference observed in Phase 2. In Test 1, both groups responded at chance, whereas in Test 2 they both responded at a higher rate on the correct than on the incorrect lever. Therefore, all animals showed that in Phase 2, cues which belonged to the stimulus dimension that was relevant during Phase 1 had a greater influence on responding than cues from the dimension that was irrelevant during Phase 1. PL lesions did not induce any deficit in attentional changes that result from discrimination learning in rats as a perceptual attentional set was formed.

The results of experiment OS 4 indicate that damage to the IL cortex had no disruptive effect on acquisition of the conditional discrimination (Phase 1) or on acquisition in Phase 2. Although a significant two-way interaction of lesion x session was observed in Phase 2, it appeared that the effect was due to a decrease in the rate of responding on both levers for the IL-lesioned animals in the 5^{th} session, leaving the level of discrimination intact (as the interaction involving lesion and lever factors was not significant). However, the results from the test trials indicated a discrepancy between sham-operated and IL-lesioned animals in their pattern of responding. In Test 1, while sham animals significantly responded more on the correct than on the incorrect lever, IL-lesioned animals presented the reverse pattern of responding, making more incorrect than correct responses (although this pattern of results was observed numerically for IL-lesioned animals subsequent simple effect analyses failed to reach significance for this group).These results showed that lesions of the infralimbic cortex abolished the optional

shift effect. As explained above, the optional design is a test that provides an assessment of the formation and/or maintenance of an attentional set; therefore experiment OS 4 presented evidence that following IL lesions the attentional set is poorly formed or/and maintained.

These results indicate that the medial prefrontal cortex in rats is involved in attentional changes that result from discrimination learning. The observation that the optional shift effect is impaired following IL, but not PL or ACC, damage provides new evidence regarding the involvement of the different subregions of the medial prefrontal cortex in behavioural flexibility. In the light of the findings presented here, a functional distinction between the different subregions of the mPFC can be proposed. On the one hand, the prelimbic cortex appears to be involved in shifting strategy to guide behaviour as lesioning this mPFC subregion results in a retardation to perform ED shifts or to shift strategies compared to sham animals (Joel et al., 1997a; Ragozzino et al., 1999a; Birrell and Brown, 2000; Floresco et al., 2008). On the other hand, the infralimbic cortex appears to be involved in forming or/and maintaining an attentional set, as lesioning this mPFC subregion resulted in disruption of the optional shift effect (experiment OS 4). Previous experiments have suggested an involvement for the prefrontal cortex in behavioural flexibility: excitotoxic lesions of the mPFC (mostly PL with fairly intact IL) induced a deficit in extradimensional shifts whereas intradimensional shifts was spared (Birrell and Brown, 2000). Although some animals suffered damage extending to ACC region, the presence of more damage dorsally was not associated with greater deficits. Ragozzino et al. (1999a; 1999b) have also found similar results. Using a place-response

learning paradigm, they demonstrated that inactivation of the PL/IL areas but not the inactivation of the ACC area impaired cross-modal shifts (learning two shifts from a spatial to a visual-cued version, and vice versa). In a study by Joel et al. (1997a), electrolytic lesions of mPFC centred on the ACC and the PL regions (but sparing the IL area) were found to impair rats' abilities in changing their strategy according to the changes in task demands. All these findings provide support for the role of the PL cortex in shifting but not in forming/maintaining an attentional set; however they do not provide any evidence regarding the role of the IL cortex as in all of these experiments the IL cortex appeared to be spared. Further evidence came from a study by Floresco et al. (2006). In their study they used the task examining behavioural flexibility of response and visual-cue discrimination similar to that described by Ragozzino et al. (1999a). They demonstrated that inactivation of the nucleus accumbens core (NAC core) left the acquisition of visual-cue or response based discrimination intact but severely disrupted the shifting from one strategy to the other. In contrast, inactivation of the nucleus accumbens shell (NAC shell) did not impair acquisition of either discriminations or shifting from one strategy to the other. Anatomically, the NAC has been shown to be widely connected with the prefrontal cortex. However, medial prefrontal cortex targets the basal ganglia nuclei in a topographical manner: axons from the ventral part (IL region) reach the NAC shell whereas axons from more dorsal prefrontal regions (PL and ACC regions) terminate in the NAC core. Results from this study are, therefore, consistent with the proposed role of the PL cortex in behavioural flexibility.

Altogether previous results and present findings have provided evidence for the involvement of the prefrontal cortex in behavioural flexibility and for a functional dissociation between the PL and IL cortices; the PL cortex is in involved attentional set shifting and the IL cortex is involved in the formation and/or maintenance of an attentional set (which might then be regarded as providing the foundation for stimulus-bound behaviour). This parallels Killcross and Coutureau's hypothesis (2003) about the role of the PL and IL regions in coordinating interface between voluntary, guided behaviours and stimulus-bound, habitual response systems. They proposed that PL cortex is involved in the execution of goal-directed behaviour (response-outcome association or R-O) whereas the IL cortex may act to override goal-directed behaviour and permit habit-based behaviour (stimulus-response associations or S-R), and the balance between the two behaviours may possibly be maintained via mutual inhibition between these two regions. To be more specific, they conceived the IL region as possibly inhibiting activity in the PL region and viewed the increasing dominance of habit-based behaviour over goal-directed behaviour as being "mediated by an increasing level of inhibitory control exerted by the infralimbic region over the prelimbic cortex" (Coutureau and Killcross, 2003). Equally, if a situation arose where goal-directed behaviour must be reinstated and reflexive behaviour suppressed, this rapid flexible switching of control should be likely to be carried out by the PL cortex inhibiting either the IL cortex or the reflexive behaviour directly (as the PL and IL regions are not hypothesized to be the location where associations that govern behaviours are stored). Similarly, in the context of behavioural flexibility, the PL and IL cortices have antagonistic roles, with the PL cortex involved in shifting strategy/shifting attentional set and the IL cortex involved in

forming/maintaining an attentional set. However, the nature of interactions (inhibitory control) between these two structures remains unclear. Therefore, it is of interest to further investigate the respective roles of the PL and the IL cortices as well as the nature of the IL/PL interaction using another paradigm that also requires some form of behavioural flexibility.

5 Neurobiological assessment of the latent inhibition effect

5.1 Introduction

The term latent inhibition (LI) was first described by Lubow and Moore (1959). This term refers to the phenomenon in which repeated, non reinforced preexposure of a stimulus retards subsequent conditioning to that stimulus. LI experiments are generally divided into two phases: a preexposure phase in which the to-be-conditioned stimulus is presented without any events of consequence and a conditioning phase in which this same stimulus is paired with the occurrence of an event of consequence (either aversive or appetitive). The latent inhibition effect is a robust phenomenon that can be demonstrated across a wide range of learning paradigms using Pavlovian (Joel et al., 1997b; Pothuizen et al., 2006) as well as instrumental (Killcross et al., 1994a, 1995) conditioning procedures. Since animals eventually learn about the preexposed stimulus, LI demonstrates a form of flexibility where by changes in attention permit conditioning to a previously irrelevant stimulus.

Theoretical accounts have suggested that LI is due to reduced associability (Mackintosh, 1975) or conditioned inattention (Lubow, 1989) to the preexposed stimulus. Other theories, however, have postulated that an expression rather than an acquisition deficit underlies LI (Kasprow et al., 1984; Joel et al., 1997b; Weiner and Feldon, 1997). An expression deficit represents the competition for behavioural expression between the stimulus-no event association acquired in preexposure and the stimulus-reinforcement association acquired in conditioning. Experiments using a range of training procedures

(Bouton and Swartzentruber, 1989; Hall and Honey, 1989) have routinely found that the retardation of conditioning produced by prior exposure to the to-be-conditioned stimulus is attenuated or even abolished when conditioning takes place in a context other than that used for preexposure. Therefore, this provides evidence that LI is context dependent and that in the preexposure phase, the stimulus-no event association receives a context modulatory influence. Furthermore, these findings also support the idea that LI reflects competition at point of behavioural expression.

In neural terms, LI has been found to be disrupted in rats with lesions of a variety of brain regions including the basolateral amygdala (Coutureau et al., 2001; Schiller and Weiner, 2004), the nucleus accumbens shell (Weiner et al., 1996) and elements of the hippocampal formation (Joel et al., 1997b). Other studies, however, have reported an enhanced LI effect following lesions of the nucleus accumbens shell (Pothuizen et al., 2006) and lesions of the hippocampal formation (Purves et al., 1995). All these regions are known to interact with the medial prefrontal cortex. A common prediction, therefore, is that lesions of the mPFC should affect LI. However, experimental findings have failed to show such an effect. Large excitotoxic lesions, as well as electrolytic lesions, of the mPFC (including PL, ACC and IL or PL and ACC) have been found to have no impact on LI in rats (Joel et al., 1997b; Lacroix et al., 2000; Schiller and Weiner, 2004). Despite the reported absence of any effect in these studies, they may nevertheless provide some evidence that lesions to the mPFC result in an enhanced LI effect (Joel et al., 1997b; Lacroix et al., 2000). Studies that selectively compare selective lesions of the mPFC (specifically PL and IL cortices) have rarely been conducted. Since LI is disrupted by

lesions of the nucleus accumbens shell and the hippocampus and these have strong connectivity with the IL region (much greater that the adjacent PL region) it is likely that selective IL lesions might result in some effect on LI. Although this might be a disruption, it might also be an enhancement of LI. In fact, the results presented by Joel et al. (1997b) do suggest that IL lesions might lead to an enhancement of LI, even though this was not discussed in their articles.

In this chapter, a series of experiments is presented examining the nature of the involvement of the mPFC in latent inhibition. First, the effect of large prefrontal cortex lesions on LI was studied in order to replicate previous findings. Secondly, selective lesions of the mPFC subregions were investigated to follow up the dissociable IL/PL effects seen in the previous chapter. A conditioned suppression procedure was used; broadly similar to that previously used by Killcross and Robbins (1993) and Killcross et al. (1994b; 1994a), employing a within-subject on-baseline procedure that allows tracking of the development of LI across conditioning trials.

Within the framework of the results presented in the previous chapter, and bearing in mind the proposed roles of the PL and IL subregions of the mPFC in behavioural flexibility and the contextual nature of the latent inhibition effect, predictions of the effect on LI following IL and PL discrete lesions can made. Since the IL cortex is assumed to be involved in the formation and/or the maintenance of an attentional set, it might be expected that following IL lesions the LI effect would be enhanced. Working on the assumption that attention is automatically allocated to a new cue and declines over the

course of preexposure, that the IL cortex mediates maintenance of attentional set is responsible for the gradual nature of the decline, then loss of the IL cortex would result in a more dramatic decline in attention during preexposure and hence to enhance LI. Conversely, PL lesions might be expected to reduce or abolish the LI effect, as this subregion is thought to be involved in learning new rules which are context dependant. That is, to the extent that the PL cortex is responsible for driving down attention to the irrelevant cue during preexposure a reduction in LI would be anticipated during the preexposure phase, attention paid to the preexposed cue should remain relatively high in the absence of this subregion (because of the IL role), resulting in a better subsequent conditioning of this preexposed cue.

5.2 Experiment LI 1: effect of large mPFC lesions on latent inhibition

In the light of the vague nature of published data (reported in the introduction of this chapter), the experiment LI 1 was conducted to determine whether or not large medial prefrontal lesions would affect latent inhibition and if so, what would be the nature of the effect (reduction/abolition or enhancement).

5.2.1 Method

Subjects

The subjects were 32 naïve male Lister Hooded rats with a mean a*d-libitum* weight of 360g (range 330-402g). Prior to the start of the experiment sixteen of the rats received bilateral excitotoxic lesions of the medial prefrontal cortex and the remaining sixteen

served as sham-operated controls. Following recovery, the rats were reduced to 85% of their *ad libitum* weights and were maintained at this level throughout the experiment by being fed a limited quantity of food following each day's training. The rats had free access to water in their home cages. They were housed in pairs in a light-proof holding room maintained on a 12h light/dark cycle (7 am to 7 pm). The subjects were tested on successive days, at the same time, during the period that the lights were on in their holding room.

Surgery &Histology

Rats were first anaesthetized with Isoflurane, their heads shaved, and placed in a stereotaxic frame (Kopf Instruments, Tujunga, CA). An incision was made in the scalp and then a skull flap overlying the prefrontal cortex was drilled out. Injections of ibotenic acid (63m M, Sigma-Aldrich Co. Ltd., UK.) were made using a 2 µl Hamilton syringe (Reno, NV) mounted on an injection pump (KDS310 Scientific). Automated injections of 0.5 µl at a rate of 0.1 µl /min were made at four sites within the medial prefrontal cortex (AP: +2.7; ML: ± 0.7; DV: −4.5 and AP: +3.7; ML: ± 0.7; DV: −4.0; Haddon and Killcross, 2006). Following each injection the needle was left in position for 5 min to allow absorption of the bolus and to minimize spread of the toxin into other brain regions. Sham-operated controls received an identical procedure with the exception that no toxin was infused. Histological procedure was as described in experiment OS 2.

Apparatus

Eight identical standard operant chambers were used; they are described in detail in experiment OS 1. For the purpose of the latent inhibition experiments, the floor of each box was connected to a shock generator that, when appropriate, delivered a scrambled shock (0.3 mA or 0.5 mA) for 0.5s. The delivery and intensity of shocks used was controlled via MED-PC software.

Behavioural procedure

Pretraining: magazine and lever press training

Prior to being used in the current procedure, animals participated in an appetitive instrumental conditioning experiment (data not presented in this thesis), therefore they did not receive magazine or lever press training. Although animals were previously exposed to auditory stimuli, it was the case that animals were counterbalanced with respect to their treatment in the previous experiment and stimuli were chosen to be maximally dissimilar to those experienced.

Preexposure

The design of the experiment is shown in Table 5.1 Animals were trained for six consecutive days and received one session per day. Each of the first five sessions of preexposure lasted 48 minutes and consisted of six, 30s presentations of a preexposed stimulus (PE) that was either a 4 kHz tone or a 20 Hz clicker, separated by a 8min inter trial interval. One lever was present throughout preexposure, and training and responses made on this lever were reinforced according to an RI 60s schedule. On the sixth session

of preexposure (unconditioned suppression phase) animals received six, 30s presentations of the PE stimulus and at the end of the session they received two, 30s presentations of the non-preexposed (NPE) stimulus; therefore, this session lasted 64 minutes. This unconditioned suppression session was introduced in order to remove the initial disruption of lever pressing and magazine approaching observed during presentation of the novel (NPE) stimulus (Killcross and Robbins, 1993; Killcross et al., 1994b, 1994a) and also provided a demonstration that animals could distinguish between the stimuli used.

Conditioning

In the conditioned suppression phase, rats were trained for five consecutive days and received one session per day. Each session lasted 48 minutes and consisted of four, 30-s presentations of the conditional stimuli (two of PE and two of NPE with a random order of presentation) terminating with the delivery of a mild foot-shock (0.3 mA, 0.5s) and each separated by a twelve-minute ITI. After the last stimulus-shock pairing, the rats were left in the operant chamber for six minutes before being returned to their home cage. During the 30-s of stimulus presentation and the 30-s prior to the stimulus presentation periods (CS and pre-CS periods, respectively), two measures of conditioning were recorded: (i) responses on the lever and (ii) magazine approaches. Suppression ratios for both measures were calculated according to the formula CS/(CS + preCS).

Preexposure (5 sessions)	**Unconditioned Suppression** (1 session)	**Conditioned Suppression** (5 sessions)
	PE (4 x 30s)	PE → shock (2 x 30s)
PE (6 x 30s)	NPE (2 x 30s)	NPE → shock (2 x 30s)

Table 5.1: Experimental design for all animals
PE was preexposed stimulus and NPE was the non-preexposed stimulus Stimuli were either a 4 kHz tone or a 20 Hz clicker. Design fully counterbalanced across animals.

5.2.2 Histological results

Contrary to the OS experiments (where the aim was to make discrete lesion to a specific structure), the aim of this experiment was to investigate the impact of large lesion of the medial prefrontal cortex, including prelimbic, infralimbic and anterior cingulate cortices. Therefore, any animals that showed damage to more than 60% of these regions were kept for this experiment. Post-histology, all animals were presented this extent of damage and therefore the final number of rats of each lesion type was 16 mPFC-lesioned and 16 sham-operated rats. Figure 5.1 depicts the maximum (striped region) and minimum (grey region) extent and location of the damage in the medial prefrontal cortex for the lesioned animals. Photomicrographs of the lesions and diagrams labelled with the region of interest are presented in the annexes.

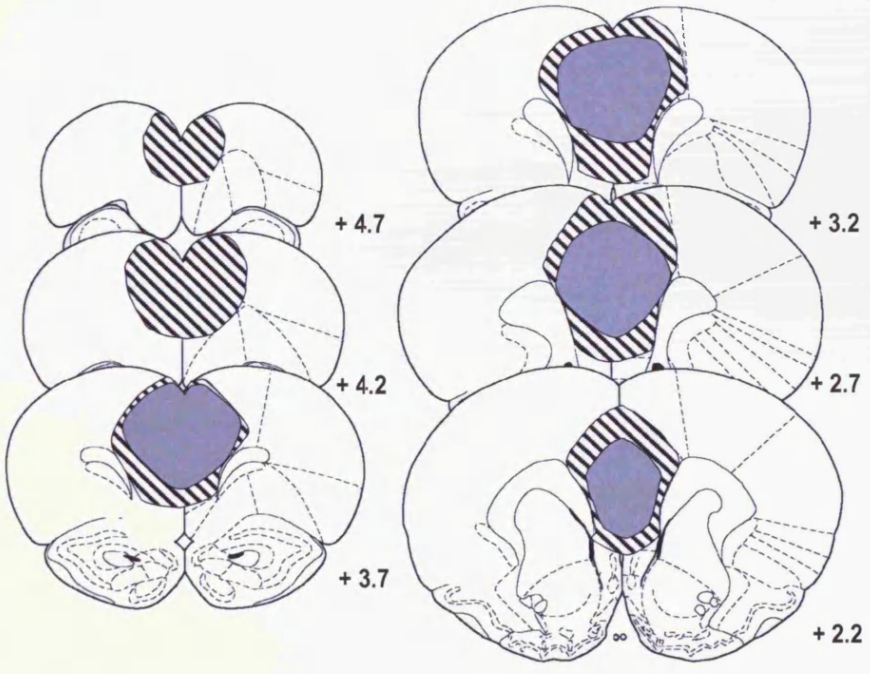

Figure 5.1: Representation of the minimum (grey) and maximum (striped) extent and location of damage within the medial prefrontal cortex. Outlines are reproduced from Paxinos and Watson (1998) and represent sections ranging from 2.7 to 4.7 anterior to bregma.

5.2.3 Behavioural results

All statistical tests are evaluated with respect to an alpha level of 0.05.

Preexposure phase

The means of suppression ratio of the preexposed stimulus across 6 sessions for lever responses and magazine approaches were calculated for both sham-operated and mPFC-lesioned animals: means of suppression ratio of lever responses were 0.53 and 0.54, respectively; and means of suppression ratio of magazine approaches were 0.57 and 0.55, respectively. A mixed ANOVA with the between-subjects factors of lesion (sham, mPFC) and of stimulus (tone, clicker) on the mean of suppression ratio of PE across 6 sessions did not revealed any significant effects or interactions for lever responses (max $F(1, 28) = 3.47$, $MSE = 0.01$) or for magazine approaches (max $F(1, 28) = 1.289$, $MSE = 0.01$).

The same calculation was made for the non-preexposed stimulus (NPE) on the 6^{th} session for both sham-operated and mPFC-lesioned animals: means of suppression ratio of lever responses were 0.30 and 0.41 in the first trial and, 0.49 and 0.59 in the second trial, respectively. The means of suppression ratio of magazine approaches were 0.26 and 0.38 in the first trial and, 0.62 and 0.50 in the second trial, respectively. Mixed ANOVAs with the between-subjects factors of lesion (sham, mPFC), of trial (Trial 1, Trial 2) and of stimulus (tone, clicker) on the mean of suppression ratio NPE on the 6^{th} session only revealed a significant effect of trial for lever responses ($F(1, 28) = 13.19$, $p < 0.001$, $MSE = 0.04$) and for magazine approaches ($F(1, 28) = 10.6$, $p < 0.01$, $MSE = 0.09$). No other

effect or interactions were significant (all Fs < 1). The slight decrease in suppression ratio observed in Trial 1 confirms that the animals could distinguish the stimuli used as preexposed and non pre-exposed cues.

Baseline rates

Baseline rates of lever pressing and magazine approaches in the sham-operated and mPFC lesioned animals were not significantly different. The mean rates of lever pressing (LP) and magazine approach (MA) during the 30-s period immediately prior to CS onsets (pre-CS period) across all conditioning trials were respectively LP/min: sham: 11.47 and mPFC: 10.92; MA/min: sham: 6.44 and mPFC: 6.47. Mixed ANOVA with the between subject factor of lesion (sham, mPFC) and the within-subject factor of exposure (PE, NPE) revealed that neither the main effect of lesion and exposure nor their interaction were significant for either lever pressing or magazine approach (all Fs < 1).

Conditioned suppression phase

The mean suppression ratios of the lever responses during presentation of PE and NPE stimuli across conditioning for both sham-operated and mPFC-lesioned animals are shown in Figure 5.2. Both groups showed a latent inhibition effect (higher suppression ratio for PE than for NPE), although this effect seemed to be bigger in mPFC-lesioned animals. A mixed ANOVA with the between-subjects factor of lesion (sham, mPFC) and the within-subject factor of exposure (NPE, PE) confirmed these observations. This analysis revealed a significant effect of exposure ($F(1, 30) = 10.82$, $p < 0.01$, MSE = 0.01) as well as a trend although the interaction of lesion x exposure failed to reach

conventional levels of probability for the rejection of the null hypothesis ($F(1, 30) = 3.65$, $p = 0.066$, $MSE = 0.01$).

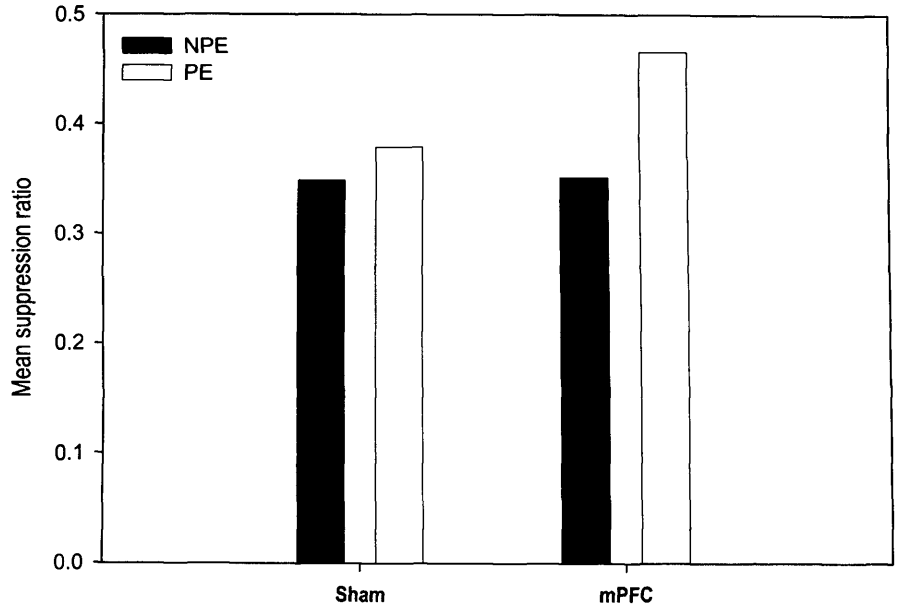

Figure 5.2: The mean of suppression ratios of the lever responses during presentation of PE and NPE stimulus across conditioning for both sham-operated (left) and mPFC-lesioned (right) animals.

The mean of suppression ratios of the magazine approaches during presentation of PE and NPE stimulus across conditioning for both sham-operated and mPFC-lesioned animals are presented in Figure 5.3. Animals with mPFC lesion showed a latent inhibition effect, showing more suppression when the NPE stimulus was presented than when PE stimulus was presented. For sham-operated animals, suppression ratios appeared to be similar for NPE and PE, therefore they did not demonstrate a latent inhibition effect. These observations were confirmed by the results of a mixed ANOVA with the between-subjects factor of lesion (sham, mPFC) and the within-subject factor of

exposure (NPE, PE). This analysis revealed a significant effect of exposure ($F(1, 30) = 5.05$, $p < 0.05$, $MSE = 0.01$) as well as a significant interaction of lesion x exposure ($F(1, 30) = 8.05$, $p < 0.001$, $MSE = 0.01$). Subsequent simple effect analysis on the lesion x exposure interaction found a significant effect of exposure for mPFC-lesioned animals ($F(1, 30) = 13.24$, $p < 0.001$, $MSE = 0.01$) but not for sham-operated animals ($F < 1$). This analysis also revealed a significant effect of lesion on NPE stimulus ($F(1, 60) = 7.06$, $p < 0.01$, $MSE = 0.02$) but not on PE ($F(1, 60) = 0.44$, $p > 0.05$, $MSE = 0.02$).

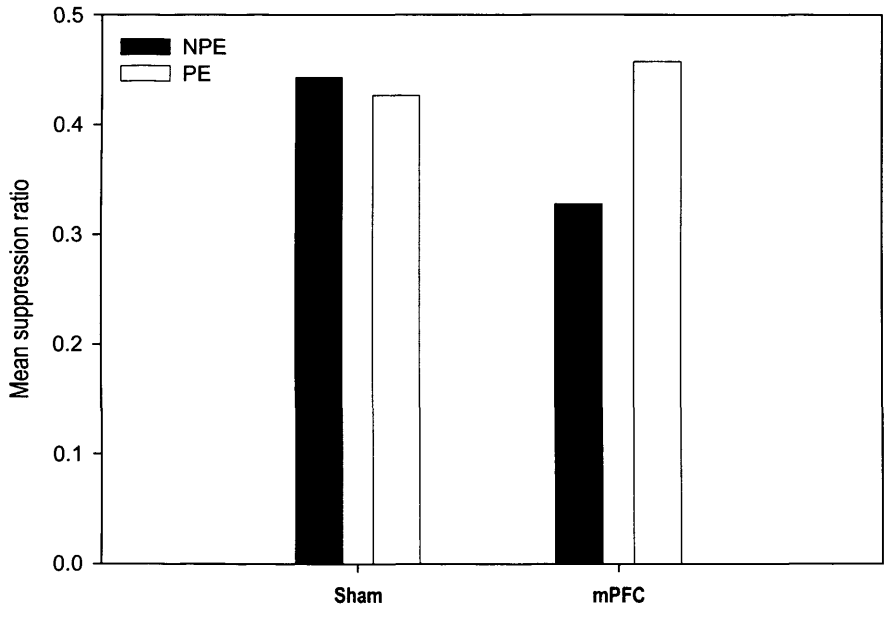

Figure 5.3: The mean of suppression ratios of magazine approaches during presentation of PE and NPE stimulus across conditioning for both sham-operated (left) and mPFC-lesioned (right) animals.

5.3 Experiment LI 2: effect of PL lesions on latent inhibition

5.3.1 Method

Subjects

The subjects were 32 naïve male Lister Hooded rats with a mean a*d-libitum* weight of 328g (range 305-358g). Before the onset of the experiment sixteen of the rats received bilateral excitotoxic lesions of the prelimbic cortex and the remaining sixteen served as sham-operated controls. After recovery, the rats were maintained and housed as described for experiment LI 1.

Surgery & histology

Injections of ibotenic acid (63m M, Sigma-Aldrich Co. Ltd., UK.) were made using a 2 µl Hamilton syringe (Reno, NV) at two sites within the prelimbic cortex (AP: +3.2; ML: ± 0.6; DV: −4.0, as previously used in Cardiff BNL). All other details about surgical and histological procedures were as described for experiment LI 1.

Apparatus

Eight standard operant chambers were used; they were as described in detail in experiment LI 1.

Behavioural procedure

Pretraining: magazine and lever press training

On the first day of training, animals received a session of magazine training learning to retrieve pellets from the magazine. This session lasted 48min and pellets were delivered on average every 120s. Following this, they were moved onto lever press training (three sessions). During each of these sessions, only one lever (always the same) was constantly inserted and reward was delivered upon lever press made. On the first day of lever press training rats were rewarded on a continuous reinforcement schedule; this was altered on the second day to a RI 15s schedule and on the 3rd day it was altered to a RI 30s schedule. Session duration was 48min.

Preexposure

Experimental details of the preexposure and unconditioned suppression phases were identical to those in experiment LI 1.

Conditioning

Experimental details of the conditioning phase were identical to those in experiment LI 1 with the exception that (i) the intensity of the shock was increased to 0.5 mA and (ii) that animals received only two presentations of CS-shock pairing (CS duration 30s – one for PE and one for NPE) during each of the six sessions of the conditioning phase.

5.3.2 Histological results

As the aim of this experiment was to investigate the impact of selective lesions of the prelimbic cortex, any animals that did show damage to surrounding regions (anterior

cingulate and infralimbic cortices) were removed from this experiment. Therefore, four animals were removed from the PL group due to unintended damage outside the prelimbic cortex. Three more animals were removed due to the small extent of damage within the PL cortex i.e due to sparing within PL. Post-histology, the final number of rats of each lesion type was 9 PL-lesioned and 16 sham-operated rats. Figure 5.4 illustrates the maximum (striped region) and minimum (grey region) extent and location of the damage in the PL cortex for the remaining animals. Photomicrographs of the lesions and diagrams labelled with the region of interest are presented in the annexes.

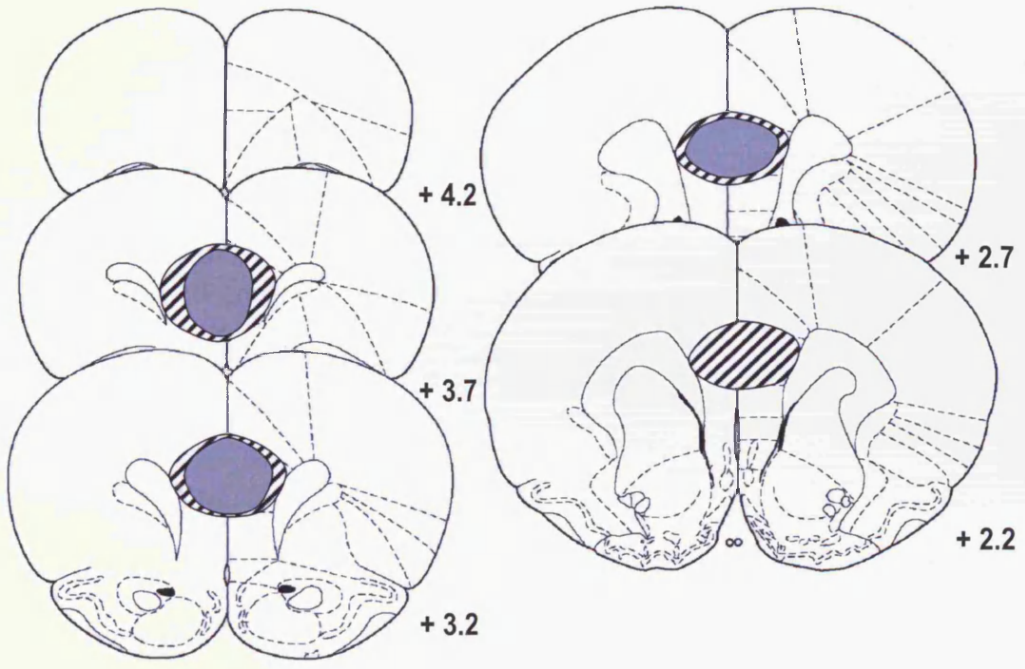

Figure 5.4: Representation of the minimum (grey) and maximum (striped) extent and location of damage within the prelimbic cortex. Outlines are reproduced from Paxinos and Watson (1998) and represent sections ranging from 2.7 to 4.7 anterior to bregma.

5.3.3 Behavioural results

Preexposure phase

The mean suppression ratios to the preexposed stimulus across 6 sessions for sham-operated and PL-lesioned animals were calculated for both lever responses and magazine approaches: mean suppression ratios for sham 0.49 and 0.50, respectively; and mean suppression ratios for PL-lesioned animals were 0.49 and 0.53, respectively. A mixed ANOVA with the between-subjects factors of lesion (sham, PL) and of stimulus (tone, clicker) on the mean of suppression ratio of PE across 6 sessions did not reveal any significant effects or interactions for lever pressing (max $F(1, 21) = 1.65$, $MSE = 0.01$) or for magazine approach (all $Fs < 1$) measures.

The same calculation was made for the non-preexposed stimulus on the 6^{th} session for both sham-operated and PL-lesioned animals: means of suppression ratio of lever responses were 0.21 and 0.22 in the first trial and, 0.42 and 0.43 in the second trial, respectively. The means of suppression ratio of magazine approaches were 0.25 and 0.22 in the first trial and, 0.51 and 0.35 in the second trial, respectively. Mixed ANOVA with the between-subjects factors of lesion (sham, PL), of trial (Trial 1, Trial 2) and of stimulus (tone, clicker) on the mean of suppression ratio NPE on the 6^{th} session only revealed a significant effect of trial for lever responses ($F(1, 21) = 9.59$, $p < 0.001$, $MSE = 0.05$) and for magazine approaches ($F(1, 21) = 7.84$, $p < 0.01$, $MSE = 0.05$). No other effect or interactions were significant (all $Fs < 1$). The slight decrease in suppression ratio observed in Trial 1 confirms that the animals could distinguish the stimuli used as preexposed and non pre-exposed cues.

Baseline rates

Baseline rates of lever pressing and magazine approaches in the sham-operated and PL-lesioned animals were not significantly different. The mean rates of lever pressing and magazine approaches during the 30s period immediately prior to CS onsets (pre-CS period) across all conditioning trials were respectively LP/min: sham: 14.14 and PL: 12.82; MA/min: sham: 10.21 and PL: 9.93. Mixed ANOVA with the between subject factor of lesion (sham, PL) and the within-subject factor of exposure (PE, NPE) revealed that neither the main effects of lesion and exposure nor their interaction were significant for either lever pressing or magazine approaches (max $F(1, 23) = 1.76$, $p > 0.1$, MSE = 17.28).

Conditioned suppression phase

The mean suppression ratios of the lever responses during presentation of PE and NPE stimulus across conditioning for both sham-operated and PL-lesioned animals are presented in Figure 5.5. In both sham and PL groups, a higher suppression ratio was observed for the PE compared with the NPE. A mixed ANOVA with the between-subjects factor of lesion (sham, PL) and the within-subject factor of exposure (NPE, PE) confirmed this observation. This analysis revealed only a significant effect of exposure ($F(1, 23) = 8.27$, $p < 0.01$, MSE = 0.01). Neither the lesion factor nor the interaction of lesion x exposure reached significance (max $F(1, 23) = 1.16$, MSE = 0.01).

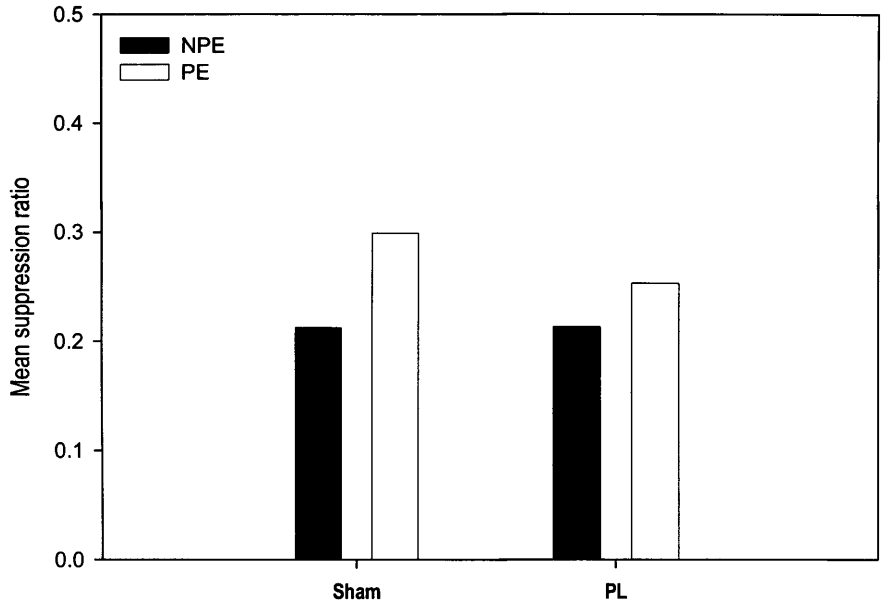

Figure 5.5: The mean suppression ratios of the lever responses during presentation of PE and NPE stimulus across conditioning for both sham-operated (left) and PL-lesioned (right) groups.

The mean suppression ratios of the magazine approaches during presentation of PE and NPE stimulus across conditioning for both sham-operated and PL-lesioned animals are shown in Figure 5.6. Animals from both sham and PL groups demonstrated a latent inhibition effect, showing more suppression when the NPE stimulus was presented than when PE stimulus was presented. Results from a mixed ANOVA analysis with the between-subjects factor of lesion (sham, PL) and the within-subject factor of exposure (NPE, PE) confirmed this observation. This analysis only revealed a significant effect of exposure ($F(1, 23) = 4.51$, $p < 0.05$, $MSE = 0.01$). Neither the lesion factor nor the interaction of lesion x exposure reached significance ($Fs < 1$).

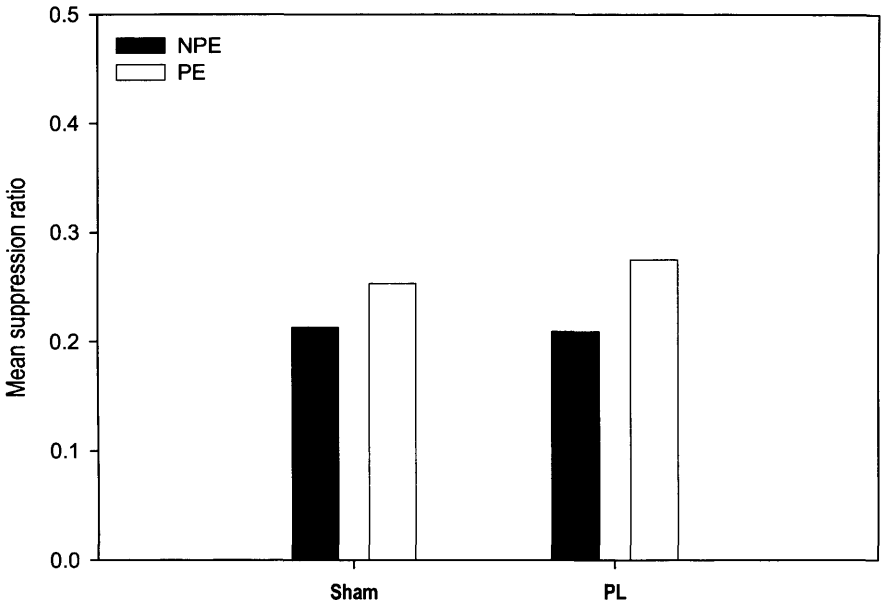

Figure 5.6: The mean of suppression ratios of magazine approaches during presentation of PE and NPE stimulus across conditioning for both sham-operated (left) and PL-lesioned (right) animals.

5.4 Experiment LI 3: effect of IL lesions on latent inhibition

5.4.1 Method

Subjects

The subjects were 32 naïve male Lister Hooded rats with a mean a*d-libitum* weight of 325g (range 305-356g). Before the onset of the experiment sixteen of the rats received bilateral excitotoxic lesions of the infralimbic cortex and the remaining animals served as sham-operated controls. After recovery, the rats were maintained and housed as described for experiment LI 1.

Surgery & histology

Injections of ibotenic acid (63m M, Sigma-Aldrich Co. Ltd., UK.) were made using a 2μl Hamilton syringe at two sites within the infralimbic cortex (AP: +2.6; ML: ± 0.6; DV: −5.4). All other details about surgical and histological procedures were as described for experiment LI 1.

Apparatus & behavioural procedure

Details about apparatus used and behavioural procedure were exactly as described in experiment LI 2.

5.4.2 Histological results

Since the aim of the experiment was to investigate the impact of selective lesions of the infralimbic cortex on LI, any animals that did show damage to surrounding regions (prelimbic cortex) or only slight damage to the IL cortex were removed from this experiment; consequently, four animals were removed from the IL group. Post-histology, the final number of rats of each lesion type was 12 IL-lesioned and 16 sham-operated rats. Figure 5.7 depicts the maximum (striped region) and minimum (grey region) extent and location of the damage in the infralimbic cortex for the remaining animals. Photomicrographs of the lesions and diagrams labelled with the region of interest are presented in the annexes.

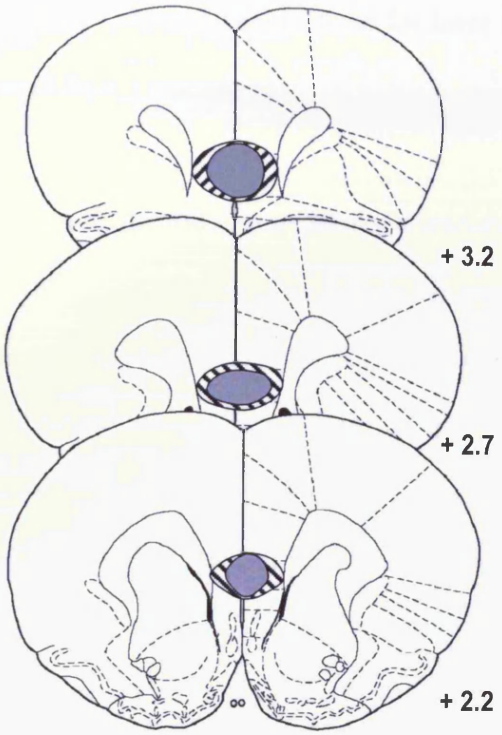

Figure 5.7: Representation of the minimum (grey) and maximum (striped) extent and location of damage within the infralimbic cortex. Outlines are reproduced from Paxinos and Watson (1998) and represent sections ranging from 2.2 to 3.2 anterior to bregma.

5.4.3 Behavioural results

Preexposure phase

The mean suppression ratios to the preexposed stimulus across 6 sessions for lever responses and magazine approaches were calculated for both sham-operated and IL-lesioned animals: mean suppression ratios of the lever response were 0.48 and 0.49, respectively; and mean of suppression ratios of magazine approaches were 0.50 and 0.50, respectively. A mixed ANOVA with the between-subjects factors of lesion (sham, IL) and of stimulus (tone, clicker) on the mean of suppression ratio of PE across 6 sessions

did not revealed any significant effects or interactions for lever responses (all Fs < 1) or for magazine approaches (all Fs < 1) measures.

The same calculation was made for the non-preexposed stimulus on the 6th session for both sham-operated and IL-lesioned animals: means of suppression ratio of lever responses were 0.21 and 0.22 in the first trial and, 0.42 and 0.39 in the second trial, respectively. The means of suppression ratio of magazine approaches were 0.25 and 0.19 in the first trial and, 0.51 and 0.35 in the second trial, respectively. Mixed ANOVAs with the between-subjects factors of lesion (sham, IL), of trial (Trial 1, Trial 2) and of stimulus (tone, clicker) on the mean of suppression ratio NPE on the 6th session only revealed a significant effect of trial for lever responses ($F(1, 24) = 15.141$, $p < 0.001$, $MSE = 0.03$) and for magazine approaches ($F(1, 24) = 14.86$, $p < 0.001$, $MSE = 0.06$). No other effect or interactions were significant (all Fs < 1). The slight decrease in suppression ratio observed in Trial 1 confirms that the animals could distinguish the stimuli used as preexposed and non pre-exposed cues.

Baseline rates

Baseline rates of lever pressing and magazine approach in the sham-operated and IL-lesioned animals were not significantly different. The mean rates of lever pressing and magazine approach during the 30s period immediately prior to CS onsets (pre-CS period) across all conditioning trials were respectively LP/min: sham: 14.41 and IL: 13.63; MA/min: sham: 10.21 and IL: 9.77. Mixed ANOVA with the between subject factor of lesion (sham, IL) and the within-subject factor of exposure (PE, NPE) revealed that

neither the main effect of lesion and exposure nor their interaction were significant for either lever pressing or magazine approaches (max $F(1, 26) = 1.45$, $p > 0.1$, $MSE = 3.53$).

Conditioned suppression phase

The mean suppression ratios of the lever responses during presentation of PE and NPE stimulus across the 6 sessions of conditioning for both sham-operated and IL-lesioned animals are shown in Figure 5.8. A latent inhibition effect (higher suppression ratio for the PE compare with the NPE) was evident in both sham and IL groups.

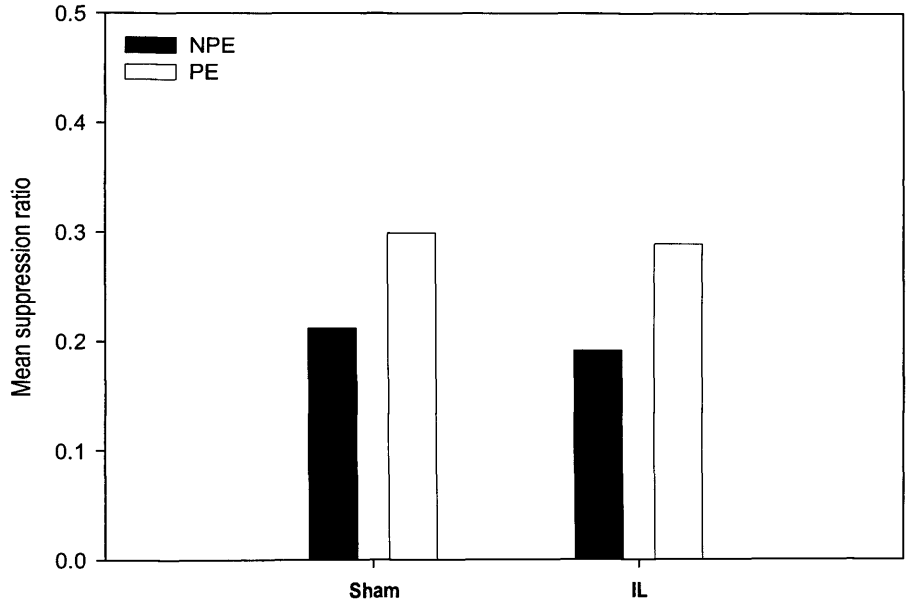

Figure 5.8: The mean suppression ratios of the lever responses during presentation of PE and NPE stimulus across conditioning for both sham-operated (left) and IL-lesioned (right) groups.

A mixed ANOVA analysis with the between-subjects factor of lesion (sham, IL) and the within-subject factor of exposure (NPE, PE) confirmed this observation. This analysis

revealed only a significant effect of exposure ($F(1, 26) = 12.37$, $p < 0.01$, $MSE = 0.01$). No other effects or interaction were significant (max $F(1, 26) = 2.27$, $MSE = 0.01$).

The mean suppression ratios of the magazine approaches during presentation of PE and NPE stimulus across conditioning for both sham-operated and IL-lesioned animals are shown in Figure 5.9. Animals from both sham and IL groups seemed to demonstrate a latent inhibition effect; however this effect appeared much bigger in IL-lesioned animals

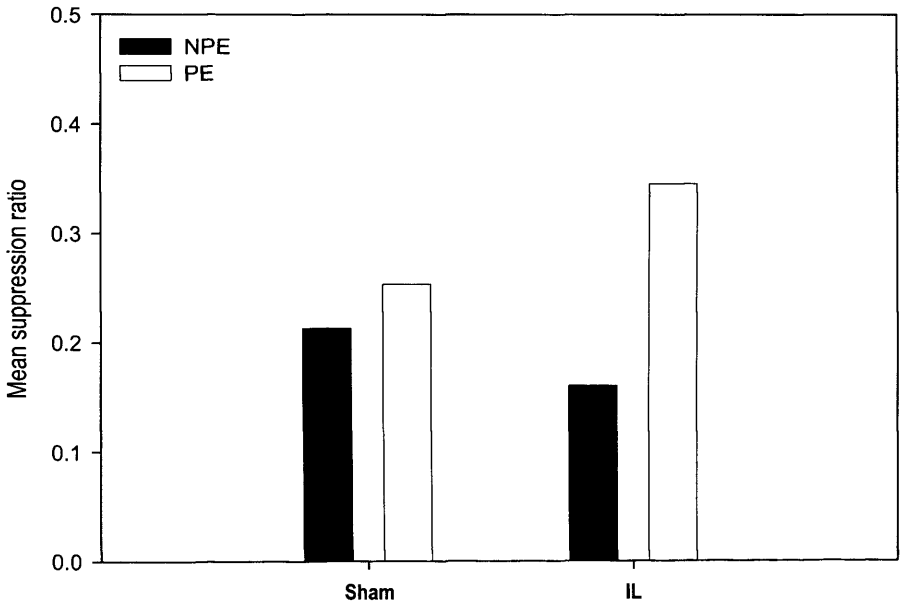

Figure 5.9: The mean suppression ratios of magazine approaches during presentation of PE and NPE stimulus across conditioning for both sham-operated (left) and IL-lesioned (right) groups.

A mixed ANOVA analysis with the between-subjects factor of lesion (sham, IL) and the within-subject factor of exposure (NPE, PE) revealed a significant effect of exposure ($F(1, 26) = 15.49$, $p < 0.001$, $MSE = 0.01$) as well as a significant two-way interaction of

lesion x exposure ($F(1, 26) = 4.65$, $p < 0.05$, MSE = 0.01). The factor of lesion did not reach significance ($F < 1$). Analysis of simple effects produced by the interaction of lesion x exposure indicated a significant effect of exposure for IL-lesioned animals ($F(1, 26) = 18.46$, $p < 0.0001$, MSE = 0.01) but not for sham-operated animals ($F(1, 26) = 1.60$, $p > 0.1$, MSE = 0.01). This analysis also revealed a significant effect of lesion on responding during the PE stimulus ($F(1,52) = 4.17$, $p < 0.05$, MSE = 0.01) but not the NPE stimulus ($F(1,52) = 0.46$, $p > 0.05$, MSE = 0.01).

5.5 Discussion

The experiments presented in this chapter investigated the effect of large medial prefrontal cortex lesions and selective lesions of the prelimbic and infralimbic cortices on aversive latent inhibition paradigm in an attempt to tease apart a functional dissociation between these subregions. Moreover, these lesions studies aimed to further investigate the role of these mPFC subregions in behavioural flexibility.

The results from experiment LI 1 indicate that all animals showed an effect of latent inhibition, presenting a higher suppression for the PE than for the NPE cue. However, mPFC-lesioned animals demonstrated a greater latent inhibition effect compared to sham-operated animals. This effect was significant for conditioned response (CR) of magazine approaches and almost significant in the case of lever-pressing. These results show that large mPFC lesions (encompassing ACC, PL and IL cortices) resulted in an enhancement of the latent inhibition effect.

The results for experiment LI 2 indicate that selective PL lesions did not result in any effect on LI. All animals (sham-operated and PL-lesioned animals) showed a LI effect, with a greater suppression of responding when the non-preexposed cue was presented than when the preexposed cue was presented for both CRs measured.

The results from experiment LI 3 demonstrate that selective IL lesions resulted in an enhancement of the LI effect compared with sham-operated animals. Furthermore, analysis of simple effects produced by the interaction of lesion x exposure indicated a significant effect of lesion on responding to the PE but not to the NPE cue. The effect of IL lesions on the latent inhibition effect was only significant for the magazine approach behaviour. Although the same pattern of results was observed numerically for lever pressing, the interaction of lesion x exposure failed to reach significance. It is perhaps surprising that the enhancement of the LI effect following IL lesions was only significant for magazine approaches and not for lever pressing. It is possible, however, that the design chosen for these experiments was such that the CR lever pressing was not sensitive to the LI effect. Indeed, development of an LI effect relies on various factors such as exposure, lever press training and shocks intensity; and a more appropriate design would reveal a LI effect in both CRs.

These results are the first demonstration that lesions of the medial prefrontal have a significant effect on latent inhibition. Moreover, based on the results of experiment LI 3, the effect observed with large lesions of the mPFC (experiment LI 1) appears to be primarily due to the damage of the IL region. However, whereas the LI effect was

significantly enhanced for magazine approaching responses, only a trend was observed for lever-press responses for both large mPFC and selective IL lesions. These results are broadly in line with previous findings (Joel et al., 1997b; Lacroix et al., 2000). Although the authors did not claim any effect, examination of their data suggests that mPFC and selective IL lesions may have enhanced LI (Figures 5 and 6 for Joel et al. – Figure 4 for Lacroix et al.). This absence of significant effect in their studies could be due to the use of an off-baseline procedure. That is, conditioning and measurement of latent inhibition occurred at different times. In the design used here, conversely, the latent inhibition effect was observed on-baseline (conditioning and measurement of the latent inhibition effect occurred in the same phase. Therefore, the present design is likely to be more sensitive and hence more likely to reveal any effect of lesions on LI. As explained in the introduction to the current chapter, a number of brain regions including the basolateral amygdala, the NAC shell and elements of the hippocampal formation (Weiner et al., 1996; Joel et al., 1997b; Coutureau et al., 2001) are believed to assume a critical role in the development and expression of LI. Moreover, all these regions are known to interact with the medial prefrontal cortex. Studies have provided evidence for an enhanced LI following lesions of the NAC shell (Pothuizen et al., 2006) and lesions of the hippocampal formation (Purves et al., 1995), regions well known to interact with the IL subregion of the mPFC.

In the introduction to this chapter, it was predicted that IL lesions would enhance LI effect whereas PL lesions would reduce or abolish the LI effect. Such predictions were based on the following assumptions: (i) if it is assumed that attention is automatically

allocated to a new cue and declines over the course of preexposure and that IL mediates maintenance of attentional set is responsible for the gradual nature of the decline, then loss of the IL cortex would result in a more dramatic decline in attention to the PE cue; (ii) if the PL is thought to be responsible for driving down attention to the PE cue during preexposure, attention to the PE cue should remain relatively high when the subregion is lesioned. Empirical findings presented in the experiments LI 2 and LI 3 support the first prediction, as the LI effect was enhanced following IL lesions but not the second prediction since no significant effects were observed following PL lesions. Therefore, it can be concluded that the IL cortex is involved in processes attempting to maintain attention to the PE cue during the preexposure phase. When the IL cortex is lesioned attention to the PE declined more rapidly, and this is in line with the proposed role of IL cortex in forming and/or maintaining an attentional set. Results following PL lesions are more puzzling. That is, it was predicted that PL lesions would abolish or reduce the LI effect as this subregion is thought to be involved in shifting attention. Therefore, it seems that, although, PL cortex is involved in shifting attention from one dimension to another, it does not seem to be involved in shifting attentional resources with respect to a single cue.

6 General discussion

The term executive function describes a set of high-level abilities that influence more basic motor, sensory and mnemonic processes. These functions include working memory, behavioural flexibility, inhibitory control, attentional processes and decision making. A large number of evidence, from human studies, non-human primates, rats and mice studies, has demonstrated a role for the prefrontal cortex in these higher cognitive processes.

The primary aim of this thesis was to develop new operant paradigms in order to investigate working memory (which is the ability to maintain a goal, or task-relevant information, over a period of time) and behavioural flexibility (which encompasses the ability to switch between different tasks according to the current goal) in rodents. A further aim was to investigate the neurobiological basis of behavioural flexibility, with particular emphasis on the roles of the subregions of the medial prefrontal cortex (prelimbic and infralimbic cortices). Final experiments also studied the functioning of the PL and IL cortices and their interaction using a neurobiological assessment of LI.

In this concluding chapter, the experimental findings are first briefly summarized. Evidence for a functional dissociation between the prelimbic and the infralimbic cortices of the prefrontal cortex in behavioural flexibility and latent inhibition is discussed with respect to the data presented here and previous findings. And finally, a hypothesis of the functioning of the PL and IL medial PFC subregions is presented.

6.1 Summary of findings

6.1.1 Behavioural experiments

Working memory

Chapter 2 introduced a novel behavioural paradigm for rats that could be utilised to study working memory. It also investigated explicitly the possible use of strategies as well as the impact of manipulations of several task parameters. Results presented in that chapter demonstrated that: (i) rats had learnt the relationship between a particular discrimination (stimulus-response pairings) and the task context defined by the occasion setter, that occasion setter influenced responding during presentation of conflicting cues and that introduction a delay between the end of the occasion setter presentation and the S_D presentation did not affect performance at any of the delays employed (Figure 2.3); (ii) animals made correct use of the occasion setter rather than the use of a stay/shift strategy which showed that the good performance observed at test in experiment WM 1 was actually based on mnemonic ability; (iii) manipulation of the ITI and the duration of the responding availability (S_D presentation) affected animals' performance other than to decrease the lever-pressing rate when the S_D presentation was reduced to 5s.

The absence of delay dependent deficit appears to be inconsistent with the literature on the working memory function in rodents. Findings from most previous research provide evidence for a delay-dependent deficit (Steckler et al., 1998) using rather short delays (below to 1 min). However, in most of these studies the paradigms used were of a delayed-response type (tasks where the response to be made was known prior to the start of the delay interval). It is generally accepted that the validity of this type of task can be

challenged because of the use of mediating strategies (Chudasama and Muir, 1997; Ennaceur and Aggleton, 1998; Steckler et al., 1998). Indeed, in such paradigms, mediating strategies (such as a particular body orientation or holding a position) have been shown to be used to bridge the delay interval: thereby reducing, or even abolishing, the cognitive task demand (i.e. online maintenance of information). It is plausible that it is the failure in maintaining the mediating strategies that is responsible for delay-dependent deficits. Interestingly, results from studies using operant delay-comparison paradigms have not provided any evidence for a consistent decline in performance (Ennaceur et al., 1997). These findings, together with the present results, provide evidence that when designs preventing the use of mediating strategies (delayed-comparison paradigms) are used no delay-dependent deficit is observed.

From human studies, theoretical accounts have long held that the decline observed in working memory tasks is a "time-based decay". That is, the amount of time between encoding and retrieval is the main determinant of performance. This theory proposes that information dissipates over time even if there is no competing information. Although very popular, this type of theory has been questioned and an alternative account have become popular (Nairne, 2002; Logan, 2004). In this theory, termed "item-based theory" or "interference theory", it is the number of items experienced between encoding and retrieval that is the main determinant of performance. It is proposed that information is lost because of competition from other information. In other words, information is held in short-term memory until it is displaced by other information. This theory accounts for results observed in humans' studies where it is common to use the same set of items

across trials or samples from a small set (Brown, 1958; Peterson and Peterson, 1959). Using a modified version of the Brown-Peterson task, Nairne et al. (1999) have demonstrated that under conditions in which interference from prior trials is kept low, very little forgetting occurs across retention intervals. Conversely, studies using the same task but where the same items were repeated across trials or drew from a restricted set, dramatic and systematic memory decline was observed (Peterson and Peterson, 1959). Extending this reasoning to studies using delayed-comparison tasks in rodents, there is no reason to expect a delay-dependent decline in performance if there are no interfering events during the delay interval. The data presented in this thesis supports this idea.

Behavioural flexibility – IDS/EDS design

In chapter 3, an operant version of the ID/ED shift procedure, designed to assess attentional changes that result from discrimination learning in rats, was presented. This between-subjects design comprised two phases: the acquisition phase where animals learnt a conditional discrimination wherein only one of the two dimensions presented was relevant for the solution of the discrimination, and the transfer phase, where they learnt a new discrimination that either involved an intradimensional or an extradimensional shift. Contrary to previous findings (Lawrence, 1949; Shepp and Eimas, 1964; Birrell and Brown, 2000), no retardation in learning of the new discrimination was observed for the ED group. Instead, both ID and ED groups learnt rapidly the new discrimination in the transfer phase (IDS/EDS 1: Figure 3.2, IDS/EDS 2: Figure 3.4).

Behavioural flexibility – Optional shift design

In experiment OS 1, a within-subject procedure that was based on an optional shift design (Kendler et al., 1964; Schwartz et al., 1971; Sirois and Shultz, 2006) was developed. Phase 1 training of this paradigm was strictly the same as the acquisition phase of the IDS/EDS operant task (conditional discrimination wherein only one of the two dimensions used was predictive of reinforcement). Then they were all transferred to a second discrimination involving audiovisual compounds comprising novel cues. During this phase of the experiment, cues from both stimulus dimensions were equally relevant to the solution of the discrimination. Finally, on probe test trials, animals were presented with compounds comprising cues that were associated with different responses during Phase 2. The results from these test trials showed that cues that belonged to the stimulus dimension that was relevant during Phase 1 had a greater influence on responding than cues from the dimension that was irrelevant during Phase 1 (Figure 3.7). These results provided evidence that at the end of Phase 1, animals were attending more to the relevant than to the irrelevant dimension. In other words, with this new operant paradigm the animals' ability to form and/or maintain a perceptual attentional set can be assessed (i.e. OS effect). Experiments OS 2, OS 3 and OS 4 further replicated this optional shift effect in sham-operated animals.

In summary, these results show that the changes in the attention paid to a stimulus over the course of discrimination training may be assessed in rats using a within-subject design and a fully automated procedure. This optional shift design overcomes some of the potential problems of other designs. Moreover, it is important to stress that this design

does not explicitly require animals to shift their attention (either to the same perceptual dimension or to the alternative perceptual dimension) but aims to assess animals' ability to form and maintain a perceptual set when trained on a conditional discrimination where one perceptual dimension is relevant for the solution of the discrimination and the other is irrelevant.

6.1.2 Neurobiological studies

Neurobiological assessment of behavioural flexibility

Chapter 4 examined the effects of selective lesions of the medial prefrontal cortex on behavioural flexibility using the optional shift paradigm described in Chapter 3. Results from experiments OS 2, OS 3 and OS 4 demonstrated that IL (figure 4.12), but not ACC or PL (figures 4.4 and 4.8), lesions induced a deficit in the formation/maintenance of a perceptual set; only IL lesions abolished the optional shift effect. These results support the idea that the medial prefrontal cortex in rats is involved in attentional changes that result from discrimination learning. Furthermore, these results, together with previous findings, provide evidence for a functional dissociation within the medial prefrontal cortex in behavioural flexibility. PL and IL cortices seem to have antagonistic roles: the PL cortex is involved in shifting attention (Birrell and Brown, 2000; Floresco et al., 2008) whereas the IL cortex is involved in forming and maintaining a perceptual attentional set.

Neurobiological assessment of latent inhibition

The final experimental chapter (Chapter 5) dealt with the effect of large medial prefrontal lesion as well as selective lesions of the prelimbic and infralimbic cortices on an aversive latent inhibition paradigm. There were two main aims of these series of experiments: (i) it was intended to clarify the nature of the involvement of the mPFC in latent inhibition, as a review of the literature showed that previous findings were unclear and hard to interpret and (ii) selective lesions of the mPFC subregions were investigated to follow up the dissociable IL/PL effects seen in the previous chapter. Results form those experiments revealed that lesions of the mPFC and IL (figures 5.2, 5.3 and 5.8, 5.9 respectively), but not of the PL (figures 5.5. and 5.6), enhanced the LI effect compared with sham-operated animals. These results are the first demonstration that lesions of the medial prefrontal have a significant effect on LI. Moreover, based on the results from experiment LI 1 and LI 3, the effect observed with large lesions of the mPFC seemed to be primarily due to the damage of the IL region rather than being dependent upon the volume of tissue damage.

In conclusion, the results presented in the thesis provide some evidence for a functional dissociation between the IL and the PL cortices. Lesions of the infralimbic cortex but not the prelimbic cortex disrupted changes in attention that resulted from discrimination learning. Similarly, IL lesions but not PL lesions had an impact of the latent inhibition effect, resulting in an enhanced latent inhibition effect. Hypotheses regarding the functioning of these subregions of the mPFC will be derived from these results and other findings in the literature.

6.2 Theoretical implications: functioning of the mPFC subregions

6.2.1 Grounds for a functional dissociation within the medial prefrontal cortex

Although there is a growing interest regarding functional dissociations between the IL and PL cortices, investigations remain rather scarce compared to the tremendous amount of research investigating the role of the PL/IL region as a whole. Based on evidence reported in the literature so far, it seems that the IL cortex is required for some aspects of the maintenance of extinction, for inhibitory control, in the maintenance of a perceptual attentional set and habit-based behaviours (Quirk et al., 2000; Coutureau and Killcross, 2003; Rhodes and Killcross, 2007b, 2007a), in contrast the PL cortex is involved in the contextual control of responding, in shifting attention from one dimension to another, in maintaining behavioural flexibility and in goal-directed behaviours (Ragozzino et al., 1999a; Birrell and Brown, 2000; Coutureau and Killcross, 2003; Marquis et al., 2007).

Some evidence of functional dissociations within the mPFC comes from studies investigating the process of inhibitory control (i.e. the ability to suppress dominant response tendencies in favour of more recently learned and appropriate behaviour). Following assessment of conditioned inhibition in an appetitive training procedure, Rhodes and Killcross (2007b) demonstrated that IL lesions disrupted inhibition as assessed by a retardation test but not a summation test[2]. Whereas the former assesses the

[2] Conditioned inhibition with test for summation:

Phase 1	Phase 2	Summation test
A+ B+	AX-	B? BX?

Conditioned inhibition with test for retardation:

Phase 1	Phase 2	Retardation test
A+	AX-	X+ Y+

ability of inhibition accrued to a stimulus to maintain behavioural control in the face of excitatory conditioning to the *same* stimulus when it was subsequently paired with reward, the summation test depends on the acquisition of an inhibitory association to one cue and its influence on behaviour in the face of direct competition from a different, excitatory stimulus. Therefore, the authors concluded that IL-lesioned animals did not have a deficit in the acquisition or expression of inhibition per se, but their behaviour failed to be controlled by the original inhibitory association to a stimulus when placed in competition with the newly formed excitatory associations to that same stimulus. The finding that IL lesions did not disrupt the ability to acquire inhibitory associations also supports other research indicating that IL-lesioned animals showed normal acquisition of extinction (Quirk et al., 2000; Rhodes and Killcross, 2007a). In these studies, however, IL lesions were shown to enhance spontaneous recovery, reinstatement (Quirk et al., 2000) and renewal (Rhodes and Killcross, 2007a) in both appetitive and aversive procedures. According to Rhodes and Killcross (2007a) such effects suggest that the IL cortex is involved in modulating or maintaining inhibitory control by a stimulus over behaviour in the face of competing excitatory associations accruing to the same stimulus. They further argued that such mechanisms are likely to be dependent on the context dependency of inhibitory associations. Findings provided by studies using other inhibitory paradigms can also be explained using the same framework. Using the 5-CSRTT, Chudasama et al. (2003) showed that IL lesions increased premature and impulsive responding. Similarly, studies considering the effect of selective PL and IL lesions on passive avoidance demonstrated that IL-lesioned, but not PL-lesioned, animals stepped down prematurely onto a floor that had previously been used to deliver footshock

(Jinks and McGregor, 1997). Finally, it has been shown that selective IL lesions (Chudasama and Robbins, 2003) but not PL lesions (Chudasama and Muir, 2001) resulted in an impairment of reversal learning. All these findings can be explained in terms failure to maintain an inhibitory influence over behaviour driven by a stimulus when in direct competition with excitatory associations that have accrued to the same stimulus. One could wonder if the opposite case is true: is the IL involved in maintaining excitatory influence over behaviour driven by a stimulus when in direct competition with inhibitory associations that have accrued to the same stimulus? A study by Nelson (2002) implied that, behaviourally at least, these effects are symmetrical. Thus, it might be the case that the same is true for the role of the IL. Alternatively, the IL might only be involved in maintaining inhibition. This interesting question could be addressed by investigating the effect of selective IL lesions using Nelson's paradigms.

Studies investigating the effect of selective lesions or inactivation of the PL cortex also provide evidence for a functional dissociation within the mPFC. For instance, using a hand-run ID/ED shift design, Birrell and Brown (2000) showed that bilateral lesions of the medial prefrontal cortex (centred on the PL cortex) induced a selective impairment in shifting attentional set between stimulus dimensions, but spared performance on initial acquisition, intradimensional shift and reversal learning. Along the same lines, Floresco et al. (2008) demonstrated that inactivation of the mPFC (cannulae implanted into the PL cortex) did not impaired initial learning of a visual-cue or a response discrimination or reversal learning of these discrimination, but impaired performance of visual-cue/response set-shifting. This suggests that the PL cortex plays a selective role in

facilitating shifts between rules, strategies or attentional sets, but is not essential for shifting between different stimulus-reward associations within a particular stimulus dimension. Using a Stroop like procedure (similar to the one used in the WM chapter) in rats, Marquis et al. (2007) also found some dissociable effects of the IL and PL mPFC subregions in the contextual control of behaviour in situations of conflicting responses. Whereas temporary inactivation of the IL cortex had no effect on the accuracy of responding in conflicting (i.e. incongruent trials) and in non-conflicting (i.e. congruent trials) situations, similar inactivation of the PL cortex led to selective deficit of performance on incongruent trials but left congruent trial performance intact. The authors concluded that the PL but not the IL subregion of the mPFC is necessary for the ability to use task-setting contextual cues to control responding in situations of conflict. Other evidence comes from studies investigating goal-directed and habit-based behaviours (Coutureau and Killcross, 2003). These findings support the idea that PL cortex is involved in goal-directed behaviours (since PL lesions led to habit-based performance, insensitive to goal devaluation after minimal training) whereas the IL cortex is responsible for behavioural dominance by habit-based systems (as IL lesions produced rats that remained goal-directed despite extensive overtraining). In further work investigating context-appropriate responding, it has also been demonstrated that a more extensively trained response will usually dominate a less-well trained response regardless of contextual control, but this dominance depends on the integrity of the IL (Haddon and Killcross, 2007).

6.2.2 IL and PL cortex functions: a dual-process system

Proposed roles of the PL and IL cortices

Killcross and Coutureau (2003) have hypothesized the existence of multiple systems within the medial PFC that may compete for behavioural expression during the course of instrumental learning. In early stages of instrumental training actions are goal-directed (depending upon R-O associations); as training proceeds performance becomes habitual (depending upon S-R associations). They proposed that the PL region is involved in the execution of goal-directed behaviour whereas the IL region may act to override goal-directed behaviour and permit habit-based behaviour. The balance between the two behaviours may be maintained via mutual inhibition.

In the light of the empirical findings reported in this thesis and the results reviewed above, the following hypothesis can be put forward. The PL cortex is involved in top-down processes responsible for maintaining and directing goal-directed behaviour and biasing behavioural control away from stimulus-bound responses that may be inappropriate to the current situation. By contrast, the IL cortex is responsible for biasing cognitive control towards innate or habit-based behaviours, in part by modulating PL function. In other words, the PL cortex brings simple cue-outcome associations and more complex behavioural patterns under the modulatory influence of contextual, or other task-relevant, information and in contrast, the IL cortex biases animals towards behaviours controlled by simple associations, or by prepotent or innate responses. In terms of the role of the IL cortex in the control of behaviour by inhibitory processes identified above, modulatory inhibitory processes are, by their nature, effective only when placed in

competition with alternative excitatory behaviours. Rhodes and Killcross (2007b) have proposed that the normal role of the functioning IL cortex is to decrease the context-specificity of such inhibitory control (i.e. to bias them towards a simple inhibitory association, opposing PL function which seeks to bring these associations under explicit contextual control), allowing inhibition to generalise across phases of learning. As such, animals with IL lesions demonstrate poor transfer of simple inhibitory associations from one context or experimental situation to another, leading to a reduction in inhibitory control.

Relation to data presented in this thesis

In the context of behavioural flexibility, a functional dissociation between the PL and the IL cortices has been described based on results presented in this thesis and previous results: the PL cortex is thought to be involved in shifting strategy to guide behaviour whereas the IL cortex is believed to be involved in the formation and maintenance of an attentional set. That is, in the case of control or sham-operated animals, after extensive training on a conditional discrimination (Phase 1 training) animals form an attentional set. Then, when they are transferred to a second phase of training that involves new exemplars, animals tend to learn more about cues belonging to the previously relevant dimension (as shown by the optional shift effect observed at test). IL-lesioned animals do not show this transfer for two potential reasons. First, in the absence of an IL cortex, animals might not form or maintain an attentional set; second in the absence of an IL cortex, PL functioning dominates, biasing animals towards learning new rules in the second stage of optional shift procedure (where animals are presented by a new set of

cues), therefore abolishing the optional shift effect. By contrast, following PL lesions, no detrimental impact on the optional shift effect is observed. That is, the IL cortex dominates, therefore enabling the formation and/or the maintenance of an attentional set.

Similarly in the context of latent inhibition, the normal function of the IL cortex tends to be to promote attention towards the PE cue whereas the PL cortex tends to encourage changes in attention when situations change. During the preexposure phase, in the case of control or sham-operated animals, attention paid to the PE cue decreases with repeated exposure, later resulting in a retardation of conditioning to that cue compared to the NPE cue. Following IL lesions, however, attention tends to decline more dramatically as the system promoting the maintenance of attention set towards cues is not functioning, resulting in a greater retardation of conditioning to the PE cue compared with sham-operated animals. In the case of PL-lesioned animals, the typical function of the PL cortex in this context would be to facilitate attentional shifts when the experienced situation changes. It is possible that in LI experiments (especially in the design used here) there is little change in the animals' experience between preexposure and conditioning, therefore there is little role for the PL cortex in the LI effect. Accounting for this hypothesis, some experiments using a range of training procedures (Bouton and Swartzentruber, 1989; Hall and Honey, 1989) have found that the retardation of conditioning produced by prior exposure to the to-be-conditioned stimulus is attenuated or even abolished when conditioning takes place in a context other than that used for preexposure.

The trade-off between processes

Daw et al. (2005) have proposed a dual-action choice model, where selection of actions is thought to be controlled by diverse neural systems referred to as "controllers": the prefrontal cortex (responsible for reflective and cognitive action planning), the striatum (responsible for reflexive and habitual behaviour) and their dopaminergic afferents. Furthermore, they suggested that a trade-off between the two controllers is determined by the uncertainty of their respective outcome predictions. With the first controller, outcome predictions are based on chaining together short-term predictions and therefore involve exploration of a branching set of possible future action situations ("tree search"). This mechanism is expensive in terms of memory, cognitive effort and time and is also error-prone due to a cumulative error at each stage of tree search. In contrast, in the second system choices are based on the long-term value of an action derived from previous experience; this mechanism is simple compared to the first, requiring little cognitive control and few mnemonic resources, but it does not allow any flexibility based on future outcome value. As this latter system relies on experience to establish a good model for the long-term future value of an action, it is initially (i.e. without experience) more unreliable than the tree search system, but over time, gains in reliability to such a point that this system generates a more reliable model than the noisy tree search. Behavioural control merely reflects the moment-by-moment fluctuations in the reliability of the two systems.

From this model and in the framework of the PL/IL dual-process hypothesis proposed above, a similar mechanism of functioning of the balance between the two systems (PL

and IL cortices) might be proposed. The interplay or transition between these control systems would be governed by the environment. If the environment is relatively fixed and constant, IL functioning dominates and behavioural control is ceded to simple associations or prepotent, first learned and innate behaviours; in contrast, when the environment is novel, changing or uncertain, the PL cortex attempts to offset uncertainty by imposing modulatory processes that allow an animal to switch between multiple different associative structures to account for the changing environment. Furthermore, the balance between these two systems can be thought as being mediated by mutual interaction. That is, in a relatively fixed environment, the ascendancy of the IL functioning could be insured via an inhibitory control over the PL functioning, as this system can be viewed as less demanding in term of cognitive control and processing. Similarly, when the environment becomes more variable, the PL functioning might dominate in the control of behaviour by inhibiting the IL functioning. Of course, this mutual inhibition may not be instantiated in the form of explicit inhibitory neural projections, but rather might come about by selective modulation of behavioural output systems via independent efferent projections.

6.3 Conclusions

In conclusion, two novel paradigms that assessed central aspects of executive functioning were introduced: one assessing working memory and another assessing attentional set behaviour in rats. In addition, experiments were presented that investigated the neurobiological basis of attentional set as measured by optional shift performance and

latent inhibition, providing evidence for a functional dissociation within the rat medial prefrontal cortex.

The optional shift design provides a novel operant paradigm to look specifically at changes in attention resulting from discrimination learning. Furthermore, it assesses the ability to form and maintain a perceptual attentional set rather than the ability to shift a perceptual attentional set. These abilities were observed to be differentially dependent upon different subregions of the medial prefrontal cortex; IL lesions impaired the formation and/or the maintenance of an attentional set, whereas PL lesions were without effect in this formation/maintenance role, but have been shown elsewhere to disrupt shifts in attentional set (Birrell and Brown, 2000; Floresco et al., 2008). Further evidence of a functional dissociation arose from experiments investigating the effect of selective mPFC lesions on LI. Whereas IL lesions induced an enhancement of the LI effect, PL lesions had no discernable effect on LI.

In the light of the findings reported here as well as previous findings it appears evident that PL and IL subregions of the mPFC play complementary and interactive roles in behavioural flexibility and more generally in executive functioning. In the grand scheme of cognitive executive control these two subregions can be viewed as complementary systems that allocate resources based on environmental conditions (i.e. the current task demands), and recruit a variety of processes including inhibitory control, and attention in order to exhibit appropriate and flexible behaviours.

Cumulative evidence from patients with frontal lobe damage (Milner, 1963; Owen et al., 1991) and functional neuroimaging studies in humans (Nagahama et al., 2001; Wang et al., 2001; Hampshire and Owen, 2006) indicate a critical role of the frontal lobe in cognitive flexibility. Moreover, functional dissociation within the frontal lobe is widely documented. Thus, the capacity to reverse affective associations is mediated by regions within the orbitofrontal cortex. In contrast, regions within the lateral prefrontal cortex may be implicated in the higher-order shifting of attention between perceptual dimensions. Furthermore, recent fMRI studies (Nagahama et al., 2001; Hampshire and Owen, 2006), using WCST or ID/ED shift paradigms, provide evidence for a functional dissociation within the lateral prefrontal cortex. According to those findings, increased activity was observed in the ventral part of the lateral PFC during switching attention between dimensions, whereas accrued activity within the dorsal lateral PFC was observed during reorganisation of stimulus-response mapping. There is also a growing interest regarding the involvement of the rat PFC in behavioural flexibility. Results from experiments using ID/ED task (Birrell and Brown, 2000; Brown and Bowman, 2002) or strategies shifting tasks (Ragozzino et al., 1999a; Ragozzino et al., 1999b) support the view that different cognitive functions are distributed across specific regions of the PFC. Thus, the orbital prefrontal cortex is involved in lower-order cognitive flexibility (reversal learning) and the medial prefrontal cortex is involved in higher-order cognitive flexibility (shift between perceptual dimensions). Moreover, results presented in this thesis together with previous experiments further support the idea of a functional dissociation within the mPFC, with the PL involves in shifting a perceptual set and the IL involves in forming/maintaining a perceptual set. Although interesting, these results do

not match the ones reported in humans studies and further investigation will be of interest (since the data regarding the functional dissociation between PL and IL reported in this thesis are unprecedented).

References

Aggleton, J. P., Neave, N., Nagle, S. and Sahgal, A. (1995). "A comparison of the effects of medial prefrontal, cingulate cortex, and cingulum bundle lesions on tests of spatial memory: evidence of a double dissociation between frontal and cingulum bundle contributions." The Journal of Neuroscience **15**(11): 7270–7281.

Baddeley, A. D. (1992). "Working memory." Science **255**: 556-559.

Baddeley, A. D., Logie, R., Bressi, S., Della Sala, S. and Spinnler, H. (1986). "Dementia and working memory." Quarterly Journal of Experimental Psychology **38**: 603-618.

Barkley, R. A. (1999). "Responses inhibition in attention-deficit hyperactivity disorder." Mental Retardation and Developmental Disabilities Research Reviews **5**: 177-184.

Birrell, J. M. and Brown, V. J. (2000). "Medial frontal cortex mediates perceptual attentional set shifting in the rat." The Journal of Neuroscience **20**(11): 4320-4324.

Bouton, M. E. (2004). "Context and behavioural processes in extinction." Learning and Memory **11**(5): 485-494.

Bouton, M. E. and Swartzentruber, D. (1989). "Slow reacquisition following extinction: context encoding and retrieval mechanisms." Journal of Experimental Psychology: Animal Behavior Processes **15**(1): 43-53.

Brown, J. (1958). "Some tests of the decay theory of immediate memory." Quarterly Journal of Experimental Psychology A **10**: 12-21.

Brown, V. J. and Bowman, E. M. (2002). "Rodent models of prefrontal cortical function." Trends in Neurosciences **25**(7): 340-343.

Carli, M., Robbins, T. W., Evenden, J. L. and Everitt, B. J. (1983). "Effects of lesions to ascending noradrenergic neurones on performance of a five-choice serial reaction task in rats: implications for theories of dorsal noradrenergic function based on selective attention and arousal." Behavioural Brain Research **9**: 361-380.

Chudasama, Y. and Muir, J. L. (1997). "A behavioural analysis of the delayed non-matching to position task: the effects of scopolamine, lesions of the fornix and the prefrontal region on mediating behaviours by rats." Psychopharmacology **134**: 73-82.

Chudasama, Y. and Muir, J. L. (2001). "Visual attention in the rat: a role for the prelimbic cortex and thalamic nuclei?" Behavioural Neuroscience **115**: 417-428.

Chudasama, Y., Passetti, F., Rhodes, S. E. V., Lopian, D., Desai, A. and Robbins, T. W. (2003). "Dissociable aspects of performance on the 5-choice serial reaction time task following lesions of the dorsal anterior cingulate, infralimbic and orbitofrontal cortex in the rat: differential effects on selectivity, impulsivity and compulsivity." Behavioural Brain Research **146**: 105-119.

Chudasama, Y. and Robbins, T. W. (2003). "Dissociable contributions of the orbitofrontal and infralimbic cortex to pavlovian autoshaping and discrimination reversal learning: further evidence for the functional heterogeneity of the rodent frontal cortex." The Journal of Neuroscience **23**: 8771-8780.

Cohen, J. D., Forman, S. D., Braver, T. S., Casey, B. J., Servan-Schreiber, D. and Noll, D. C. (1994). "Activation of the prefrontal cortex in non-spatial working memory task with functional MRI." Human Brain Mapping **1**: 293-304.

Condé, F., Maire-Lepoivre, E., Audinat, E. and Crepel, F. (1995). "Afferent connections of the medial frontal cortex of the rat. II. Cortical and subcortical afferents." Journal of Comparative Neurology **352**(4): 567-593.

Coull, J. T. (1998). "Neural correlates of attention and arousal: insights from electrophysiology, functional neuroimaging and psychopharmacology." Progress in Neurobiology **55**: 343-361.

Coutureau, E., Blundell, P. J. and Killcross, A. S. (2001). "Basolateral amygdala lesions disrupt latent inhibition in rats." Brain Research Bulletin **56**(1): 49-53.

Coutureau, E. and Killcross, A. S. (2003). "Inactivation of the infralimbic prefrontal cortex reinstates goal-directed responding in overtrained rats." Behavioural Brain Research **146**: 167-174.

D'Esposito, M., Aguirre, G. K., Zarahn, E., Ballard, D., Shin, R. K. and Lease, J. (1997). "Functional MRI studies of spatial and non-spatial working memory." Cognitive Brain Research **7**: 1-13.

Dalley, J. W., Cardinal, R. N. and Robbins, T. W. (2004). "Prefrontal executive and cognitive functions in rodents: neural and neurochemical substrates." Neuroscience & Biobehavioral Reviews **28**(7): 771-784.

Daw, N. D., Niv, Y. and Dayan, P. (2005). "Uncertainty-based competition between prefrontal and dorsolateral striatal for behaviour control." Nature Neuroscience **8**(12): 1704-1711.

Dias, R. and Aggleton, J. P. (2000). "Effects of selective excitotoxic prefrontal lesions on acquisition of nonmatching- and matching-to-place in the T-maze in the rat: differential

involvement of th prelimbic-infralimbic and anterior cingulate cortices in providing behavioural flexibility." European Journal of Neuroscience **12**: 4457-4466.

Dias, R., Robbins, T. W. and Roberts, A. C. (1996). "Dissociation in prefrontal cortex of affective and attentional shifts." Nature **380**(6569): 69-72.

Ding, D. C. D., Gabbott, P. L. A. and Totterdell, S. (2001). "Differences in the laminar origin of projections from the medial prefrontal cortex to the nucleus accumbens shell and core regions in the rat." Brain Research **917**(1): 81.

Dunnett, S. B. (1990). "Role of prefrontal cortex and striatal output systems in short-term memory deficits associated with ageing, basal forebrain lesions, and cholinergic rich grafts." Canadian Journal of Psychology **44**: 210-232.

Dunnett, S. B. (1993). Oxford, OIRL Press, Oxford University Press.

Eagle, D. M. and Robbins, T. W. (2003). "Lesions of the medial prefrontal cortex or nucleus accumbens core do not impair inhibitory control in rats performing a stop-signal reaction time task." Behavioural Brain Research **146**: 131-144.

Ennaceur, A. and Aggleton, J. P. (1998). "An attempt to overcome the problem of motor mediation by rats in the delayed non-matching to position in rats." Neuroscience Research Communications **22**(3): 153-162.

Ennaceur, A., Aggleton, J. P. and Fray, P. J. (1997). "Delayed non-matching to sample in a novel automated visual memory apparatus using mixed retention intervals." Neuroscience Research Communications **20**(2): 103 -111.

Eslinger, P. J. and Grattan, L. M. (1993). "Frontal lobe and frontal-striatal substrates for different forms of human cognitive flexibility." Neuropsychologia **31**(1): 17-28.

Floresco, S. B., Block, A. E. and Tse, M. T. L. (2008). "Inactivation of the medial prefrontal cortex of rat impairs strategy set-shifting, but not reversal learning, using a novel, automated procedure." Behavioural Brain Research **190**: 85-96.

Floresco, S. B., Ghods-Sharifi, S., Vexelman, C. and Magyar, O. (2006). "Dissociable roles for the nucleus accumbens core and shell in regulating set shifting." The Journal of Neuroscience **26**(9): 2449-2457.

Fuster, J. M. (2000). "Executive frontal functions." Experimental Brain Research **133**(1): 66-70.

Gevins, A., Smith, M. E., McEvoy, L. and Yu, D. (1997). "High-resolution EEG mapping of cortical activation related to working memory: effects of task difficulty, type of processing and practice." Cerebral Cortex **7**: 374-385.

Ghods-Sharifi, S., Haluk, D. M. and Floresco, S. B. (2007). "Differential effects of inactivation of the orbitofrontal cortex on strategy set-shifting and reversal learning." Neurobiology of Learning and Memory **89**(4): 567-573.

Giguere, M. and Goldman-Rakic, P. S. (1988). "Mediodorsal nucleus: Areal, laminar, and tangential distribution of afferents and efferents in the frontal lobe of rhesus monkeys." Journal of Comparative Neurology **277**(2): 195-213.

Gisquet-Verrier, P. and Delatour, B. (2006). "The role of the rat prelimbic/infralimbic cortex in working memory: not involved in the short-term maintenance but in monitoring and processing functions." Neuroscience **141**: 585-596.

Givens, B. and McMahon, K. (1997). "Effects of ethanol on non spatial working memory and attention in rats." Behavioural Neuroscience **111**(2): 275-282.

Goldman-Rakic, P. S. (1987). "Circuitry of primate prefrontal cortex and regulation of behaviour by representational memory". In F. Plum & V. Mountcastle (Eds.), *Handbook of Physiology:* The nervous system (pp. 373-417). Bethesda: American Physiological Society.

Granon, S., Vidal, C., Thinus-Blanc, C., Changeux, J.-P. and Poucet, B. (1994). "Working memory, response selection and effortful processing in rats with medial prefrontal lesions." Behavioural Neuroscience **108**: 883-891.

Haddon, J. E. and Killcross, A. S. (2006). "Prefrontal cortex lesions disrupt the contextual control of response conflict." The Journal of Neuroscience **26**(11): 2933-2940.

Haddon, J. E. and Killcross, A. S. (2007). The effect of infralimbic inactivation on the contextual control of response conflict in rats. 11th Associative Learning Symposium., Gregynog, Wales.

Hall, G. and Honey, R. C. (1989). "Contextual effects in conditioning, latent inhibition and habituation: associative and retrieval functions of contextual cues." Journal of Experimental Psychology. ABp **15**(3): 232-241.

Hampshire, A. and Owen, A. M. (2006). "Fractionating attentional control using event-related fMRI." Cerebral Cortex **16**: 1679-1689.

Heidbreder, C. A. and Groenewegen, H. J. (2003). "The medial prefrontal cortex in the rat: evidence for a dorso-ventral distinction based upon functional and anatomical characteristics." Neuroscience & Biobehavioral Reviews **27**(6): 555.

Herremans, A. H. and Hijzen, T. H. (1997). "The delayed-conditional discrimination task improves measurement of working memory in rats." Neuroscience and Biobehavioral Reviews **21**(3): 371-379.

Hohl-Abrahao, J. C. and Creutzfeldt, O. D. (1991). "Topographical mapping of the thalamocortical projections in rodents and comparison with that in primates." Experimental Brain Research **87**: 283-294.

Hurley, K. M., Herbert, H., Moga, M. M. and Saper, C. B. (1991). "Efferent projections of the infralimbic cortex of the rat." Journal of Comparative Neurology **308**(2): 249-276.

Jinks, A. L. and McGregor, I. S. (1997). "Modulation of anxiety-related behaviours following lesions of the prelimbic or infralimbic cortex in the rat." Brain Research **772**: 181-190.

Joel, D., Weiner, I. and Feldon, J. (1997a). "Electrolytic lesions of the medial prefrontal cortex in rats disrupt performance on an analogue of the Wisconsin Card Sorting Test, but do not disrupt latent inhibition: implications for animal models of schizophrenia." Behavioural Brain Research **85**(2): 187-201.

Joel, D., Weiner, I. and Feldon, J. (1997b). "Electrolytic lesions of the medial prefrontal cortex in rats disrupt performance on an analogue of the Wisconsin Card Sorting Test, but do not disrupt latent inhibition: implications for animal models of schizophrenia." Behavioral Brain Research **85**(2): 187-201.

Kasprow, W. J., Catterson, D., Schachtman, T. R. and Miller, R. R. (1984). "Attenuation of latent inhibition by a post-acquisition reminder." Quarterly Journal of Experimental Psychology **36b**: 53-63.

Kendler, T. S., Kendler, H. H. and Silfen, C. K. (1964). "Optional shift behavior of albino rats." Psychon. **1**: 5-6.

Killcross, A. S., Dickinson, A. and Robbins, T. W. (1994a). "Amphetamine-induced disruptions of latent inhibition are reinforcer mediated: implications for animal models of schizophrenic attentional dysfunction." Psychopharmacology **115**(1-2): 185-195.

Killcross, A. S., Dickinson, A. and Robbins, T. W. (1994b). "Effects of the neuroleptic alpha-flupenthixol on latent inhibition in aversively- and appetitively-motivated paradigms: evidence for dopamine-reinforcer interactions." Psychopharmacology (Berl) **115**(1-2): 196-205.

Killcross, A. S., Dickinson, A. and Robbins, T. W. (1995). "The on-baseline latent inhibition effect is not counterconditioning." Psychopharmacology **118**(1): 104-106.

Killcross, A. S. and Robbins, T. W. (1993). "Differential effects of intra-accumbens and systemic amphetamine on latent inhibition using an on-baseline, within-subject conditioned suppression paradigm." Psychopharmacology **110**(4): 479-489.

Kolb, B., Buhrmann, K., McDonald, R. and Sutherland, R. J. (1994). "Dissociation of the medial prefrontal, posterior parietal, and posterior temporal cortex for spatial navigation and recognition memory in rats." Cerebral Cortex **6**: 664-680.

Kolb, B. and Tees, R. (1990). "The cerebral cortex of the rat". In B. Kolb & R. Tees (Eds.), The Cerebral Cortex of the Rat (pp. 439-458). Cambridge, Massachusetts London, England: MIT Press.

Krettek, J. E. and Price, J. L. (1977). "The cortical projections of the mediodorsal nucleus and adjacent thalamic nuclei in the rat." Journal of Comparative Neurology **171**: 157-192.

Lacroix, L., Spinelli, S., White, W. and Feldon, J. (2000). "The effects of ibotenic acid lesions of the medial and lateral prefrontal cortex on latent inhibition, prepulse inhibition and amphetamine-induced hyperlocomotion." Neuroscience. **97**(3): 459-468.

Lansbergen, M. M., van Hell, E. and Kenemans, J. L. (2007). "Impulsivity and conflict in the Stroop task. An ERP study." Journal of Psychophysiology **21**(1): 33-50.

Lawrence, D. H. (1949). "The acquired distinctiveness of cues: I. Transfer between discriminations on the basis of familiarity with the stimulus." Journal of Experimental Psychology **39**: 770-784.

Leonard, C. M. (1969). "The prefrontal cortex of the rat. I. Cortical projections of the mediodorsal nucleus. II. Efferent connections." Brain Research **12**: 321-343.

Logan, G. D. (2004). "Working memory, task switching, and executive control in the task span procedure." Journal of Experimental Psychology **133**(2): 218-236.

Lubow, R. E. (1989). Latent inhibition and conditioned attention theory. New-York, Cambridge University Press.

Lubow, R. E. and Moore, A. U. (1959). "Latent inhibition: the effect of nonreinforced preexposure to the conditioned stimulus." Journal of Comparative Physiology and Psychology **52**: 415-419.

Mackintosh, N. J. (1975). "A theory of attention: variations in the associability of stimuli with reinforcement." Psychological Review **82**(4): 276-298.

Mackintosh, N. J. and Little, L. (1970). "An analysis of transfer along a continuum." Canadian Journal of Psychology **24**(5): 362-369.

Marquis, J. P., Haddon, J. E. and Killcross, A. S. (2007). "Inactivation of the prelimbic, but not infralimbic, prefrontal cortex impairs the contextual control of response conflict in rats." European Journal of Neuroscience **25**: 559-566.

McCarty, G., Puce, A., Constable, R. T., Krystal, J. H., Gore, J. C. and Goldman-Rakic, P. S. (1996). "Activation of human prefrontal cortex during spatial and non-spatial working memory tasks measured by functional MRI." Cerebral Cortex **6**: 600 - 611.

McDonald, A. J., Mascagni, F. and Guo, L. (1996). "Projections of the medial and lateral prefrontal cortices to the amygdala: a Phaseolus vulgaris leucoagglutinin study in the rat." Neuroscience **71**(1): 55-75.

Milner, B. (1963). "Effects of different brain lesions on card sorting." Archives of Neurology **9**: 100-110.

Muir, J. L., Everitt, B. J. and Robbins, T. W. (1996). "The Cerebral Cortex of the Rat and Visual Attentional Function: Dissociable Effects of mediofrontal, cingulate, anterior dorsolateral, and parietal cortex lesions on a five-choice serial reaction time task." Cerebral Cortex **6**: 470-481.

Mumby, D. G., Pinel, J. P. J. and Wood, E. R. (1990). "Nonrecurring-items delayed non-matching to sample in rats." Psychobiology **18**: 321-326.

Nagahama, Y., Okada, T., Katsumi, Y., Hayashi, T., Yamauchi, H., Oyanagi, C., Konishi, J., Fukuyama, H. and Shibasaki, H. (2001). "Dissociable mechanisms of attentional control within the human prefrontal cortex." Cerebral Cortex **11**: 85-92.

Nairne, J. S. (2002). "Remembering over the short-term: the case against the standard model." Annual Review of Psychology **53**: 53-81.

Nairne, J. S., Whiteman, H. L. and Kelley, M. R. (1999). "Short-term forgetting of order under conditions of reduced interference." Quarterly Journal of Experimental Psychology A **52**(1): 241-251.

Nelson, J. B. (2002). "Context specificity of excitation and inhibition in ambiguous stimuli." Learning and Motivation **33**: 284-310.

Nigg, J. T. (2000). "On Inhibition/Disinhibition in Developmental Psychopathology: Views From Cognitive and Personality Psychology and a Working Inhibition Taxonomy." Psychological Bulletin **126**(2): 220-246.

Olton, D. S. and Markowska, A. L. (1993). "Mazes: their use in delayed conditional discriminations and place discriminations." Methods in Behavioural Pharmacology 195-216.

Öngür, D. and Price, J. L. (2000). "The organization of networks within the orbital and medial prefrontal cortex of rats, monkeys and humans." Cerebral Cortex **10**(3): 206-219.

Owen, A. M., Downes, J. D., Sahakian, B. J. and Polkey, C. E. (1990). "Planning and spatial working memory following frontal lobe lesions in man." Neuropsychologia **28**(10): 1021-1034.

Owen, A. M., Evans, A. C. and Petrides, M. (1996). "Evidence for a two-stage model of spatial working memory processing within the lateral frontal cortex: a positron emission tomography study." Cerebral Cortex **6**: 31-38.

Owen, A. M., McMillan, K. M., Laird, A. R. and Bullmore, E. (2005). "N-back working memory paradigm: a meta-analysis of normative functional neuroimaging studies." Human Brain Mapping **25**: 46-59.

Owen, A. M., Roberts, A. C., Hodges, J. R., Summers, B. A., Polkey, C. E. and Robbins, T. W. (1993). "Contrasting mechanisms of impaired attentional set-shifting in patients with frontal lobe damage or Parkinson's disease." Brain **116**: 1159-1175.

Owen, A. M., Roberts, A. C., Polkey, C. E., Sahakian, B. J. and Robbins, T. W. (1991). "Extra-dimensional versus intra-dimensional set shifting following frontal excisions, temporal lobe excisions or amygdalo-hippocampectomy in man." Neuropsychologia **29**(10): 993-1006.

Paule, M. G. and Bushnell, P. J. (1998). "Symposium overview: the use of delayed matching-to-sample procedures in studies of short-term memory in animals and humans." Neurotoxicology Teratology **20**(5): 493-502.

Paxinos, G. and Watson, C. (1998). "The rat brain in stereotaxic coordinates. 4th ed.", San Diego, CA: Academic Press.

Perry, R. J. and Hodges, J. R. (1999). "Attention and executive deficits in Alzheimer's disease." Brain **122**: 383-404.

Peterson, L. R. and Peterson, M. J. (1959). "Short-term retention of individual verbal items." Journal of Experimental Psychology **58**: 193-198.

Petrides, M. (1996). "Specialized systems for the processing of mnemonic information within the primate frontal cortex." Philosophical Transactions of the Royal Society of London B **351**: 1455-1462.

Petrides, M., Aliviastos, B., Meyer, E. and Evans, A. C. (1993). "Functional activation of the human frontal cortex during the performance of verbal working memory tasks." Proceedings of National Academy of Sciences **90**: 878-882.

Petrides, M. and Milner, B. (1982). "Deficits on subject-ordered tasks after frontal- and temporal-lobe lesions in man." Neuropsychologia **20**(3): 249-262.

Pontecorvo, M. J., Sahgal, A. and Steckler, T. (1996). "Further developments in the measurement of working memory in rodents." Cognitive Brain Research **3**: 205-213.

Pothuizen, H. H. J., Jongen-Relo, A. L. and Feldon, J. (2006). "Latent inhibition of conditioned taste aversion is not disrupted, but can be enhanced, by selective nucleus accumbens shell lesions in rats." Neurosciences **137**: 1119-1130.

Purves, D. Bonardi, C. and Hall, G. (1995). "Enhancement of latent inhibition in rats with electrolytic lesions of the hippocampus." Behavioural Neuroscience **109**: 366-370.

Quirk, J. Q., Russo, G. K., Barron, J. L. and Lebron, K. (2000). "The role of the ventromedial prefrontal cortex in the recovery of extinguished fear." The Journal of Neuroscience **20**(16): 6225-6231.

Ragozzino, M. E., Detrick, S. and Kesner, R. P. (1999a). "Involvement of the prelimbic-infralimbic areas of the rodent prefrontal cortex in behavioural flexibility for place and response learning." The Journal of Neuroscience **19**(11): 4585-4594.

Ragozzino, M. E., Wilcox, C., Raso, M. and Kesner, R. P. (1999b). "Involvement of rodent prefrontal cortex subregions in strategy switching." Behavioural Neuroscience **113**(1): 32-41.

Rhodes, S. E. and Killcross, A. S. (2007a). "Lesions of rat infralimbic cortex enhance renewal of extinguished appetitive pavlovian responding." European Journal of Neuroscience **25**: 2498-2503.

Rhodes, S. E. and Killcross, A. S. (2007b). "Lesions of rat infralimbic cortex result in disrupted retardation but normal summation test performance following training on a pavlovian conditioned inhibition procedure." European Journal of Neuroscience **26**: 2654-2660.

Robertson, I. H., Manly, T., Andrade, J., Baddeley, B. T. and Yiend, J. (1997). "'Oops!' Performance correlates of everyday attentional failures in traumatic brain injured and normal subjects." Neuropsychologia **35**: 747-758.

Roitblat, H. L. and Harley, H. E. (1988). "Spatial delayed matching to sample performance by rats: learning, memory and proactive interference." Journal of Experimental Psychology: Animal Behavior Processes **14**: 71-82.

Rose, J. E. and Woolsey, C. N. (1948). "The orbitofrontal cortex and its connections with the mediodorsal nucleus in rabbit, sheep and cat." Res. Publ. Assn. Nerv. Dis. **27**: 210-132.

Rosvold, H., Mirsky, A., Bransome, E. and Beck, L. (1956). "A continuous performance test of brain damage." Journal of Consulting Clinical Psychology **20**: 343-350.

Sarter, M. and McGaughy, J. (1995). "Behavioural vigilance in rats: task validation and effects of age, amphetamine, and benzodiazepine receptor ligands." Psychopharmacology **117**: 340-347.

Sarter, M. and McGaughy, J. (1998). "Sustained attention performance in rats with intracortical infusions of 192 IgG-saporin-induced cortical cholinergic deafferentiation: effects of physostigmine and FG 7142." Behavioural Neuroscience **112**(6): 1519-1525.

Schiller, D. and Weiner, I. (2004). "Lesions of the basolateral amygdala and the orbitofrontal cortex but not to the medial prefrontal cortex produce an abnormally persistent latent inhibition in rats." Neuroscience **128**: 15-25.

Schwartz, R. M., Schwartz, M. and Tees, R. C. (1971). "Optional intradimensional and extradimensional shifts in the rat." Journal of Comparative Physiological Psychology **77**(3): 470-475.

Sesack, S. R., Deutch, A. Y., Roth, R. H. and Bunney, B. S. (1989). "Topographical organization of the efferent projections of the medial prefrontal cortex in rats; an anterograde tract-tracing study with phaseolus vulgaris leucoagglutinin." The Journal of Comparative Neurology **290**): 213-242.

Shepp, B. E. and Eimas, P. D. (1964). "Intradimensional and extradimensional shifts in rat." Journal of Comparative Physiological Psychology **57**: 357-361.

Sirois, S. and Shultz, T. R. (2006). "Preschoolers out of adults: discriminative learning with a cognitive load." Quarterly Journal of Experimental Psychology **59**(8): 1357-1377.

Sloan, H. L., Good, M. and Dunnett, S. B. (2006). "Double dissociation between hippocampal and prefrontal lesions on an operant delayed matching task and a water maze reference memory task." Behavioural Brain Research **171**: 116-126.

Steckler, T., Drinkenburg, W. H. I. M., Sahgal, A. and Aggleton, J. P. (1998). "Recognition memory in rats - I. Concepts and classification." Progress in Neurobiology **54**: 289-311.

Stroop, J. R. (1935). "Studies of interference in serial verbal reaction." Journal of Experimental Psychology **18**: 643-662.

Uylings, H. B., Groenewegen, H. J. and Kolb, B. (2003). "Do rats have a prefrontal cortex?" Behavioural Brain Research **146**(1-2): 3-17.

Uylings, H. B. and van Eden, C. G. (1990). "Qualitative and quantitative comparison of the prefrontal cortex in rat and in primates, including humans." Progress in Brain Research **85**: 31-62.

Van Hest, A. and Steckler, T. (1996). "Effects of procedural parameters on response accuracy: lessons from delayed (non-) matching procedure in animals." Cognitive Brain Research **3**: 193-203.

Vertes, R. P. (2002). "Analysis of projections from the medial prefrontal cortex to the thalamus in the rat, with emphasis on nucleus reuniens." Journal of Comparative Neurology **442**(2): 163-187.

Vertes, R. P. (2004). "Differential projections of the infralimbic and prelimbic cortex in the rats." Synapse **51**: 32-58.

Wang, L., Kakigi, R. and Hoshiyama, M. (2001). "Neural activities during Wisconsin Card Sorting Test - MEG observation." Cognitive Brain Research **12**: 19-31.

Weiner, I. and Feldon, J. (1997). "The switching model of latent inhibition: an update of neural substrates." Behavioural Brain Research **88**(1): 11-25.

Weiner, I., Gal, G., Rawlins, J. N. P. and Feldon, J. (1996). "Differential involvement of the shell and core subterritories of the nucleus accumbens in latent inhibition and amphetamine-induced activity." Behavioural Brain Research **81**: 123-133.

Whyte, J., Grieb-Neff, P., Gantz and Polansky, M. (2006). "Measuring sustained attention after traumatic brain injury: Differences in key findings from the sustained attention to response task (SART)." Neuropsychologia **44**: 2007-2014.

Annexes

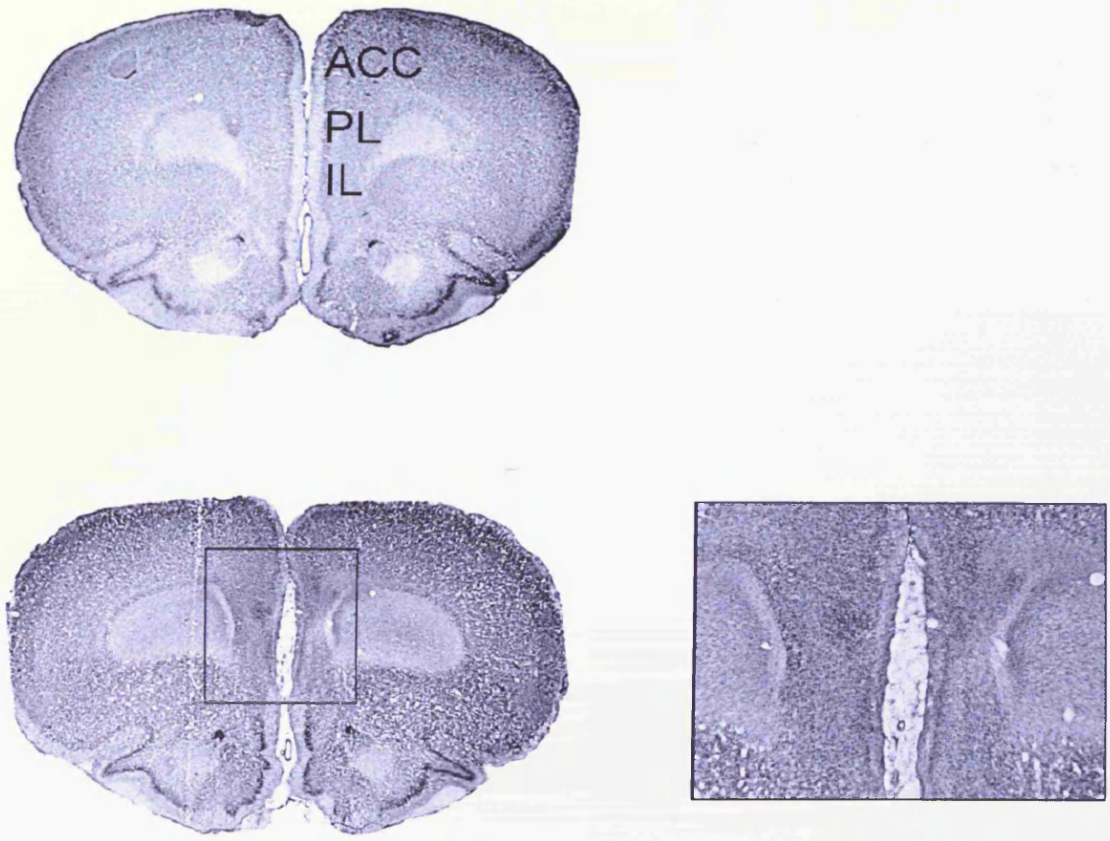

Figure A.1: Photomicrographs of bilateral sections through a Sham-lesioned (top) and mPFC-lesioned (bottom) brain at approximately 3.7 mm anterior to bregma.

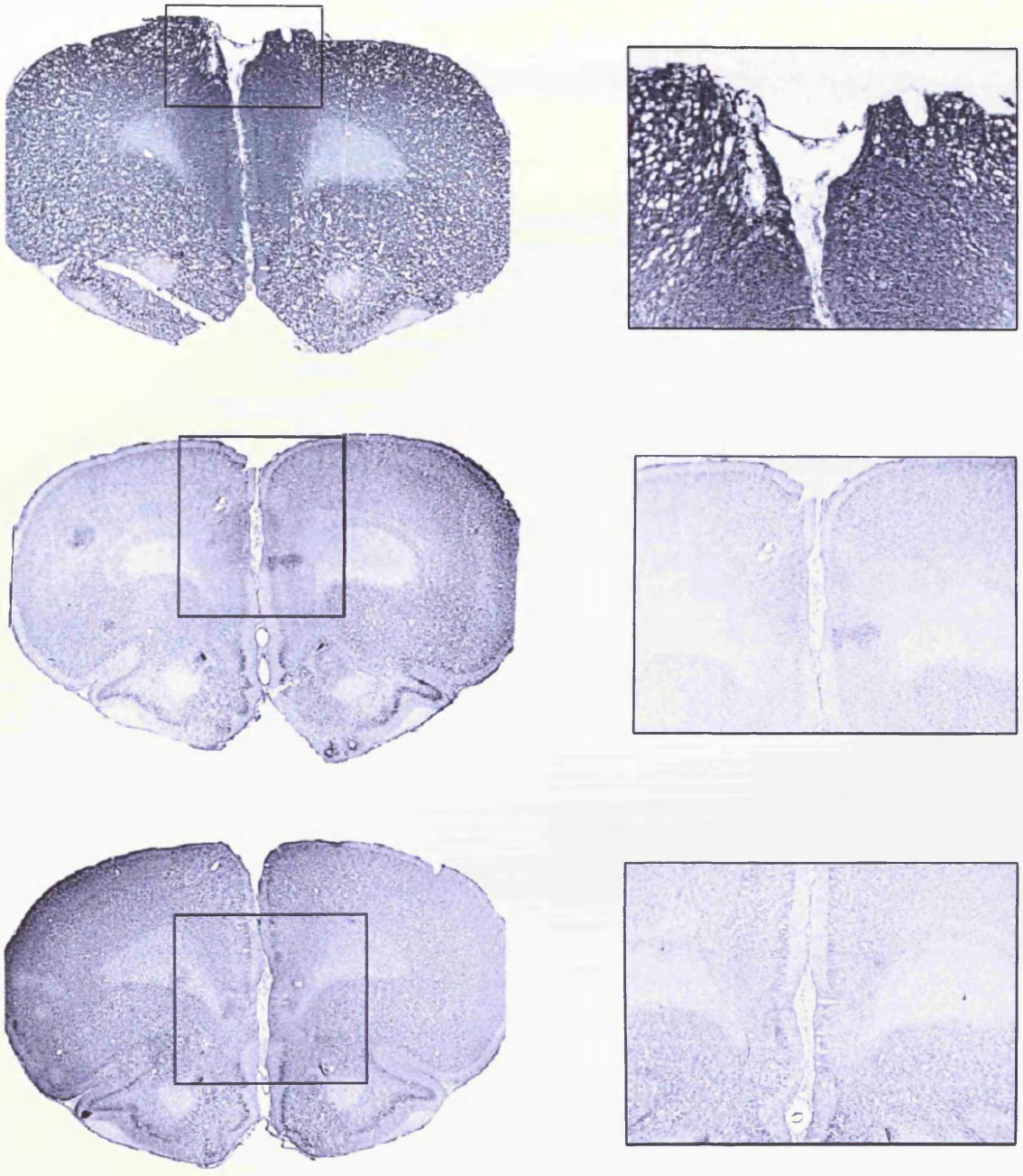

Figure A.2: Photomicrographs of bilateral sections through a ACC-lesioned (top), and PL-lesioned (middle) and a IL-lesioned (bottom) and ACC-lesioned (middle right) brain at approximately 3.2 mm, 3.7 mm and 3.2 anterior to bregma respectively.

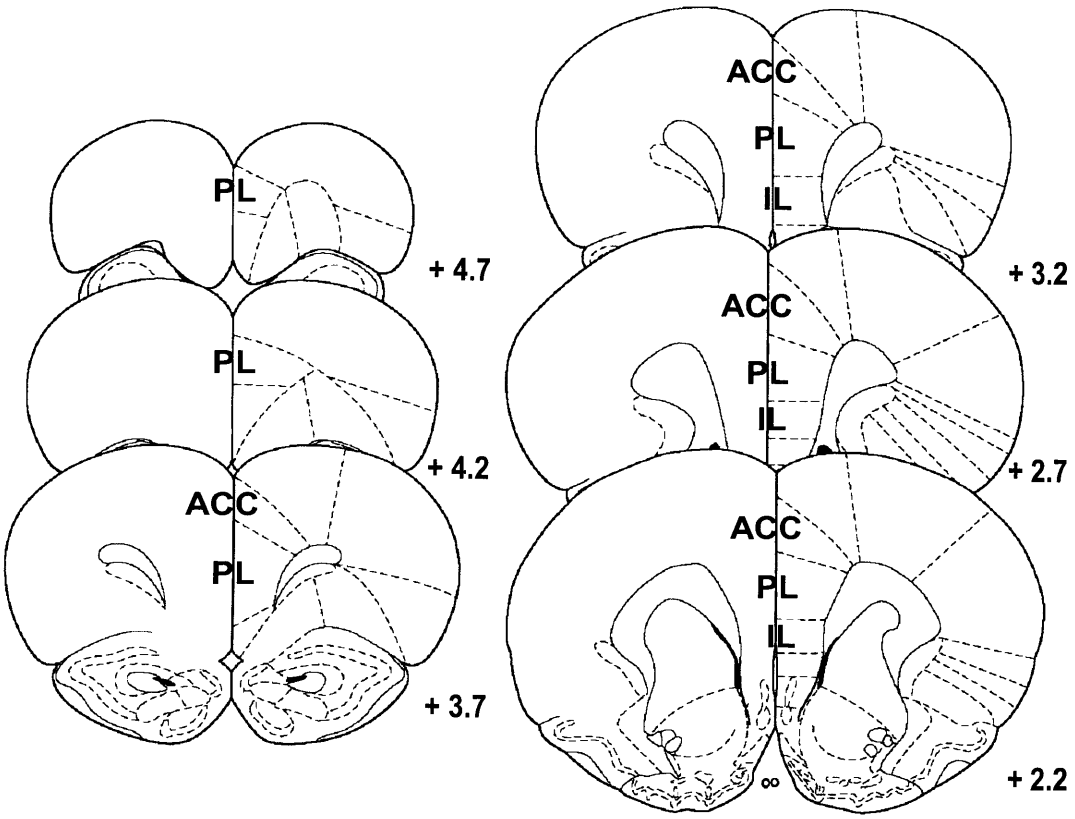

Figure A.3: Diagrams depicting the various medial prefrontal regions. Outlines are reproduced from Paxinos and Watson (1998) and represent sections ranging from 2.2 to 4.7 anterior to bregma.